GENETIC DIVERSITY AND POPULATION STRUCTURE OF COMMON BEAN (*Phaseolus vulgaris* L.) GERMPLASM FROM ETHIOPIA

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This is to certify that the thesis prepared by **Zelalem Fisseha Gebreegziabher**, entitled: **Genetic Diversity and Population Structure of Common Bean (*Phaseolus vulgaris* L.) Germplasm from Ethiopia**‘ and submitted in fulfillment for the requirements for the Degree of Doctor of Philosophy (Biology: Applied Genetics) complies with the regulations of the University and meets the accepted standards with respect to the originality and quality.

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Genetic Diversity and Population Structure of Common Bean (*Phaseolus vulgaris* L.) Germplasm from Ethiopia

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Abstract

The common bean is one of the most important diet components and cash crops in Ethiopia and Africa. However, despite having such major benefits, the production and productivity of the crop has been highly constrained by: inadequacy of improved varieties; complex myriads of biotic and abiotic constraints, and narrow genetic base of germplasm used in breeding. The multiple centers of domestication accompanied by heterogenous farming practices adopted by farmers, ever since its introduction to Ethiopia, and recent variety development projects have resulted in a range of morphologically diverse landraces via the preservation and exploitation of useful alleles. To this end, assessing the morphological and molecular diversity; and population structure of common bean landrace accessions in Ethiopia is sine qua non vis-à-vis establishing an efficient breeding and conservation scheme, nationally.

Genetic diversity and population structure of 125 common bean accessions were studied using agro-morphological and SSR DNA markers. The morphological diversity assessment was conducted in the main rainy season of 2013 at Melkassa Agricultural Research Center, main research station, Ethiopia. The molecular diversity study was conducted from August 2012 to February 2013 at the lab of the BecA-ILRI hub, Nairobi, Kenya, using 24 fluorescent SSR markers.

Higher and significant genetic variability among accessions was evidenced, with respect to 8 quantitative and 11 qualitative agro-morphological traits. On the other hand, results of the association analyses revealed that 100-seed weight and seed diameter had positively-significant correlation and higher positive direct effects on the seed yield of the common bean accessions. Hence, these two traits, 100-seed weight and seed diameter, were recommended, as important traits to be used in the indirect selection of high-yielding common bean cultivars, in conjunction with seed yield. On the other hand, results of the morphological (phenotypic) diversity analyses revealed that both the Tocher and Neighbor-joining clustering methods identified similar five clusters with almost identical members in each. Of these, three and two clusters were predominated by Andean and Mesoamerican accessions, respectively. However, a comparable proportion of accessions had intermediate features between the two gene pools, suggesting the significant presence of inter-gene pool introgressions. The Mahalanobis (D^2) analysis of the five clusters indicated mostly significant differences between all the combinations of cluster pairs. This indicated there may be a great opportunity to obtain transgressive segregants and maximum heterosis in future common bean breeding programs. With regards to the molecular genetic diversity using SSR markers among the common bean accessions sampled from different geographical regions, it indicated that most allelic parameters had values comparable to previous similar study results. Specifically, the geographical populations, ‗Amhara' and SNNP had the highest number of effective alleles; Shannon's diversity index (I); and heterozygosity values. Furthermore, results of Analysis of Molecular Variance (AMoVA) indicated that the greater proportion of the genetic variation was explained between individuals from different populations (58%) and between individuals within the same population (40%).

In comparison, only 2% of the genetic variation was due the variation among the populations themselves. In addition, the calculated F_{st} value was small (F_{st}=0.015), associated with a high gene flow value (N_m=16.282), indicating lower differentiation of the populations, which, in turn, implied significant exchange of planting materials among farmers in the studied populations. Finally, Neighbor-Joining (NJ) cluster and Principal Coordinate analyses (PCoA) revealed that accessions from different collection sites tended to cluster together, probably owing to the high gene flow among the populations. Moreover, five groups of clusters were identified in the NJ dendrogram.

In addition to the aforementioned, analysis of structure of genetic diversity of the Ethiopian common bean accessions was undertaken with respect to the Andean and Mesoamerican gene pools of origin. Results indicated at cluster K=2, accessions separated into the Mesoamerican and Andean gene pools. In view of this, the number of accessions from the Mesoamerican gene pool was higher than that of their Andean counterparts. Moreover, STRUCTURE identified K=5, as the optimum cluster number. At K=5, five clusters: three from the Andean gene pool; and two from the Mesoamerican gene pool were identified. On the other hand, based on calculations of hybrid/non-hybrid accessions (from membership coefficient values), 72 out of the 125 accessions were found to be inter-gene pool introgressions. Moreover, results of NJ cluster analysis and PCOA done with the remaining 55 non-hybrid accessions identified at K=5 revealed common bean genetic diversity in Ethiopia, as organized into the Andean and Mesoamerican gene pools. This was exhibited by the clustering of accessions with either of the Andean or Mesoamerican control genotypes. The other peculiar event was mixed membership of Andean/Mesoamerican accessions in some of the clusters. Finally, yet equally importantly, Principal Component Analysis (PCA); stepwise discriminant and canonical correlation analyses; and data recorded in some agro-morphological traits distinguishing the Andean and Mesoamerican gene pools were used in conjunction, in order to determine the identities of the five cluster groups, identified at K=5 of the molecular structure analysis into the ecogeographic races of the Andean or Mesoamerican gene pools. To this end, PCA, done with the 125 accessions identified at K=2 of the molecular analysis, revealed that Mesoamerican and Andean groups of accessions separated along the first Principal Component (PC) axis. Nonetheless, several accessions occupied intermediate positions between the Andean and Mesoamerican control genotypes, which supported the presence of inter-gene pool introgressions detected in both the morphological and molecular analyses. Furthermore, stepwise discriminant and canonical correlation analyses, using the non-hybrid accessions at the molecular analysis STRUCTURE preset K=5, displayed that there was fair level of separation among the Andean and Mesoamerican cluster groups, with some overlaps. Ultimately, the identities of the five cluster groups identified in the molecular structure analysis were determined. In line with this, it was concluded that Ethiopian common bean accessions from the Andean gene pool had a broader base, belonging to ecogeographic races in the gene pool. In other words, the three Andean cluster groups were found to be from two of the three races in the Andean gene pool-‗Nueva Granada' and ‗Peru', whereas the Mesoamerican accessions had a narrower genetic base, belonging only to race ‗Mesoamerica' in the same gene pool.

In summary, results of the present study showed that common bean landrace genotypes in Ethiopia had adequate genetic diversity, organized in to the Andean and Mesoamerican gene pools. Importantly, this rich genetic diversity should be harnessed in order to maximize genetic variability in future common bean improvement programs in Ethiopia. On the other hand, the significant presence of inter-gene pool introgressions, by itself, can be used as opportunity, for such introgressions were previously reported to be rich in many adaptation and nutritional genes. This, in turn, can be used as an advantage in prospective common bean breeding works. On the other hand, the higher/significant variations among accessions vis-à-vis qualitative and quantitative agro-morphological traits should be directed towards broadening genetic variability and developing transgressive genotypes, excelling their parents in relation to these traits.

Key words: *AMoVA, Genetic Diversity, landrace, accessions NJ, PCA, PCoA, Population Structure,STRUCTURE,*

Dedication

This thesis is dedicated to my parents, who, through their relentless hard work and inspiration, have shown me the right path.

About the author

Zelalem Fisseha was born in January 23, 1979 in Addis Ababa. He, first, went to Hale Luya Elementary and Junior Highschool. Thenafter, he attended the Entoto Academic, Technical, and Vocational School to pursue his highschool education. In September 1996, he jouned the then Alemaya University, and graduated with a B.Sc in Plant Sciences in July 2000. After attaining his undergrad education, he was employed by the then Ethiopian Agricultural Research Organization (EARO) in April 2001. During his tenure as junior researcher from April 2001 to September 2002, he served at the positions of Head of the Lowland Pulses and Oilcrops Research Departments at Pawe Agricultural Research Center. In September 2002, he started his graduate studies at Alemaya University, and graduated with an M.Sc degree in Plant Breeding in July 2005. Upon completing his graduate education, he went back to Pawe Research Center, where he served at various demanding responsibilities, including the post of the National Rice Research Project Leader and Crop Research Department Head. In May 2006, he joined the Somali Region Pastoral and Agro-pastoral Research Institute (SoRPARI), where he has been serving as a senior researcher and regional crop research director to date. Subsequently, he joined the Department of Microbial, Cellular, and Molecular Biology in Addis Ababa University (AAU) to pursue his PhD education in Applied Genetics. In July 2015, he completed his PhD education. During his research and graduate/post-graduate education career, he has produced various journal manuscripts in local and international journals. Moreover, he has developed various rice; common bean; and soybean improved varieties

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Acronyms and abbreviations

ACOS: Agricultural Commodity Supply;
AFLP: Amplified Fragment Length Polymorphism;
AMoVA: Analysis of Molecular Variance;
ANoVA: Analysis of Variance;
asl: above sea level;
C.V.: Coefficient of Variation;
CACC: Central Agricultural Census Commission;
CIAT: International Center for Tropical Agriculture
CSA: Central Statistics Agency;
CTAB: Cetyltriethylammonium bromide;
DAP: Diammonium phosphate;
df: degrees of freedom;
DTF: Days to Flowering;
EIAR: Ethiopian Institute of Agricultural Research;
EIB: Ethiopian Institute of Biodiversity;
ENBRP: Ethiopian National Bean Research Project;
ESE: Ethiopian Seed Enterprise;
G x E: Genotype by Environment;
GD: Genetic Distance;
HSW: Hundred Seed Weight;
IBPGR: International Board for Plant Genetic Resources;
ISSR: Inter Simple Sequence Repeats;
MAS: Marker Assisted Selection;
MS: Mean Square;
MSD: Mean Seed Diameter;
NBPT: Number of Branches/plant;
NJ: Neighbor-Joining;
NoPP: Number of Pods per Plant;
NoSPPL: Number of Seeds per Plant;
NoSPPO: Number of Seeds per Pod;

PC: Principal Component Axis;

PCA: Principal Component Analysis;

PCoA: Principal Coordinate Analysis;

PGR: Plant Genetic Resources;

PHT: Plant Height;

PIC: Polymorphic Information Content;

PLYLD: Plot Yield;

PrinComp: Principal Component;

PVPP: PolyVinyl PolyPyrrolidone

RAPD: Random Amplified Polymorphic DNA;

RFLP: Restricted Fragment Length Polymorphism;

RVG: Released Varieties Group;

SAS: Statistical Analysis System;

SDIA: Seed Diameter;

SH: Seed Height;

SNNP: Southern Nations Nationalities and Peoples Regional State;

SS: Sum of Squares;

SSR: Simple Sequence Repeats;

UPGMA: Unweighted Pair-Group Method;

1 Introduction

1.1 Background and justification

The genus *Phaseolus,* which is comprised of some 70 species (Freytag and Debouck, 2002), has contributed to human welfare with five cultigens domesticated in pre-Columbian times: the common bean (*P. vulgaris*); the year bean (*P. dumosus* Macfad.); the runner bean (*P. coccineus* L.); the tepary bean (*P. acutifolius* A. Gray-); and the lima bean (*P. lunatus* L.). The common bean is the most widely distributed and consumed species of the genus *Phaseolus*, It is seed-propagated, a true diploid (2n=22) and has a relatively small genome (650 Mb) (Broughton et al., 2003). Originating in the Neo-Tropics, common bean was domesticated in at least two major centers in Mesoamerica and the Andes (Gepts, 1988), and, possibly in a third minor center in the Northern Andes (Islam et al., 2002).

Common bean, harvested as dry grain, is one of the most important food grain legumes in Eastern and Southern Africa, occupying more than 4 million ha annually, and providing food for more than 100 million people (Wortmann et al., 1998; Asfaw et al., 2009). As a source of dietary protein, it is the second most important food crop in Africa next to faba bean (Broughton et al., 2003). Moreover, it is the third most important source of calories for lower income African households, after cassava and maize (Broughton et al., 2003; Asfaw et al., 2009). Out of the total production in Sub-Saharan Africa, 62% of the production in 2005 originated in the Eastern African countries of Burundi, DR Congo, Ethiopia, Kenya, Rwanda, Tanzania, and Uganda, making this the most important region for the crop within the continent (Asfaw et al., 2009). It became established as a food crop in Africa, before the colonial era (Allen et al., 1989). It is assumed that common bean was introduced to Ethiopia in the 16th century by the Portuguese and has, since then, become an important component of the human diet in the same (Imru, 1985; Zelalem, 2005). Moreover, it is among the most important food legumes produced in Ethiopia (Amare, 1987; Zelalem, 2005; Kassaye, 2006). In addition, it has become an export crop for more than 40 years with a rapidly increasing export value, highlighting its importance among major pulse crops (Ferris and Kaganzi, 2008; Asfaw et al., 2009).

Two geographic centers of domestication are reported in relation to bean, namely the Mesoamerican and Andean centers. The multiple centers of domestication of the crop have endowed it with relatively high diversity that is broadly classified into two gene pools, Mesoamerican and Andean (Gepts and Bliss, 1986; Singh et al., 1991a, b). Ever since its introduction into Ethiopia, farmers have developed farming practices adapted to local conditions by preservation and exploitation of useful alleles, which have resulted in a range of morphologically diverse landraces (Wortmann et al., 1998; Sperling, 2001; Kwak and Gepts, 2009). Moreover, recent efforts of regional/national bean-breeding programs in Ethiopia targeted towards improving on-farm productivity have resulted in the continuous introduction of new germplasm from different parts of the world, since the 1980s (Asfaw et al., 2009; CIAT, 2009). The existence of both gene pools (Andean and Mesoamerican) in Africa has furthermore been documented (Martin and Adams, 1987; Asfaw et al., 2009) and probably is a result of original introductions and subsequent imports of novel germplasm. Given the wide range of landraces on the continent, East Africa is considered a secondary center of diversity for the common bean (Purseglove, 1968; Allen and Edje, 1990; Wortmann et al., 1998; Sperling, 2001; Asfaw et al., 2009).

Ethiopia is among the major bean producers in Sub-Saharan Africa. However, national average bean yield is still lagging behind the global average. This can be attributed largely to low-yielding capacity of cultivars under use, biotic/abiotic stresses, and low soil inorganic nutrients (Habtu, 1990; Zelalem, 2005; Asfaw et al., 2009). To this end, it is essential to tap the potential of landrace genetic resources in order to introgress novel genes of adaptation; resistance; and tolerance. Landraces grown by small farmers and collected by plant exploration missions are abundant and rich sources of valuable genes and genetic diversity (Mondini et al., 2009). Nevertheless, information on their origin, pedigree, and other characteristics is usually not known and their identity and difference from the previously obtained genotypes is also questionable. The occurrence of duplicates within the maintained collection cannot be excluded either. To this end, the knowledge about the extent of genetic diversity, identification, differentiation, and characterization of genotypes and populations, respectively, provides information for the detection of duplicates in the collection, their effective extension, a better

characterization and utilization in breeding (Horňáková et al., 2003; Mondini et al., 2009). Even though the rich genetic diversity of common beans in Ethiopia has been frequently reported (Purseglove, 1968; Kassaye, 2006; Asfaw et al., 2009), the controversy remains whether the observed variations in and between landraces are the results of the original differences between the various introductions or were brought about by a continuous process of natural hybridization and selection by farmers and the environment (Blair et al., 2011). Moreover, it is not clear, if gene flow within and between gene pools and races via spontaneous out-crossing in farmers‘ fields or crossing programs in formal breeding could result in intermediate phenotypes that do not correspond well to any of the single race or gene pool divisions (Beebe et al., 2001; Islam et al., 2002; Díaz and Blair, 2006; Blair et al., 2007; Blair et al., 2009).

Classical methods of estimating diversity among groups of plants have relied chiefly upon morphological characters, which still play a central role in the analysis of genetic variability in crop species and their relatives (Newbury and Ford-Lloyd, 1997; Islam et al., 2002). However, because of the strong environmental influence on morphological traits, mainly on those of a quantitative nature, new techniques which analyze diversity at biochemical or molecular level have been developed (Karp et al., 1997) and successfully applied in evolutionary and diversity studies of different crops (Gepts, 1993; Bretting and Widrlechner, 1995). Molecular techniques are more expensive than most morphological approaches to the study of genetic or species diversity (Newbury and Ford-Lloyd, 1997; Mohammadi and Prasanna, 2003), and consequently they should be used only where other techniques are less powerful or not feasible (Bisby, 1995). Molecular analyses in conjunction with morphological and agronomic evaluation of germplasm are recommended because these provide complementary information and increase the resolving power of genetic diversity analyses (Gomez et al., 2004; Mondini et al., 2009) as molecular markers often represent neutral evolution and, hence, actual genetic distances (Paredes and Gepts, 1995; Gomez et al., 2004; Mondini et al., 2009).

Although morphological features are indicative of genotypes, they are represented by only a few loci, because there are not a large enough number of characters available

(Islam et al., 2005). Moreover, they can also be affected by environmental factors and growth pattern. Consequently, it may be erroneous to completely rely on them, per se (Ullah et al., 2010). According to Mondini et al. (2009), molecular markers may or may not correlate with phenotypic expression of a genomic trait. Nonetheless, they offer numerous advantages over conventional, phenotype-based alternatives, as they are stable and detectable in all tissues regardless of growth, differentiation, development, or defense status of the cell. Additionally, they are not confounded by environmental, pleiotropic and epistatic effects (Ullah et al., 2010)

Molecular markers based on PCR amplification are efficient tools for plant breeding programs (Powell et al., 1995; Gupta and Varshney, 2000). Different molecular marker technologies have been developed, including Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSRs), and Inter-Simple Sequence Repeats (ISSRs). Microsatellites or Simple Sequence Repeats (SSR) are sets of repeated sequences found within eukaryotic genomes (Dietrich et al., 1992; Bell and Ecker, 1994; Morgante and Olivieri, 1993). These consist of sequences of repetitions, comprising basic short motifs generally between 2 and 6 base-pairs long. Polymorphisms associated with a specific locus are due to the variation in length of the microsatellite, which in turn depends on the number of repetitions of the basic motif. Variations in the number of tandemly-repeated units are mainly due to strand slippage during DNA replication where the repeats allow matching via excision or addition of repeats (Schlotterer and Tautz, 1992). As slippage in replication is more likely than point mutations, microsatellite loci tend to be hypervariable. Microsatellite assays show extensive inter-individual length polymorphisms during PCR analysis of unique loci using discriminatory primers sets. Microsatellites are highly popular genetic markers as they possess: co-dominant inheritance, high abundance, enormous extent of allelic diversity, ease of assessing SSR size variation through PCR with pairs of flanking primers and high reproducibility (Mondini et al., 2009). These are ideal genetic markers for detecting differences between and within species of genes of all eukaryotes (Farooq and Azam, 2002; Jonah et al., 2011). Due to the aforementioned merits microsatellite (SSR) markers do possess, they have been successfully used in the genetic analysis of the

common bean worldwide, and have become first choices in discerning the diversity of common bean genotypes so far (Asfaw et al., 2009; Kwak and Gepts, 2009; Blair et al., 2010; Blair et al., 2011). According to Kwak and Gepts (2009), microsatellite markers are more polymorphic (Blair et al. 2006) than markers used earlier to characterize genetic diversity, such as phaseolin seed protein (Gepts et al., 1986), allozymes (Koenig and Gepts, 1989; Singh et al., 1991c), RFLP (Becerra Vela´squez and Gepts, 1994), and RAPD (Freyre et al., 1996). They are also more widely distributed in the bean genome (Freyre et al., 1998; Blair et al. 2003). In common bean, around 400 microsatellite markers have been developed and mapped (Yu et al., 2000; Gaitán-Solí´s et al., 2002; Blair et al., 2003;Masi et al., 2003; Yaish and Pérez de la Vega, 2003; Guerra-Sanz, 2004; Caixeta et al., 2005; Buso et al., 2006). Even though, molecular markers, such as SSR markers have been proven very useful in plant genetic studies, they, themselves, have their own drawbacks (such as, often no correlation with phenotypic markers). Hence, molecular analyses in conjunction with morphological and agronomic evaluation of germplasm are recommended, because they provide complementary information and increase the resolving power of genetic diversity analyses (Gomez et al., 2004).

Understanding the pattern and level of genetic diversity of bean landraces and cultivars (accessions) and their relationships with the Andean and Mesoamerican gene pools can therefore shed light on the level of gene flow, and eventually be of paramount importance to future bean breeding and conservation initiatives in Ethiopia. Furthermore, the systematic study of identifying the ecogeographic races present among Ethiopian landraces may be of paramount importance in shedding light on the structure of genetic diversity for common bean germplasm of Ethiopia. To this end, integrating phenotypic evaluation with molecular marker diversity assessment is a useful tool to ascertain whether the subpopulations identified with phenotypic markers will also be distinguished in the molecular analysis or vice versa (Burle et al., 2011). However, the works on genetic diversity and population structure of Ethiopian landrace common bean genotypes have been so far limited to a few genotypes and use of only molecular markers. Consequently, the present study was proposed with the following general/specific objectives.

1.2 General objective

- Determine the genetic diversity within and between common bean germplasm of Ethiopia; discern their corresponding population structure with respect to the Andean and Mesoamerican gene pools using morphological and molecular markers.

1.3 Specific objectives

- Determine the variability of common bean landrace germplasm of Ethiopia with respect to important qualitative and quantitative traits;
- Assessing the association of yield with yield-related and component traits to recommend useful indexes for indirect selection in common bean breeding/improvement in Ethiopia;
- Determine the morphological diversity within and between common bean accessions of Ethiopia with phenotypic markers;
- Determine/assess the genetic diversity within and between common bean accessions from Ethiopia with microsatellite (SSR) DNA markers;
- Determine the populations structure of genetic diversity in Ethiopian common bean (*Phaseolus vulgaris* L.) germplasm in relation to the Mesoamerican and Andean gene pools for backing up conservation and use strategy;
- Determining the presence/identity of ecogeographic races of the Mesoamerican and Andean gene pools in Ethiopian common bean landrace accessions towards delineating scope, gaps, and future breeding/conservation needs vis-à-vis genetic diversity in Ethiopia; and
- Determine to what extent the subpopulations identified at the molecular level could also be distinguished at the morphological and agronomic levels, and ascertain the synchrony between the cluster groups identified by the morphological and molecular analyses.

2 Literature Review

2.1 Genetic diversity analysis: essence, applications and uses

The importance of plant genetic resources to humanity stems from their use as a source of genetic material to develop crops and medicinal plants fundamental to world population‘s nutrition and health. These resources also fulfill vital ecosystem functions related to soil structure, nutrient cycles, hydrological flows, agro-ecosystem stability, and defense against pests and diseases (Saad et al., 2013).

Understanding the molecular basis of the essential biological phenomena in plants is crucial for the effective conservation, management, and efficient utilization of plant genetic resources (Mohammadi and Prasanna, 2003). In particular, an adequate knowledge of existing genetic diversity, where in plant population it is found and how to best utilize it, is of fundamental interest for basic science and applied aspects like the efficient management of crop genetic resources. The improvement of crop genetic resources is dependent on continuous infusions of wild relatives, traditional varieties and the use of modern breeding techniques. These processes all require an assessment of diversity at some level, to select resistant, highly productive varieties (Mondini et al., 2009).

The assessment of genetic diversity within and between populations is routinely performed at the molecular level using various laboratory-based techniques, such as allozyme or DNA analysis, which measure levels of variation directly. Genetic diversity may be also gauged using morphological, and biochemical characterization and evaluation:

i. Morphological characterization does not require expensive technology, but large tracts of land are often required for these experiments, making it possibly more expensive than molecular assessment. These traits are often susceptible to phenotypic plasticity; conversely, this allows assessment of diversity in the presence of environmental variation.
ii. Biochemical analysis is based on the separation of proteins into specific banding patterns. It is a fast method which requires only small amounts of biological

material. However, only a limited number of enzymes are available and thus, the resolution of diversity is limited.

iii. Molecular analyses comprise a large variety of DNA molecular markers, which can be employed for analysis of variation. Different markers have different genetic qualities (they can be dominant or co-dominant, can amplify anonymous or characterized loci, can contain expressed or non-expressed sequences, etc.) (Mondini et al., 2009).

The importance of genetic diversity/relationship between crop plants can be explained by the fact that it serves to provide information about genetic diversity, and is a platform for stratified sampling of breeding populations (Mohammadi and Prasanna, 2003). Accurate assessment of the levels and patterns of genetic diversity can be invaluable in crop breeding for diverse applications including: analysis of genetic variability in cultivars (Smith, 1984; Cox et al., 1986; Mohammadi and Prasanna, 2003); identifying diverse parental combinations in the selection of segregating progenies to harness the prevailing maximum genetic variability (Barrett and Kidwell, 1998; Mohammadi and Prasanna, 2003); and introgressing desirable genes from diverse germplasm into the available genetic base (Thompson et al., 1998; Mohammadi and Prasanna, 2003). Determination of genetic relationships among inbred lines or pure lines can be particularly useful in planning crosses, in assigning lines to specific heterotic groups, and for precise identification with respect to plant varietal protection (Hallauer and Miranda, 1988; Mohammadi and Prasanna, 2003). Furthermore, genetic diversity analysis in germplasm collections can facilitate reliable classification of accessions and identification of subsets of core accessions with possible utility for specific breeding purposes. More recently, significant emphasis is being paid to comprehensive genetic diversity analysis in numerous crops, including major field crops such as wheat (*Triticum aestivum* L.) (Barrett et al., 1998), rice (*Oryza sativa* L.) (Xiao-ping et al., 2007), maize (*Zea mays* L.) (Ajmone-Marsan et al., 1998; Lubberstedt et al., 2000), barley (*Hordeum vulgare* L.) (Russell et al., 1997), and soybean (*Glycine max* (L.) Merr.) (Thompson et al., 1998).

Study of genetic diversity is the process by which variation among individuals or groups of individuals or populations is analyzed by a specific method or a combination of methods. The data often involve numerical measurements and in many cases, combinations of different types of variables. Diverse data sets have been used by researchers to analyze genetic diversity in crop plants; most important among such data sets are pedigree data (Bernardo, 1993; Messmer et al., 1993; van Hintum and Haalman, 1994), passport data-morphological data (Smith and Smith, 1992; Bar-Hen et al.,1995), biochemical data obtained by analysis of isozymes (Hamrick and Godt, 1997) and storage proteins (Smith et al., 1987), and, recently, DNA-based marker data that allow more reliable differentiation of genotypes. Since each of these data sets provide different types of information, the choice of analytical method(s) depends on the objective(s) of the experiment, the level of resolution required, the resources and technological infrastructure available, and the operational and time constraints, if any (Karp et al., 1997, Mohammadi and Prasanna, 2003).

2.2 The common bean crop

2.2.1 Evolutionary origin and domestication

The genus *Phaseolus* originated in the American continent and a large number of its species is found in Mesoamerica (Delgado-Salinas, 1985; Freytag and Debouck, 2002). The genus is a member of the tropical tribe Phaseoleae, which also includes cowpea, pigeon pea, and soybean. The Phaseoleae tribe is part of the Phaseoloid-Millettioid clade, and; diverged some 45–50 million years ago from the Hologalegina clade, which contains most temperate crop legumes, such as pea, alfalfa, chickpea, and lentil (Lavin et al., 2005). Synteny between *Phaseolus* and other legumes is negatively correlated with phylogenetic distance (Gepts et al., 2008). Thus, the highest synteny levels are observed with the genus *Vigna* (cowpea and mung bean), followed by the genus *Glycine* (soybean), and distantly, the Hologalegina clade (Boutin et al., 1995; Lee et al., 2001; Choi et al., 2004;Yan et al., 2004; Moffet and Weeden, 2006). For example, the region marked by the Bng122-D0140-Bng171-Bng173 markers on linkage group Pv01 of common bean is syntenic with a region on LG-G of soybean. This region harbors a cluster of disease and

nematode resistance genes (Freyre et al., 1998; Foster-Hartnett et al., 2002; Kelly et al., 2003).

The intra-specific genealogy and geographical origins of the common bean are now well understood (Gepts, 1998; Chacón et al., 2005), as is the phylogeny of the genus *Phaseolus* (Delgado-Salinas et al., 1999) and the centers of primary diversification in America (Debouck, 1986; Kami et al., 1995; Chacón et al., 2005).

Studies of the evolution of common bean have uncovered several noteworthy features that are of interest to other crop plants. Unique among crop plants, common bean consists of two geographically distinct, evolutionary lineages (Andean and Mesoamerican) that predate domestication and trace back to a common, still extant ancestor located in Ecuador and northern Peru (Debouck et al., 1993; Kami et al., 1995). Patterns of marker diversity and virulence in pathogens and *Rhizobium* parallel those in the bean host, suggesting host-microbe co-evolution (Guzmán et al., 1995; Geffroy et al., 2000; Kelly and Vallejo, 2004; Araya et al., 2004; Mkandawire et al., 2004; Aguilar et al., 2004). Geffroy et al. (2000) have shown that Andean and Mesoamerican resistance specificities appeared in the same, presumably ancestral gene cluster.

The inheritance of the domestication syndrome in common bean was the second among all crop plants and the first one in the legumes to be investigated (Koinange et al., 1996). The traits involved, such as growth habit (e.g., determinacy), photoperiod sensitivity and phenology, pod and seed size, and seed color, were not only important in domestication, but remain crucial agronomic traits determining farmer and consumer acceptability. The higher polymorphism in common bean relative to soybean is presumably related to its diversification into the two geographic gene pools in the Andes and Mesoamerica, mentioned earlier (Gepts et al., 2008).

2.2.2 Organization of genetic diversity of *Phaseolus* species

Phaseolus is a diploid genus with most species having 2n=2x=22 chromosomes (some species have 2n=2x=20) (Gepts, 2001). The genome size of *P. vulgaris* (580Mbp/haploid

genome) is comparable to that of rice (490Mbp/haploid genome) (Bennett and Leitch, 2005). In common bean, the levels of duplication and the amount of highly repeated sequences are generally low. Mapping experiments demonstrated that most loci are single copy (Vallejos et al., 2006; Freyre et al., 1998; McClean et al., 2002). Gene families tend to be small, and the traditionally large families such as resistance gene analogs (Rivkin et al., 1999) and protein kinases (Vallad et al., 2001) are of moderate size. Further experiments are needed, however, to confirm these conclusions and compare these results to those of other legumes.

Debouck (1991, 1999) and Debouck and Smartt (1995) discussed the taxonomy and phylogenetic relationship among members of the *Phaseolus* species in relation to the common bean. Freytag and Debouck (2002) described in considerable detail the taxonomy, distribution, and ecology of the genus *Phaseolus* in North America, Mexico, and Central America. However, genetic diversity among *Phaseolus* species is organized into primary, secondary, and tertiary gene pools, based on the ability to cross with the common bean (Miklas and Singh, 2007; Porch et al. 2013). The primary gene pool of each species comprises both the wild populations (i.e., the immediate ancestor of cultivars) and cultivars. *P. coccineus* L. (scarlet runner), *P. costaricensis* Freytag & Debouck, and *P. polyanthus* Greenman (synonymous with *P. dumosus*, year-long bean) form the secondary gene pool. The tertiary gene pool comprises *P. acutifolius* A. Gray (tepary bean) and *P. parvifolius* Freytag. Lima bean (*P. lunatus* L.) and other species compose the quaternary gene pool. However, in addition to the common bean, only Lima, scarlet runner, tepary, and year-long bean are cultivated (Ullah et al., 2010).

2.2.3 The two gene-pool concept- the Andean and Mesoamerican gene pools- in the common bean

The wild common bean has been documented to be the ancestral origin of the cultivated common bean (*Phaseolus vulgaris* L.), based on archaeological, morphological, and biochemical (phaseolin seed protein and allozymes) evidence (Miranda Colin, 1967; Gentry, 1969; Gepts et al., 1986; Briicher, 1988; Delgado-Salinas et al., 1988; Kaplan and Kaplan, 1988; Gepts, 1988, 1990; Gepts and Bliss, 1986; Singh et al., 1991a, b;

Gepts and Debouck, 1993; Blair et al., 2011; Burle et al., 2011). According to Singh et al. (1991a) both wild populations and cultivated forms are self-pollinating, and they hybridize with each other easily, producing viable and fertile individuals. Nonetheless, some reproductive isolation has been reported with respect to both the wild and domesticated forms in the two gene pools (Burle et al., 2011). Common bean was domesticated independently in the southern Andes and Mesoamerica, resulting in two distinct major domestication gene pools in the Andes and Mesoamerica, respectively (Burle et al., 2011).

Acosta-Gallegos et al. (2007) noted that since wild progenitors are the foundation of landraces and landraces are the foundation of modern cultivars, it is important that breeders and conservationists define the sites of domestication. Furthermore, multiple domestications in time and space have been one of the key determinants in shaping the diversity observed in modern crops (Chacón et al., 2005). At least two domestication events occurred in the Americas with regards to the common bean, with an area bordering the states of Jalisco and Guanajuato as the areas where Mesoamerican beans were domesticated (Gepts et al., 1986; Gepts, 1988b). This suggestion was based on the similarity in phaseolin, a reserve protein found in the seed, between wild and domesticated beans from the area. Long before, this region has been recognized as an area of abundant genetic variation in both wild and landrace populations (Miranda-Colin, 1967; Gentry, 1969). Gepts et al. (1986), with phaseolin, and Chacón et al. (2005), with chloroplast sequences, have shown that domestication led to a reduction in genetic diversity. This suggests a founder effect probably due to a strong selection pressure practiced by humans during domestication and the fact that only few wild bean populations were included in the process. Therefore, a large amount of genetic variation is still untapped in the wild form of *P. vulgaris*, whose variation could be used to improve and enhance the diversity in the domesticated form. Chacón et al. (2005) also suggested that there were actually multiple domestications in the Mesoamerican gene pool based on the existence of four major cpDNA haplotypes. However, their data are inconsistent with phaseolin seed protein (Gepts et al., 1986; Gepts, 1988a) and amplified fragment length polymorphism (AFLP) data (Papa and Gepts, 2003). Instead, their data could be

explained by post-domestication capture of chloroplast DNA caused by gene flow from domesticated to wild-types (Papa et al., 2005).

Subsequently, several investigations along the distribution area of wild *P. vulgaris* have shown the existence of gene flow between wild and domesticated beans in spite of the fact that common bean is a predominantly self-pollinated species (Acosta-Gallegos et al., 1994; Beebe et al., 1997; Papa and Gepts, 2003; Papa et al., 2005; Payró de la Cruz et al., 2005; Zizumbo-Villarreal et al., 2005; Acosta-Gallegos et al., 2007). These investigations have shown that gene flow is widespread and has altered the distribution of genetic diversity among wild and domesticated populations. Gene flow takes place predominantly from domesticated to wild-types (Papa and Gepts, 2003) over short distances (<100m; Payró de la Cruz et al., 2005). As a consequence of this gene flow, genetic diversity in wild populations tends to be displaced by that in cultivars, potentially leading to a reduction in genetic diversity (Papa et al., 2005).

Since the 1980s, inter-gene pool reproductive barriers have been described that cause difficulties in crossing between gene pools; that is, reduced viability and fertility (Singh and Gutierrez, 1984; Gepts and Bliss, 1985; Koinange and Gepts, 1992; Acosta-Gallegos et al., 2007), a situation that suggests that the gene pools have reached the subspecies level (Becerra-Velásquez and Gepts, 1994; Acosta-Gallegos et al., 2007). In contrast, crosses between wild and domesticated common bean within each gene pool are compatible (Koinange and Gepts, 1992). The crossing of distinct cultivars from different gene pools is pursued to widen the reduced variation observed in the domesticated form (Voysest et al., 1994; Singh, 2001), particularly in those cases where breeders have used a limited set of related elite parents within the same seed commercial class (Acosta-Gallegos et al., 2007).

Singh et al. (1991a) further divided the Andean and Middle American gene pools into six races-three Andean gene pool (all large-seeded): Chile, Nueva Granada, and Peru races; and three Middle American gene pool: Durango (medium-seeded semi-climber), Jalisco (medium-seeded climber), and Mesoamerica (all small-seeded) races (Fig. 1).

Furthermore, the existence of additional diversity within the Middle American gene pool, especially within a group of Guatemalan climbing bean accessions that were distinct from previously defined races has been reported (Beebe et al., 2000; Miklas and Singh, 2007).

2.2.3.1 Differences between Mesoamerican and Andean cultivated landraces

Singh et al. (1991a) noted that landraces between and within the two gene pools of origin have been distinguished by biochemical markers (phaseolin, allozymes) and vegetative and reproductive traits still found in cultivated species. For example, it was possible to distinguish the two groups of landraces by the shape of the terminal leaflet of the trifoliolate leaf (Fig. 2), the density and length of the straight hairs, the shape and size of the flower bracteoles (Fig. 2), the presence or absence of stripes on the outer base of the standard petal, the number of nodes of pod-bearing inflorescence, the pod beak position, and the size and shape of dry seeds (Fig. 1 and 2).

1. Middle American races

A. Mesoamerica

This race includes:

- small-seeded (< 25 g/100 seed) landraces of all seed colors and growth habits;
- small, intermediate, or large leaf size and internode length;
- often characterized by an ovate, cordate, or hastate terminal leaflet of the trifoliolate leaves and large, broad cordate or lanceolate bracteoles;
- Flower standard often possessing marked stripes at the outer base.
- Color of petals can be white, white with pink stripes, or purple.
- Inflorescences are multinoded.
- Pods are 8-15 cm long, slender, fibrous or parchmented, and easy to thresh; they contain six to eight seeds (Fig. 3) (Singh et al., 1991a).

Figure 1: Characteristics of dry seeds of different races of cultivated common bean.

The race is distributed throughout the tropical lowlands and intermediate altitudes of Mexico, Central America, Colombia, Venezuela, and Brazil (Fig. 4). Two subgroups could be identified within this race, based on growth habit, number of nodes to flower, and days to flowering and maturity, phaseolin types, and/or differences in allelic frequencies at some allozyme loci. One group was represented by indeterminate, erect, type II landraces, whereas the other subgroup was formed by indeterminate, prostrate, type III early maturing landraces. The latter group appears to be more primitive than the former, but both were considered subgroups within race Mesoamerica and did not warrant independent race status, at least based on review of literature up until now. Landraces of this race can carry the Dl-I gene, leading to F_1 hybrid dwarfism or lethality

in the presence of the Dl-2 gene of Andean origin (Gepts and Bliss, 1985; Shii et al., 1980; Singh and Gutirrrez, 1984; Vieira et al., 1989), or give rise to deformed leaflets (virus-like symptoms) in segregating generations of interracial crosses (Singh and Molina, 1991; Singh et al., 1991a). In addition, insensitivity to photoperiod and resistance to bean common mosaic virus (H gene) and tolerance to angular leaf spot, bean golden mosaic virus, high temperatures, moisture stress, and low soil fertility can be found in this race (Singh et al., 1991a).

B. Durango

Landraces are predominantly of:

- indeterminate, prostrate growth habit III;
- characterized by relatively small to medium ovate or cordate leaflets, thin stems and branches, short internodes, and fruiting commencing from and concentrated in the basal nodes;
- possess small ovate bracteoles with a pointed tip; with four to five flattened rhombohedric seeds of medium size (25-40 g/100 seeds);
- Seed colors are often tan-like ('bayo'), but may also be yellow, cream, gray, black, white, red, or pink, with or without spots or stripes (Fig. 3).

The race is distributed in the semiarid central and northern highlands of Mexico and the southwestern USA. As with the other eco-geographic races, it is also a source of many valuable traits, such as: early maturity, drought tolerance, high harvest index, positive general combining ability (GCA) for seed yield (Nienhuis and Singh, 1988), and tolerance to some viral diseases (Morales and Singh, 1991) and anthracnose. Members of this race are often non-carriers of the DI- 1 allele (Singh, 1990; Singh et al., 1991a).

C. Race Jalisco

This race is often characterized by:

- Indeterminate growth habit IV;
- Plant height can be over 3 m in its natural habitat;
- Terminal leaflet of trifoliolate leaves is hastate, ovate, or rhombohedric and sometimes relatively large.

- Stems and branches are weak and have medium-sized or long internodes.
- Most germplasm from this race possesses medium-sized, cordate, ovate, or lanceolate bracteoles.
- Fruiting is distributed either along the entire length of the plant or most mostly in its upper part.
- Pods are 8-15 cm long and have five to eight medium-sized seeds, whose shape is round, oval, or slightly elongated and cylindrical or kidney-shaped (Fig. 1; Fig. 3) (Singh et al., 1991a).

Their natural habitat is the humid highlands of central Mexico and Guatemala, where maximum diversity is found. Some small-seeded landraces of growth habit III (e.g., 'Carioca' (G 4017) and 'San Cristrbal 83' (G 17722)) fell into this group, as determined by multivariate statistical analyses. Thus, some heterogeneity was found, and its small-seeded members were included in race Mesoamerica (Fig. 4).

High seed yield, positive GCA for yield, high levels of resistance to Apion spp. and anthracnose, and tolerance to angular leaf spot and low soil fertility can be found in this race (Singh et al., 1991a).

2. South American (Andean) races

A. Race 'Nueva Granada'

Germplasm from this race are:

- Growth habits I, II, and III with medium (25-40 g/100 seeds) and large seeds (>40 g/100 seeds) of often kidney or cylindrical shapes which vary greatly in color (Fig. 1; Fig.3).
- Leaves are often large with hastare, ovate, or rhombohedric central trifoliolate leaflets and long, dense, straight hairs; and stem internodes are intermediate or long.
- Having small or medium, and ovate, lanceolate, or triangular bracteoles.
- Dry pods are fibrous, hard, medium to long (10-20 cm), and leathery, and possess four to six seeds.
- The pod beak often originates between the placental and ventral sutures.

This race is distributed mostly at intermediate altitudes (<2000 m) of the northern Andes in Colombia, Ecuador, and Peru, but it is also found in Argentina, Belize, Bolivia, Brazil, Chile, Panama, and some Caribbean countries, including the Dominican Republic, Haiti, and Cuba (Lioi et al., 1990). Some landraces of this group may carry the Dl-2 allele (Gepts and Bliss, 1985; Shii et al., 1980; Singh and Gutirrrez, 1984; Vieira et al., 1989) or produce deformed leaflets in the segregating generations upon crossing with races of Middle American origin (Fig. 4) (Singh and Molina, 1991).

Insensitivity to photoperiod, early maturity, and resistance to bean common mosaic virus, halo blight, anthracnose, and angular leaf spot can be found in this race (Singh et al., 1991a).

B. Race 'Chile'

Landraces are predominantly:

- Of indeterminate growth habit III, with leaves often relatively small or medium hastate, rhombohedric, or ovate.
- Short internodes; small or medium, and narrowly triangular, spatulate, or ovate bracteoles;
- Of light pinkish or white flower; medium-sized (5-8 cm) pods, often with reduced fiber content; and round to oval seeds (three to five per pod).
- Morphologically, these landraces largely resemble germplasm from race Durango, except that seeds of race Chile are round or oval, and fruiting is sparser.
- In some of the landraces, pods exhibit an attractive anthocyanin striping, and in many countries these are harvested for green seeds (green shelled or "granados") before physiological maturity.
- Some members of this race carry the Dl-2 allele (Fig. 3).

Distribution-wise, this race is frequent in relatively drier regions at lower altitudes in the southern Andes (southern Peru, Bolivia, Chile, and Argentina) (Fig. 4) (Singh et al., 1991a).

C. Race 'Peru'

Morphological characteristics of germplasm belonging to this race are:

- With large hastate or lanceolate leaves (often basal) and long and weak internodes having either indeterminate or determinate type IV climbing growth habit (Fig. 3) (Debouck et al., 1988; Singh et al., 1991a).
- Pods are often long (10-20 cm) and leathery and distributed fruiting either along the entire stem length or only in the upper part of the plants (Fig. 3).
- Seeds are large and often round or oval but can also be elongated (Fig. 1).

Race members are distributed from the northern Colombian highlands (>2000 m altitude) to Argentina. Its members in the southern Andes (e.g., 'overitos', 'nufias', 'tiachos') occur at relatively lower altitudes, are earlier maturing, and possess comparatively smaller seeds with distinctive speckling and spotting (Fig. 4) (Singh et al., 1991a).

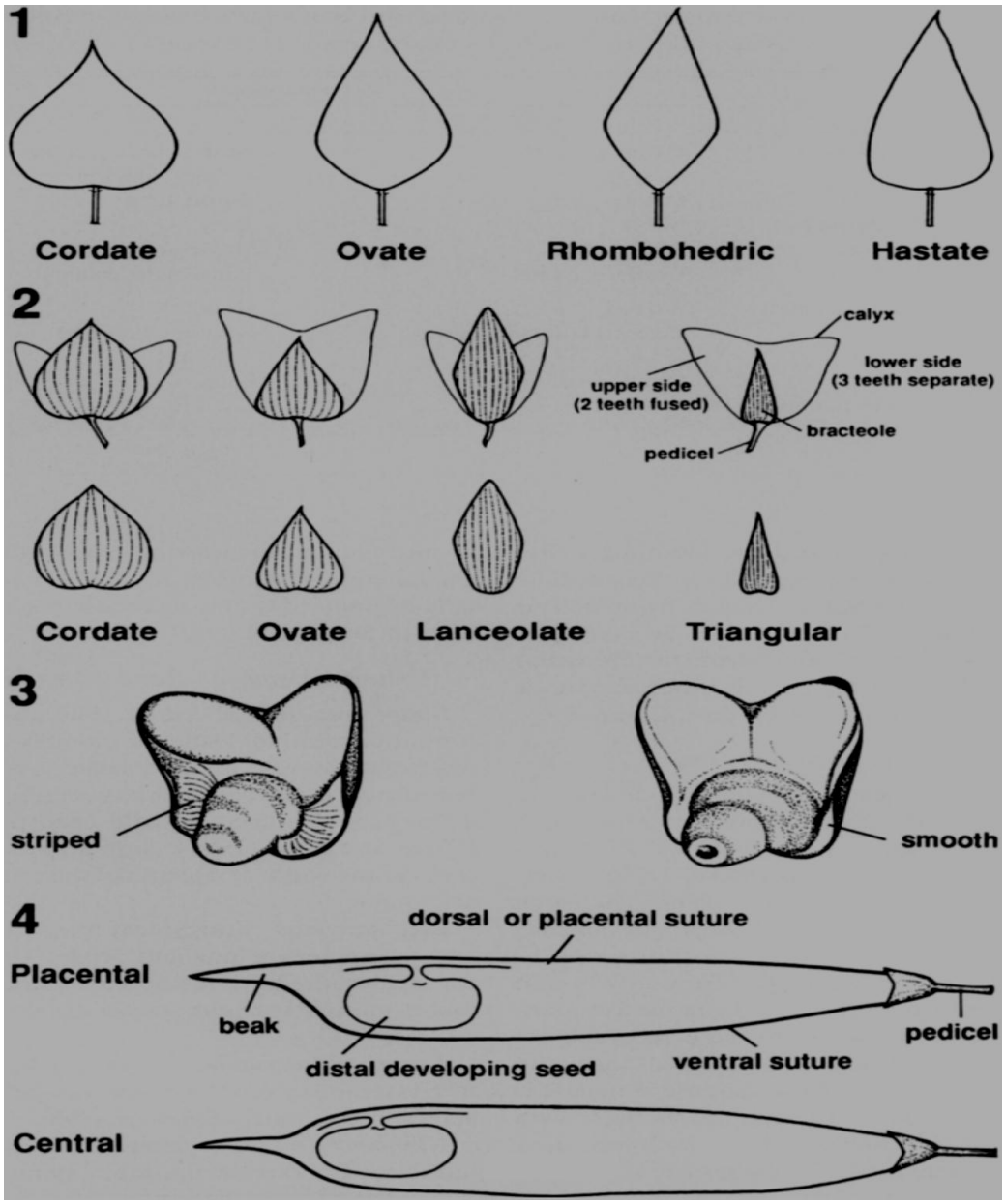

Figure 2: Variation in shape of the central leaflet of the trifoliolate leaves (1); principal bracteole types (2); Striped versus smooth basal outer surface of flower standard (banner petal) (3); and placental versus central pod beak position (4) in cultivated common bean.
(Source: Singh et al., 1991a)

Race	Seed		Bracteole	Phaseolin[b]	Allozyme[c]	Growth habit[d]	Gene pool[e]	Distribution[f]	Landraces
	Size[a]	Shape							
Middle American									
Mesoamerica	Small	Cylindrical, kidney, oval	Large cordate	S, Sb, B	*Me*^{98} or *Diap-2*^{105}	Determinate I; indeterminate II, III, and IV	1, 2, 3, 4	Lowlands of Latin America	Brazil 2, Jamapa, Porrillo, Rojo de Seda, Mulatinho, Magdalena 3
Durango	Medium	Rhombohedric	Small ovate	S, Sd	*Me*^{102}	Indeterminate III	5	Semiarid highlands of Mexico	Pinto, Morado de Agua, Bayo, Ojo de Cabra
Jalisco	Medium	Oval, round, cylindrical	Medium lanceolate	S	*Me*^{100} or *Mdh-2*^{102}	Indeterminate IV	6	Humid highlands of Mexico	Garbancillo Zarco, Frijola, Flor de Mayo, Apetito, Conejo
Andean South American									
Nueva Granada	Medium and large	Cylindrical, kidney	Small lanceolate or triangular	T	*Rbsc*^{100} or *Me*^{100}	Determinate I, indeterminate II and III	7, 8, 9	Intermediate altitudes of Andes	Cargabello, Antioquia 8, Radical, Jalo, Bagajo
Chile	Medium	Oval, round	Small triangular	C, H	*Mdh-1*^{100}	Indeterminate III	10	Southern Andes	Frutilla, Tórtolas, Coscorrón, Bolita Cristal
Peru	Medium and large	Oval, round	Large lanceolate or cordate	T, C, H	*Mdh-1*^{103}	Indeterminate or determinate IV	11, 12	Andean highlands (>2000 m)	Overitos, Caballeros, Nuñas, Bolón Bayo, Bola Roja, Cargamanto

[a] Weight of 100 seeds (hence, size) of small is <25 g, medium varies between 25 and 40 g, and large is >40 g. [b] According to Gepts and Bliss 1986, Gepts et al. 1986, Koenig et al. 1990. [c] According to Singh et al. 1991b. [d] According to Singh 1982. [e] According to Singh 1988, 1989, 1991b. [f] See also Fig. 6.

Figure 3: Some characteristics of the races of cultivated common bean (*Phaseolus vulgaris* L.), their relationships with gene pools, and distribution with in the primary centers of domestication in the Americas
(Source: Singh et al., 1991a)

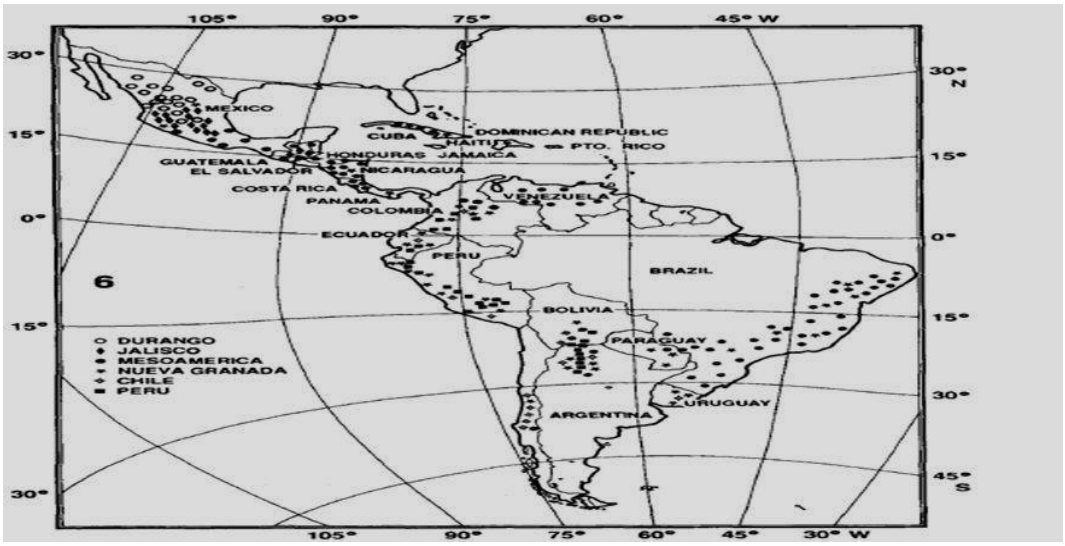

Figure 4: Distribution of races of cultivated common bean in Latin America. Races Mesoamerica, Jalisco, and Durango are from Middle America and races Nueva Granada, Peru, and Chile are from the Andes.
(Source: Singh et al., 1991a)

2.2.4 Botany, taxonomy, cytogenetics, and production ecologies of the common bean-a global perspective

Freytag and Debouck (2002) described in considerable detail the taxonomy, distribution, and ecology of over 25 *Phaseolus* species, including *P. vulgaris*, which are native to North America, Mexico, and Central America. Cultivated and wild *P. vulgaris* (Brücher, 1988) and other *Phaseolus* species (Debouck, 1999) are also native to Andean South America.

According to Miklas and Singh (2007), the natural habitat of wild common bean ranges between ca. 800 to 2750 m elevations. Indeterminate climbing populations have a perennial tendency in the wild, but when planted in the field they may behave, as an annual similar to most cultivated types. Taxonomically, the genus *Phaseolus* belongs to family Leguminosae, subfamily Papilionoideae, and *P. vulgaris* belongs to its section Phaseoli. There is continuous variation in growth habit from determinate bush to indeterminate climbing cultivars. Singh (1982), however, classified growth habits into four major classes using the type of terminal bud (vegetative vs. reproductive), stem strength (weak vs. strong), climbing ability (non-climber vs. strong climber), and fruiting patterns (mostly basal vs. along entire stem length or only in the upper part). These are the Type I=determinate upright bush, Type II=indeterminate upright bush, Type III=indeterminate, prostate, non-climbing or semi-climbing, and Type IV=indeterminate, strong climbers/determinate strong climbers. Roots are generally fibrous with a marked tap or main root. Under most field conditions, especially in cool subtropical and temperate environments, they may bear nitrogen-fixing nodules from a few weeks after emergence through flowering. The main stem derives from the axis of the seed embryo. The number of branches and branching pattern may vary greatly depending upon the genotype and environment. Often more than 50% of the pods are borne on branches. The two unifoliolate leaves borne above the cotyledonary node are opposite to each other followed by one trifoliolate leaf at each node in an alternate phyllotaxy. The fully developed trifoliolate leaf has a long (> 7 cm) petiole, a small (< 3 cm) petiolule, very small pulvini, and three leaflets of which the central one is often symmetrical and chordate, ovate, or lanceolate. On the other hand, the inflorescence is a pseudoraceme,

often with several flowers of which only the basal few bear pods; an exception are small-diameter snap bean that bear a profusion of pods. Also, dry bean of outrigger types bearing six or more pods can be rarely found. Flowers can be pink, purple, white, or bicolor with or without stripes at the outer base of a very pronounced standard. Sessile bracteoles often are larger in Middle American compared to Andean genotypes and may be chordate, ovate, or lanceolate. Bilabiate calyx is small (<5 mm) with the upper two teeth united. The two keels may be coiled up to two times. There is a single vexillary stamen on the upper side and nine stamen united into a long sheath or tube around the style. The introrse stigma tends to extend around the tip of the style. Flowers are cleistogamous and normally are highly self-pollinated (<1% outcrossing) (Miklas and Singh, 2007). Nonetheless, outcrossing rates ranging from 0.0 to 78% for individual families with a mean rate for six dry bean genotypes ranging from 4.4 to 10.2% in California have also been reported (Ibarra-Pérez et al., 1997). Anthesis occurs in early morning hours, and crosses are made with or without emasculation of anthers prior to anthesis. Mature pods are straight to slightly-curved with five to eight seeds. There is considerable variation in size, shape, and color of pods and seeds. Germination is epigeal with cotyledons dropping off a couple of weeks after emergence.

Common bean is a short-day crop (White and Laing, 1989). Cultivars adapted to higher latitudes either have evolved during dissemination from the primary centers of domestication or have been developed by breeding. Mildly cool environments favor growth and development. Thus, under non-stressed environments with 18 to 22^{0}C mean growing temperatures and about 12-h day-length, most cultivars, in Eco-geographic races like _Peru‘, complete their growing cycle from germination to seed maturity in 70 to 120 days. In the highlands (above 2000 m elevation) of Bolivia, Colombia, Ecuador, and Peru, climbing cultivars often require more than 250 d to mature. In the humid highlands of Guatemala and Mexico and in Principado de Asturias, Spain, climbing cultivars require ca. 150 d to mature (Miklas and Singh, 2007).

At higher latitudes in temperate climates, dry bean cultivars of growth habit Types I, II, and III predominate. These are harvested within 90 to 120 days of planting. Cultivars of

growth habit Types I, II, and III are grown in monoculture as well as under different relay, strip, and intercropping systems throughout the world (Singh 1992). Type IV cultivars always require support. Thus, these are grown in association with maize (*Zea mays* L.) and other crops or on trellises or stakes. Although dry bean is grown in a wide range of soil types, light loamy soils with pH 7.0 and rich in organic matter are more suitable for production. A 90- to 120-d crop with a yield of 2500 kg ha-1 will usually remove 60 to 80 kg of soil nitrogen and 40 kg of phosphorus (Miklas and Singh, 2007).

P. vulgaris and a great majority of other cultivated and wild *Phaseolus* species have 2n=2x=22 chromosomes, but few have 2n=2x=20 (Gepts, 2001). The *P. vulgaris* chromosomes are extremely small, and all 11 chromosomes have been identified (Mok and Mok, 1977; Cheng and Bassett, 1981). They were also recently assigned to the respective linkage groups (LGs) (Table 1) using the fluorescence in situ hybridization (FISH) (Pedrosa et al., 2003). The common bean has one of the smallest genomes in the legume family with 0.65 pg/haploid genome or 635 mbp (Arumuganathan and Earle, 1991). Debouck (1999) provided the number of available accessions for some species and reviewed their useful traits and the extent of chromosome pairing between interspecific hybrids. Despite the fact that the common bean chromosomes are extremely small, Mok and Mok (1977) at the prophase of somatic cells, and Cheng and Bassett (1981) at the diplotene stage of meiosis in pollen mother cells, identified all 11 chromosomes. Ashraf and Bassett (1986) induced translocations. Ashraf and Bassett (1987) also reported five primary trisomics, four tertiary trisomics, and two tetrasomics.

2.3 The common bean in Ethiopia

2.3.1 The common bean as a dietary component in Ethiopia

Production of common bean is expanding slowly, based on population growth, with highest usage in poor developing countries, where beans provide an alternative to meat as a source of low-cost protein. Beans are well suited to low input systems as they can be stored for long periods without refrigeration and provide an excellent nutritional complement to maize, which is one of the most important grain cereals (Ayele, 1990; Ferris and Kagnazi, 2008).

The common bean is high in starch, protein and dietary fiber and is an excellent source of minerals and vitamins including iron, potassium, selenium, molybdenum, thiamine, vitamin B6, and folic acid. Dry beans will keep for 3–4 years if stored in a cool, dry place, but as time passes, its nutritive value and flavor degrades and cooking times lengthen as they desiccate and harden. Dried beans are almost always cooked by boiling, often after having been soaked for several hours. While the soaking step is not essential it shortens the cooking. Common beans take longer to cook than most pulses, and depending on the variety, cooking times vary from one to four hours (Ferris and Kagnazi, 2008).

There is a growing domestic and regional demand for red beans. In the future, there is potential to expand into new export markets as there are trends for richer consumer segments in industrialized countries to adopt vegetarian diets. In Ethiopia there are strong cultural bonds with pulse crops which are closely associated with the dietary customs of the majority Orthodox Christian community. Moreover, most traditional vegetarian dishes are prepared from highland pulses, such as chickpeas, split peas, faba beans and lentils. *Phaseolus* beans are considered to be a lower value and lower esteem pulse crop, but there is increasing interest in *Phaseolus* beans, particularly among the low income segments for reasons of food security and income generation (Ferris and Kagnazi, 2008; Karanja et al., 2011).

Recent studies indicated that there is considerable genetic variation in iron and zinc concentration in bean landraces and breeding lines from Eastern Africa and other countries (CIAT, 2008, 2010; Blair et al., 2010b). This implies that the mineral density of local varieties can be enhanced by more than 80% for iron and 60% for zinc by transferring genes controlling grain mineral accumulation to these varieties. Studies in humid and sub-humid environments have shown that agronomic management practices such as application of macro and micronutrients, organic amendments and supplemental irrigation can further enhance nutrient accumulation in grain (Rengel et al., 1999; Okonda et al., 2007; Ferris and Kagnazi, 2008; Felix, 2009; Karanja et al., 2011).

Although canning beans have been grown in Eastern Africa, since the early 1950s, little work has been done to develop improved bean varieties that combine tolerance to biotic and abiotic stresses with canning quality. Consequently, bean varieties such as Mexican-142 dominated production in the first decades of the century, despite its susceptibility to rust, anthracnose, common bacterial blight and drought. Preliminary studies suggest that agronomic management practices may influence canning quality and productivity (Loggernberg, 2004; Teshale, 2010). The potential of many new bean varieties for canning is still unknown due to lack of capacity in the region to assess their canning characteristics during variety development. This is now urgent due to changing eating habits, preference for fast cooking off-shelf products and high cost of cooking fuel (Karanja et al., 2011).

2.3.2 The common bean in Ethiopia from marketing and varietal preference perspectives

Common bean is grown throughout Ethiopia and is an increasingly important commodity in the cropping systems of smallholder producers for food security and income. Farmers grow a wide range of bean types, in terms of color and size, but the most common types are the pure red and pure white beans (Fig. 5). Most of the beans produced, traded and consumed in the domestic Ethiopian bean markets are the medium and small red beans, whereas white beans are virtually all exported (Ferris and Kagnazi, 2008; Karanja et al., 2011). In Ethiopia, common bean is one of the most important cash crops and source of

protein for farmers in many lowlands and mid-altitude zones. Between the periods from 2007-2010, the country‘s export earnings from the crop was estimated to be over 85 % of export earnings from pulses, exceeding that of other pulses such as lentils, faba bean and chickpea (Negash, 2007; Katungi et al., 2010). Overall, common bean ranks third as an export commodity in Ethiopia, contributing about 9.5 % of total export value from agriculture (FAOSTAT, 2010). Total national production was estimated at 421,418 ton in 2008, with a market value of USD 132,900,609 million (FAOSTAT, 2010; Katungi et al., 2010). During the 2012/13 growing season, the production was estimated at 463,008.49 ton and this has a 19.3% increment from the previous growing season, i.e. 2011/12 (CSA, 2013). According to Buruchara et al. (2011), since 1996, over 550 new bean varieties have been released by the alliance across Africa, many of which have gone on to transform beans from a subsistence crop to a cash crop, such as the white pea bean in Ethiopia, which grew from an annual export industry of USD 8.5 million in 2004 to USD 50 million in 2010. On the other hand, this market is a foreign exchange annual value in the range of USD 25–30 million in 2012-13 (Ronner and Giller, 2013).

Prospects for increased regional trade of red beans are somewhat dependent upon drought, conflict, and food aid needs. The recent policy shift towards higher levels of local procurement means that more food aid will be sourced from the East African region and therefore it is likely that demand for red beans will grow. Red beans are mainly produced for domestic consumption, while, white beans are almost exclusively grown to supply a longstanding export market from Ethiopia. There are good prospects for continued growth in this export market with the arrival of several major processing companies that are investing in the white bean sector (Spilsbury et al., 2003; Ferris and Kagnazi, 2008; Karanja et al., 2011).

Despite growth in the bean markets, there is little evidence of large-scale bean farming in Ethiopia and virtually all beans are produced on smallholder plots, with minimal inputs. The average plot-size of farmers in Ethiopia is 1.5 ha and up to 83% of the farming households in Ethiopia have an area of less than 2 ha, with 56% of farming households having less than 1 ha. This multitude of smallholder farmers is unlikely to change as the

high population density in Ethiopia limits the amount of land available for agricultural expansion into large-sized farms (World Bank, 2006; Karanja et al., 2011).

There is a wide range of common bean varieties grown in Ethiopia (Fig. 5), including mottled, red, white and black varieties. The most common commercial varieties are pure red and pure white-colored beans and these are becoming the most commonly grown types with increasing market demand (Ferris and Kagnazi, 2008; Ronner and Giller, 2013).

To support both the growth in domestic and export bean markets, the Ethiopian Institute of Agricultural Research (EIAR) has developed a range of high-yielding, multi-disease resistant bean varieties. The focus of this genetic improvement program has been on the pure red and white beans to support the commercial sector. Within the red bean types, the most favored and most commercially accepted varieties include Red-Melka, a mottled medium sized red; Red-Wolayta, a medium sized pure light red; and Nasser, a small pure dark red variety (Fig.6) (Ferris Kagnazi 2008; Karanja et al. 2011).

Figure 5: *Phaseolus vulgaris* bean types grown in Ethiopia
(Source: Ferris and Kagnazi, 2008)

In Ethiopia, red beans are preferred by rural consumers, and there is a wide range of reds, in the form of red mottled varieties that are produced and sold in the rural markets. White beans are sold almost exclusively for the export markets. The leading white bean varieties include Awash 1, Awash-Melka and Mexican 142, all of which are small white beans (Ronner and Giller, 2013) (Fig. 6). The white beans are often referred to as white pea

beans, due to their small size and round shape; they are otherwise known as navy beans. White beans are popular in industrialized nations, such as the USA and UK, as they are used to prepare pre-cooked canned _baked beans' (Miklas and Singh, 2007). The baked bean market is growing in many parts of the world, as it is low cost, nutritious snack food that is easy and quick to prepare. Although an important export crop, the white pea bean is not consumed by many Ethiopians, probably owing to being new to the Ethiopian food preparation recipe, and often reserved for export purposes (Ferris and Kagnazi, 2008).

The level of production and sales of beans are highly dependent upon rainfall, and in years with drought, yields are significantly reduced, by more than 50%. To this end, it was noted in some previous literatures that a comparison of yield in a good rainfall year (1996) with that of a drought year (2001/2002) indicate that the national average yields drop by 40-50% during drought years (FAOSTAT, 2010; Katungi et al., 2010).Usually, no irrigation is applied to the bean crop, although simple irrigation systems are used by these farmers in the rift valley region for higher value crops such as vegetables and khat (Ferris and Kagnazi, 2008). The arrival of two international bean trading companies in Ethiopia in the past decades has introduced increased demand for internationally recognized varieties that are not grown in Ethiopia and rapid evaluation of existing commercial varieties, thus, has placed additional demands on the national bean research system. Poortman plc imported AR04 RGY, which has been released for production and a rival company ACOS has been seeking permission to begin multiplication of two additional white bean varieties, i.e. Avanti and Christod, and one red variety, McMillan (Ronner and Giller, 2013).

These varieties were developed for canning and are currently mainly grown in USA. The export and processing companies particularly want supplies of these varieties as their canning factories are geared to process these specific varieties, and they suggest it is easier to change the production in Ethiopia to meet this market rather than changing the processing system for the factories (Ferris and Kagnazi, 2008; Karanja et al., 2011).

2.3.3 Production and distribution of the common bean in Ethiopia

Common bean is widely grown in Ethiopia in areas with altitude between 1400-2000m asl. The main production areas include eastern Ethiopia, the south and the south west, the west and, largely, the Rift Valley (Fig.7; Table 1). The latter area accounts for more than half of the country‘s bean production, mainly of the white pea bean type that is grown for export. Other bean types are for national use (Ali et al., 2006; Ronner and Giller, 2013).

Beans are grown as sole crop or intercropped with sorghum, maize or other crops. Productivity of common beans has increased over the last years; while the area planted remains more or less stable (Table 1) (Ronner and Giller, 2013). Hence, yields per hectare have improved (Fig.8). Important constraints for further yield increase is the lack of improved varieties (Gurmu, 2007), low soil fertility (N and P), moisture stress, pests and diseases and weeds (Ali et al., 2006; Ronner and Giller, 2013).

In mono-cropping systems, bush types are grown (mainly in Central and Southern Ethiopia. In west and southwestern Ethiopia, areas with higher rainfall and extended growing seasons, climbing beans are intercropped with maize, planted along the borders of maize fields, or along homestead fences (Gebeyehu et al., 2006; Ronner and Giller, 2013).

In Southern Ethiopia, an experiment with climbing beans indicated that higher yields for bush types than climbing types (Worku, 2008). A study in Eastern Ethiopia showed that in intercropping, climbing varieties were not preferred because they caused lodging in maize (after which evaluation in sole stands was followed) (Assefa et al., 2005; Ronner and Giller, 2013).

Planting dates are between mid- and end-June gave highest grain yields around the Sidama zone, with a plant density of 400,000 seeds/ha. In the Belg season, sowing between mid-February and mid-March resulted in the highest grain yields (Ali et al., 2006; Ronner and Giller, 2013).

The two major bean-producing regions are Oromiya and Southern Nations, Nationalities and People's Region (SNNPR), which, on average, produce 70 and 60 thousand tons per year, respectively, and these two regions make up 85% of the total production (CSA, 2005; Ronner and Giller, 2013). The Rift valley contributes to 48% out of 163,688ha and 55% of 138.4216 tons production of the country (Teshale et al., 2006; Ronner and Giller, 2013). The Hararghe highland is one of the major common bean-producing areas in the country (Wortmann and Allen, 1994; Ronner and Giller, 2013). It is estimated to cover 11,696.4 ha of land with a production of 1 ton/ha (CACC, 2001; Ronner and Giller, 2013). It is becoming important as short duration crop because of the recurrent late onset and early termination of rainfall in these areas. Bean production in Ethiopia ranges widely from 100–200 thousand tons per year. The wide range in yields is due to the considerable and regular losses that farmers suffer due to drought, which is a regular and severe event (Table 1; Fig. 9) (Ronner and Giller, 2013). The main producing areas of common bean in the country are in the Rift Valley area, which runs diagonally across the centre of Ethiopia from top right to bottom left of the country.

Figure 6: Main commercial bean varieties grown in Ethiopia

Table 1: Land size, yield/ha, production and consumption, and percentage from national total

Region	Land size (ha)	Yield (Kg/ha)	Production		Total consumption	
			Production (tons)	% from national total	Consumption (tons)	% from regional total
Afar	62	286	18	0.01	16	91
Amhara	29,983	595	17,848	12	10,913	61
Benishangul-Gumuz	3,954	894	3,534	2	2,610	74
Gambella	221	499	110	0.07	91	83
Oromiya	98,217	710	69,699	46	45,820	66
SNNPR	72,898	814	59,339	39	45,435	77
Somali	1,224	210	2,573	0.17	160	62
Tigray	2,310	432	999	0.66	822	82
Total Country	208,872	4,440	151,805	100	105,870	70

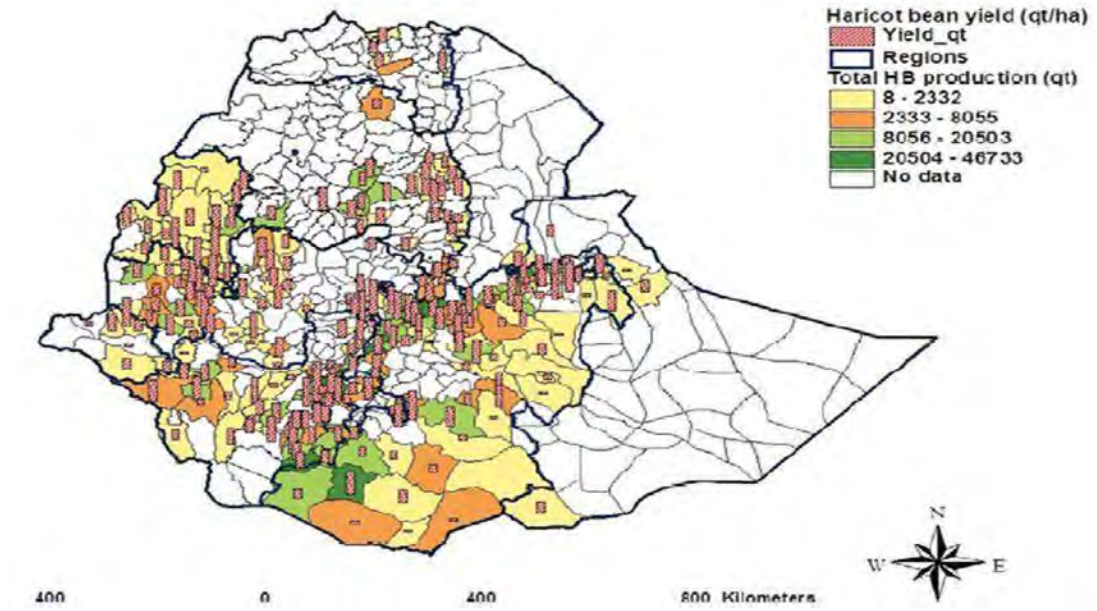

Figure 7: Main production zones for red and white beans
Source: Ferris and Kaganzi (2008)

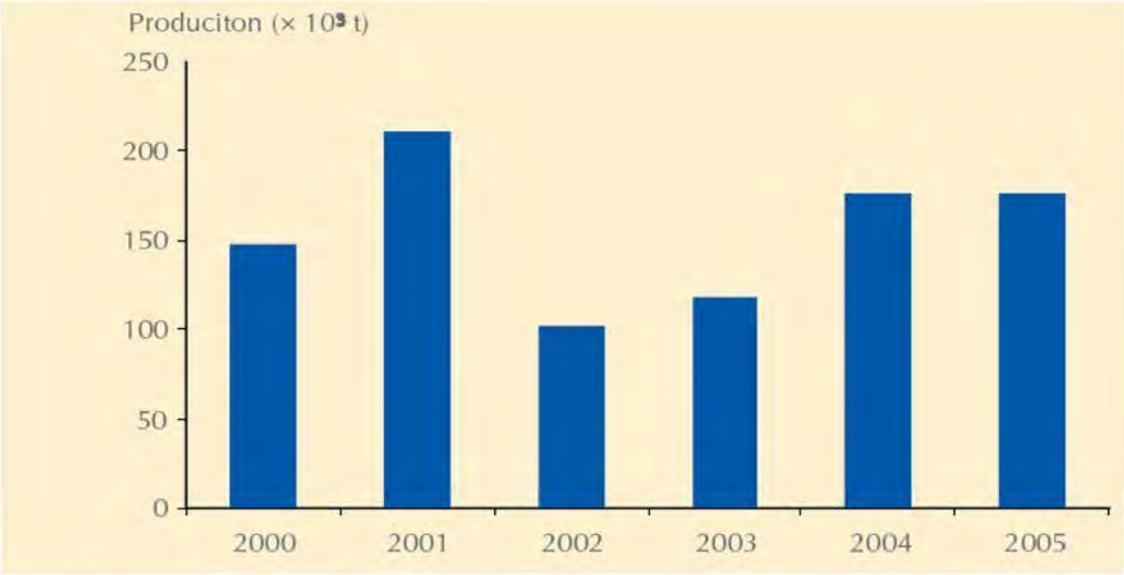

Figure 8: Production levels for common bean in Ethiopia in 2000–05.
Source: Ethiopian Central Statistical Authority: Agricultural Abstracts 2000–05.

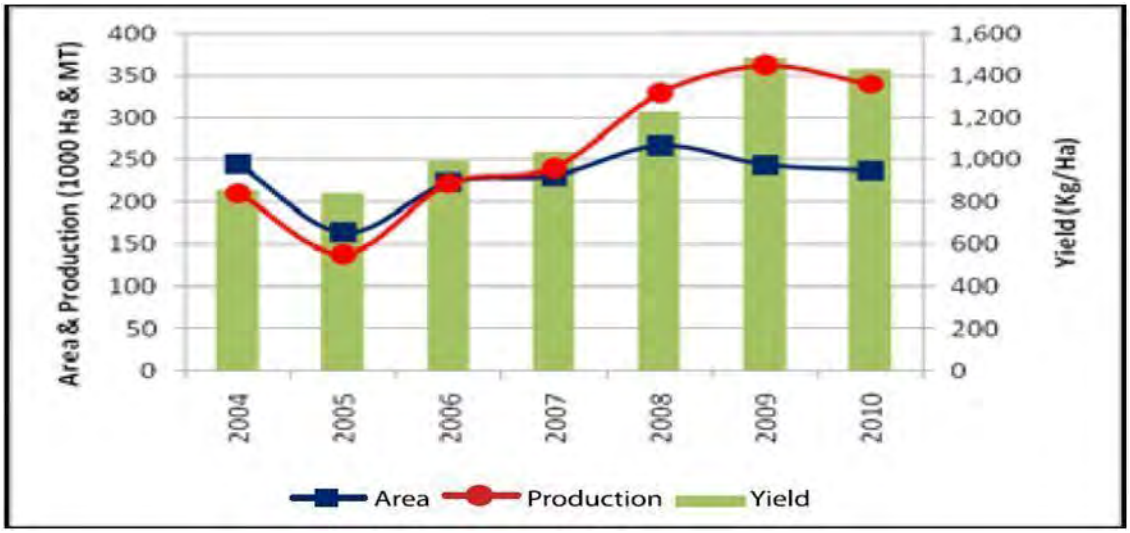

Figure 9: Production trends of common bean in Ethiopia for the period from 2004 to 2010
Source: ICRISAT (2011); Ronnie and Giller (2013)

2.3.4 Trends in common bean improvement research and seed system in Ethiopia

In sub-Saharan Africa, bean improvements, which combine breeding and improved agronomic practices, began in 1960s (Singh, 1992; Katungi et al., 2010). In the 1980s, breeding programs were expanded to cover the major bean-producing areas supported by CIAT. Areas were of high production potential, relatively fertile soils and favorable climatic conditions, nevertheless production was constrained by pests and diseases. As a

result, the initial breeding emphasized developing varieties with resistance to biotic stresses, especially seed-transmitted diseases (Singh, 1992; Katungi et al., 2010). Efforts contributed to production and distribution of disease-free seed, stabilization of yields, and reduced disease outbreaks, ultimately contributing to the positive yield growth through 1980s (Katungi et al., 2010).

According to Kassaye (2006) research on the genetic improvement of the common bean in Ethiopia has been characterized by conservative breeding strategies designed to adhere to rigorous consumer preferences mainly market qualities and resistance to diseases that affect common bean production in the country. These factors, argued the author, have reduced the germplasm sources used in hybridization and have limited the genetic variability available for breeding programs.

Varietal adaptation under the Ethiopian Institute of Agricultural Research (EIAR) and Regional Agricultural Institutes (RARIs) with the support from the International Center of Tropical Agriculture (CIAT) under the umbrella of the Pan Africa Bean Research Alliance (PABRA) resulted in the release of high yielding improved common bean varieties that are potentially suitable for a range of ecologies (from lowlands to highlands). These improved varieties were also highly appreciated by consumers and market (Assefa et al., 2006), but farmers continued to grow low yielding old varieties, instead. The analysis of constraints hindering use of improved varieties with stakeholders revealed that the main constraint to adoption of bean improved varieties was associated with limited accessibility to seed (Rubyogo et al., 2011). Before 2004, EIAR and RARIs had been relying only on a few farmer research groups (around research centers/stations) and Ethiopia Seed Enterprise (ESE), a government agency to disseminate improved varieties to farmers (Alemu and Spielman, 2010). These sources could only meet less than 1% of farmers‘ seed needs, without even considering the setback that they furthermore had focused on just a few already popular and old bean varieties (mainly Mexican 142, released in 1972) (Rubyogo et al., 2011). Therefore relatively few farmers had the opportunity to access the seeds of the newer varieties. In 2004, the Ethiopian National Bean Research Program (ENBRP) with technical support from the International

Center for Tropical Agriculture, (CIAT)-PABRA framework, initiated a partnership with a broader range of organizations from government, non-governmental and farmers' organizations and individual farmers aiming to overcome these seed bottlenecks. The intervention encompassed both the production and distribution of seeds, and enhancing the skills and knowledge of service providers to backstop a growing seed supply sector (Rubyogo et al., 2011). As a result of the intervention, a longer-lasting partnership on bean technology transfer was developed among the participating agencies (including bean exporters and traders), and farmers in impressive numbers have gained access to new bean varieties (PABRA, 2005; Rubyogo et al. 2011). As farmers' access to quality seeds of the varieties of their choices increased, and they started to appreciate their characteristics, the ENBRP in partnership with service providers (farmers' cooperative unions, public extension and NGOs) equally promoted complementary improved agronomic practices to optimize the use of varieties. The improved agronomic practices included timely weeding, judicious use of fertilizers, spacing etc. In addition, ENBRP decided to engage the policy makers and interacting with bean value chain actors more particularly the exporters of canning beans. The engagement of government policy makers was translated into support which aligned the bean subsector development with its policy of facilitating a free and competitive export market. Subsequently, the Ethiopian government initiated market development including Ethiopian Commodity Exchange and beans have been listed as one of commodities. All these efforts have led into transparent market and ultimately fair return to farmers. For instance the farm-level income of bean increased from USD 120 in 2004 to USD 450 in 2008 and in USD 650 in 2010 (Rubyogo et al., 2011). Table 2 describes the names; year of release; and use category of improved common bean varieties released within the Ethiopian agricultural research system. The ones released after 2004 were released under the umbrella of PABRA.

Although the release of the aforementioned bean variety technologies has, by itself, been a great success story, the following bottleneck production constraints are still hindering the effort towards fully harnessing the genetic potential of common bean via delivering varieties with high yield and related qualities.

- Lower genetic base vis-à-vis the genetic base used in the national bean improvement program due to specific breeding strategies adopted;
- Recurrent occurrence of disease and insect epidemics, due to narrow genetic base and evolution of pathogenic races;
- Little orchestrated efforts to mine the rich genetic potential of common bean landrace accessions in Ethiopia;
- Use of poor quality seed;
- Use of old and traditional varieties (no longer adapted);
- Inaccessibility to quality of seed of new preferred/marketable varieties;
- Lack of appropriate extension packages and support to farmers to increase bean productivity;
- Lower marketability of beans;
- Lower grain prices;
- Inadequate linkages/connections among the bean value chain actors; and etc. (Kassaye, 2006; Rubyogo et al., 2011; Ronner and Giller, 2013).

Hence, future research directions with respect to common bean improvement, so as to efficiently benefit small-scale bean producers, should unequivocally address the aforementioned constraints.

2.4 Variability of common bean genotypes in quantitative and qualitative traits

Mohammadi and Prasana (2003) noted that analysis of genetic variability of accessions in crop plants with respect to qualitative and quantitative traits is one important precursor of determining genetic diversity. In relation to this, various studies were done in the past on common bean genotypes/landrace accessions/improved cultivars in order to determine variability in qualitative and quantitative traits. The following section reviews the major findings of these studies.

Oscar et al. (2004) analyzed the variability in nine red-seeded Nicaraguan common bean landrace accessions using 14 phenotypic traits. Consequently, they reported that ANoVA

showed agroecological zones where the landraces were collected from and the experimental sites had a great impact on the majority of the traits studied.

Table 2: Improved varieties released up to 2010 by the Ethiopian Common Bean Research Program (ECBRP); RARIs; and HLUs

S/N	Name of variety	Year of release	Use/Food types
1	Mexican 142	1972/73	Canning
2	Red Wolaita	Early 80s	Food
3	Black Dessie	Early 80s	Food
4	Brown speckled	Early 80s	Food
5	Awash-1	1989	Canning
6	Roba-1	1989	Food
7	Atendaba	1997	Food
8	Zebra	1997	Food
9	PAN 173	1999	Canning
10	Awash Melka	1999	Canning
11	Goberasha	1999	Food
12	MX 2500-19	1999	Food
13	Tabor	1999	Food
14	Melkie	1998	Food
15	CIAT-Line A 776	1999	Food
16	GX 1175-3	1999	Food
17	TY 3396-7	1999	Food
18	AFR 504	2001	Food
19	RWR 719	2002	Food
20	Gofta	1997	Food
21	Ayenew	1997	Food
22	Beshbesh	2003	Food
23	Nasir	2003	Food
24	Dimtu	2003	Food
25	Ibbado	2003	Food
26	Omo 95	2003	Food
27	Chore	2006	Canning
28	Melka Dima	2006	Canning
29	DRK	2007	Food/canning/export
30	Argene	2007	Canning
31	Cranscope	2007	Food/export
32	Sugar 131	2008	Food/export
33	Hawassa Dume	2008	Food types

Interactions between these factors were significant for some traits (100-seed weight, leaf surface area, and plant growth habit). In addition, significant differences among the landrace accessions within agroecological zones were found for phenological traits (days to flowering and days to physiological maturity). Furthermore, accessions from different agroecological zones showed variability with respect to agronomic, morphological, and phonological traits, such as grain yield, pod length, stem length, days to flowering, and days to physiological maturity. Nonetheless, the authors reported that no variation in the proportion of plants showing either of the variants of flower wing or standard was

observed among agroecological zones. On the other hand, considerable differentiation of accessions from some agroecological zones was observed in terms of earliness, plant growth habit, and standard color.

Duran et al. (2005) analyzed the variability of common bean landrace accessions from Haiti, Dominican Republic, Puerto Rico, and other regions in close geographical proximity with the three. As a result, they reported that higher yields were recorded for accessions from Haiti and Dominican Republic in the years 2001 and 2002, respectively. In summary, the authors noted that spatial and temporal variations governed the variability in yield and related agronomic/phonological traits among the common bean landrace accessions from these countries. Furthermore, considerable variability among the landrace accessions were recorded in qualitative character traits, such as standard (flower) color, seed coat color, seed shape, plant growth habit, etc.

Zelalem (2005) studied the variability of advanced breeding lines of common bean in the Northwestern part of Ethiopia with respect to 11 quantitative traits. Consequently, significant genetic variations were recorded for most of the traits at each location. Furthermore, combined analysis over locations indicated significant genotype, environment, genotype x environment variations for all the traits, except days to maturity, number of branches/plant, and 100-seed weight. Finally, yet importantly, the author remarked that even though released varieties, Zeber and Roba-I, topped the rank in yield, many advanced lines outyielded other released varieties in yielding potential. This, the author argued, may pinpoint the prospective breeding potential the advanced lines would offer in future common bean breeding/improvement endeavors.

Kassaye (2006) studied variability among one hundred forty-four (144) common bean germplasm introductions to Ethiopia with respect to 18 agronomic and morphological traits. To this end, high genotypic coefficient of variation (GCV) was observed for plant height, number of nodes on the main stem, number of pods per plant, internode length and hundred seed weight. Furthermore, it was reported that phenotypic coefficients of variation (PCV) were higher than corresponding genotypic coefficients of variation

(GCV), indicating the significance of environmental influence. On the other hand, higher genetic advance and heritability was observed for plant height, number of nodes on the main stem, and hundred seed weight.

Barelli et al. (2009) studied the variability of 35 common bean landrace accessions from *Malto Grosso do Sul* state in nine agrono-morphological traits. As a result, significant to highly significant variations among the accessions were recorded for all the traits evaluated. Moreover, up on comparing the coefficient of variation (C.V.) recorded for the traits, they found out that days to emergence and flowering, pod length, seed weight, and cycle had C.V. values below 10%. In contrast, traits, such as insertion height of the first pod, number of pods/plant, number of seeds/pod, and number of seeds/plant had C.V. values in the ranges between 10.30% and 24.41%. These C.V. values, noted the authors, were less than reported by Duran et al. (2005).

Lima et al. (2012) analyzed the genetic variability among 100 common bean landrace accessions from Brazil using 22 qualitative and quantitative agro-morphological traits. Their results indicated that highly significant variability was recorded for days to flowering, plant architecture, yield, and 100-seed weight. Furthermore, 7 groups of accessions were seen with respect to values of days to flowering, whereas, five, seven, and four groups were evident for plant architecture, grain yield, and 100-seed weight, respectively.

Okii et al. (2014a) studied the variability among 284 Ugandan common bean landrace accessions in 7 quantitative traits during two growing seasons. Consequently, accessions showed highly significant variability in all the traits evaluated: mean leaflet length, days to flowering, mean number of nodes on the main stem, mean number of flower buds, mean length of pods, number of locules/pod, and 100-seed weight.

In conclusion, there are various accounts as to the presence of adequate genetic variability among common bean germplasm worldwide with respect to major qualitative and quantitative morph-agronomic traits. This opportunity, in turn, can be effectively

harnessed to genetically improve common bean through international germplasm exchange channels.

2.5 Association of yield and yield-related/component traits

Selection is an integral part of breeding by which the productivity of common bean can be increased. However, selection of genotypes based on seed yield alone has been shown to have little efficiency (Alem et al., 1998; Miklas and Singh, 2007). This is because, yield is a complex trait, and is highly influenced by genotypic, environmental and Genotype x Environment variations. The more effective approach is indirect selection, which involves selection for yield based on yield and yield related characters. The efficiency and dependability of this approach has been elucidated by several investigators (Ebong, 1972; Yassin, 1973; Sharma et al., 1977; Lal and Tomer, 1979; Rohewal and Pandya, 1980; Saewar et al., 1982; Seifu, 1988; Katiyar and Singh, 1990; Negahi et al., 2014). The extent of association of the characters among themselves and with seed yield can be measured by correlation analysis (Alem et al., 1998; Salehi et al., 2010). However, yield is a complex trait, and it is difficult from correlations alone to determine which traits contribute more to grain yield. Therefore, it is important to carry out other analyses to establish the direct and indirect contribution of each trait on grain yield (Nakawaka and Adiapala, 1999; Kulaz and Ciftci, 2013). The most recent studies done on the association of yield and yield-related and component traits in the common bean are summarized in the following section.

Zelalem (2005) reported that due to the significance of genotype x environment interactions in the traits evaluated, component analysis was carried out for each of the locations included in the study. Consequently, seed yield/plant and pod length had higher and significant genotypic and phenotypic correlations with the seed yield of the common bean accessions studied, while having higher positive direct effects on the same. In view of this, the author noted that indirect selection using seed yield and aforementioned traits, in combination, would improve the accuracy, precision, and efficiency of breeding-by-selection in the common bean.

Kassaye (2006) studied the association of yield with yield-related/component traits in common bean germplasm introductions in Ethiopia. Consequently, it was reported that seed yield had significant and positive correlations with biological yield, number of pods/plant, number of seeds/pod, plant height, stem diameter, number of nodes on the main stem, days to flowering, and days to maturity, while number of pods/plant, 100-seed weight, and number of seeds/pod had the highest direct effect on seed yield.

Roy et al. (2006) studied the association seed yield with yield-related and component traits in 27 common bean genotypes. To this end, they reported that genotypic correlation coefficient values, in most of the cases, were higher than the corresponding phenotypic correlation coefficient values, indicating inherent associations between these traits and seed yield in the common bean genotypes studied. Moreover, seed yield had significantly positive correlations with days to 50% flowering, duration of flowering, plant height, number of pods/plant, and number of seeds/pod. As association of characters, argued the authors, determined by correlation coefficients, alone, may not indicate the relative importance of direct and indirect effects of each individual yield-contributing character on yield, direct and indirect effects were determined using path coefficient analysis. As a result, number of pods/plant, days to flowering, 100-seed weight, number of seeds/pod, plant height, and pod length had higher positive direct effects on seed yield in their order of appearance. Finally, they concluded that number of pods/plant, number of seeds/pod, plant height, and days to flowering were the main contributors to seed yield, and thus, emphasis should be given to selecting positively for plants with these characters jointly with seed yield in order to enhance the efficiency of selection of genotypes in common bean breeding.

Salehi et al. (2008) studied the association of yield and yield-contributing characters/traits. Consequently, they reported that there were positive and significant correlation between number of seeds/pod, number of pods/plant, and pod length with seed yield. On the other hand, factor and path coefficient analyses revealed number of pods/plant, seed yield/plant, and number of seeds/pod, as the most important traits in relation to seed yield in the common bean.

Karasu and Oz (2010) studied the association of seed yield with yield-related and component traits in 30 common bean varieties from Bulgaria. According to the results, highest positive significant correlation was observed between seed yield and biomass yield, number of pods/plant, and number of branches/plant. Furthermore, path analysis indicated that seed yield/plant had the highest direct effect on seed yield/ha, followed by 100-seed weight, and plant height. Furthermore, percentages of direct effects on seed yield/ha were 63.1%, 51.7%, and 48.9%, respectively, for seed yield/plant, 100-seed weight, and plant height.

Salehi et al. (2010) reported that regression analysis revealed that number of pods/plant was the only effective trait on seed yield, explaining 83.2% of the total variation of the latter. Furthermore, path coefficient analysis showed that number of seeds/pod and harvest index had the maximum positive direct effects on seed yield in the common bean.

Sadeghi et al. (2011) reported that correlation analysis demonstrated that seed yield in the common bean was significantly positively correlated with harvest index, number of seeds per pod, number of seeds/plant, number of pods/plant, seed length, straw weight, days to 50% flowering, pod length and 100-seed weight. On the other hand, path coefficient analysis showed that number of seeds/plant, harvest index, seed length, number of pods/plant, and straw weight had the highest positive direct effects on seed yield in the common bean.

Akintunde (2012) stated that:

1. If the correlation coefficient between a causal factor and the effect is almost equal to its direct effect, then correlation explains the true relationship.
2. If the correlation coefficient is positive, but the direct effect is negative or negligible, the indirect effects seem to be the cause of correlation. In such cases, the indirect causal factors are to be considered.
3. The correlation coefficient may be negative but the direct effect is positive and high. In these circumstances, a way to selectively drop the undesirable indirect effects will have to be introduced (Singh and Chaudhary, 1977).

4. The residual effect determines how best the causal factors account for variability of the dependent variable. If the residual accounts for a large portion of the variability in the dependent variable, it then means that other causal variables have to be brought into the study as those being considered are not the causal factors directly responsible for the effect.

Ahmed and Kamaluddin (2013) reported that seed yield in the common bean was correlated significantly positively with days to 50% flowering, plant height, pod length, number of pods/plant, and number of seeds/pod. On the other hand, path coefficient analysis revealed that days to 50% flowering, number of pods/plant, pod length, and 100-seed weight had the highest positive direct effects on the seed yield in the studied common bean germplasm.

Cokkizgn et al. (2013) examined associations between seed yield and yield-contributing traits in common bean accessions from Turkey. As a result, it was reported that seed yield was positively significantly correlated with all traits, except plant height and 100-seed weight. Furthermore, correlations between seed yield and number of branches/plant, pod length, number of seeds/pod, number of pods/plant, number of seeds/plant, and seed yield/plant were positive, whereas that between seed yield and first pod height was negative. Path coefficient analysis, on the other hand, depicted that the highest positive direct effects on seed yield was exerted by number of seeds/plant, whereas 100-seed weight had the highest negative direct effect on seed yield of the studied common bean accessions.

Kulaz and Ciftci (2013) reported that positively significant correlations were found among seed yield and biological yield/unit, seed yield/plant, number of branches/plant, and number of pods/plant. Furthermore, path coefficient analysis revealed higher positive direct effects of biological yield, 100-seed weight, seed yield/plant, plant height, and number of seeds/pod on the seed yield of common bean accessions studied.

Kumar et al. (2014) studied the associations between marketable seed yield and 18 yield-related and component traits in 44 accessions of common bean from India. Results showed that marketable seed yield had highly significant positive correlations with length of inflorescence, number of inflorescence/plant, number of flowers/inflorescence, pod length, pod width, and number of seeds/pod. On the other hand, path coefficient analysis depicted that number of pods/plant was the chief contributing character to seed yield with favorable indirect effects through number of inflorescence/plant, number of seeds/pod, number of flowers/inflorescence, and length of inflorescence. Finally, the authors recommended that seed yield/plant and number of pods/plant were the most important traits to be used in conjunction with seed yield for the indirect-selection scheme in common bean breeding for Indian accessions in the target environments to maximize accuracy, precision, and efficiency.

Negahi et al. (2014) documented that biomass yield, number of seeds/plant, and number of pods/plant had significant positive correlations with seed yield in common bean genotypes from Iran. Path coefficient analysis indicated that number of seeds/plant, 100-seed weight, and biomass yield were considered as the first-order variables among the various seed yield-contributing traits evaluated. Hence, concluded the authors, these traits should be jointly used with seed yield in selection schemes for the improvement of seed yield in the common bean accessions studied.

In summary, cognizant of the importance of selecting multiple yield-related and component traits to enhance selection accuracy and efficiency in the common bean, several authors identified different sets of traits (varying depending on accessions included and study environments).

2.6 Analysis of genetic diversity and population structure of common bean: a global perspective

2.6.1 Analyses for genetic diversity in common bean with morphological markers

Characterization of genetic diversity of accessions can be achieved with phenotypic traits and molecular markers. Phenotypic traits have the advantage that they may be directly related to the fitness of the populations and usefulness for plant breeding (Miklas and

Singh, 2007). Joint analyses of molecular and phenotypic diversity as well as attempts at predicting the breeding value for different phenotypic traits depending on the molecular marker diversity or genotype of the parents; generally show a poor correlation between the two types of data (Reed and Frankham, 2001). The situation can be attributed to a variety of reasons: the lack of tight linkage between molecular markers (mainly neutral) and genes coding for phenotypic traits that may be subjected to selection. Other possible reasons include the lack of correspondence in gene action between phenotypic traits (additive, dominance or epistatic action) and molecular markers (indirect measure of additive gene action), differences in heritability (low to high for phenotypic traits versus high for molecular markers), mutation rate and mutational input (high for polygenic phenotypic traits versus low for molecular markers). Various authors have therefore, proposed to assess and select for genetic diversity by analyzing genes directly involved in the traits of interest (Delaney and Bliss, 1991; van Trienderen et al., 2002). Such studies include agronomic and morphological traits. If phenotypic observations are based on adequately large sample sizes and the physical traits measured show significant differences among populations, they can provide a reasonable representation of overall genetic performance (Humphreys, 1991).

Singh et al. (1991a) reported that principal component analysis showed that Mesoamerican and Andean cultigens had distinct morphology and that the Mesoamerican group was morphologically more diverse than its Andean counterpart. Furthermore, up on undertaking discriminant analysis to further examine the differences between Mesoamerican and Andean cultigens, the following variables had the strongest effect on the discriminant function: length of fifth internode; node to first flower; leaflet length; leaflet width; seed length; seed height; and seed yield.

Horňáková et al. (2003) analyzed the genetic diversity among 82 common bean landrace accessions from the Western and Eastern Carpatien. Consequently, they reported that cluster analysis using phenotypic/morphological data grouped the accessions into two main branches, reflecting growth type, seed-size parameters, and 100-seed weight.

Gomez et al. (2004) indicated most of the genetic variation, with in common bean landrace accessions from Nicaragua, at the molecular level was explained by differences within or among landraces, but not among agro-ecological zones, whereas at the phenotypic level most of the variation was attributed to differences among agro-ecological zones. This suggests that molecular differentiation of landraces [coancestry coefficient (F_{ST}=0.34)] was due to founder effect while phenotypic differentiation was due to the effect of adaptation.

Duran et al. (2004) studied the genetic diversity of common bean landrace accessions from the Caribbean. To this end, they noted that the landraces were grouped into two clusters morphologically: one with Mesoamerican characteristics, which included all the red mottled lines from Haiti and three landraces from the Dominican Republic collected near the Haitian border and the other with Andean characteristics, which included all the lines from Puerto Rico and the remaining lines from the Dominican Republic.

A phenotypic diversity analysis of common bean accessions from Bulgaria showed the formation of five groups with lower similarity values. Nonetheless, the analysis failed to show a clear separation among genotypes. The cluster analysis identified five groups, consisting from five to eight accessions. The populations with climbing growth habit, having high value of weight of plant and low number of pods and seeds per plant, are clustered together. Furthermore, a clear separation among genotypes was not found. Bulgarian and Portuguese populations were dispersed all over the dendrogram (Stoilova et al., 2005).

Kassaye (2006) reported of two major clusters of common bean lines, which belong to the Mesoamerican and Andean pools and with a clustering pattern free of geographical distribution of germplasm, after studying the morphological diversity of introduced bean lines in Ethiopia. In conclusion, the author noted that exploiting the diversity among clusters would broaden the genetic base of dry bean breeding populations in Ethiopia. According to the same, analysis of genetic diversity in germplasm collections can

facilitate reliable classification of accessions, and identification of subsets of core accessions with possible utility for specific breeding purposes.

Barelli et al. (2009) reported that, upon clustering Brazilian bean landraces using the Tocher and Nearest-Neighbor methods, breeding programs in Brazil, and also in other tropical countries, are based on Mesoamerican with limited Andean germplasm introgression. They reached to this conclusive remark after the clustering analyses showed a dominant percentage of the cultivars fell into the Mesoamerican category.

According to Burle et al. (2011) the Andean and Mesoamerican gene pools were clearly separated in two groups along the first principal component (PC1; 39% of total variation). The Andean group showed, in general, larger seeds, an un-striped flower standard, a range of flower colors, and a central pod beak position, while the Mesoamerican group showed smaller seeds, a striped flower standard, purplish or white flowers, and a placental pod beak position. They noted that their results go in conformity with earlier observations made by Gepts et al. (1986). Moreover, the study identified 12 variables for a model in a stepwise discriminant analysis, which enabled the separation of the genotypes into the Andean and Mesoamerican gene pools of domestication. These were: seed weight, standard color, pod beak position, seed coat color pattern, pod beak pattern, seed shape, determinacy, standard surface texture, flower wing color, growth habit, pod maturity color, and seed color (Burle et al. 2011).

Lima et al. (2012) reported that eight groups were evident with cluster analysis, while results of principal component analysis revealed that nine of the 22 descriptors were redundant or little variability, and thus, eliminated. Consequently, it was suggested that 10-20 morphological descriptors can be used in studies of characterization of genetic variation in Brazilian common bean landrace accessions.

Cluster analysis based on different agro-morphological traits revealed important classification regarding genetic diversity for studied traits among genotype. Two local cultivars proved to be best among indigenous and exotic genotypes, respectively. The

cultivars with high grain yield, biological yield and 100-seed weight were grouped into same clusters and these genotypes could prove useful resources for common bean genetic improvement program through hybridization and as direct introduction, after further evaluation in different agroecological zones of the country, especially in the mountainous areas of Pakistan (Awan et al., 2014).

2.6.2 Analyses of genetic diversity in common bean with biochemical (isozymes and allozymes) method

As with all plant species, diversity levels and organization of genetic diversity (–structure") in common bean were initially estimated with a wide array of molecular marker types. The majority of these marker analyses were designed to understand the organization of diversity in the species, which is now one of the best known among crop species. Based on phaseolin, seed storage protein variation and partial reproductive isolation, Gepts and colleagues developed the _two gene pool' concept for *P. vulgaris* (Gepts and Bliss, 1985, 1986; Gepts et al., 1986; Koenig et al., 1990; Gepts, 1990). This result was confirmed by an extensive isozyme analysis (wild species: Koenig and Gepts, 1989; and domesticated: Singh et al., 1991b), and mtDNA restriction fragment length polymorphisms (RFLP) (Khairallah et al., 1990, 1992). These analyses also found that the within-gene pool variation was less than that found between gene pools. Allozyme diversity was a key component utilized to define: three Mesoamerican (Durango, Jalisco, Mesoamerica-1); and three Andean (Nueva Granada, Peru, and Chile) domesticated races in common bean (Singh et al., 1991a). Allozyme data provided important ancestry information regarding the origin of genetic materials from South Western Europe by demonstrating that the patterns of variation in this region are similar to those found in the Americas (Santalla et al., 2002). Essentially the allozyme data provided an important hypothesis regarding the origin of common bean and the levels of genetic diversity within the species.

2.6.3 Genetic diversity analyses using molecular markers in the common bean

Jonah et al. (2011) stated that diversity based on agro-morphological traits, usually varies with environments and evaluation of traits requires growing the plants to full maturity prior to identification, but now the rapid development of biotechnology methods and tools allows easy analysis of large number of loci distributed throughout the genome of

the plants. Molecular makers have proven to be powerful tools in the assessment of genetic variation and in elucidation of genetic relationships within and phenotypically similar cultivars are genetically similar would therefore be of great interest in crop breeding programs (Duzyaman, 2005).

Molecular genetics or the use of molecular technique for detecting differences in the DNA of individual plants has many applications of value to crop improvement (Wammanda and Jonah, 2006). The differences are called molecular markers, because they are often associated with specific gene and acts as a _sign posts' to those genes and such markers when very tightly linked to genes of interest, can be used to select indirectly for the desirable allele and this represents the simplest form of marker-assisted selection (MAS) (Hoisington, et al, 2002) among species (Chakravarthi and Naravaneni, 2006). Molecular markers for classification of genotype are abundant, but unlike morphological traits, markers are not affected by environment (Staub et al., 1997).

Summaries of the major genetic diversity and population structure studies done on the common bean world-wide using molecular markers are presented in the following sections.

Masi et al. (2002) developed and run seven set of multiplex microsatellite markers, based on 30 SSR markers selected from gene bank sequences. From a sample of 264 common bean landrace accessions, they detected a total of 135 alleles, equivalent to 4.3 alleles per SSR. Null alleles were observed in each of the three landraces analyzed. The authors concluded that the procedures used in their study may be applicable in the study of genetic diversity in common bean germplasm collections consisting of a significant number of accessions, and should be transferable to similar analyses of any species.

Payro de la Cruz et al. (2005) analyzed the diversity, genetic structure, and gene flow of wild populations of *Phaseolus vulgaris* L. within its Mesoamerican area of domestication by means of inter-simple sequence repeat molecular markers. Overall, 89% of the loci studied were polymorphic, 35% in the least diverse population and 65% in the most

diverse. Genetic diversity in the populations was high, between h=0.14 and 0.29, as was the maximum distance between populations (D=0.3). Between 40% and 45% of the diversity was explained by the differences among populations. Consequently, the authors postulated that a large number of populations may be necessary to represent the wild gene pool in the germplasm collections. Uniformity in allele frequencies among the populations was observed, suggesting presence of out-crossing. Furthermore, no correlation was observed between genetic and geographic distances, but, remarked the authors, the dendrogram topology suggested geographical isolation due to the mountainous topography. On the other hand, negative correlations were observed between the coefficient of variation of seed size and the distance between wild populations and fields. Similarly, highly negative correlation between percentage of polymorphic loci and distance to the nearest crop field was observed, which also suggested gene flow from the domesticated populations. These observations may suggest that genetic flow was taking place from domesticated toward wild populations and that the farmer, through his agricultural activities, could be influencing the magnitude and the characteristics of the gene flow, and along with this, the differentiation of wild populations. Hence, they suggested that new approaches should be established for conservation *in situ* and maintaining bio-safety, given the risk of introducing genotypes from the Andes and transgenic varieties and causing genetic assimilation.

Martínez-Castillo et al. (2006) studied the genetic diversity/population structure of 11 wild populations of lima bean (*Phaseolus lunatus* L.) in four regions of traditional agriculture in the Yucatan Peninsula, Mexico, part of the putative domestication area of its Mesoamerican gene pool. Furthermore, with eight microsatellite loci examined, the populations showed high values of diversity: observed heterozygosity (H_o) 0.46 to 0.9; Nei‘s index of diversity (H) 0.35 to 0.59 and average number of alleles per locus (A) 2.37 to 3.38. Both Nei‘s index of populations‘ differentiation (G_{st}) and analysis of molecular variance (AMoVA) indicated strong differentiation. The Bayesian analysis of grouping and the Mantel‘s test suggested isolation among agricultural regions as a major factor for population differentiation. Even though a low long-term gene flow (N_m=50.66), and low

rates of recent migration among populations were observed, there were some cases where the accidental transport of seeds could be favoring a gene flow at a long distance.

Kumar et al. (2008) used eight AFLP markers to assess genetic diversity among 44 common bean accessions that included 6 exotic accessions, 15 Indian landraces and 23 released varieties. The AFLP primers used had 820 products, of which 698 were polymorphic (85.12%). Wide variations were observed among all the accessions for the number of amplification products, percent polymorphism and average polymorphism information content (PIC). The Jaccard‘s similarity indices (J) based on the AFLP profiles were subjected to UPGMA cluster analysis. The dendrogram generated revealed seven major groups. Out of 23 released varieties 17 were restricted to clusters VI and VII. The value of r=0.934 in Mantel‘s test applied to the cluster analysis indicated the high fitness of the accessions to a group. Consequently, the authors concluded that the germplasm used in their study had narrow genetic base, although moderate to high genetic diversity was observed.

Kwak and Gepts (2009) analyzed the genome-wide genetic composition at 26, mostly unlinked microsatellite loci, in 349 accessions of wild and domesticated common bean from the Andean and Mesoamerican gene pools. The authors identified nine wild or domesticated populations in common bean, including four of Andean and four of Mesoamerican origins using a model-based approach, implemented in the software STRUCTURE. The ninth population was the putative wild ancestor of the species, which was classified as a Mesoamerican population. A neighbor-joining analysis and a principal coordinate analysis confirmed genetic relationships among accessions and populations observed with the STRUCTURE analysis. Furthermore, geographic and genetic distances in wild populations were congruent with the exception of a few putative hybrids identified in this study, suggesting a predominant effect of isolation by distance. Domesticated common bean populations possessed lower genetic diversity, higher F_{ST}, and generally higher linkage disequilibrium (LD) than wild populations in both gene pools; their geographic distributions were less correlated with genetic distance, probably reflecting seed-based gene flow after domestication. The LD was reduced when analyzed

in separate Andean and Mesoamerican germplasm samples. The Andean domesticated race ‗Nueva Granada' had the highest F_{st} value and widest geographic distribution compared to other domesticated races, suggesting a very recent origin or a selection event, presumably associated with a determinate growth habit, which predominates in this race.

Cabral et al. (2011) evaluated the genetic diversity of 57 common bean accessions, including 31 landraces, propagated by small-scale farmers, 20 accessions supplied by the Brazilian Agricultural Research Agency, and six commercial accessions, using 16 microsatellite markers. Among these markers, 13 were found to be polymorphic, giving 29 polymorphic alleles. The largest number of alleles per locus was observed for the SSR marker, BM141, which had four alleles. The polymorphic information content varied from 0.11 to 0.51, observed the markers BM212 and BM141, respectively. Grouping analysis revealed four groups, according to the place of origin. This tendency was also found in the principal coordinate analysis. The landrace accessions were found to have relatively high genetic diversity, while the released and commercial cultivars had a relatively narrow genetic basis.

Blair et al. (2011) evaluated a wide collection of commercial cultivars of common beans from Mexico, including Azufrado, Bayo, Flor de Mayo, Flor de Junio, Pinto, and black bean types, to discover their level of relatedness with 32 simple sequence repeat (SSR) markers. A total of 204 alleles were detected in their study, showing the diversity among the Mexican cultivars. Through population structure and principal components analyses, Andean and Mesoamerican genotypes as well as three subgroups within the Mesoamerican gene pool were distinguished. The divisions corresponded to a group of Andean cultivars, two subgroups of the ‗Durango–Jalisco' (DJ) complex, and one group equivalent to race ‗Mesoamerica'. This latter race was much less diverse than the DJ complex and had little gene flow with other groups. This study showed the high value of fluorescent SSR markers for evaluating commercial cultivars of common beans. The authors inferred that given the importance of Mexico as a primary center of origin for common beans, as a major market for the crop, and as the second largest producer in

Latin America, this study has implications for the global evaluation of common bean cultivars.

Ince et al. (2011) compared the efficiency of Td-DAMD-PCR, Td-SSR and CAPS-microsatellite techniques using the same PCR amplification profile and reagents towards discerning the genetic diversity present in Turkish landraces of common bean. In conclusion the authors remarked that Td-DAMD-PCR markers amplified with selected 13 mini-satellite primers can be effectively used in identification and preservation of common bean landraces that Td-SSR and CAPS-microsatellite markers could not reveal polymorphisms. Furthermore, they noted that common bean landraces grown in Turkey contain great genetic variations.

Hegay et al. (2012) evaluated 28 common bean accessions from Kyrgyzstan, including control genotypes from the Mesoamerican and Andean gene pools with nine polymorphic microsatellite markers. To this end, the number of alleles per microsatellite locus ranged from 2 to 4, and there were a total of 24 alleles. The observed heterozygosity of each accession over all loci ranged from 0 to 0.11 (with an average of 0.01); while the expected average heterozygosity was 0.05, which could reflect the self-pollinating breeding behavior of common beans. The analysis of molecular variance further revealed that 94.71% of the total variation was accounted by differences among accessions (F_{st} =0.947; $p<0.001$). Cluster analysis grouped accessions in two gene pools: 16 belong to the Andean and 12 to the Mesoamerican gene pool. The microsatellites separated accessions in Mesoamerican gene pool from ‗Durango' and ‗Jalisco' races, which were grouped together. They also reported that Kyrgyzstani accessions, probably from the Mesoamerican race, were the most divergent group. Furthermore, Andean accessions were found to be less diverse than Mesoamerican accessions.

Generally, studies related to determining structure of genetic diversity in various common bean genotypes can be summarized as in the following section:

- SSR-DNA markers are powerful and efficient tools with respect to discerning genetic diversity and population structure in the common bean;

- Joint application of morphological and molecular markers enhance precision and efficiency of studies related to genetic diversity and population structure in the common bean;
- Common bean accessions from similar collection sites tend to cluster together (hence higher genetic differentiation in the studied populations) or cluster differently (indicating possible gene flow between regions/populations studied); and
- The presence of genetic diversity, organized into the Andean and Mesoamerican gene pools of origin, has frequently been evidenced in the majority of the studies, which took this aspect into consideration.

2.7 Genetic diversity and population structure of common bean germplasm from Eastern and Central Africa into the two gene pools

Cultivated common beans originated in Latin America from two centers of domestication about 7,000 to 8,000 years ago (Gepts and Debouck, 1993; Asfaw et al., 2009). The higher diversity, brought about the multiple centers of domestication, is broadly classified into two gene pools: Mesoamerican and Andean (Gepts et al., 1986; Singh et al., 1991a, b, c; Asfaw et al., 2009). The two gene pools further differentiate into different races, such as ‗Mesoamerica', ‗Durango', ‗Jalisco', and ‗Guatemala' in the Mesoamerican gene pool and Nueva Granada, Peru and Chile in the Andean gene pool (Singh et al., 1991b; Beebe et al., 2000). The gene pool and race differences have been validated using various marker systems including seed size, phaseolin (seed storage protein) patterns, plant morphology, isozymes, RFLP, RAPD, AFLP and microsatellite markers (Singh et al., 1991a, b, c; Becerra and Gepts, 1994; Beebe et al., 2000, 2001; Islam et al., 2002; Asfaw et al., 2009; Blair et al., 2006, 2010a).

Common beans are believed to have been introduced into the East coast of Africa by Portuguese and Spanish traders in the sixteenth and seventeenth century (Gentry, 1969). Since then farmers have developed farming practices adapted to local conditions by preservation and exploitation of useful alleles which have resulted in a range of morphologically diverse landraces (Wortmann et al., 1998; Sperling, 2001). Moreover, with recent efforts to improve on-farm level productivity by many national bean-breeding

programs in Africa, new germplasm sources have been continually introduced to African farming systems from different parts of the world since the 1980s (CIAT, 2009). The existence of both gene pools (Andean and Mesoamerican) in Africa has furthermore been documented (Martin and Adams, 1987) and, probably, is a result of original introductions and subsequent imports of novel germplasm. Given the wide range of landraces on the continent, Africa can be considered to be a secondary center of diversity for common beans (Allen and Edje, 1990; Wortmann et al., 1998; Sperling, 2001; Asfaw et al., 2009). The grouping of common bean genotypes from East Africa into the Andean and Mesoamerican gene pools of domestication, though with some level of introgression, was observed in some previous studies (Asfaw et al., 2009). However, many accessions occupied intermediate positions between the two gene pools and the control genotypes for the two gene pools, probably due to introgression and/or shared morphological markers, such as seed color and growth habit of the accessions in them. Although a considerable level of intermixing between different cultivars within individual countries in similar groups was observed in the same study, accessions from the same country of origin tended to cluster together especially with the Andean genotypes indicating distinct germplasm at the national level and perhaps some cross-border gene flow between the countries (Asfaw et al., 2009). After comparing the level of separation of the genotypes with morphological and molecular markers, the same authors remarked that SSR markers were stronger than morphological markers, indicating the success of this marker type in detecting gene pools in the common bean. Nonetheless, despite the limitations of morphological analysis, the similarity distance matrices obtained using SSR markers were significantly correlated with that obtained with morphological markers (Asfaw et al., 2009).

Blair et al. (2010b) reported that common bean diversity in central Africa has been under threat due to a variety of circumstances. First among these, economic and agronomic developments have led to some emphasis on single-component varieties over multi-component mixtures. This is driven by more urban consumers demanding pure lines rather than mixtures, since they are easier to prepare due to uniformity. Secondly, the introduction of new varieties has displaced some traditional varieties. In this regard, new

climbing beans brought into the region since the 1980s have been widely adopted due to their high yield potential and this has led to farm intensification. Furthermore, they documented that compared to other regions; the allelic diversity found in the Central African germplasm was high with an average of ten alleles per locus, but not as high as that found across the entire range of common beans throughout the world. Among the elements that maintain diversity in Central Africa, seed mixing appears to be very important. Seed mixtures are used for various agronomic and cultural reasons (David and Sperling, 1999; Blair et al., 2010b). For example, seed mixtures 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. Strikingly, some genotypes showed inter-gene pool introgression both in their position in the principal coordinate analysis and in their seed characteristics: grouping with one gene pool but having characteristics of the other gene pool. The authors argued that such a preservation of the products of inter-gene pool introgression is a more likely happenstance in farmers who produce for home consumption and save the seed of all the segregants resulting from any natural hybridization for planting in subsequent generation. Highly commercial farmers who rouged out off-types in the field or remove seed mixture components from their harvests are less likely to preserve these segregants, but subsistence farmers would likely save them. Subsistence farmers would also more likely keep seed mixtures intact and practice less strict rouging or selection in their landraces, which in turn, would maintain the high level of diversity and encourage additional rounds of hybridization between divergent genotypes and gene flow between the gene pools. This study identified a high level of genetic diversity in landraces and varieties from Central Africa. As such, germplasm from this region was reported as an integral part of the secondary diversity of common beans found in Eastern and Southern Africa as a whole. They further remarked that this diversity is especially valuable considering the high level of bean production in the region, which, compared to other

regions of the world, is among more concentrated and important to its inhabitants (Blair et al., 2010a).

Fivawo and Msolla (2011) evaluated the genetic diversity of 45 common bean landrace accessions from Tanzania, which were evaluated for agronomic/yield and resistance against six *Phaeoisariopsis griseola* (Sacc) Ferr isolates. Consequently, they found out that considerable diversity in all the ten variables studied particularly in angular leaf spot resistance, six adapted genotypes, and the four checks, BAT332, G5686, Amendoim, Mexico54 exhibited resistance to all the six *Phaeoisariopsis griseola* isolates, whereas 31 genotypes exhibited intermediate resistance and four showed susceptibility to angular leaf spot disease. Much variability was also shown in seed sizes, seed weights, seed colors, and growth habits. The 45 genotypes were also diversified in the seed types collected from the three zones of Tanzania. In addition, the bean genotypes were divergent in each zone, especially, among large and medium large bean types.

Finally, yet equally importantly, a morphological diversity analyses comprising 284 Ugandan common bean landrace cultivars showed there was a moderate genetic diversity, i.e. a mean Shannon-Weaver diversity index value (H) of 0.56±0.19 (Okii et al., 2014a). Principal component analysis (PCA) clustered the germplasm into three major groups (G1, G2 and G3). The genotypes differed mostly for growth habit, pod cross-section, pod curvature, hypocotyl color, days to flowering, node number on the main stem, number of flower buds, and 100 seed weight (Okii et al., 2014a).

3 Rationale and Relevance of the Study

Genetic variation is essential for the development of improved cultivars, as well as survival of the species. Knowledge, access, and use of the available diversity in domesticated and wild relatives are essential for broadening the genetic base of cultivars to sustain improvement (Singh, 2005). The process of domestication inherently reduces genetic variation (Ladizinsky, 1985; Gepts, 2004) and the intensive modern breeding practiced in industrial countries has further diminished their available variability.

The common bean (*Phaseolus vulgaris* L.) is a food crop of a high nutritive value for people in five continents. It has been concluded that the common bean first originated from the New World (Beaver and Osorno, 2009). To this end, two centers of origin were identified–Andean and Mesoamerican. Consequently, domestication and subsequent evolution of the common bean effected changes in morphological, physiological, and other traits. A reduction of genetic variation of cultivated beans, in comparison with wild beans, accompanied this process (Gepts and Debouck, 1993). In Ethiopia, bean breeders have developed and released about three dozens improved bean varieties to date (Bean Research Coordination Office, personal communication). Traditional landraces play a very important role in common bean variety development, as they are deemed to be rich sources of various adaptation and resistance genes (Asfaw et al., 2009; Mondini et al., 2009). Nevertheless, the information about their origin, pedigree, and other characteristics is usually not known or is not available (Asfaw et al., 2009; Blair et al., 2010b).

Knowledge/information on the variability of landrace genotypes with respect to qualitative and quantitative traits helps in identifying accessions with higher potential for yield, disease resistance, and nutritional qualities (Awan et al., 2014). Nonetheless, despite the immense potential landraces possess in enhancing adaptation and resistance/tolerance to major biotic and abiotic stresses, little has been done so far vis-à-vis documenting the variability found in them with respect to important qualitative and quantitative traits in the common bean. This gap can only be bridged via analyzing the

extent and nature of variability present in the landrace accessions with respect to important qualitative and quantitative traits.

Furthermore, as important quantitative traits, such as yield, are very complex in their nature (influenced greatly by genotypic, environmental, genotype x environment effects), selecting singly for traits (direct selection), like grain yield, cannot be relied upon (Zelalem, 2005). Hence, quantifying and determining the association of grain yield with important yield-contributing traits, using correlation and path coefficient analysis is of paramount importance (Negahi et al., 2014; Okii et al., 2014a). However, these important Ethiopian common bean landrace accessions have not so far been studied, so as to determine traits with higher positive association and direct effects on seed yield via component analyses. Hence, there is an urgent need to bridge this gap via determining the association of seed yield with important yield-contributing traits/characters in Ethiopian common bean landrace accessions.

The knowledge about the extent of genetic diversity, identification, differentiation, and characterization of genotypes and populations, their effective extension, leads to better characterization and utilization in breeding (Asfaw et al., 2009). Mondini et al. (2009) argued that a comparison of plant morphology is the simplest approach for the assessment of genetic diversity. This strategy, they noted, is sensitive to environmental influences and cannot always distinguish between closely related samples. Molecular methods of identification have the distinct advantage of being independent of climatic variables but can be limited by other considerations. For example, it is important that the technique used must be able to distinguish most or all genotypes held in a collection and also be able to provide evidence of genetic erosion. At the same time, the urgent need of identifying accessions held in gene banks dictates that the protocol used should be quick, uncomplicated and cheap. Among the various marker types, simple sequence repeat (SSR) loci are ideal for visualizing the genetic distinctiveness of common bean cultivars due to their high inherent variability, co-dominant inheritance, and allelic diversity (Blair et al., 2006). In addition, SSR markers are highly repeatable and provide room for automization; as has been elucidated in some recent studies in the common bean (Kwak

and Gepts, 2009; Blair et al., 2010b). Finally, SSR markers are considered to be very useful for determining population structure within both inbreeding (Coburn et al., 2002) and cross-breeding species (Liu et al., 2003). In the common bean, SSR fingerprinting has been used to determine cultivar differences within each of the gene pools based on landraces (Díaz and Blair, 2006; Blair et al., 2007), national germplasm collections (Asfaw et al., 2009; Becerra et al., 2010; Blair et al., 2010b), or core collections (Blair et al., 2010a), but less often in released cultivars.

The existence of the Andean and Mesoamerican gene pools in common bean and the multiple domestications associated with them are a unique situation among crops, rice being an exception (Vitte et al., 2004; Londo et al., 2006), pepper (e.g., Kraft et al., 2014), anc cotton (Page et al., 2013). Knowledge on the level of genetic diversity and population structure in common bean landrace genotypes with respect to the two gene pools of origin is the crux of utilizing the potential of these important germplasm collections towards future genetic improvement and conservation endeavors. The knowledge generated, in turn, helps answer a number of questions, such as the origin and relationships between these two gene pools, the qualitative and quantitative differences in genetic diversity between them, the respective levels of linkage disequilibrium, and the extent to which different loci have been the subject of selection during and after the two major domestications in the species. This can be justified, as this strong division of the domesticated common bean gene pool in to Andean and Mesoamerican groups, presents clear implications for the use of common bean germplasm, such as in disease resistance breeding (Guzmán et al. 1995; Crous et al., 2006).

The body of knowledge garnered in the areas of morphological and molecular genetic diversity and population structure with respect to the Andean and Mesoamerican gene pools can be of limited use, if not accompanied by knowledge and information gathered through integrating molecular data with agro-morphological information. Such integration helps clearly understand the true identities of the cluster groups identified at both molecular and morphological levels. Nonetheless, there has been no systematic study toward determining the morphological and molecular genetic diversity/population

structure; and identifying the ecogeographic races present among Ethiopian common bean landraces.

This strong division of the domesticated commonbean gene pool in Andean and Mesoamerican groups presentsclear implications for the use of common bean germplasm, such as in disease resistance breeding. Guzmán et al. (1995) identified two major groups of isolates of thefungus responsible for angular leaf spot disease (*Pseudocercospora griseola*, formerly *Phaeoisariopsis griseola*; Crouset al., 2006). The two groups of isolates were recovered, respectively, in Andean and Mesoamerican accessions of common bean, suggesting co-evolution between the fungusand its common bean host. Singh et al. (1991a) alsoproposed a further genetic classification of each majorgene pool into three ecogeographic races on the basis ofmorphological characteristics, ecological distribution, and phaseolin and allozyme types of domesticated commonbean genotypes. The integration of molecular marker data about the genetic diversity/population structure with agronomic/morphological data has been used to ascertain the coherence between grouping of accessions identified with phenotypic/molecular markers, and identify the sub-groups identified in each to the eco-geographic races, belonging to the Mesoamerican/Andean races (Singh et al., 1991a, b; Blair et al., 2010b; Burle et al., 2011). Nonetheless, there has been no systematic study toward determining the morphological/phenotypic and molecular genetic diversity/population structure; and identifyingthe ecogeographic races present among Ethiopian common bean landraces.

Consequently, owing to the aforementioned rationale, this Ph. D research was undertaken, in order to, mainly, determine the genetic diversity of common bean germplasm from Ethiopia, discern the prevailing population structure with respect to the Andean and Mesoamerican gene pools of origin, and identify sub-groups identified in the molecular analysis with the integration of agronomic/morphological marker data.

4 Materials and Methods

4.1 Field experiment for genetic trait-variability, trait associations, and phenotypicdiversity studies

4.1.1 Planting materials

Of the 121 common bean accessions used in this study, 115 were landraces, which were acquired from the gene bank of the Ethiopian Institute of Biodiversity Conservation (EIB). In addition, six released varieties, which were provided by the Ethiopian National Bean Research Project (ENBRP), based at Melkassa Agricultural Research Center, were used as control genotypes and/or for comparison purposes. Selection of the landrace accessions used in the study was done on the basis of the importance of the regions in terms of size of bean production from the passport data of the landrace accessions. Thus, a total of 121 landrace accessions, among them 6 released cultivars, were included in the study. Summary of collection sites, names, and numbers of accession are presented in Table 3. Furthermore, the geographic regions, from which sampling was done for the landrace accessions, are displayed in Figure 10. Among the released varieties, _Awash-1‘ and _Mdka-Dima‘, were used as Mesoamerican and Andean control genotypes, respectively. Both the landrace accessions and the released varieties were grouped into 5 regional states and one additional group named the released varieties group, respectively, based on geographical proximity and possibility of sharing common seed systems (Table 3). Passport and other related data of the accessions for the collection sites are presented in Appendix 1.

4.1.2 Test environments and locations

The 121 accessions, including the released cultivars, used in the study were grown in a field experiment at the main research station of Melkassa Agricultural Research Center, Nazareth, Ethiopia, during the main rainy season of 2013 (i.e., June-November 2013). The center is geographically located at a latitude of 78^0 24‘ North; a longitude of 39^0 21‘ East; and 107 km and 17 km from Addis Ababa and Adama, respectively, in the Oromiya regional state. It has an altitude of 1550 m above sea level (asl) (http://www.eiar.gov.et/index.php/melkassa-agricultural-research-center, accessed on

January 14, 2015). Climatic conditions of the study site during the planting period are presented in Appendix 2.

4.1.3 Experimental design and field management

Planting of the genotypes was done in Mid-June 2013. Experimental management practices, such as fertilizer application, inter and intra-row spacings, seed rate, etc. were done, according to the nationally-recommended cultivation practices for common bean in the semi-arid areas of Ethiopia. The experimental design was simple lattice (11 X 11). Each plot consisted of four rows spaced 60 cm, and plants spaced 15 cm apart, with a plot length of 4m. The two outer most rows were used as border rows, to minimize associated effects of neighboring plots.

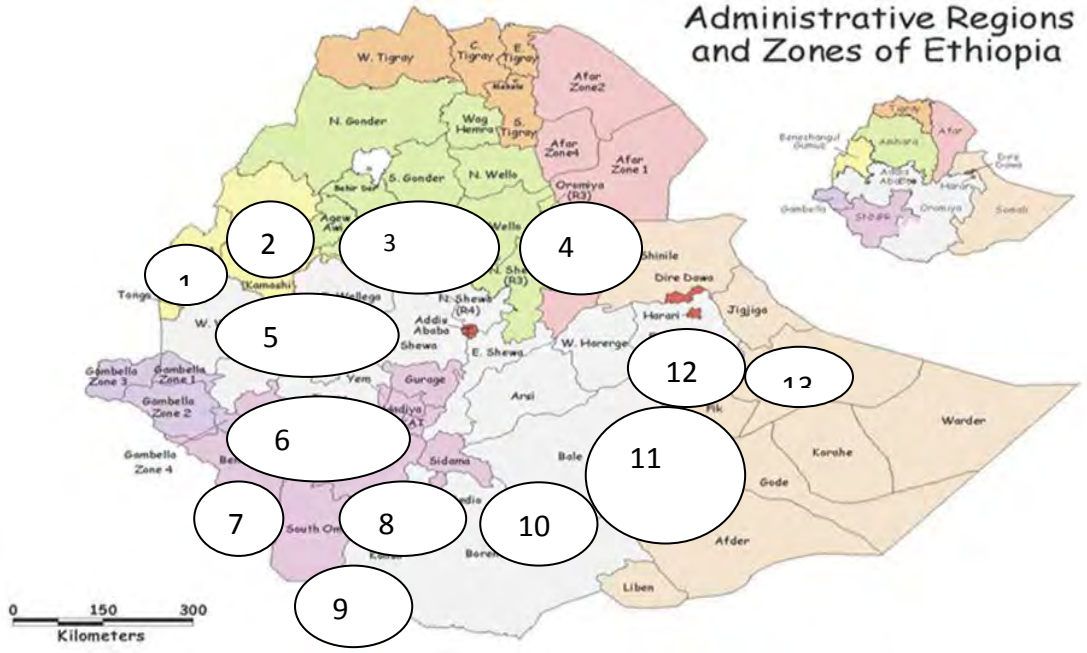

Figure 10: Map showing the collection sites

(key: 1 = Assosa; 2 = Metekel; 3 = Gojam; 4 = North Shewa & South Wello;; 5 = Wellega Gojam; 6 = Jimma and Illubabor; 7=Bench Maji; 8 = North Omo;; 9 = South Omo; 10 = Sidama and Others around; 11 = Bale & Arsi; 12 = East Hararghe;13 = West Hararghe) size of bubbles doesnot correspond to number of genotypes sampled in each location

Table 3: List of accessions, collection regions, and sites for the 121 common bean (*Phaseolus vulgaris* L.) genotypes used in the study

No	Regions	Administrative Zone	ID	No	Location	Administrative Zone	ID
1	Oromiya	Jimma/Illubabor	201666	38	Oromiya	E.Hararghe	211319
2	Oromiya	W.Hararghe	201293	39	Oromiya	E.Hararghe	211320
3	Oromiya	W.Hararghe	201294	40	Oromiya	E.Hararghe	211322
4	B.Gumuz	Assosa	207933	41	Oromiya	E.Hararghe	211323
5	B.Gumuz	Assosa	207934	42	Oromiya	W.Hararghe	211325
6	B.Gumuz	Assosa	207938	43	Oromiya	W.Hararghe	211327
7	Oromiya	Jimma/Illubabor	207949	44	Oromiya	W.Hararghe	211329
8	Oromiya	W.Hararghe	208638	45	Oromiya	E.Hararghe	211331
9	Oromiya	E.Hararghe	208646	46	Oromiya	E.Hararghe	211332
10	Oromiya	E.Hararghe	208647	47	Oromiya	E.Hararghe	211334
11	Oromiya	Wellega	208695	48	Oromiya	Wellega	211337
12	Oromiya	Wellega	208698	49	Oromiya	Wellega	211338
13	Oromiya	Wellega	208702	50	Oromiya	Wellega	211339
14	Oromiya	Wellega	208703	51	Oromiya	Wellega	211340
15	Oromiya	Wellega	208705	52	Oromiya	Wellega	211341
16	Amhara	Gojam	211266	53	Oromiya	Wellega	211342
17	Amhara	Gojam	211267	54	B.Gumuz	Metekel	211344
18	Amhara	Gojam	211269	55	B.Gumuz	Metekel	211345
19	Oromiya	Wellega	211271	56	B.Gumuz	Metekel	211347
20	SNNP	S.Omo	211277	57	B.Gumuz	Metekel	211348
21	SNNP	S.Omo	211278	58	B.Gumuz	Metekel	211349
22	SNNP	S.Omo	211279	59	B.Gumuz	Metekel	211350
23	SNNP	S.Omo	211286	60	B.Gumuz	Metekel	211356
24	SNNP	Benchmaji	211290	61	B.Gumuz	Metekel	211361
25	SNNP	Benchmaji	211291	62	B.Gumuz	Metekel	211362
26	SNNP	Benchmaji	211292	63	Oromiya	Bale & Arsi	211377
27	SNNP	N.Omo	211293	64	Oromiya	Bale & Arsi	211378
28	SNNP	N.Omo	211294	65	Oromiya	Bale & Arsi	211379
29	Oromiya	W.Hararghe	211298	66	Amhara	North Shewa/South Wello	211382
30	Oromiya	W.Hararghe	211299	67	Amhara	North Shewa/South Wello	211386
31	Oromiya	W.Hararghe	211300	68	Amhara	North Shewa/South Wello	211387
32	Oromiya	W.Hararghe	211301	69	Oromiya	Wellega	211388
33	Oromiya	W.Hararghe	211304	70	Amhara	North Shewa/South Wello	211389
34	Oromiya	W.Hararghe	211305	71	SNNP	S.Omo	211394
35	Oromiya	E.Hararghe	211315	72	SNNP	Benchmaji	211481
36	Oromiya	E.Hararghe	211317	73	SNNP	Benchmaji	211483
37	Oromiya	E.Hararghe	211318	74	SNNP	N.Omo	211546

Table 3 (cont'd)

No	Region	Administrative Zone	ID	No	Region	Administrative Zone	ID
75	Amhara	North Shewa/South Wello	211550	106	SNNP	Sidama	241748
76	Amhara	North Shewa/South Wello	211551	107	SNNP	N.Omo	241750
77	SNNP	N.Omo	211552	108	SNNP	Benchmaji	241752
78	SNNP	S.Omo	212860	109	SNNP	Benchmaji	241753
79	Oromiya	Bale & Arsi	212861	110	SNNP	Benchmaji	241756
80	SNNP	N.Omo	212978	111	SNNP	Benchmaji	241757
81	SNNP	Benchmaji	213046	112	SNNP	Benchmaji	241765
82	Amhara	North Shewa/South Wello	215719	113	Amhara	Gojam	241807
83	Gambella	Gambella	216730	114	Amhara	Gojam	241814
84	Oromiya	E.Hararghe	216819	115	Southern Nations	Sidama	244805
85	Oromiya	E.Hararghe	216820	116	Released	Released	Andean Control
86	Oromiya	W.Hararghe	219233	117	Released	Released	CHERCHER
87	Oromiya	E.Hararghe	219234	118	Released	Released	GOBERASHA
88	Oromiya	E.Hararghe	219235	119	Released	Released	MA Control
89	Oromiya	E.Hararghe	218235	120	Released	Released	NASER
90	Oromiya	Bale & Arsi	230779	121	Released	Released	MEX-142
91	SNNP	Benchmaji	235692				
92	Oromiya	Bale & Arsi	235697				
93	Oromiya	Bale & Arsi	237078				
94	SNNP	N.Omo	237993				
95	Oromiya	Jimma/Illubabor	240173				
96	Oromiya	Jimma/Illubabor	240187				
97	Oromiya	Jimma/Illubabor	240190				
98	B.Gumuz	Metekel	240512				
99	Oromiya	Bale & Arsi	240552				
100	Oromiya	Bale & Arsi	241730				
101	SNNP	Sidama	241733				
102	SNNP	Sidama	241736				
103	SNNP	Sidama	241737				
104	SNNP	N.Omo	241738				
105	SNNP	N.Omo	241739				

Key: B.Gumuz=Benishangul-Gumuz Regional State; E. Haraghe=Eastern Hararghe; MA=Mesoamerican;N.Omo=North Omo; SNNP=Southern Nations, Nationalities, and Peoples Regional Stat; S.Omo=South Omo; W.Hararghe=West Haraghe

4.1.4 Data collection for agro-morphological genetic variability and association analyses

4.1.4.1 Data collected for quantitative and qualitative trait variability

A total of ten qualitative and eight qualitative traits were recorded following IBPGR (2002) and Singh et al. (1991a) morphological descriptors (Table 4). Details on the ranges and labels of all parameters used in qualitative data collection are given in Table 4. Morphological descriptors were evaluated according to IBPGR (2002) and Singh et al. (1991a) (Table 4).

Quantitative trait data were collected on the following agronomic/phenological variables:

- **Days to (50%) flowering**: the number of days taken by each accession from the day of sowing to the day on which 50% of the plants in the harvestable rows of a plot opened their first flower in a plot;
- **100-seed weight**: the average weight of 100 seeds taken randomly from five randomly-selected plants measured at 12-14% seed moisture content and expressed in grams;
- **Seed yield per plot**: the weight of seeds harvested from all the plants in the harvestable rows of a plot measured in grams;
- **Seed height**: average height in millimeter of 15 seeds from five randomly-selected plants measured from the hillium to the opposite side, using Vernier Caliper;
- **Seed diameter**: average diameter of 15 seeds from five plants measured using Vernier Caliper and recorded in millimeter;
- **Number of pods per plant**: the number of fertile pods from five randomly-selected plants counted and averaged;
- **Plant height**: height of the plant from the ground surface to the tip of the main guide recorded in centimeters;
- **Number of seeds per pod**: the average number of seeds from 30 randomly-taken pods from five randomly selected plants counted and averaged;
- **Number of branches per plant**: the number of branches on the main stems of five randomly taken plants counted and averaged; and

- **Number of seeds per plant**: number of seeds in all the pods of five randomly taken plants counted and averaged.

On the other hand, the eight morphological descriptors of common bean, presented below in Table 4, were evaluated on the third (central) plant of each field plot in each of the two replications, according to Singh et al. (1991a), IBPGR (2002), and Burle et al., (2011). Traits scored were: seed coat color; plant growth habit; standard (flower) color; seed shape; seed brilliance; pod color; color of flower wings (in freshly-opened flowers); and user category (Table 4).

Table 4: Morphological (qualitative) character traits evaluated in the sample of Ethiopian common bean (*Phaseolus vulgaris* L.) landraces and their ranges

No.	Traits	States, Ranges	Remark
1.	Seed coat color	White; dull; cream; yellow; brown; pink; red	The ranges included are only the ones observed
2.	Plant growth habit	I; II; III; IV	I=Determinate Bush; II=Indeterminate bush; III=Indeterminate Prostrate; IV=Determinate/Indeterminate Climbing
3.	Standard (flower) color	White; green; lilac; white with lilac edges; white with red stripes; dark lilac; carmine red; purple	The ranges included are only the ones observed
4.	Seed shape	Round; oval; cuboid; kidney; markedly-truncate or truncate fastiagte	The ranges included are only the ones observed
5.	Seed brilliance	Dull; medium; shiny	The ranges included are only the ones observed
6.	Pod color	Dark purple; dark pink rose; pale yellow to white	The ranges included are only the ones observed
7.	Color of flower wings (in freshly-opened flowers)	White; carmine stripe on green; purple	The ranges included are only the ones observed
8.	User‘s category	Dry bean/snap bean	

4.1.4.2 Data collected for the association study

In view of elucidating the association of seed yield with other related and component traits among landrace accessions from different growing areas of Ethiopia, eight important agronomic traits were analyzed, as independent variables, and seed yield/plot, as the dependent variable.

- Seed yield per plot (g/m^2);
- Days to (50%) flowering;
- 100-seed weight (g);
- Seed diameter (mm);
- Number of pods per plant;
- Plant height (cm);
- Number of seeds per pod;
- Number of branches per plant;
- Number of seeds per plant.

4.1.5 Data analysis for agro-morphological genetic variability and association analyses

4.1.5.1 Data analysis for the quantitative/qualitative variability study

Transformation was done for all the qualitative character variables according to Fisher (1938), cited in Burle et al. (2011), optimal scoring method using the OPSCORE transformation option in the SAS 9.1 PRINQUAL procedure (SAS Institute, 2009). To avoid distortions, observations with more than one missing value or observations that presented missing values transformed to outlier values were excluded from the analysis. Subsequently, all the agro-morphological variables (transformed qualitative variables and original quantitative variables) were checked for normality using the SAS 9.1 (http://support.sas.com/documentation/onlinedoc) UNIVARIATE procedure (PLOT option).

I. Analysis of variance (ANOVA) for quantitative traits

The plot mean values were subject to statistical analysis of variance as per the simple lattice design for each character separately. The relative efficiencies of simple lattice over

RCBD were calculated as per the method described by Gomez and Gomez (1984). To this end, as the relative efficiency values of lattice over RCBD calculated for all the traits did not exceed 1.15 (Appendix 3), further analyses were done using RCBD model.

II. Analysis of qualitative traits

As none of the transformed qualitative variables had normal distributions, a Fisher's exact test was done and calculated with the PROC FREQ procedure in the SAS software package, SAS 9.1 (SAS Institute, 2009), in order to compare frequencies for qualitative character traits.

4.1.5.2 Data analyses for the association study

I. Correlation analysis

Correlation coefficients between the parameters were calculated from the variance and covariance components using the formula;

$$r_{(x,y)} = Cov(x,y)/(\sigma_x^2\sigma_y^2)^{1/2}$$

Where:

r (x, y) is the phenotypic correlation between variables x and y.

Cov (x, y) is the phenotypic co-variance between the two variables, σ_x^2 is the phenotypic variance of the variable x, and σ_y^2 is the phenotypic variance of the variance of the variable y. Subsequently, phenotypic correlation coefficients were tested for their significance with tabulated r-values at t-2 degrees of freedom (Singh and Chaudhary, 2001).

II. Path coefficient analysis

Path coefficient analysis was carried out using phenotypic correlation values to determine the direct and indirect effects of the yield components and other morphological characters on seed yield per plot using the general formula of Dewey and Lu (1959).

$r_{ij} = P_{ij} + \sum r_{ik}P_{jk}$:

Where:

r_{ij} is the mutual association between the independent character (i) and dependent character (j);

P_{ij} is the direct effect of independent character (i) on the dependent variable (j); and $\sum r_{ik}P_{jk}$ is summation of components of indirect effects of a given independent character (i) on a given dependent character (j) via other independent characters (k).

To determine P_{ij} values, square matrices of phenotypic correlation coefficients between independent characters in all possible pairs were inverted and then multiplied by the correlation coefficient between the independent and dependent characters following complete descriptions of the method in Singh and Chaudhary (2001).

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4.1.5.3 Genetic diversity analysis using agro-morphological data

The landrace accessions were clustered by the Tocher and Nearest Neighbor methods, using the R-software program (version 3.1.1). The Tocher method uses dissimilarity matrix as an input, based on which the most similar pair of individuals, composing the initial group would be identified. Then, the possibility of inclusion of new individuals was evaluated, adopting the requirement that the average intra-group distance was lower than the average intergroup distance (Cruz and Carneiro, 2003).

The genetic diversity among clusters was determined using the generalized Mahalanobis-D^2 statistics. This method determines divergence prevailing among populations in terms of generalized group distance (Sharma, 1998; Olika et al., 2011). Testing the significance D^2 values obtained for a pair of clusters were considered as calculated chi-square (X_2) values, and tested against the tabulated X_2 values for P degrees of freedom (P being the number of characters) (Singh and Chaudhary, 1985), at an appropriate probability level that was considered.

Principal component analysis was performed using generated correlation matrices by employing the R-program among 17 agro-morphological correlated traits, and subsequently converting those into new uncorrelated variables, called principal components, were identified as per the formulae suggested by Johnson and Wichern (1988).

4.2 Molecular genetic diversity and population structure study

4.2.1 Plant materials

A total of 116 landrace accessions collected from a range of common bean production agro-ecologies in Ethiopia, four Ethiopian cultivars, three Kenyan cultivars, and other two cultivars, used as control genotypes for the Andean and Mesoamerican gene pools, respectively, were grown in a greenhouse at the Biosciences eastern and central Africa (BecA-ILRI) hub, Nairobi, Kenya, for DNA extraction and analysis. The Ethiopian accessions were sampled from potential bean growing areas in the country (Table 5; Fig. 10). The seeds of the control and commercial cultivars were acquired from the Ethiopian National Bean Research Project, based at Melkassa Agricultural Research Center, Adama, Ethiopia. On the other hand, the landrace accessions were provided by the Gene Bank of the Ethiopian Biodiversity Institute, (EIB). A total of ten plants per each accession were planted in a single row in the screen house of BecA-ILRI hub, Nairobi, Kenya in August 2012 for DNA extraction.

4.2.2 Genomic DNA extraction

For the molecular diversity assessment, total genomic DNA for each accession was isolated from a bulked leaf tissue sample of one week old plants from five randomly selected plants per accession using cetyltriethylammonium bromide (CTAB) method (Doyleand Doyle, 1990) with some minor modifications, as described in the following sections.

About 200mg of fresh leaf tissue samples/leaf were placed in a 2ml autoclaved and labeled Eppendrof tubes, covered by paraffin paper with a small slot at one side for circulation, and freeze-dried for two days at -80^{0}C. Subsequently, a drop of Polyvinyl PolyPyrrolidone (PVPP) was added to the Eppendrof tubes to increase the probable breakup of cellular materials for dry grinding. Then after, 500µl of 1X CTAB was added to each tube to break open cells and soluble cellular contents. Next, the contents in each tube were mixed using vortex, and kept in gently-shaking water bath for 1 hour at 65 ^{0}C. After the samples were taken out of the water bath, they were centrifuged at 14,000 rpm for 30 minutes, using Eppendrof centrifuge (5417R). Afterwards, the supernatant solution

was transferred into new Eppendrof tubes, and 250µl of potassium acetate was added. A total of 400µl of ice-cold isoproanol was added to the supernatant solution harvested, after centrifuging the samples at 14,000 rpm for 30 minutes. At this point, the samples were left to incubate at -20^{0}C overnight. The following day, the samples were removed from the -20^{0}C freezer; centrifuged at 14,000 rpm for 30 minutes at -4^{0}C, and then, the supernatant harvested. Then after, the supernatant was poured off, and dried, in order to remove the remaining isopropanol drops, by placing the tubes upside down on a paper towel, and air-drying the pellet for 30 minutes at room temperature.

Subsequently, 200µl of TE and 3µl of RNAse were added to each tube, and they were left to incubate in a water bath at 37 ^{0}C. Following this, chlorophyll and some denatured proteins were removed by dissolving in 200µl mixture of phenol, chloroform, and isoamylalcohol at a ratio of 25:24:1, then it was mixed with manual inversions from 5 to 10 times. Next, the samples were incubated at room temperature for 10 minutes. Subsequently, fixed volume of supernatant (180µl) was harvested from each tube into new sets of 1.5ml Eppendrof tubes, and 300µl of ice-cold 100% ethanol plus 15µl of sodium acetate (at PH 5.2) was added to each. Then, they were incubated at -80^{0}C for 5 minutes. Centrifuging was done at 14,000 rpm for 30 minutes, the supernatant poured off, the inside of each tube washed with 200µl of 70% ethanol, and another centrifuging done at 14,000 rpm for 30 minutes at -4 ^{0}C. Following this, DNA pellets were air-dried for an hour, and re-suspended with 30µl of low salt buffer. DNA quality and quantity were measured by gel electrophoresis (using 1% agarose gel for 1 hour using λ-DNA marker) (Fig. 11).

However, 47 accessions did not produce enough, genomic DNA, probably due to poor leaf sample qualities, which, in turn, imposed the need to re-do DNA extraction for the same, using zymo plant seed DNA extraction kit (details on the protocol are presented in appendix 4).

Table 5: ID number and names of collection site for the germplasm used in the study

No.	Accession ID	Region/Collection Area	No.	Accession ID	Region/Collection Area	No.	Accession ID	Region/Collection Area
1	211315	East Hararghe	28	NASER	Standard Variety	70	241738	North Omo
2	211317	East Hararghe	29	237993	North Omo	71	241739	North Omo
3	211318	East Hararghe	30	240173	Jimma and Illubabor	72	241748	Sidama et al
4	211349	Metekel	31	240512	Metekel	73	216819	East Haraghe
5	241736	Sidama et al	32	241730	Bale & Arsi	74	211278	South Omo
6	241756	Bench Maji	33	207934	Assosa	75	211292	Bench Maji
7	241757	Bench Maji	34	207938	Assosa	76	237078	Bale & Arsi
8	244805	Sidama et al	35	207949	Jimma and Illubabor	77	211348	Metekel
9	211286	South Omo	36	208638	West Hararghe	78	211356	Metekel
10	211294	North Omo	37	212861	Bale & Arsi	79	211378	Bale & Arsi
11	211293	North Omo	38	212978	North Omo	80	211387	North Shewa & South Wello
12	211301	West Hararghe	39	213046	Bench Maji	81	208646	Somali
13	211331	Somali	40	215719	North Shewa & South Wello	82	208695	Wellega
14	211340	Wellega	41	219233	West Hararghe	83	208698	Wellega
15	211341	Wellega	42	230779	Bale & Arsi	84	208702	Wellega
16	211345	Metekel	43	235692	Bench Maji	85	208703	Wellega
17	208647	Somali	44	235697	Bale & Arsi	86	211266	Gojam
18	208705	Wellega	45	201066	Jimma and Illubabor	87	211267	Gojam
19	211269	Gojam	46	201293	West Hararghe	88	211277	South Omo
20	211271	Wellega	47	201294	West Hararghe	89	211279	South Omo
21	211389	North Shewa & South Wello	48	207933	Assosa	90	211290	Bench Maji
22	211394	South Omo	49	MWITEMA	Kenyan	91	211291	Bench Maji
23	211551	North Shewa & South Wello	50	E7	Kenyan	92	211299	West Hararghe
24	211552	North Omo	51	WANJIRU	Kenyan	93	211300	West Hararghe
25	MELKADIMA	Andean Control	52	211298	West Hararghe	94	211305	West Hararghe
26	CHERCHER	Standard Variety	53	211294	North Omo	95	211319	East Hararghe
27	GOBERASHA	Standard Variety	54	211304	West Hararghe	96	211320	East Hararghe

Table 5 (cont'd)

No.	Accession ID	Region/Collection Area	No.	Accession ID	Region/Collection Area
97	211322	East Haraghe	113	211349	Metekel
98	211323	East Haraghe	114	212860	South Omo
100	211327	West Haraghe	115	216819	East Hararghe
101	211329	West Haraghe	116	216820	East Hararghe
102	211332	East Haraghe	117	211337	Wellega
103	211295	East Haraghe	118	240522	Metekel
104	211337	Wellega	119	241733	Sidama et al
105	211338	Wellega	120	241750	North Omo
106	211339	Wellega	121	241752	Bench Maji
107	211342	Wellega	122	241753	Bench Maji
108	211344	Metekel	123	241755	Bench Maji
109	211350	Metekel	124	241814	Gojam
110	211377	Bale & Arsi	125	MEXICAN-142	Standard Variety
111	211388	Wellega			
112	211546	North Omo			

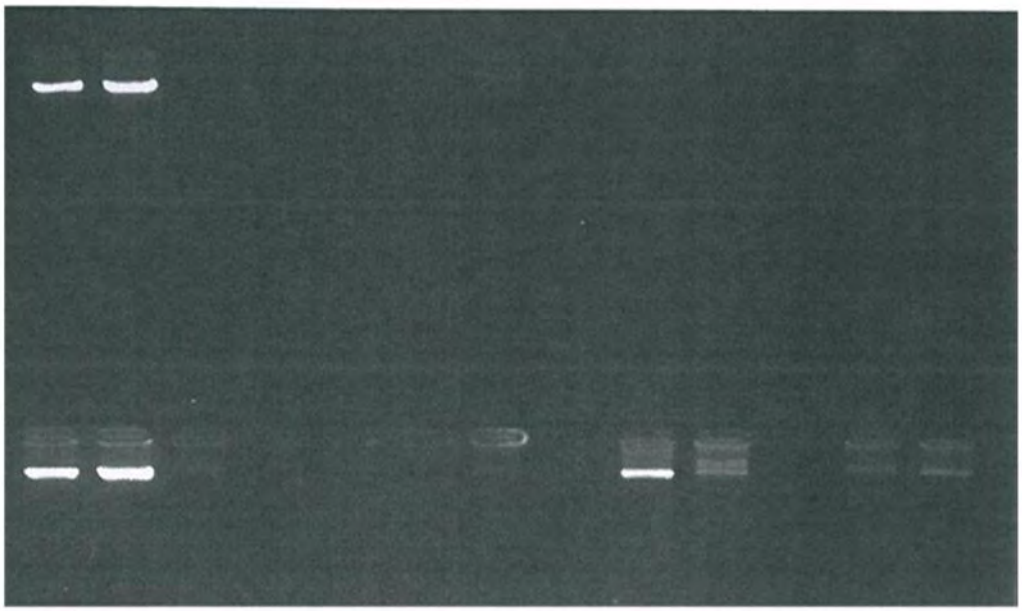


Figure 11: Gel picture of the genomic DNA extraction done with λ-uncut DNA ladder

Upon completion of DNA extraction from seeds, DNA quality and quantity were measured, similar to the aforementioned techniques, using gel electrophoresis and nano-drop readings, respectively. Figure 12 show the results of gel electrophoresis.

4.2.3 Microsatellite amplification

Twenty-four (24) microsatellite markers from all the 11 linkage groups were selected based on their Polymorphic Information Content (PIC) values and dispersed map locations (Yu et al., 2000; Pedrossa-Haren et al., 2008; Kwak and Gepts, 2009). Out of the 24 SSR markers, 15 were genomic, and the remaining nine were non-genomic (genic) markers. Sequences, repeat motifs, and color (dye) of the markers are presented in Table 6.

Markers were PCR amplified with 6-FAM, NED, PET or VIC 5‘-labeled forward primers and unlabeled reverse primers. The primers were run in multiplexes, based on their fluorescence dye and allele size using BIONEER ACCUPOWER® Multiplex PCR Premix Kits (Appendix 3). Out of the 24 SSR markers, seven were dropped after preliminary evaluation, because either producing no amplification or being monomorphic. These were: BM172 and BMd1 (no amplification) and BM188, BM183, BMd16, PV-AG001, and PV-AT001 (monomorphic). PCR products were run on an ABI PRISM 3730xl fragment analyzer (Applied Biosystems, Foster

City, CA, USA) at the BecA-ILRI hub (Sequencing, genotyping, and Oligo unit, SegoLip), and allele sizes were determined by comparing with Genescan LIZ500 size standard using GeneMapper v. 3.7.3.7 software. The observed allele size was then adjusted for the discrete allele size using AlleloBin software (http://test1.icrisat.org/gt-bt/download_allelobin.htm).

4.2.4 SSR genetic diversity analysis

Genalex 6.5b3 (http://biology.anu.edu.au/GenAlEx/) was used to calculate genetic diversity parameters, such as genetic distance, number of alleles (N_A); number of effective alleles (N_E); number of private alleles (N_{PA}); observed heterozygosity (H_O); Shannon's information index (I); Analysis of Molecular Variance (AMoVA); and Principal Coordinate Analysis (PCoA).

Genetic associations were determined with Darwin V. 5.0 (http://darwin.cirad.fr/darwin), using neighbor-joining coefficient. Genepop V.4 (http://www.cecill.info/index.en.html) and Popgene32 (http://www.ualberta.ca/~fyeh/fyeh) programs were also used to determine genetic diversity, polymorphic loci, gene flow, levels of heterozygosity, fixation index, and F-values,. Finally, PowerMarker v. 3.25 (Liu and Muse, 2005) was used to estimate the number of alleles, Polymorphic Information Content (PIC) values, genetic distance matrices, and observed heterozygosity (H_o) for each marker across all genotypes and then across the genotypes within and between gene pools.

4.2.5 Analysis of population structure

The software program STRUCTURE was run for K values ranging from 2 to 8. Each run was performed using the admixture model and 5,000 replicates for burn-in and 50,000 during the analysis (Pritchard et al., 2000). The K = 2 analysis was done with a particular interest of distinguishing between Andean and Mesoamerican accessions (Kwak and Gepts, 2009). To this end, five independent runs were performed with the admixture model and 5,000 replicates for burn-in and 50,000 replicates during analysis. The clustering in different runs was almost identical (similarity coefficient 0.9914). The run with the lowest likelihood value was selected among the five runs, and the accessions with more than 50% posterior assignment probability for the Mesoamerican cluster were assigned to the Mesoamerican gene pool (and vice versa for the Andean gene pool) (Table 26). Lower posterior assignment probability values (e.g., between 50

and 80%) may actually indicate hybrids rather than ‗pure'' accessions (Kwak and Gepts, 2009). Nonetheless, such accessions were included in the K=2 analysis, as they are important in future studies towards shedding light on the population structure of the common bean in Ethiopia, and as baseline information in breeding/improvement programs.

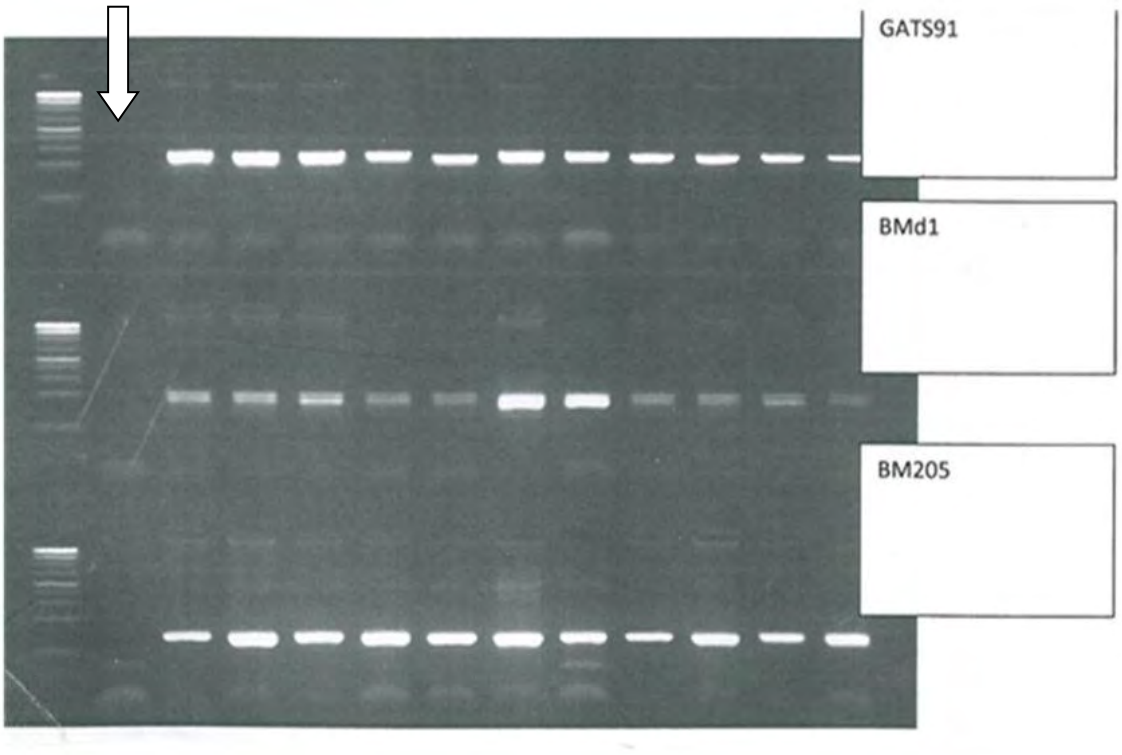


Figure 12: Gel electrophoresis picture for PCR done with DNA extracted from seeds of some accessions (arrow indicates DNA lane extracted from leaf tissue sample taken from the accession similar to the next one to the right)

Subsequently, ten simulations per K value were then performed from K=2 to 8 using 5,000 replicates for burn-in and 50,000 replicates during the analysis. The Δ statistical test showed that K=5 was optimal in this analysis (as shown in Figure 14) (Rosenberg et al., 2002; Evanno et al., 2005; Ehrich, 2006). At K=5, the membership coefficient from the run with the lowest likelihood value (-4286) was used to assign each accession to the K=1 to 5 populations for each accession based on the highest membership coefficient. Accessions with a membership coefficient less than 0.8 or 0.9 were identified as putative hybrids. A graphical plot of membership coefficients

was generated using the Distruct program (Fig. 13; Rosenberg, 2004). F_{st} coefficients among the five selected populations were calculated using STRUCTURE.

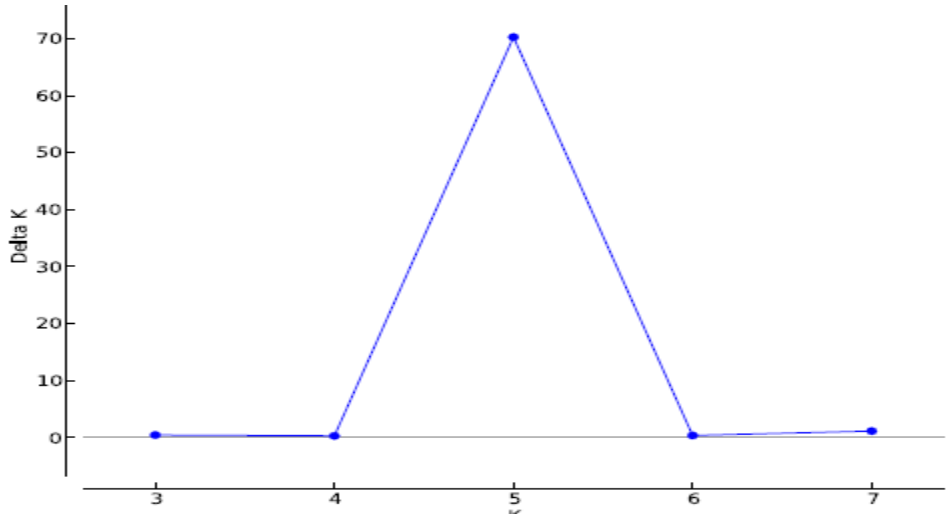


Figure 13: Results of the Evano et al. (2005) test for ΔK between different sub-groupings of 123 common bean accessions/cultivars and two control genotypes based on analysis of allelic diversity at 17 microsatellite loci.

Table 6: List of microsatellite (SSR) markers with forward/reverse nucleotide sequence; dye color; repeat motif; chromosomal location; and annealing temperature

No.	SSR marker	Nucleotide Sequence	Dye Color	Repeat motif	Chromosomal location	Annealing temperature
1.	BM139-F	TTAGCAATACCGCCATGAGAG	NED	$(CT)_{25}$	2	55 ^{0}C
	BM139-Reverse	ACTGTAGCTCAAACAGGGCAC				
2.	BM140-F	TGCACAACACACATTTAGTGAC	PET	$(GA)_{30}$	4	55 ^{0}C
	BM140-Reverse	CCTACCAAGATTGATTTATGGG				
3.	BM-141-F	TGAGGAGGAACAATGGTGGC	VIC	$(GA)_{29}$	9	55-58 ^{0}C
	BM-141-Reverse	CTCACAAACCACAACGCACC				
4.	BM143-F	GGGAAATGAACAGAGGAAA	6-FAM	$(GA)_{35}$	2	55-58 ^{0}C
	BM143-Reverse	ATGTTGGGAACTTTTAGTGTG				
5.	BM151-F	CACAACAAGAAAAGACCTCCT	NED	$(TC)_{14}$	8	55 ^{0}C
	BM151-Reverse	TTATGTATTAGACCACATTACTTCC				
6.	BM156-F	CTTGTTCCACCTCCCATCATAGC	NED	$(CT)_{32}$	10	55-58 ^{0}C
	BM156-Reverse	TGCTTGCATCTCAGCCAGAATC				
7.	BM165-F	TCAAATCCCACACATGATCG	VIC	$(TA)_3(CA)_9$	8	52 ^{0}C
	BM165-Reverese	TTCTTTCATTCATATTATTCCGTTCA				
8.	BM172-F	CTGTAGCTCAAACAGGGCACT	6-FAM	$(GA)_{23}$	2	50 ^{0}C
	BM172-Reverse	GCAATACCGCCATGAGAGAT				
9.	BM183-F	CTCAAATCTATTCACTGGTCAGC	NED	$(TC)_{14}$	7	52 ^{0}C
	BM-Reverse	TCTTACAGCCTTGCAGACATC				
10.	BM187-F	TTTCTCCAACTCACTCCTTTCC	PET	$(CT)_{10}$ T $(CT)_{14}$	6	50-52 ^{0}C
	BM187-Reverse	TGTGTTTGTGTTCCGAATTATGA				
11.	BM188-F	TCGCCTTGAAACTTCTTGTATC	VIC	$(CA)_{18}(TA)_7$	9	55 ^{0}C
	BM188-Reverse	CCCTTCCAGTTAAATCAGTCG				

Table 6 (cont'd)

No.	SSR marker	Nucleotide Sequence	Dye Color	Repeat motif	Chromosomal location	Annealing temperature
12.	BM205-F	CTAGACCAGGCAAAGCAAGC	6-FAM	$(GT)_{11}$	7	50 ^{0}C
	BM205-Reverse	TGAGCTGGGATTTCATTTCTG				
13.	AG1-F	CATGCAGAGGAAGCAGAGTG	NED	$(GA)_8$ GGTA $(GA)_5$ GGGGACG	3	50 ^{0}C
	AG1-Reverse	GAGCGTCGTCGTTTCGAT				
14.	GATS54-F	GAACCTGCAAAGCAAAGAGC	PET	$(GA)_5$ AACAGAGTC $(GA)_8$ $(AG)_4$	10	56 ^{0}C
	GATS54-Reverse	TCACTCTCCAACCAGATCGAA				
15.	GATS91-F	GAGTGCGGAAGCGAGTAGAG	VIC	$(GA)_{17}$	2	58 ^{0}C
	GATS91-Reverse	TCCGTGTTCCTCTGTCTGTG				
16.	BMd53-F	TGCTGACCAAGGAAATTCAG	6-FAM	$(GTA)_5$	5	50 ^{0}C
	BMd53-Reverse	GGAGGAGGCTTAAGCACAAA				
17.	BMd36-F	CATAACATCGAAGCCTCACAGT	NED	$(TA)_8$	3	50 ^{0}C
	BMd36-Reverse	ACGTGCGTACGAATACTCAGTC				
18.	BMd42-F	TCATAGAAGATTTGTGGAAGCA	PET	$(AT)_5$	10	55 ^{0}C
	BMd42-Reverse	TGAGACACGTACGAGGCTGTAT				
19.	BMd1-F	CAAATCGCAACACCTCACACAA	VIC	$(AT)_9$	3	54 ^{0}C
	BMd1-Reverse	GTCGGAGCCATCATCTGTTT				
20.	BMd16-F	ATGACACCACTGGCCATACA	6-FAM	$(CATG)_4$	4	55 ^{0}C
	BMd16-Reverse	GCACTGCGACATGAGAGAAA				
21	BMd18-F	AAAGTTGGACGCACTGTGATT	NED	$(TGAA)_3$	2	50-53 ^{0}C
	BMd18-Reverse	TCGTGAGGTAGGAGTTTGGTG				
22	PV-AG001-F	CAATCCTCTCTCTCTCATTTCCAATC	PET	$(GA)_{11}$	11	50 ^{0}C
	PV-AG001-Reverse	GACCTTGAAGTCGGTGTCGTTT				
23	PV-AT001-F	GGGAGGGTAGGGAAGCAGTG	VIC	(TA)22	11	53 ^{0}C
	PV-AT001-Reverse	GCGAACCACGTTCATGAATGA				
24	PV-CTOO1-F	CCAACCACATTCTTCCCTACGTC	6-FAM	$(CTT)_3$	4	56 ^{0}C
	PV-CT001-Reverse	CGCAGGCAGTTATCTTTAGGAGTG				

4.3 Integrating phenotypic evaluations with a molecular diversity assessment of an Ethiopian collection of common bean (*Phaseolus vulgaris* L.) landraces

4.3.1 Plant Materials

The same plant materials in section 4.1.1 were used as plant materials in this study.

4.3.2 Morphological evaluation

Data parameters and procedures of data collection used in 4.1.4 were also used in this experiment set.

4.3.3 Genetic grouping on the basis of molecular markers

The study samples were classified into genetic groups (two, i.e., Andean Vs Mesoamerican; three; four and five groups), and genetic grouping was performed on the basis of the molecular characterization of the accessions, using 17 microsatellite markers. Details on the microsatellite markers evaluation are presented in Table 6.

4.3.4 Data analysis

Qualitative character traits were transformed, as per the method described in section 4.1.5. Subsequently, means were calculated per accession for all the transformed variables and for the original quantitative variables to perform the multivariate analysis of the agro-morphological data. Principal component analyses were performed for the agro-morphological data using the PRINCOMP procedure of SAS 9.1 (http://www.sas.com/). Graphics with score plots of the first principal components were prepared using JMP 7 (http://www.jmp.com/software/jmp7/).

A stepwise discriminant analysis was performed using the STEPDISC procedure of SAS 9.1 (F values measured by Wilks‘ Lambda likelihood ratio criterion), in order to identify the agro-morphological variables that discriminate the two major gene pools (Andean and Mesoamerican). Subsequently, the variables selected in this stepwise procedure were used in a canonical discriminant analysis that was performed using the CANDISC procedure of SAS.

To determine, if the populations or subpopulations identified with molecular analysis and Structure modeling (Chapter 5.5) also showed distinguishing morphological traits, tables with character states and ranges for some of the morphological descriptors in each group are presented for two (K=2) and five (K=5) groups as defined in Chapter 5.5. The morphological descriptors

presented in these tables are important descriptors for the classification of common bean races (Singh et al., 1991a, b, c; Burle et al., 2011).

On the basis of the Structure simulations, 72 accessions were not allocated in any of the five groups, when the Structure model was run at K=5 on marker data (posterior membership coefficient below a threshold of 80%); those accessions were classified as potential hybrids (section 5.5). Because of their presumed hybrid nature, these potential hybrids were not included in the analysis to facilitate recognition of potential morphological differences distinguishing the different groups.

5 Results and Discussion

5.1 Qualitative and quantitative trait variability in Ethiopian common bean (*Phaseouls vulgaris* L.) germplasm

Overall, the sample of the studied common bean accessions showed considerable variability with respect to the qualitative and quantitative traits evaluated. Nonetheless, none of the qualitative character traits presented a normal distribution. The non-normal distribution presented by qualitative traits was also reported in a previous study in common bean (Burle et al., 2011). On the other hand, the quantitative (continuous) traits showed a clear normal distribution (seed diameter, seed length, yield etc.) or a distribution very close to normal (days to flowering). In support of the present findings, Burle et al. (2011) reported similar pattern in the quantitative (continuous) traits examined in Brazilian common bean landrace accessions. Consequently, the comparison of frequencies for the qualitative traits was carried out, using Fischer‘s exact test in the PROCFREQ procedure of the SAS software program, SAS 9.1 (SAS Institute, 2009). This result agrees with that reported by Burle et al. (2011). Overall, the sample of common bean accessions studied showed variability with respect to qualitative and quantitative traits evaluated.

The variability among accessions with respect to qualitative and quantitative traits is presented in detail in the following sections.

5.1.1 Qualitative character variability

The pattern of variability of the 121 landrace accessions (including six released cultivars) was discerned using two approaches. The first approach was: analyzing the percentages of observed frequencies on a character-by-character basis. The second one involved analyzing the frequency of different labels of each of the characters, on the basis of the sites the accessions were collected from. Two major patterns could be discerned from the frequency values in Table 7, considering the prevalence of variation vis-à-vis the qualitative character traits studied.

The most frequent character classes observed for each qualitative character trait were:

- Red seed coat color (39.58%);
- White standard (flower) color (64.95%);
- Type-II (indeterminate bush) growth habit (60.15%);

- Oval-shaped seeds (36.55%);
- Shiny seed brilliance (68.60%);
- Dark purple pod color (47.63%); and
- White color of flower wings in freshly-opened flowers (62.18%) (Table 7).

The second most frequent class-combination in the qualitative traits evaluated variation was:

- White seed coat color (29%), and ;
- Dark lilac-colored flowers (24.77%);
- Type IV (indeterminate-climbing) growth habit (17.52%);
- Cuboid (28.15%) or round (25.21%) seed shape;
- Medium (11.98%) and other type (18.60%) of seed brilliance; and
- Lilac with dark lilac veins in freshly-opened flowers (26.94%) (Table 7).

In view of the variations observed the following remarks were made:

I. Variation in seed-coat color

Across all regions (or collection sites), the majority of the landrace accessions studied had red seed coat color (39.58%), followed by white seed coats (29%). Region-wise analysis showed that red seed coats were the most prevalent types (in descending order) in the accessions collected from ‗Gambella' region (100%), followed by those the ‗Amhara' region (45.83%) (Table 7). On the other hand, accessions from the Southern Nations, Nationalities, and Peoples region (SNNP) (33.33%), followed by those from ‗Benishangul-Gumuz' (BG) (30.77%), and ‗Oromiya' (28.32%) regions had white seed coat color (the second most prevalent color type) (Table7). The predominance of white and red-colored seeds in Ethiopian common bean landrace accessions was reported previously (Asfaw et al., 2009). Moreover, Duran et al. (2005), Blair et al. (2010b), and Burle et al. (2011) reported the dominant prevalence of Central African and Brazillian common bean landrace accessions with red seed coat color, which concur with the present result. The predominance of red and white seed coat colors might have emanated from users' and market preferences (i.e. red accessions preferred for consumption, while white accessions fetch the maximum market income).

II. Variation in (flower) standard color

The majority of the accessions had white (flower) standards (64.95%), followed by a considerable proportion of dark-lilac white (flower) standard color (24.77%) (Table 7).

III. Variation in plant growth habit

Considerable variation in plant growth habit was also observed among the accessions studied. Generally, Type-II (indeterminate bush) was the most prevalent among the accessions (60.15%), followed by Type-IV (indeterminate climbing) (15.71%); Type-I (determinate bush) (13.41%) growth habits. Across regions (collection sites), accessions from _Oromiya' (66.37%), SNNP (51.56%), and _Gambella' (50%) had the highest frequencies of Type-II (indeterminate bush) growth habit. Interestingly, Type-IV (determinate climbing) accessions comprised the largest group in the _Amhara' region. Another worth mentioning result is the considerable occurrence of Type-I (determinate bush) growth habit in accessions from _Gambella' (50%), and _Amhara' (20.83%), (Table 7). Similarly, Okii et al. (2014a) reported that Type-II and I were the most dominant ones in common bean landrace accessions from Uganda. Furthermore, Asfaw et al. (2009) reported that landrace accessions with Type-II, and IV were more frequent in Ethiopian common bean genotypes. Nonetheless, the most dominant plant type identified in their study (i.e. Type-III) was not evident in the present study. This may be explained by the differences in plant material sampling and environmental factors between the two related studies.

IV. Variation in seed shape

Generally, oval (36.55%); cuboid (28.15%); and round (25.21%) seeds were the most prevalent seed shape types, amongst others, in the common bean accessions studied. Regionally, in populations: 50% in BG and _Gambella' (39.74%) in _Oromiya' had oval-shaped seeds. On the other hand, cuboid-shaped seeds were higher in occurrence in _Gambella' (50%), SNNP (30.65%), and _Amhara' (29.17%). Round seeds were most frequent in accessions from BG (29.17%) (Table 7). Similarly, Awan et al. (2014) reported cuboid seeds were predominant in Pakistani exotic common bean accessions. On the other hand, Bulre et al. (2011) reported that cuboid and oval seeds were among the predominant seed shapes in common bean landrace accessions from Brazil, which is in agreement with the present findings.

V. Variation in seed brilliance

With respect to the observed variability in seed brilliance, shiny types (69.16%) were most frequent, with seed brilliance types, labeled as ‗other', were the second most frequent types (Table 7). Region-wise, the same trend was followed. The present result goes in harmony with that reported in Burle et al. (2011), where shiny, medium, and matt (referred to as ‗others' in this study) were the most prevalent seed brilliance labels in Brazilian landrace common bean accessions.

VI. Variation in pod color

Dark-purple pods (47.64) were the most prevalent in accessions from all regions, followed by accessions with dark-pink rose pods. Region-wise, dark purple pods were dominant in all, with the maximum of 100% in the released varieties' group, and minimum of 70.42% in accessions from ‗Oromiya'. On the other hand, dark-pink rose pods were the second most prevalent types, especially in ‗Oromiya' and SNNP (23.95% and 21.1%, respectively). Especially, those from ‗Gambella' and BG had the largest frequencies (100% and 52.17%, respectively) of accessions with dark-purple pods (Table 7). The considerable presence of purple and pink pod colors in common bean landrace accessions were previously reported in previous studies (Burle et al., 2011).

VII. Variation in color of flower wings (in freshly-opened flowers)

Overall, white (62.18%) and purple (26.94%) were the most prevalent color of flower wings in freshly-opened flowers (Table 7). On the other hand, across regions, the frequency of white flower wings (in freshly-opened flowers) ranged from 100% in ‗Gambella' to 40% in ‗Amhara' region. On the other hand, purple flower wings (in freshly-opened flowers) constituted from 60% in the ‗Amhara' region to 12.5% in accessions from SNNP. Meanwhile, flower wings (in freshly-opened flowers) colored white-with-carmine-stripes had considerable frequencies in landrace accessions from SNNP (14.6%) and ‗Oromiya' (13.04%). Similarly, Asfaw et al. (2009) and Awan et al. (2014) reported white and purple flower wings (in freshly-opened flowers) were the most predominant types in landrace common bean accessions from Ethiopia and Pakistan, respectively. Furthermore, Okii et al. (2014) reported that white, purple, and white-with-carmine-stripes were common flower wing colors in common bean landrace accessions from Uganda, which concurs with the results of the present study.

Table 7: Frequencies of different labels of morphological descriptors in 121 common bean landrace accessions/cultivars from Ethiopia

Variations in the total column indicate the total number of accessions with the trait value accounted for (excluding missing values)

Trait	Labels	Origin					
		Amhara	**BG**	**Gambella**	**Oromiya**	**Released**	**SNNP**
Seed Coat Color	1=White	5	8	0	32	4	21
	2=Cream	4	3	0	11	1	6
	3=Dull	2	4	0	17	0	6
	4=Brown	1	1	0	6	0	1
	5=Medium/pink	0	0	0	0	0	1
	6=Red	11	10	2	37	7	28
	7=Shiny/yellow	1	0	0	10	0	0
Total		**24**	**26**	**2**	**113**	**12**	**63**
User Category	1=Dry bean	24	26	2	114	12	64
	2=Snap bean	0	0	0	0	0	0
	3=Green-shelled bean	0	0	0	0	0	0
	4=Popping bean	0	0	0	0	0	0
Total		**24**	**26**	**2**	**114**	**12**	**64**
Standard (Flower) Color	1=White	15	20	2	54	9	39
	2=Green	0	0	0	0	0	0
	3=Lilac	0	0	0	0	0	0
	4=White with lilac edges	1	2	0	11	0	3
	5=White with red stripes	2	0	0	0	0	2
	6=Dark lilac	4	4	0	30	1	14
	7=Dark lilac with purplish spots	0	0	0	1	0	0
	8=Carmine red	0	0	0	0	0	0
	Purple	0	0	0	0	0	0
Total		**22**	**26**	**2**	**96**	**10**	**58**
Plant Growth Habit	I=Determinate Bush	5	3	1	14	2	10
	II=Indeterminate Bush	7	15	1	75	6	33
	III=Indeterminate Prostate or Viny	1	0	0	3	0	2
	IV=(Indeterminate) Climbing	8	4	0	15	0	14
	IV=(Determinate) Climbing	3	1	0	4	4	3
		24	**23**	**2**	**111**	**12**	**62**

Table 7 (cont'd)

Trait	Labels	Origin					
		Amhara	**BG**	**Gambella**	**Oromiya**	**Released**	**SNNP**
Seed Shape	1=Round	9	7	0	22	5	17
	2=Oval	5	12	1	45	4	20
	3=Cuboid	7	5	1	32	3	19
	4=Kidney-shaped	3	0	0	13	0	6
	5=Markedly Truncate or Truncate-fastigate	0	0	0	2	0	0
		24	**24**	**2**	**114**	**12**	**62**
Seed Color	1=White	5	8	0	35	4	20
	2=Cream	3	3	0	12	1	6
	3=Dull	3	3	0	12	1	8
	4=Brown	1	1	0	6	0	1
	5=Medium	0	0	0	0	0	0
	6=Red	11	11	2	38	6	28
	7=Shiny	1	0	0	0	0	0
	8=Yellow	0	0	0	11	0	0
	9=Pink	0	0	0	0	0	1
		24	**26**	**2**	**114**	**12**	**64**
Seed Brilliance	7=Shiny	12	20	2	83	8	41
	8=Medium	6	3	0	11	0	9
	9=Others	6	3	0	18	4	14
		24	**26**	**2**	**112**	**12**	**64**

Table 7 (cont'd)

Trait	Labels	Origin					
		Amhara	**B/G**	**Gambella**	**Oromiya**	**Released**	**SNNP**
Pod Color	1=Dark-purple	11	12	2	50	6	30
	2=Carmine-red	2	0	0	4	0	0
	4=Carmine stripe on green	0	0	0	0	0	0
	5=Pale-red stripe on green	0	0	0	0	0	0
	6=Dark-pink rose	1	1	0	17	0	8
	7=Normal green	0	0	0	0	0	0
	8=Shiny green	0	0	0	0	0	0
	9=Dull green to silver gray	0	0	0	0	0	0
	10=Golden or deep yellow	0	0	0	0	0	0
		14	**13**	**2**	**71**	**6**	**38**
Color of flower wings	1=White	8	14	2	54	7	35
	2=Green	0	0	0	0	0	0
	3=Lilac	0	0	0	0	0	0
	4=White with carmine stripes	0	2	0	12	0	7
	5=Strong-veined in red to dark lilac	0	0	0	0	0	0
	8&9=Purple	12	6	0	26	2	6
	99=Others	0	0	0	0	0	0
		20	**22**	**2**	**92**	**9**	**48**

BG=Benishangul-Gumuz; SNNP=Southern Nations, Nationalities, and Peoples Region

5.1.2 Quantitative trait variability

Significant to highly significant variability among the studied accessions were observed in: days to flowering, 100-seed weight, plot yield (PLYLD), number of pods/plant (NoPP), plant height (PHT), and number of seeds per plant (NoSPPL) (Table 8), indicating there were adequate variability vis-à-vis these traits in common bean accessions from different parts of Ethiopia. Furthermore, it implied that the underutilized genetic variability of the common bean landrace accessions studied should be future priority cornerstone in prospective common bean breeding/improvement/genetic conservation programs. Several previous studies reported similar results on the significant variability among common bean genotypes from different parts of the world with regard to the above-mentioned important traits (Duran et al., 2005; Zelalem, 2005,

Kassaye, 2006; Lima et al., 2012; Awan et al., 2014). The minimum and maximum values recorded for DTF were 51.5 and 61 days, respectively with a mean value of 55.52 days (Table 9). Considering the range of values in DTF in the present study, it seemed to be narrower than that reported in previous studies (Zelalem, 2005; Kassaye, 2006; Burle et al., 2011). Genetic and environmental differences among the samples included in the respective studies may be possible explanation behind the difference in the results. Specifically, accessions from ‗Amhara' and SNNP topped the rank with the highest DTF, whereas those from ‗Oromiya', ‗Benishangul-Gumuz', and ‗Amhara' regions had the lowest DTF values (Table 10). In general, the variability in DTF was, more or less, evenly distributed within and between regions (each population/region/collection site had various ranges of values vis-à-vis DTF).

With respect to the observed variation in HSW, it ranged from a minimum of 15.5g to a maximum of 70g, with a mean value of 32.77g. The broader range of HSW values may indicate the possible presence of both the Mesoamerican and Andean gene pool genotypes, as seed weight is one of the most important traits discriminating between the two gene pools of origin (Singh et al., 1991a, b, c; Kwak and Gepts, 2008, Asfaw et al., 2009; Blair et al., 2010a,b; Burle et al., 2011) (detailed accounts on the variability in seed weight versus genetic diversity and population structure of the study accessions are given in sections 5.3 and 5.6). ‗Chercher', a released variety from the Andean gene pool, topped the rank with a seed weight of 70g, whereas accessions from ‗Oromiya' and ‗Amhara' had the lowest values in HSW. Similarly, several authors reported significant variation in HSW values among common bean accessions from various parts of the world (Singh et al., 1991a, b, c; Zelalem, 2005; Kassaye, 2006; Kwak and Gepts, 2008; Asfaw et al., 2009; Blair et al., 2010b; Blair et al., 2011; Awan et al., 2014; Okii et al., 2014a).

Significant variability among the accessions/varieties was observed for PLYLD in the present study (Table 8). The significant variability among the accessions may indicate there was ample genetic variability among the landrace accessions, which is yet to be exploited in future breeding programs in the common bean in Ethiopia. The mean PLYLD recorded was 167.35g, with minimum and maximum values of 44.2g and 537.5g (plot size of 4.8m^2) (Table 9). As can be expected, accessions from the released varieties group topped the rank in yield, whereas those

from ‗Benishangul-Gumuz' gave the lowest yields (Table 10). Nonetheless, considering the LSD value estimated (205.96) (Table 12), several accessions had statistically at par yields with the highest yielding released variety (‗Chercher') (Table 10). This may imply the common bean landrace genotypes of Ethiopia possess tremendous genetic potential useful in future breeding programs to improve seed yield in common bean. Duran et al. (2005); Zelalem (2005); Kassaye (2006); and Lima et al. (2012) reported significant variation among common bean accessions with respect to yield, which supports the results of this study.

On the other hand, highly significant variation was observed among accessions in NoPP (Table 8). Minimum and maximum NoPP values recorded were 101.5 and 22.5, respectively, whereas the mean was 45.14 (Table 9). Highly significant variation in NoPP among common bean genotypes was also reported in some previous studies in Ethiopia as well as other parts of the world (Duran et al., 2005; Zelalem, 2005; Kassaye, 2006; Lima et al., 2012; Awan et al., 2014). A group of accessions from ‗Oromiya' had the highest NoPP values, whereas those from SNNP had the lowest values (Table 10).

Values in PHT ranged from 31.95cm to 119.11cm with a mean of 64.94cm (Table 8). Highly significant variation was also observed among accessions with respect to PHT, which is in agreement with results of some previous studies (Table 8) (Zelalem, 2005; Kassaye, 2006). Region-wise, accessions from SNNP had the highest PHT, whereas those from ‗Benishangul-Gumuz', ‗Oromiya', and SNNP were at the bottom of the rank (Table 9).

Finally, highly significant variation among accession was observed for NoSPPL, ranging from 57.59 to 479 and a mean value of 198.21 (Table 8 and 9). Results of some previous studies supported the present finding (Duran et al., 2005; Zelalem, 2005; Kassaye, 2006; Lima et al., 2012). This trait had positive significant and highly significant correlation with NBPT and NoPP, respectively (details on the next section), which may make joint use of all the traits for common bean improvement.

In summary, accessions showed significant variability, distributed, often evenly, across populations/regions/collection sites. Moreover, considerable number of accessions had

comparable, and even in some cases higher, values in important quantitative traits. Such important inherent genetic variability should be among the most important priorities vis-à-vis future common bean breeding conservation. To this end, Horňáková et al. (2003) and Mondini et al. (2009) stated that crop landrace accessions are abundant and rich sources of valuable genes and genetic diversity. In line with this, further detailed accounts on the association of quantitative traits with yield; and the contribution of traits (qualitative and quantitative) to genetic diversity are given in sections 5.2, 5.3, and 5.6.

Table 8: Mean Squares and significance of variance components of the Analysis of Variance of 10 (ten) quantitative traits in 121 common bean accessions from Ethiopia

Variable	Mean Square (Treatment)	F-Value	Significance	C.V (%)
DTF	13.198	2.28	**	4.336
HSW	257.04	1.75	**	27.02
PLYLD	15273.745	1.42	*	32.033
SH	17.672	1.22	NS	28.251
SDIA	12.369	1.26	NS	24.041
NoPP	491.366	1.62	**	38.615
PHT	571.635	2.49	**	23.33
NoSPPO	9.035	1.24	NS	27.07
NBPT	63.58	1.34	NS	29.36
NoSPPL	11521.2	1.99	**	30.01

Where: DTF=Days to Flowering; HSW=Hundred Seed Weight; PLYLD=Plot Yield; SH=Seed Height; SDIA=Seed Diamter; NoPP=Number of Pods/plant; PHT=Plant Height; NoSPPO=Number of Seed/pod; NBPT=Number of Branches/plant; NoSPPL=Number of seeds/plant

Table 9: Mean, minimum, and maximum values recorded for six quantitative variables in 121 common bean accessions from Ethiopia

No	Variable	Mean	Maximum	Minimum
1.	**DTF**	55.52	61	51.5
2.	**HSW**	32.77g	70g	15.5g
3.	**PLYLD**	167.35g	537.5g	44.2g
4.	**NoPP**	45.14	101.5	22.5
5.	**PHT**	64.94cm	119.11cm	31.95cm
6.	**NoSPPL**	190.21	479	57.59

Where: DTF=Days to Flowering (50%); HSW=100-Seed Weight; PLYLD=Plot Yield; NoPP=Number of Pods/Plant; PHT=Plant Height; and NoSPPL=Number of Seeds/Plant

Table 10: Mean values in six quantitative triats recorded for the 121 common bean accessions/varieties, and their respective LSD values

ID/Acces.No	Population	Mean DTF	Mean HSW	Mean PLYLD	Mean NoPP	Mean PHT	Mean NoSPPL
211266	Amhara	60	25	227	39.5	56.35	159.45
211267	Amhara	53	50	321.5	48	52.75	479
211269	Amhara	59.5	44.5	168	34.55	48.61	128.84
211386	Amhara	56.5	46	157	35	46	114
211387	Amhara	56.5	15.5	90.5	44	73.25	162.5
211389	Amhara	55	40	273.5	27	68	218
211550	Amhara	55	41	211	40	71.5	169
211551	Amhara	53	34.5	165	47	61.5	143.5
215719	Amhara	58	32.5	101.5	31	46.81	145.49
241807	Amhara	59	25	57	29.5	85.75	155
241814	Amhara	51.5	29.5	254	42.25	49.89	69.91
207933	B.Gumuz	60	47.25	168.5	44.5	47.45	197
207934	B.Gumuz	53.5	32.5	73.5	51.5	31.95	200.62
207938	B.Gumuz	58	30.5	153	49	43.5	144.5
211344	B.Gumuz	53	20	127	43.5	57.5	202.5
211345	B.Gumuz	53	33.5	136	42.5	59.5	190
211347	B.Gumuz	58	24.5	170	52	54.89	169.51
211348	B.Gumuz	53	61	168.5	30	73.5	172.5
211349	B.Gumuz	56	40	173	44	54.5	117.5
211350	B.Gumuz	53	22.25	130	38	48	224
211356	B.Gumuz	51.5	46.5	224.5	45.5	81.5	160.5
211361	B.Gumuz	55	27	101.5	30.5	41.5	160

Table 10 (cont'd)

ID/Acces.No	Population	Mean DTF	Mean HSW	Mean PLYLD	Mean NoPP	Mean PHT	Mean NoSPPL
216730	Gambella	56	32.5	149.5	38	89.5	161.4
201666	Oromiya	55	22	63.8	37	55.5	240.5
201293	Oromiya	58	39.5	144.5	25.5	49.88	57.59
201294	Oromiya	58	31.5	127.2	27.5	61.1	129
207949	Oromiya	51.5	33	161.5	37.5	39.25	190.5
208638	Oromiya	51.5	21	285	75.5	57	274
208646	Oromiya	56	61	174	27	36.06	341.12
208647	Oromiya	58	21.5	88.5	30	83.2	86.5
208695	Oromiya	57	20.5	49.5	26.4	51.12	147.4
208698	Oromiya	56	36.5	247.5	41	55.25	247.5
208702	Oromiya	56	38	249	47.5	86	199
208703	Oromiya	58	19.5	187	33.5	57	114
208705	Oromiya	60.5	36.61	44.2	29.5	70.5	67
211271	Oromiya	56	32.5	64.5	101.5	76.65	451
211298	Oromiya	55	31	61	76.5	96.35	203
211299	Oromiya	52.5	24.25	142.3	43	71.5	234.5
211300	Oromiya	55	23.5	179	77.5	69.85	188
211301	Oromiya	52.5	27	129.5	43.5	69.3	291
211304	Oromiya	53	62.5	203.5	91	80.25	208
211305	Oromiya	55	48.5	183	45.5	45	279.5
211315	Oromiya	56	39.5	90.5	52.95	68.9	186
211317	Oromiya	53	28.5	128	87	70.42	200.14
211318	Oromiya	52	29.5	123	40.5	50	358
211319	Oromiya	54	22	67.5	82.5	57.5	307.5
211320	Oromiya	55	21.5	111	41.5	54	304
211322	Oromiya	58	26.5	181.5	50	50.47	282.76
211323	Oromiya	55	29	264	94.5	64	179.5
211325	Oromiya	60	31	186	26.5	95.1	395.09
211327	Oromiya	57.5	42.5	92	38.5	71.8	95
211329	Oromiya	57	43.5	96	45	66.62	188.66
211331	Oromiya	55	36	111.8	58.5	69.5	162.5
211332	Oromiya	52	21.5	147	47.5	59.75	196
211334	Oromiya	53	50.5	237.5	51.5	61	205
211337	Oromiya	55	43.5	96.5	25	76.7	197
211338	Oromiya	53	34.5	214	65.5	37.5	199.35
211339	Oromiya	53	23.5	162	73	53.55	323

Table 10 (cont'd)

ID/Acces.No	Population	Mean DTF	Mean HSW	Mean PLYLD	Mean NoPP	Mean PHT	Mean NoSPPL
211340	Oromiya	59.5	29	168.5	48	58.5	122.7
211341	Oromiya	57	34.5	251.5	34	66.4	266.5
211342	Oromiya	56	21	118.5	59	77	153.5
211377	Oromiya	51.5	21.5	136.5	66	51.5	167.5
211378	Oromiya	54	33.5	125	45.5	53	150
211379	Oromiya	55	35.2	176	37	72.89	198.18
211388	Oromiya	53	16	182.5	49	89	177
212861	Oromiya	56	23	178.5	36.5	58	149
216819	Oromiya	55	30.7	69.5	37.5	81	192
216820	Oromiya	55	15.5	105	24.5	90	143
219233	Oromiya	59	18.5	128.5	49	92	103
219234	Oromiya	60.5	19.5	188.5	31.5	69.5	150
219235	Oromiya	55	19	46	29	65.5	136.5
218235	Oromiya	56	31	87.5	51	58.5	96
230779	Oromiya	54	38.5	224	46	88.5	207
235697	Oromiya	55	31.5	137.5	47	46	273
237078	Oromiya	53	33	217	32	80.7	160.5
240173	Oromiya	55	30.5	130	43	68	150.5
240187	Oromiya	55	19	123	30	67	299.5
240190	Oromiya	59.5	24	99.5	24.5	84	93.5
240552	Oromiya	58.5	27.5	198	59	48	214.5
241730	Oromiya	54	46	217.5	31.5	65.25	220.85
Andean Control	Released	56	30.5	181.5	43.5	69.5	88.36
CHERCHER	Released	56	70	338	34	64	207.5
GOBERASHA	Released	53	54.5	481	57	59.5	121
MA Control	Released	55	23	329.5	50	87.5	190.5
NASER	Released	55	58.5	537.5	69	56	227.5
MEX-142	Released	52	29.5	238	67	82.5	198.5
211277	SNNP	59	44	80	54	82.75	237.85
211278	SNNP	55	33.15	46	29.5	86.5	202
211279	SNNP	53.5	30	154	36.5	69.7	173
211286	SNNP	52	48	322	41	44.5	341
211290	SNNP	53.5	24.25	174	59.5	41.5	163
211291	SNNP	52.5	24	56	44.5	68.09	286.85
211292	SNNP	60.5	26	189	34.5	49	113.52
211293	SNNP	58	52	169.5	71.5	83.5	125.68
211294	SNNP	57	36	148.5	60	45	224

Table 10 (cont'd)

ID/Acces.No	Population	Mean DTF	Mean HSW	Mean PLYLD	Mean NoPP	Mean PHT	Mean NoSPPL
211394	SNNP	55	32	99	44	107.88	101
211481	SNNP	57	26.4	243.5	52.5	51.05	297.83
211483	SNNP	60	41	90	27	66.44	198.32
211546	SNNP	53	35.1	76.2	49	35.5	153
211552	SNNP	56	21	171	36	84	181
212860	SNNP	61	21	104.5	40.5	65.25	143.5
212978	SNNP	53	46	333	23.25	45	187.5
213046	SNNP	55	34.5	96.5	38.5	69.88	109.9
235692	SNNP	60.5	18.5	76	60	55	178
237993	SNNP	59.5	28	416.5	38	61	120.5
241733	SNNP	55	27	179	32	55.5	**130.5**
241736	SNNP	57	32.5	204.5	44	119.11	85.72
241737	SNNP	53.5	25.25	151.8	30	103.93	241.48
241738	SNNP	56.5	19	372.5	41	82	108
241739	SNNP	55	48	135	22.5	49	165
241748	SNNP	52	22	138	43	50	99
241750	SNNP	55	32.5	92.5	53	56	189.5
241752	SNNP	55	43	106.9	55	75.6	307.06
241753	SNNP	52	28.5	221.5	53.5	51	209.5
241756	SNNP	53	65.5	326	59.5	57.7	260
241757	SNNP	52	35.25	179	33.5	104	297
241765	SNNP	58	35.5	169.5	49.5	69.35	162.85
244805	SNNP	56	23	64	35.5	71.05	168.39

5.2 Association of seed yield with yield component and yield-related characters in common bean germplasm from Ethiopia

5.2.1 Correlation analysis

5.2.1.1 Correlation of yield and yield components and related traits

Yield is a complex trait governed by many genes. Therefore, estimation of correlation coefficients of yield with yield-related traits is of paramount importance to utilize the available genetic variability through selection.

Table 11 shows that PLYLD had significant to highly significant correlations with MSD and HSW (r=0.342** and r=0.147*, respectively). On the other hand, it had negative significant correlation with DTF (r=-0.142*). The significant positive correlation PLYLD had with HSW and MSD may indicate selecting for accessions with larger seeds and higher seed diameter favors the simultaneous improvement of these traits and seed yield in the common bean. Kassaye (2006) reported similar results in common bean that PLYLD correlated significantly positively with MSD. Though, the same author reported a contradictory result with this one in which PLYLD had significant positive and negative correlations with DTF and HSW, respectively. In contrast, Zelalem (2005); Roy et al. (2006); Karasu and Oz (2011); Sadeghi et al. (2011); Negahi et al. (2014) reported a similar result in that PLYLD had a significant positive correlation with HSW, whereas, it had negative significant correlation with DTF. Furthermore, several authors reported opposite results in which PLYLD had negative and positive significant correlations with HSW and DTF, respectively (Bhushan et al., 2008; Kumar et al., 2009; Sofi et al., 2011; Ahmed and Klamuddin, 2013). Such different results may be explained by the variation brought about by genotypic and environmental factors.

The significant positive correlation between PLYLD and HSW; and PLYLD and MSD may indicate selecting for accessions with larger seeds and higher seed diameter favors the simultaneous improvement of these traits and seed yield in the common bean. On the other hand, selecting against DTF may contribute, partially, to the improvement in the seed yield of the common bean. Nonetheless, as correlation analysis, alone, cannot explain the association among characters, the aforementioned inferences may need to be verified via path coefficient analysis, as it delineates the direct and indirect effect each trait has on yield. Seed yield is a polygenic

character; hence direct selection for this character may often be misleading (Singh and Chaudhary, 2001) (detailed account of the results of path coefficient analysis is given in the final parts of this section).

5.2.1.2 Correlation among other yield-related and component traits

In view of the association among phenological and agronomic yield-related/component traits, the following results were evidenced in this study.

DTF had a positive highly significant correlation with MSD (r=0.209**), whereas it had significant negative correlation with PLYLD (r=-0.141*) and NoSPPL (r=-0.155*) (Table 11). As stated in the sections above, several authors reported a significant positive correlation between DTF and PLYLD (Kassaye, 2006; Roy et al., 2006; Bhushan et al., 2008; Kumar et al., 2009; Sofi et al., 2011; Ahmed and Klamuddin 2013), which contradicts the findings in this study. On the other hand, Sadeghi et al. (2011) reported a similar significant negative correlation between DTF and PLYLD.

Even though, HSW correlated positively/negatively with the other traits, the only significant (positive) correlation it had was with PLYLD (r=0.342**). All the other correlations it had with the rest of the traits was non-significant, which may imply the trait had little associations with yield-related and component traits in common bean. In contradiction with this result were the reports of Kassaye (2006) and Roy et al. (2006). On the other hand, similar results showing a positive significant correlation between HSW and PLYLD were reported on common bean by various other authors (Zelalem, 2005; Karasu and Oz, 2011; Sadeghi et al., 2011; Negahi et al., 2014).

MSD, on the other hand, had significant to highly significant positive correlation with DTF (r=0.205**) and PLYLD (r=0.147*). Though still non-significant, it had a considerable (numerically) negative correlation with PHT (r=-0.114) (Table 11). Sadeghi et al. (2011) reported a result that contradicts with this finding, in which MSD had a negatively significant correlation with DTF, and non-significant positive correlation with PLYLD.

In the case of NoPP, highly significant correlations were recorded for this trait with NBPT (r=0.215**) and NoSPPL (r=0.421**). Similarly, significant positive correlations of NoPP with NoSPPL was reported by several authors (Zelalem, 2005; Karasu and Oz, 2010; Sadeghi et al., 2011; Cokkizigin et al., 2013; Negahi et al., 2014). Furthermore, the significant positive correlation between NoPP and NBPT was also reported in previous studies on common bean (Zelalem, 2005; Karasu and Oz, 2010; Cokkizigin et al., 2013) (Table 11).

PHT did not have any significant correlation with any of the other traits. However, numerically-speaking, it had a higher negative correlation with MSD (r=-0.114) (Table 11). The present result dose not concur with that reported in some previous studies, in which PHT had significant positive or negative correlations with a number of yield-related and component traits in common bean (Kassaye, 2006; Roy et al., 2006; Karasu and Oz, 2010; Ahmed and Kamaluddin, 2013; Negahi et al., 2014). On the other hand, non-significant positive or negative correlations of PHT with other traits, with the one with MSD as higher (numerically) was also evidenced in some previous studies (Salehi et al., 2008; Sadeghi et al., 2011)

NoSPPO had a highly significant correlation with NoSPPL (r=0.875**) (Table 11). Apart from this one, none of the other correlation coefficients it had with the rest of the studied traits were significant. Results from several previous studies concur with the present results (Zelalem, 2005; Roy et al., 2006; Salehi et al., 2008; Karasu and Oz, 2010; Sadeghi et al., 2011; Cokkizigin et al., 2013; Negahi et al., 2014).

Table 11 shows there were significant to highly significant positive correlations between NBPT and NoPP (r=0.215**) and NoSPPL (r=0.150*). This result agrees with the one reported by Zelalem (2005) in common bean in Ethiopia. Significant correlations between NBPT and NoPP/NoSPPL had also been documented by Karasu and Oz (2011) and Cokkizigin et al. (2013).

Finally, yet equally importantly, NoSPPL exhibited positive significant/highly significant correlations with NoPP (r=0.421**) and NoSPPO (r=0.150*), which agrees with previous

similar studies (Table 11) (Zelalem, 2005; Karasu and Oz, 2010; Sadeghi et al., 2011; Cokkizigin et al., 2013; Negahi et al. 2014).

5.2.2 Path coefficient analysis of yield and yield-related and component traits

Sometimes, correlation coefficients give misleading results, because the correlation between two variables may be due to a third factor. It is therefore necessary to analyze the cause and effect relationship between dependent and independent variables to untangle the nature of relationship between the variables (Sidramappa et al., 2008). Path coefficient analysis (Dewey and Lu, 1959) is often used to elicit the nature of relationship of dependent variable (yield) with closely associated independent variables in common bean. The selected traits are those, not only having high positive correlation, but also exerting high direct effects that are expected to be useful as selection criteria in selection program (Sadeghi et al., 2011).

The results of path coefficient analysis done by taking PLYLD as a dependent variable and DTF, HSW, MSD, NoPP, PHT, NoSPPO, NBPT, and NoSPPL, as independent variables are presented in Table 12. The table shows that four of the eight traits studied had higher positive direct effects on PLYLD. These were HSW (0.3216); NoSPPO (0.2733); MSD (0.1723); and NoPP (0.1675). This may imply the importance of using these parameters, simultaneously with seed yield, in the indirect selection to improve PLYLD on common bean. On the other hand, higher negative direct effects on PLYLD were exerted by NoSPPL (-0.3275) and DTF (-0.1608), which may indicate selecting against these two traits as a key tool in improving the seed yield of the common bean.

The higher positive direct effect exerted on PLYLD by MSD was also reported in common bean by Salehi et al. (2010); Sadeghi et al. (2011); Ahmed and Kalamuddin (2013); and Kumar et al. (2014). Furthermore, Salehi et al. (2010) reported similar results, in relation to the higher positive direct effect NoSPPO exerted on PLYLD. Mean while, the higher positive direct effect exerted by HSW on PLYLD was also reported by Cokkizigin et al. (2013) and Kulaz and Ciftici (2013).

On the other hand, contrary to the findings of the present study, higher positive direct effect exerted by DTF on PLYLD was reported in some related previous studies (Sadeghi et al., 2011;

Ahmed and Kalamuddin, 2013). Furthermore, NoSPPL, as having higher positive direct effect on PLYLD, negating the present result, was reported previously (Cokkizigin et al., 2013; Kulaz and Ciftici, 2013; Negahi et al., 2014). In addition, Cokkizigin et al. (2013) reported a contradicting result with this study in which NoSPPO and NoPP exerted higher negative direct effects on PLYLD in the common bean. The difference among the present result and the aforementioned authors may be an expected phenomenon, as differences in genotype and environment often bring about differences in the results of different studies.

The aforementioned accounts suggest the importance of looking into the direct and indirect effects of each variable in detail is paramount. The following sections emphasize on the detailed interpretation of the path coefficient analysis carried out in the study.

In the case of HSW, the direct effect it exerted on PLYLD accounted for ≈94.12% of its correlation with the same (Tables 11 and 12). This may imply, as per the assumption (decision rules) discussed in section 2.5, correlation explained the true relationship the trait had with PLYLD (Akintunde, 2012).

Although, Roy et al. (2006) reported a result agreeing with this one with respect to HSW having a higher positive direct effect on PLYLD, their results differed from the present results, in such a way that it had a significant negative correlation with the latter. This, contrary to the present results, might have called for the use of restrictive simultaneous selection to nullify the undesirable effects of some variables through which HSW exerted a higher negative indirect effect on PLYLD. Furthermore, Sadeghi et al. (2011) reported a partially differing result in which HSW had significant positive correlation with and smaller (negligible) negative direct effect on PLYLD. This, the same authors argued, might be explained by the higher positive indirect effects the trait exerted on PLYLD via other traits, like harvest index. In contrast, the present result differs with theirs, as the higher positive direct effect of HSW on PLYLD explained about 94% of its correlation with the same. Variation in genotypic; environmental; and genotype by environment (G x E) interaction effects may explain the differences between ours and other related previous studies. On the other hand, Kulaz and Ciftci (2013) had a harmonious result in that HSW had higher positive direct effect on and significant positive correlation with

PLYLD. Similarly, Karasu and Oz (2011) reported that HSW had one of the highest positive direct effects on PLYLD, which explained for a larger portion of its correlation with the same. Consequently, the authors stated that seed weight exerted lower and insignificant indirect effect on PLYLD, while it had a positive and higher direct effect to the positive significant correlation it had with PLYLD.

In comparison, correlation explained about 85% of the direct effect MSD exerted on PLYLD (Tables 11 and 12), whereas the rest 15% is explained by summation of negligible positive and negative indirect effects the former exerted on the latter via most of the other independent variables. Sadeghi et al. (2011) had a result that supported the present findings in that MSD exerted a higher positive direct effect on PLYLD, with smaller indirect effects via other traits like HSW.

Considering NoSPPO, its correlation with PLYLD only explained for about 5% its direct effect on the latter. Even though it had a smaller negative correlation with PLYLD (r=-0.0147) (Table 13), its direct effect on the same was higher and positive (0.2733) (Table 12). Employing the same decision rules discussed in section 2.5, a selection scheme, commonly referred to as restrictive simultaneous selection, may have to be employed in future breeding-by-selection in order to improve seed yield in the common bean (Akintunde, 2012).

Restrictive simultaneous selection refers to a method to minimize (or drop) undesirable effects, which may hamper the usefulness of the trait in selecting for higher yielding genotypes in the common bean. In this regard, it can be seen from Table 12 that however higher and favorable the direct effect of NoSPPO on PLYLD might have offset the narrowly higher negative indirect effect it exerted through NoSPPL (-0.2867). This suggested the aforementioned use of restrictive simultaneous selection to nullify the undesirable negative effect NoSPPO exerted on PLYLD via NoSPPL (Shah et al., 1996; Roy et al., 2006).

Roy et al. (2006) reported a similar result in which the higher direct effect NoSPPO exerted on PLYLD accounted only for about 39% of its correlation with the same. This implied that its higher direct effect may have been masked by undesirable negative effects it exerted on PLYLD

via other traits, like pod length and HSW. Nonetheless, it had higher significant correlation with seed yield, more probably due to the higher positive indirect effects it had on the same via DTF and NoPP. In contrast, the present result shows that although negative or positive indirect effects were exerted by NoSPPO through various other traits, none of them was significant enough to mask its direct effect, except the one it exerted through NoSPPL (Table 12). On the other hand, Kulaz and Ciftci (2013) reported a disagreeing result in that the direct effect NoSPPO exerted on PLYLD was negative and lower, but rather accompanied by higher positive indirect effects it had through NoSPPL and HSW. The higher positive direct effect, higher positive indirect effect via NoSPPL; and higher negative indirect effect NoSPPO exerted via NoPP has also been documented previously (Cokkizigin et al., 2013). Moreover, insignificant lower direct effect of NoSPPO on PLYLD was also reported by Karasu and Oz (2011).

NoPP had a higher positive direct effect on PLYLD (0.1675) (Table 14). Nonetheless, the insignificant positive correlation it had with PLYLD (r=0.0358) (Table 13) was only about one-fifth of its direct effect on the same. This implied indirect effects had higher importance in this case. Obviously, the higher negative indirect effect it had on PLYLD through NoSPPL (-0.1378) (Table 12) played the major role in offsetting the positive higher direct effect, ergo, an insignificant correlation with the response variable (PLYLD). Cokkizigin et al. (2013) presented an opposing result where NoPP had the highest negative direct effect on PLYLD with higher negative and positive indirect effects through NoSPPO and NoSPPL, respectively. The common genetic and environmental sources of variation may be causing such stark differences between the results of such similar studies. Furthermore, Kulaz and Ciftci (2013) reported a contradicting result with the present study in that NoPP exerted an insignificant direct effect on PLYLD, whereas, it had highly significant positive correlation with seed yield. To this end, the authors argued that the most important effect it had and explained for the significant positive correlation may be the higher positive indirect effect it exerted via NoSPPL. In contrast, Sadeghi et al. (2011) also reported a practically similar result with this one in which NoPP exerted one of the highest direct effects on PLYLD. However, the direct effect in their case, was not accompanied by, as was in the present result, with any other negative indirect effects through the other traits. Finally, Roy et al. (2006) documented that the direct effect NoPP had on seed yield comprised about 59% of its correlation with the same. They further pointed out that the remaining 41% was

accounted for by higher negative and positive indirect effects it exerted on PLYLD through other traits.

In the case of DTF, its significant negative correlation with PLYLD (r=-0.1414*) accounted for about 88% of the higher negative direct effect it exerted on the latter (-0.1608) (Tables 11 and 12). Hence this suggested that correlation explained its true relationship with the response variable (i.e., PLYLD). In line with this, the negative or positive indirect effect it had through other traits was not significant with respect to explaining its relationship with PLYLD. In contradiction with this result, Roy et al. (2006) noted that there was a highly significant positive correlation between DTF and PLYLD, accompanied by higher positive direct effect and corresponding higher positive indirect effects it exerted on the latter through PHT, NoPP, and NoSPPO. A different type of relationship between DTF and PLYLD was reported by Kumar et al. (2014) where DTF had lower positive direct effect accompanied by insignificant positive correlation with seed yield in the common bean. Zelalem (2005), however, had a similar result in which there had been higher negative direct effect exerted by DTF on PLYLD, which explained a significant portion of the correlation between the two traits.

NoSPPL, on the other hand, exerted the highest negative direct effect on yield in this study, whereas, its correlation with the latter was insignificant and negative (-0.3275 and r=-0.0183) (Tables 11 and 12). This implied the higher importance that should be attached to the indirect effects it exerted via the other traits on PLYLD can account for the larger portion of its correlation with the same. In view of this, the higher positive indirect effect it had through NoSPPO (0.2393) (Table 12) may be the most important one in defining its actual relationship with PLYLD. Higher negative direct effect of NoSPPL on PLYLD was also reported by Zelalem (2005), supporting the present finding. On the other hand, contradicting results, in which NoSPPL had higher positive direct effects with PLYLD was reported in several previous studies (Kassaye, 2006; Cokkizigin et al., 2013; Kulaz and Ciftci, 2013; Sadeghi et al., 2013; Negahi et al., 2014). Such contradictions may be resulted in owing to genetic and environmental differences, which in turn, may bring about variation in the direct and indirect relationship the causal traits have on the response variable.

Finally, looking into the other two variables included in the path coefficient analysis, NBPT and PHT, had insignificant correlation with and lower direct effects on PLYLD. Hence, it can be concluded that these traits were of very little importance towards being used in the indirect selection for higher yield in common bean.

Consequently, HSW and MSD were the most important traits, owing to having significant positive correlation with yield. In addition, they had the highest direct effects on PLYLD, which accounted for 85-95% of their respective correlation with the same. Hence, it can be noted that due attention should be given to these traits, in order to augment seed yield and the traits, could be used in future breeding programs involving common bean landrace accessions from Ethiopia.

Table 11: Correlation coefficients among seed yield and eight yield-related/component traits in common bean accessions from Ethiopia

	DTF	**HSW**	**PLYLD**	**MSD**	**NoPP**	**PHT**	**NoSPPO**	**NBPT**	**NoSPPL**
DTF	1	-.078	-.141*	.209**	-.129	.083	-.077	.101	-.155*
HSW	-.078	1	.342**	.037	-.034	-.045	-.053	.109	-.064
PLYLD	-.141*	.342**	1	.147*	.036	-.036	-.015	.021	-.018
MSD	.209**	.037	.147*	1	-.068	-.114	-.009	-.024	-.030
NoPP	-.129	-.034	.036	-.068	1	.062	.028	.215**	.421**
PHT	.083	-.045	-.036	-.114	.062	1	.041	.001	.054
NoSPPO	-.077	-.053	-.015	-.009	.028	.041	1	.060	.875**
NBPT	.101	.109	.021	-.024	.215**	.001	.060	1	.150*
NoSPPL	-.155*	-.064	-.018	-.030	.421**	.054	.875**	.150*	1

Where: DTF=Days to Flowering; HSW=Hundred Seed Weight; PLYLD=Plot Yield; MSD= Mean Seed Diamter; NoPP=Number of Pods/plant; PHT=Plant Height; NoSPPO=Number of Seed/pod; NBPT=Number of Branches/plant; NoSPPL=Number of seeds/plant

Table 12: Direct and indirect effects of eight independent variables on the seed yield of common bean genotypes from Ethiopia

	DTF	**100-Seed Weight**	**MSD**	**NoPP**	**PHT**	**NoSPPO**	**NBPT**	**NoSPPL**
DTF	**-0.1608**	-0.0250	0.0360	-0.0216	-0.0001	-0.0211	0.0003	0.0509
HSW	0.0125	**0.3216**	0.0064	-0.0058	0.0001	-0.0144	0.0003	0.0210
Mean Seed Diameter	-0.0336	0.0119	**0.1723**	-0.0114	0.0002	-0.0025	-0.0001	0.0098
NoPP	0.0208	-0.0111	-0.0117	**0.1675**	-0.0001	0.0076	0.0006	-0.1378
PHT	-0.0142	-0.0145	-0.0195	0.0108	**-0.0016**	0.0119	0.0000	-0.0185
NoSPPO	0.0124	-0.0169	-0.0016	0.0046	-0.0001	**0.2733**	0.0002	-0.2867
NBPT	-0.0163	0.0351	-0.0041	0.0360	0.0000	0.0164	**0.0028**	-0.0492
NoSPPL	0.0250	-0.0206	-0.0051	0.0704	-0.0001	0.2393	0.0004	**-0.3275**

Where: DTF=Days to Flowering; HSW=Hundred Seed Weight; PLYLD=Plot Yield; MSD= Mean Seed Diamter; NoPP=Number of Pods/plant; PHT=Plant Height; NoSPPO=Number of Seed/pod; NBPT=Number of Branches/plant; NoSPPL=Number of seeds/plant

5.3 Phenotypic diversity of common bean (*Phaseolus vulgaris* L.) landraces from Ethiopia

5.3.1 Cluster analyses

Cluster analysis of genotypes using the Tocher method grouped the accessions into five major clusters (Table 13 and 14). Originally, Tocher clustering yielded ten clusters, with six clusters having one member each. However, using bootstrap value of 1000 and comparing the clusters with the ones identified by Neighbor-joining analysis, five main cluster groups were eventually identified. With the same accord, clustering of the genotypes with the neighbor-joining (NJ) method, similarly, yielded five (5) clusters, with almost the same assignment of accessions in each cluster with Tocher's clustering method (Fig. 15). Detailed results of the cluster analysis are given in the following sections. Importantly, the two clustering methods similarly identified the presence of both the Andean and Mesoamerican gene pools in Ethiopia. Similar results in Ethiopian and other African common bean landrace accessions using agro-morphological characters were reported in related previous studies (Asfaw et al., 2009; Blair et al., 2010b; Okii et al., 2014a, b).

Cluster I (n=25) contained accessions with white/cream/red medium-sized seeds. Moreover, seeds had round/oval/cuboid/kidney shapes. Furthermore, Type-II was the dominant plant type, followed by accessions with Type-I, IV, and III growth habits. Seed weights in this cluster ranged from 21.5-58.5g, with mean value of 39.13 g. Based on the identifiers of major gene pool (Mesoamerican Vs Andean) and respective races in each gene pool used in some previous studies (Singh et al., 1991a, b, c, Burle et al., 2011), this cluster seemed to be predominantly from one of the ecological races in the Andean gene pool (medium/large seed weight; cuboid/kidney-shaped seeds). Subsequently, accessions with medium-large sized round/oval seeds of a Type-IV growth habit may have belonged to another different race in the Andean gene pool. Finally, yet importantly, the rest members of the cluster with medium-sized oval/round seeds and Type-III plant type may correspond to another ecogeographic race in the Andean gene pool (Tables 13 and 14). Even though the presence of Andean genotypes was significant in the cluster, considerable number of accessions, however, did have intermediate features between the two gene pools (small seed weight and different plant types, for instance), which may indicate they were inter-gene pool introgressions. Kwak and Gepts (2008); Blair et al. (2010b); Burle et

al. (2011) and Okii et al. (2014a, b) reported similar observations in which significant presence of hybrid genotypes in the common bean.

Cluster II (N=8) was comprised of mainly large-seeded accessions (mean seed weight = 54.19 g) (Table 13, 14) with growth habits of Type-II, I, and IV (determinate). Seeds had white/red/cream colors. Apparently, this may be an Andean cluster with a heterogeneous representation of ecogeographic races. Member accessions with Type-I and II growth habits, and cuboid/kidney seeds may probably be from one of the races in the gene pool, where as accessions with Type-IV growth habit, and oval/round seeds might have been from a different Andean race (Singh et al., 1991a, b). Nonetheless, many of the remaining member accessions had features of the Mesoamerican gene pool, for instance, smaller seeds (<25g), which may imply considerable presence of inter-gene pool introgressions in the cluster. Singh et al. (1991a) reported a similar result entailing the presence of more than one Andean ecogeographic race in common bean cluster groups identified through morphological markers.

On the other hand, Cluster III (N=27) was, most probably, a Mesoamerican group, owing to the average seed weight recorded (i.e., 25.16 g) (Table 13 and 14). Small-seeded accessions with Type-II growth habit may belong to race _Mesoamerica'. On the other hand, accessions of medium-sized seeds and Type-IV plant types may be from a different race in the Mesoamerican group. However, the presence of outlier genotypes, having Andean features was commoner in the cluster, as many larger-seeded genotypes were also observed (Appendix 5). Results reported by Burle et al. (2011) in Brazilian common bean accessions are in agreement with our present finding. Presences of heterogeneous accession groups of common bean landraces from two different races of the Mesoamerican gene pool was, similarly, reported by Burle et al. (2011).

Cluster IV had 38 accessions having predominantly white seeds and Type-I and II growth habits. Overall, accessions in the cluster had medium seed weights (mean seed weight = 29.13 g) (Table 13 and 14). Probably, accessions with small seeds may be grouped under the race _Mesoamerica', whereas those with medium-sized seeds and Type-IV (indeterminate) plant types may belong to either of the other two races in the Mesoamerican gene pool. On the other hand, one accession (240173) had Type-III (indeterminate) growth habit, and white medium-sized

seeds (Appendix 5). This specific accession may be from one of the races in the Andean gene pool. Kwak et al. (2009) made a similar observation with this one, where mixed membership of Andean genotypes in Mesoamerican clusters, and vice versa using molecular markers in common bean genotypes.

Cluster V (n=23) was an Andean group, which consisted of accessions that clustered with the Andean control genotype. Most of the accessions in this cluster had Type-II growth habit and medium-sized seeds. From these, it may be implied that most of the member accessions in this cluster belonged to race _Nueva Granada' in the Andean gene pool. Nonetheless, a number of accessions had values, with respect to identifier morphological/agronomic characters, exactly similar to neither of the two gene pools, which may indicate that they were inter-gene pool introgressions/hybrids (Table 13 and 14).

Generally, the two clustering: Tocher and neighbor-joining; identified almost similar pattern of classification of accessions into two groups (Andean and Mesoamerican). Nonetheless, further sub-grouping in each of the five clusters was observed in the NJ dendrogram (Fig. 15). Such further sub-grouping with neighbor-joining analysis in cluster groups, initially identified by both the methods, was also observed by Barelli et al. (2009).

In summary, the identification of five clusters, with three predominated by Andean genotypes, and the other two by accessions from the Mesoamerican gene pool, was also observed in the molecular genetic diversity and population structure analyses described in subsequent sections (5.4, 5.5, and 5.6). Moreover, the significant presence of inter-gene pool introgressions, having characters between the two gene pools, was also reported in previous findings (Asfaw et al., 2009; Blair et al., 2010b; Okii et al., 2014a, b). Mahalanobis distance (D^2) of the five clusters of Ethiopian common bean landrace accessions based on the morphological and agronomic traits studied is given in Table 15. The inter-cluster distance (D^2) analysis showed a highly significant ($p<0.01$) difference between cluster II and III (42.37), cluster II and IV (37.94), and cluster III and V (39.74) (Table 15).

Table 13: Distributions of 121 Ethiopian common bean landrace accessions over the five clusters identified with Tocher and neighbor-joining clustering methods

Cluster I	**Cluster II**	**Cluster III**	**Cluster IV**		**Cluster V**
201293	211293	201666	207938	235697	201294
207933	211304	208638	208695	237993	207934
208698	211305	208646	208703	240173	207949
211266	211334	208647	211290	240190	211271
211267	211348	208702	211291	240552	211278
211269	241756	208705	211294	241733	211279
211277	244805	211298	211299	241737	211325
211286	CHERCHER	211319	211300	241753	211327
211292		211320	211317	241765	211329
211301		211338	211318	241807	211340
211315		211342	211322	GOBERASHA	211341
211332		211344	211323	MEX-142	211345
211337		211350	211331		211378
211349		211386	211339		211394
211382		211387	211347		211546
211389		211388	211356		211551
211483		211552	211361		212861
211550		212860	211362		216730
212978		219233	211377		237078
230779		219234	211379		241730
240512		219235	211481		241736
241739		235692	213046		241750
241752		240187	215719		Andean Control
241757		241738	216819		
NASER		241748	216820		
		241814	218235		
		MA Control	235697		
25 (20.66%)	**8 (6.6%)**	**27 (22.31%)**		**38 (31.4%)**	**23 (19.01%)**

Table 14: Mean values observed in the clusters identified by Tocher and neighbor-joining clustering methods in 121 Ethiopian common bean accessions studied

Cluster	Number of accessions	Seed color	Growth habit	Seed shape	100-seed weight
I	25	White, cream, and red	Type-II (dominant), Types-I, IV, and, III	Round, oval, cuboid, kidney-shaped	21.5 g-58.5 g Mean 39.13 g
II	8	White, red, and cream	Type-II, I, and IV (determinate)	Oval, round, cuboid, and kidney	23 g-70 g 54.1 g
III	27	White, red, cream, and brown	Type-II (dominantly), Type-I, and IV	Oval, round, and cuboid	15.5 g- 60.5 g 25.16 g
IV	38	White (dominant), red, and cream	Types-I & II (92%), IV, and III	Round, oval, cuboid, and markedly-truncated	15.5 g- 50.2 g 29.13g
V	23	White, red, brown/light brown, and cream	Type-II (dominant), I, and IV	Oval, round, cuboid, kidney	23 g-46 g 33.5 g

The smallest inter-cluster distance was between I and IV (2.20), where as the highest and highly significant distance was that between cluster II and III (Table 15). The significant inter-cluster distances indicated that there may be a great opportunity for obtaining transgressive segregates and maximizing heterosis by crossing accessions belonging to different clusters, as there is higher probability that unrelated genotypes would contribute unique desirable alleles at different loci (Olika et al., 2011).

5.3.2 Principal component analysis (PCA)

Eigen values, percent of total variance, percent of total cumulative variance, and eigen vectors for the agro-morphological in the common bean landrace accessions are shown in Table 16. Principal Component Analysis (PCA) was performed to assess the relative importance of each character towards explaining the prevalent genetic diversity in the studied accessions. The first eight principal components explained 69.4% of the variation. The first principal component which accounted for 20.4% of the variation was, chiefly, due to the discriminatory effects of traits like, seed color, seed shape, 100-seed weight; and seed brilliance (Table 16). This result is in agreement with that of Burle et al. (2011), where seed weight exerted major effect on the variation explained by Principal Component (PC) 1. Color of flower wings (in freshly-opened flowers), standard color, days to flowering, and 100-seed weight contributed chiefly to the variation explained by PC 2 (13.6%). Burle et al. (2011) reported, similarly to the present result, that days to flowering was one of the traits exerting major effects on the variation explained by PC2 in their study. The third principal component accounted for 7% of the total variation, and mainly caused by days to flowering, plant growth habit, pod color, and seed brilliance (Table 16). Owing to being the largest portion of the total variation presented in PCA, the characters that brought about significant variation in the first two principal components were selected to be the most important ones in their application vis-à-vis improvement/breeding of the common bean. Hence, these traits: seed color, seed shape, 100-seed weight, seed brilliance, color of flower wings, standard color, and days to flowering are recommended to be given higher emphasis in future breeding/conservation programs involving Ethiopian common bean accessions. Similarly, these traits have been reported in previous studies to be the most important ones in identifying/determining the genetic diversity/population structure of common bean germplasm into the two gene pools (Singh et al., 1991a; Burle et al., 2011). The present study confirmed that Ethiopian common bean accessions showed variation for the characters studied. This trait diversity evident among the accessions suggests the presence of opportunities for genetic improvement through selection directly from the accessions and/or selection of diverse parents for hybridization programs and conservation of the germplasm for further utilization. Such an existence of broad agro-morphological genetic diversity among common bean accessions is in agreement with the results of related previous studies (Asfaw et al., 2009; Blair et al., 2010b; Okii et al., 2014b).

Summarizing, the present study was unique in such a way that it generated new information on the morphological genetic diversity of Ethiopian common bean landrace accessions. Both Tocher and NJ clustering methods identified similar five groups, which agree with the genetic diversity and population structure revealed in the same set of genotypes with SSR markers (sections 5.4, 5.5, and 5.6). Furthermore, clustering with Tocher and neighbor-joining methods confirmed the results of the molecular genetic diversity and population structure (sections 5.4, 5.5, and 5.6). That is, both the Mesoamerican and Andean gene pool germplasm are present in Ethiopian common bean landrace germplasm, which was also reported in another similar study by other authors (Asfaw et al., 2009). Furthermore, the significant presence of inter-gene pool introgressions in common bean accessions in this study was also reported by several other authors (Kwak and Gepts, 2009; Asfaw et al., 2009; Blair et al., 2010b).

Table 15: Mahalanobis distance (D^2) of the 5 clusters of 121 Ethiopian common bean accessions based on 15 morphological and agronomic traits

	Distance between clusters				
	I	**II**	**III**	**IV**	**V**
I		8.30	5.69	2.20	11.58
II			42.37**	37.94**	18.90
III				9.83	39.74**
IV					9.83

** Highly significant at $p<0.01$ (X^2) = 29.141.

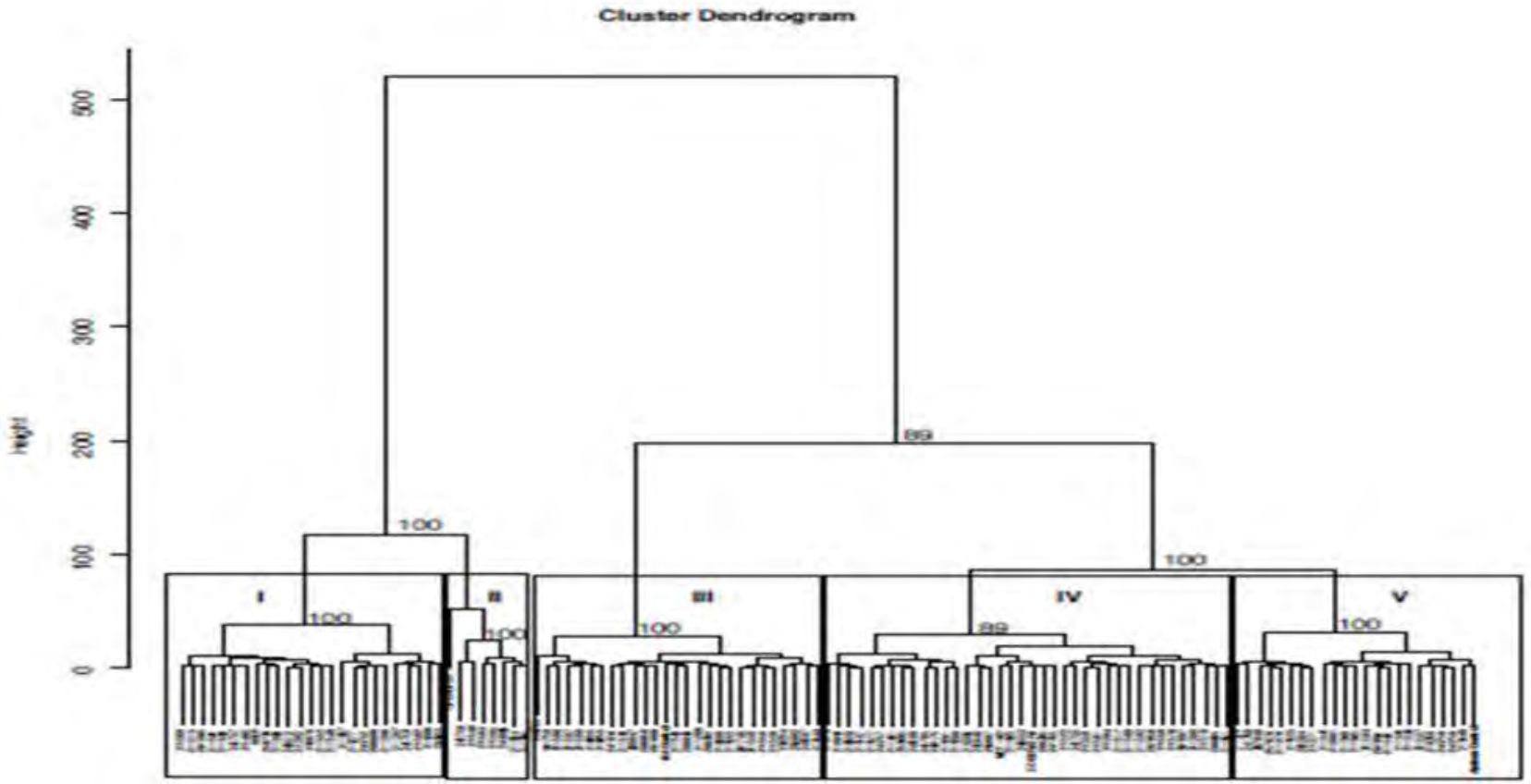


Figure 14: Neighbor-joining dendrogram in 121 Ethiopian common bean accessions

Table 16: Eigen values, total variance, cumulative variance, and eigen vectors for nine morphological and agronomic characters in 121 Ethiopian common bean germplasm

Traits/Variables	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**
Seed Color	0.6572	-0.0308	0.0809	-0.0776	0.1449	-0.1640	0.0159	0.0006
plant growth habit	-0.1165	-0.0243	0.4149	-0.3893	0.4577	0.4702	-0.4318	-0.1789
Days to flowering	0.0921	0.4653	0.3979	-0.1411	-0.2606	0.2056	0.5225	-0.4118
Color of Flower (Standard)	0.0958	0.5080	-0.2623	0.3373	0.1418	-0.0154	-0.4904	-0.2540
Seed Shape	0.2425	-0.3055	-0.3931	-0.2310	-0.0426	0.5691	0.1529	0.1734
100-Seed Weight	-0.1261	0.2916	0.0906	-0.6051	0.0159	-0.4147	-0.0705	0.4703
Seed Brilliance	-0.1569	0.0812	-0.3830	-0.1202	0.7120	-0.1624	0.4644	-0.2370
Pod Color	-0.0077	-0.0122	-0.4506	-0.5172	-0.3901	-0.1111	-0.2245	-0.4981
Color of Flower Wings (in Freshly Opened Flowers)	0.0843	0.5820	-0.2755	-0.0140	-0.0267	0.3870	0.0393	0.4214

PC=Principal Component Axis

5.4 Molecular genetic diversity of Ethiopian common bean accessions with respect to collection sites using microsatellite markers

5.4.1 Allelic patterns/diversity

A total of 149 alleles were identified, giving an average of 8.8 alleles per locus for the 17 microsatellites evaluated, of which 12 were genomic, whereas five were genic (gene-based) markers (Table 6). The range in allele number was 4–15, with the marker BM143 having the highest number of alleles, followed by GATS91, GATS54 and BM140, with 14, 13, and 13 alleles, respectively. These markers were genomic. The highest number of alleles found for a gene-based microsatellite was for BMd53 with nine alleles, followed by BMd36 and BMd42 having six alleles each. The mean number of alleles for genomic microsatellites was 1.5 times more than that of genic microsatellites. The observed heterozygosity on average was 0.51 across all the 17 markers evaluated. The markers with the highest levels of observed heterozygosity were GATS91 (0.684) and BM143 (0.67), whereas, the genic marker PV-CCTT001 had the lowest value, 0.013 (Table 17). In other words, the number of alleles, genetic diversity, and observed heterozygosity recorded were as high as those reported in previous bean diversity studies involving Eastern and Central African bean landraces (Asfaw et al., 2009; Blair et al., 2010b). Though the recorded number of alleles may be high, they were not higher than those reported in studies involving common bean landraces from across the world. Specifically, genomic SSR markers like GATS91 and BM143 had the highest number of alleles (15), observed heterozygosity (0.684), and genetic diversity (0.845), in this study. Blair et al. (2010b) reported a similar result with the aforementioned genomic SSR markers having the highest number of alleles, observed heterozygosity; and genetic diversity, when used to evaluate common bean landrace accessions from Central Africa. On the other hand, the allelic patterns across the studied populations are presented in Fig. 15. The figure also depicts the number of alleles, number of effective alleles, Shanon‘s diversity index, number of private alleles, and number of less common alleles in bars of different colors. As the line above the bars showed pattern of variation in expected heterozygosity among the different groups of accessions, ‗Amhara‘ and Southern Nations, Nationalities, and People (SNNP) had the highest expected heterozygosity. Nonetheless, the overall variation observed in

accessions from different populations (collection sites) vis-à-vis expected heterozygosity values was moderate. Furthermore, the calculated values for each of the aforementioned allelic measures are given in Table 17. The table corroborates the patterns depicted by Fig. 15. According to Table 18, accessions from ‗Oromiya' and SNNP had the highest average number of alleles (6.88 and 6.35, respectively). On the other hand, accessions from ‗Amhara' and the released varieties' group had the highest number of alleles with frequencies ≥5% (measurement taken to alleviate the sampling error associated with the sampling of race or distinct alleles, i.e., with frequencies ≤ 5%) (N_a Freq. >=5%), whereas accessions from ‗Amhara' and SNNP had the highest number of effective alleles (N_e) (Table 18). In essence, ‗Amhara' and SNNP may be the most important populations, owing to the higher number of alleles with frequencies ≥5% (excluding rare alleles) and number of effective alleles. As with the scenario observed regarding number of effective alleles (N_e), ‗Amhara' and SNNP had the highest genetic diversity measures (Shannon's index=I) (Table 18). This may further strengthen the argument made above regarding the two populations: ‗Amhara' and SNNP, being the most important ones with respect to having the highest number of alleles corresponding to polymorphic loci and levels of allelic diversity. Furthermore, accessions from ‗Oromiya' and SNNP had the highest numbers of private alleles (0.824 and 0.588, respectively), and less common alleles with frequencies less than 50% (1.765 and 1.588, respectively). This may imply that, upon further determination of what functional traits, if any, these private or less common alleles encode for, it may be possible to harness the potentials of accessions in the population in future common bean improvement and genetic conservation endeavors in Ethiopia. The highest values for both expected and unbiased expected heterozygosity were recorded for accessions from ‗Amhara', SNNP, and the released varieties' group. In summary, the results presented in Table 18 and Fig.15, showed that, of the populations/collection sites considered, ‗Amhara' and SNNP were the most important ones, owing to the highest values they had in terms of number of effective alleles (N_e), Shannon's diversity index (I), and expected/unbiased expected heterozygosity. It is more probable that higher diversity observed in the geographical populations: ‗Amhara' and SNNP may be attributed to the cross-border exchange of planting materials with Sudan and Kenya, respectively.

Furthermore, it may imply these two populations should be of the highest priority towards acquiring common bean accessions to be used as parents for future breeding programs in Ethiopia. The present result supports the arguments made by various authors on the importance of landrace accessions vis-à-vis being rich reservoirs of useful adaptation and resistance genes (Horňáková et al., 2003; Mondini et al., 2009).

Table 17: Observed/effective number of alleles, genetic diversity, PIC, total number of alleles, and Shannon index of the 17 SSR markers used in the study

Locus	**Sample Size**	**n_a***	**n_e***	**I***	**Average heterozygosity**	**F_{st}**	**Genetic Diversity**	**PIC**
BM205	258	8	2.69	1.4	0.5428	0.25	0.618	0.591
AG-1	224	4	1.66	0.76	0.3716	0.33	0.409	0.376
GATS91	234	14	6.57	2.19	0.684	0.28	0.845	0.831
GATS54	254	13	3.06	1.52	0.5277	0.27	0.665	0.627
BMd42	242	6	2.89	1.38	0.567	0.28	0.638	0.608
PV-CCTT001	250	4	1.07	0.08	0.0126	0.11	0.025	0.025
BMd53	258	9	3.03	1.40	0.6134	0.13	0.664	0.605
BM156	250	11	2.98	1.40	0.56	0.284	0.655	0.595
BM187	216	11	2.86	1.35	0.651	0.421	0.648	0.584
BMd18	216	5	1.69	0.8	0.35	0.423	0.420	0.384
BMd36	220	6	3.06	1.30	0.53	0.34	0.669	0.621
BM151	218	8	3.44	1.43	0.56	0.336	0.705	0.656
BM140	232	13	4.1	1.72	0.64	0.31	0.752	0.717
BM141	242	7	2.56	1.18	0.48	0.31	0.603	0.536
BM143	242	15	4.17	1.91	0.67	0.223	0.757	0.736
BM165	226	6	3.51	1.39	0.54	0.366	0.717	0.671
BM139	244	9	1.88	1.08	0.41	0.251	0.457	0.437
Mean	**237**	**8.8**	**3.01**	**1.31**	**0.51**	**0.289**	**0.603**	**0.565**
St. Dev		**3.527**	**1.245**	**0.471**	**0.16**			

Where: Na=Number of alleles; Ne=Number of Effective Alleles; I=Shannon‘s Diversity index; and PIC=Polymorphic Information Content

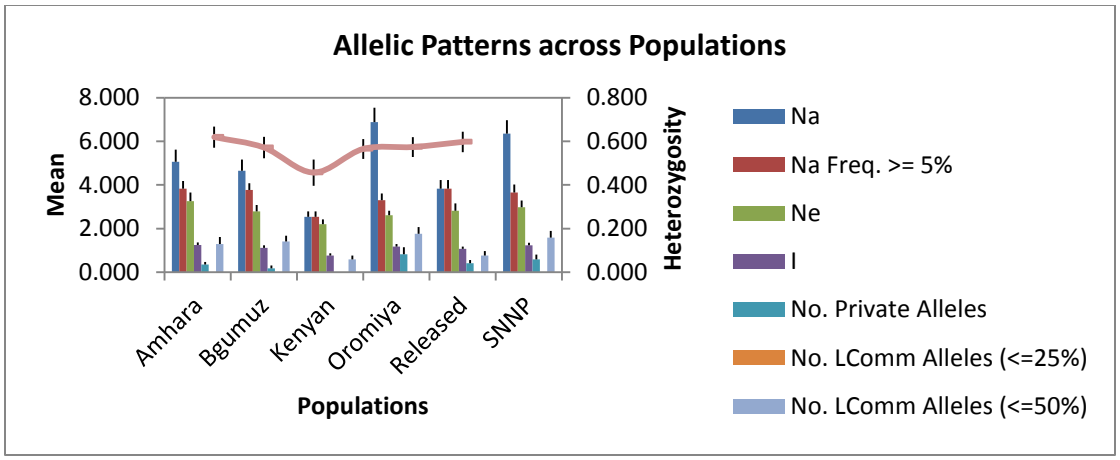


Figure 15: Patterns of allelic variation observed in the study populations along with important allelic values

N_a (number of alleles), N_a Freq >= 5% (number of alleles with frequencies greater than or equal to 5%, N_e (number of effective alleles), I (Shannon's index), number of private alleles, number of less common alleles (with frequencies less than or equal to 25% and 50%, and He (expected heterozygosity).

Table 18: Important allelic values recorded in the landrace and control genotypes in six population groups

Paraneters	**Populations**					
	Amhara	**Bgumuz**	**Kenyan**	**Oromiya**	**Released**	**SNNP**
Na	5.059	4.647	2.529	**6.882**	3.824	**6.353**
Na Freq. >= 5%	**3.824**	3.765	2.529	3.294	**3.824**	3.647
Ne	**3.262**	2.791	2.195	2.612	2.815	**2.970**
I	**1.239**	1.114	0.757	1.179	1.065	**1.236**
No. Private Alleles	0.353	0.176	0.000	**0.824**	0.412	**0.588**
No. Less common Alleles (<=50%)	1.294	1.412	0.588	**1.765**	0.765	**1.588**
He	**0.619**	0.571	0.457	0.565	**0.574**	**0.597**
uHe	**0.652**	0.593	0.578	0.571	**0.627**	**0.607**

N_a (number of alleles), N_a Freq >= 5% (number of alleles with frequencies greater than or equal to 5%, N_e (number of effective alleles), I (Shannon's index), number of private alleles, number of less common alleles (with frequencies less than or equal to 25% and 50%, and He (expected heterozygosity). Populations refer to geographical administrative regions from which accessions had been collected.

5.4.2 Analysis of Molecular Variance (AMOVA) and genetic distances

5.4.2.1 Analysis of molecular variance

Results of AMOVA are presented in Table 19 and Fig.16. Fig.16 shows that 58% of the total variation was attributed to genetic variability among individuals from different populations, whereas 40% was due to variation among individuals within the same population. In contrast, a smaller portion (2%) of the total variation was among populations. These results were also evident in the AMoVA output displayed in Table 19. According to IPGRI and Cornell University (2003), the F-statistics allows analysis of structure of subdivided populations, which may also be used to measure the genetic distance among sub-populations. Moreover, the overall concept behind the whole F-statistics theory is that those sub-populations that are not intermating will have different allele frequencies with the total population. In light of these, the statistical indexes involved measure:

- F_{is}: the deficiency or excess of average heterozygosity in each population;
- F_{st}: the degree of genetic differentiation among populations in terms of allele frequencies and;
- F_{it}: the deficiency or excess of average heterozygosity in a group of populations.

Of the aforementioned statistical indexes, F_{st} has got more importance vis-à-vis discerning genetic differentiation among the studied populations. To this end, decision rules dictate that F_{st} values ranging from 0 to 0.05 imply small genetic differentiation among populations. On the other hand, F_{st} values from 0.05 to 0.15 correspond to moderate, from 0.15 to 0.25 imply large, and those greater than 0.25 mean very large genetic differentiation among populations in terms of allele frequencies. In line with these rules, the extent of genetic differentiation among the six populations in terms of allele frequencies measured was small ($F_{st}=0.015^{*}$), which implied intermating among individuals within similar populations was not significant. The value recorded for the number of migrants (gene flow between and within populations) ($N_m=16.282$) (Table 20) could be the cause of the lower population differentiation observed. Similarly, Asfaw et al. (2009) and Blair et al. (2010b) reported the presence of gene flow between

and within regions (populations), which, ultimately, caused common bean landraces from different collection sites to cluster together. Furthermore, pair-wise N_m values among the six populations studied were calculated and presented in Table 20. As can be seen from this table, the highest gene flow values were recorded for the following pair of populations: _Benishangul-Gumuz‘ and SNNP (N_m=63.186); _Benishangul-Gumuz‘ and Kenya (N_m=54.601); and _Oromiya‘ and SNNP (N_m=30.790). Although the higher gene flow recorded between accessions from _Benishangul-Gumuz‘ and Kenya may be unexpected, it can be explained by the higher gene flow between accessions from _Benishangul-Gumuz‘ and SNNP, which may, indirectly, contribute largely in bringing it about. This assumption may be strengthened by the geographical proximity between the latter (SNNP) and Kenya. Furthermore, the higher gene flow recorded between accessions from _Benishangul-Gumuz‘ and SNNP; and _Oromiya‘ and SNNP may be brought about by geographical proximity, cross-region farmers‘ seed exchanges, and/or possible expansion of germplasm via the interventions of governmental/non-governmental organizations. The higher gene flow between regions (or populations) observed in the present study supports previous findings of Asfaw et al. (2009), who reported that there was significant gene flow between regions and collection sites for common bean germplasm from Ethiopia.

5.4.2.2 Genetic distance among of the common bean accessions

Average unbiased genetic distances among the landrace accessions were estimated in Nei and displayed in Table 21. According to results in the table, the genetic distances recorded ranged from 0.073 (between genotypes from Benishangul-Gumuz and Oromiya) to 0.329 (between genotypes from the released group and Kenya). Peculiarly, the genetic distance between accessions from Kenya and all the rest of the populations were higher (Table 21).

Table 19: Values of sum of squares; mean squares; and F-values among populations; among individuals in a population; and among individuals in all the populations

Source	df	SS	MS	Est. Var.	%	F-Statistics	Value	P(random >= data)
Among Pops	5	53.742	10.748	0.085	2%	F_{st}	0.015	0.020
within pops	119	924.562	7.769	2.247	40%	Fi_s	0.407	0.010
among Indiv	125	409.500	3.276	3.276	58%	F_{it}	0.416	0.010
Total	249	1387.804		5.608	100%	N_m	16.282	

Df=Degrees of Freedom; SS=Sum of Squares; MS=Mean Square; Est. Var.=Estimated Variability

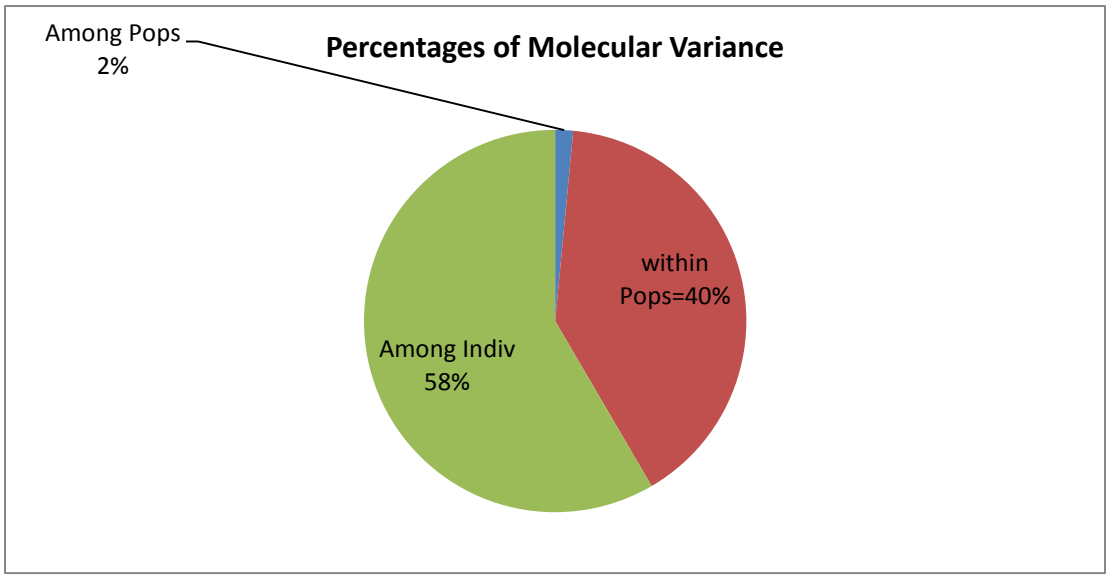


Figure 16: AMoVA variation pie chart for 125 common bean accessions from six populations in Ethiopia

Table 20: Pair-wise Number of Migrants (N_m) values based on F_{st} Values

	Amhara	Bgumuz	Kenyan	Oromiya	Released	SNNP
Amhara	0.000					
Bgumuz	27.570	0.000				
Kenyan	19.226	**54.601**	0.000			
Oromiya	14.973	14.168	5.207	0.000		
Released	10.537	3.838	2.738	6.193	0.000	
SNNP	0.000	**63.186**	9.461	**30.790**	6.480	0.000

Bgumuz: Benishangul-Gumuz; SNNP=Southern Nations, Nationalities, and Peoples Regional State; bold values indicate the highest N_m values observed

Even though there were a few instances of relatively higher genetic distances recorded for some combinations of populations, the overall magnitude of genetic distance recorded in this study was lower in relation to previously-reported values (Hegay et al., 2012). Asfaw et al. (2009) reported that average Nei‘s unbiased genetic distance was lower between Ethiopian and Kenyan common bean genotypes, in agreement with findings of the present study.

Table 21: Average Nei‘s unbiased genetic distance calculated among accessions from different populations

	Amhara	Bgumuz	Kenyan	Oromiya	Released	Southern
Amhara	0.000					
Bgumuz	0.112	0.000				
Kenyan	0.272	0.244	0.000			
Oromiya	0.086	0.073	0.243	0.000		
Released	0.234	0.212	0.329	0.174	0.000	
Southern	0.101	0.070	0.228	0.045	0.206	0.000

Bold values indicate the highest genetic distance values observed in the study

5.4.3 Cluster and principal coordinate analyses (PCoA)

5.4.3.1 Cluster analysis

Cluster analysis with respect to populations (collection sites) was performed on the allelic frequency data using neighbor-joining method, with the Darwin and PowerMarker V3.25 software programs. Figure 17 shows the dendrogram clustering pattern for individual accessions (of the Darwin software program) in different populations (collection sites). As can be seen from the dendrogram, five different groups were identified. Furthermore, accessions from different populations (collection sites) clustered together, which may imply the presence of gene flow between and within populations/regions/collection sites. The present results support previous finding in common bean accession populations from East and Central Africa, where mixed clusters composed of accessions from different collections sites had been observed (Asfaw et al., 2009; Blair et al., 2010b). On the other hand, when the genetic diversity of the accessions is seen from the angle of the gene pools of origin (Mesoamerican and Andean), the considerable presence of inter-gene pool introgressions may be one of the reasons behind mixed clustering of accessions from different populations/collection

sites (detailed explanation on the structure of genetic diversity among Ethiopian common bean landrace germplasm with respect to Andean and Mesoamerican control genotypes is given in section 5.5). Similarly, the presence of inter-gene pool introgressions, which, in turn, brought about mixed clusters composed of Andean and Mesoamerican genotypes was reported in several previous studies in common bean (Asfaw et al., 2009, Kwak and Gepts, 2009, Blair et al., 2010b, Blair et al., 2011).

On the other hand, Figure 18 shows the results of the cluster analysis done based on Nei's average unbiased genetic distance (Nei, 1983) among the accessions studied. Based on these results, four groups of populations were identified among the common bean landrace accessions from six different populations. Group 1 belonged to accessions from the _Amhara' region, which was relatively more of an outlier group. Geographical barriers/isolation may be one of the most probable reasons behind this distinct clustering present in this group. Similarly, Martinez-Castillo et al. (2006) reported that wild populations of lima bean (*P. lunatus*) were outliers, owing to the existence of geographical isolations among the populations studied. On the other hand, the second group comprised accessions from the SNNP and _Oromiya' regions. The close-grouping of these two populations may be explained by the geographical proximity (ergo less geographical isolation/barrier). The third group contained accessions from the _Benishangul-Gumuz' region, whereas group four was composed of accessions from Kenya and the released varieties' group. Especially, the closer-clustering of the latter two populations of accessions may be explained by the possible existence of some trans-boundary gene flow between the two countries. This present results further support the arguments made by Asfaw et al. (2009), who noted that despite accessions from Kenya and Ethiopia clustered together with other accessions from the same country of origin (Kenya and Ethiopia), there had been some considerable cross-border gene flow between the two countries. Another neighbor-joining dendrogram was constructed based on the shared-allele frequency genetic distances measured (Fig. 19). In comparison, this dendrogram identified five groups (compared to the four groups identified in the Nei's genetic distance NJ dendrogram), with _Oromiya' and SNNP in the farthest end and _Benishangul-Gumuz' and _Amhara',

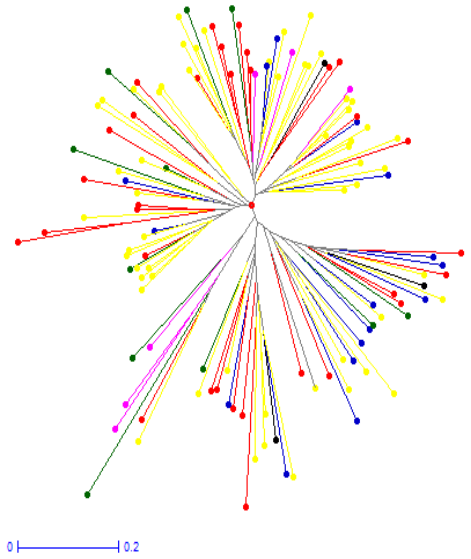
being group 3 and 4. Finally, yet importantly, the shared-allele frequency NJ dendrogram, similarly with the Nei‘s NJ dendrogram, clustered accessions from Kenya and the released varieties‘ group together. This similarity in the grouping of accessions from the latter two populations further strengthens the presence of some cross-border gene flow between the two countries vis-à-vis common bean germplasm.

5.4.3.2 Principal coordinate analysis (PCoA)

The first three axes of the PCoA accounted together 64.47% of the total variation, with 26.66%, 21.01%, and 16.80% explained by PC axis 1, 2, and 3, respectively. Results of the PCoA are displayed in Fig. 20. It can be seen from this figure that accessions from different collection sites often clustered together. This, in turn, agrees with the results of the NJ dendrogram (of the Darwin 5 software program) in that there was no unique clustering among accessions from the same population/collection site. The presence of gene flow between and within populations/collection sites, accompanied by the prevalence of inter-gene pool introgressions/hybrids between the Mesoamerican and Andean gene pools of origin may be the most probable explanations behind the mixed clustering of accessions from different populations/collection sites together. The repetition of this result in the PCoA further supports previous results of Asfaw et al. (2009) and Blair et al. (2010b), where mixed clustering was observed among accessions from different origin populations clustering together, owing to gene flow between and within populations, and presence of inter-gene pool hybrids between the Mesoamerican and Andean gene pools.

Generally, the results of the present study showed that there was ample allelic diversity among the common bean landrace accessions studied, based on the values recorded for all the SSR markers used in the study (like heterozygosity, Shannon‘s index, PIC etc). Most of the values recorded for the SSR markers used in the study were comparable (or even some times higher) to previous studies on common bean genetic diversity in Africa. Moreover, geographical populations with higher genetic diversity and other allelic values were identified, which will be important baseline information for future common bean breeding/genetic resource conservation endeavors in Ethiopia. In light of

these, the lower emphasis so far given towards harnessing the rich genetic potential of landrace germplasm in common bean breeding/improvement should be reversed in the near future, in order to develop superior cultivars with higher yield/resistance/quality. On the other hand, cluster analyses and PCoA jointly showed the presence of gene flow and inter-gene pool introgressions among the landrace accessions and control varieties, which should be carefully considered in future genotype sampling and collection vis-à-vis biodiversity evaluation and conservation in the common bean.



Legend
Amhara = green;
Benishangul Gumuz = blue;
Oromiya = yellow;
Southern region= red;
Released = purple;
Kenyan = orange

Figure 17: Neighbor-joining dendrogram of the 125 common bean accessions constructed by Darwin 5 software program.

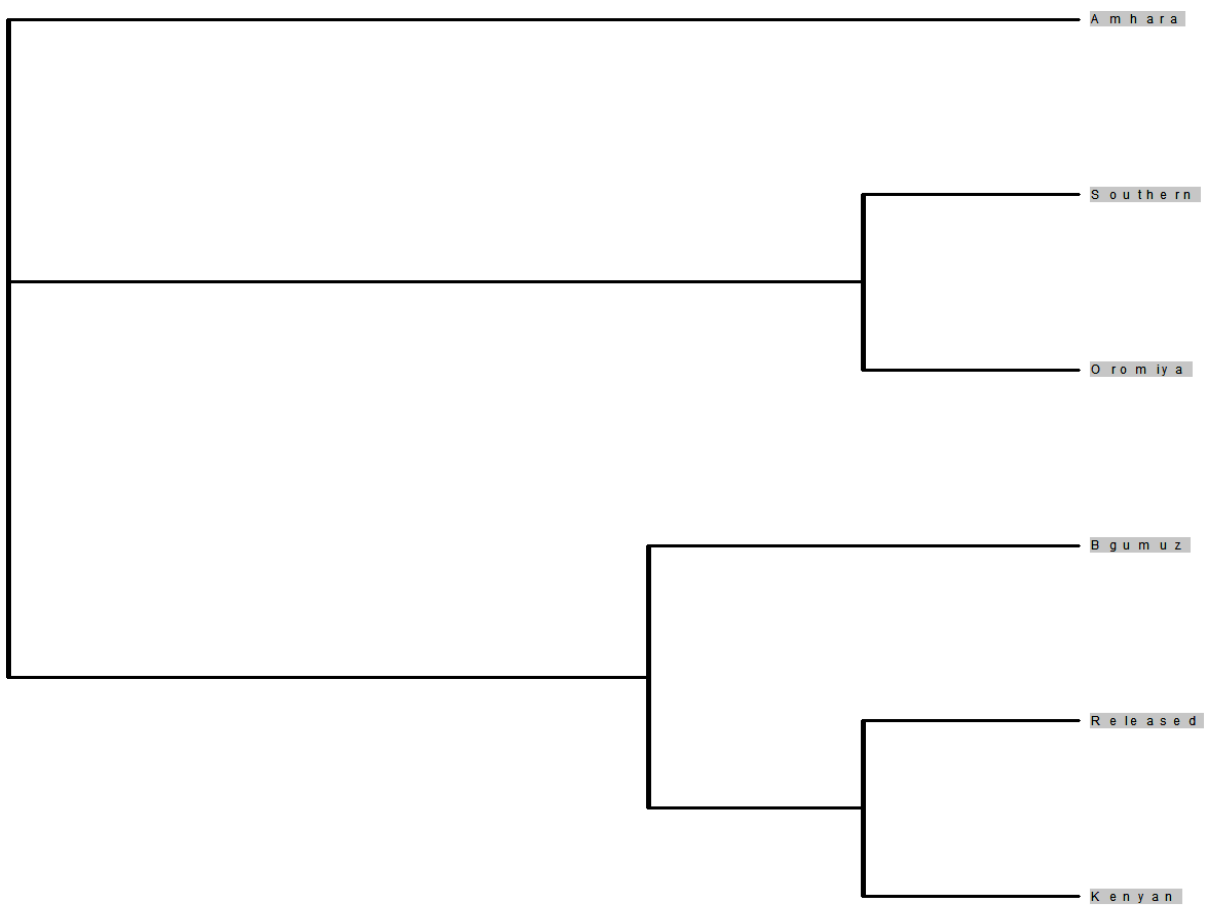


Figure 18: Neighbor-joining dendrogram for the six (geographical) populations based on Nei‘s unbiased genetic distance (Nei, 1983) measured

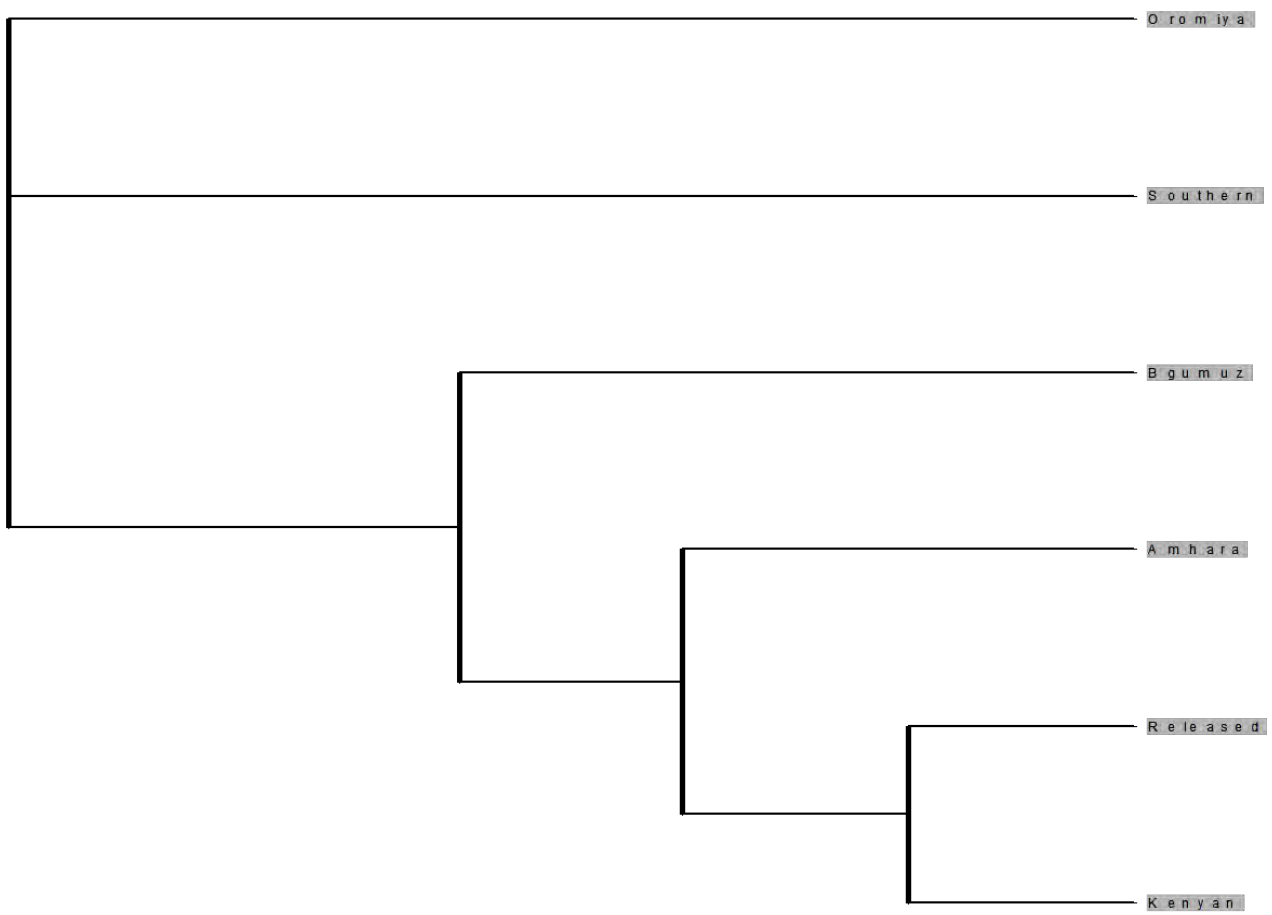


Figure 19: Neighbor-joining dendrogram for the six (geographical) populations based on shared-allele genetic distance values measured.

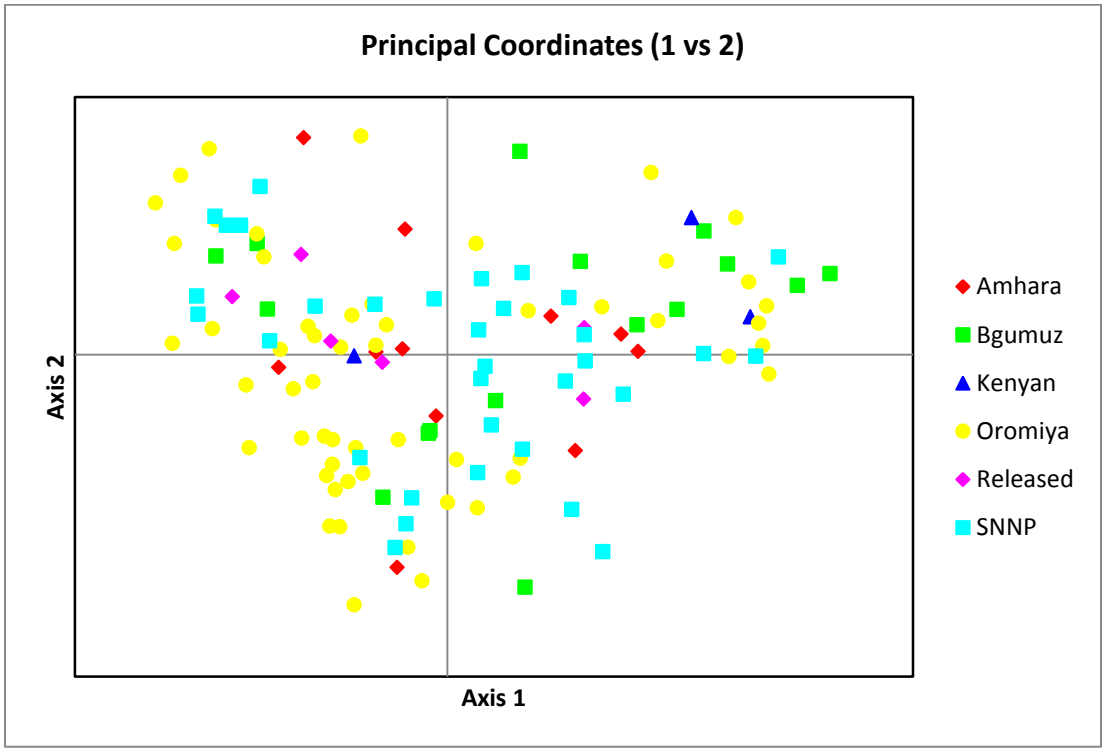


Figure 20: PCoA graph of the 125 common bean accessions from 6 populations

5.5 Population structure of Ethiopian common bean (*Phaseolus vulgaris* L.) landrace germplasm into the Mesoamerican and Andean gene pools

5.5.1 Population structure and genetic differentiation among populations

The population subdivision (as determined by STRUCTURE) (Fig. 21), the NJ tree (Fig. 24), and the PCoA (Fig. 23; Table 26) showed significant Andean–Mesoamerican gene pool divergence as well as racial differentiation within these two gene pools. The accessions were assigned to the respective gene pools of origin, as per the methods explained in the –Materials and Methods" for K=2. Consequently, 78 accessions out of the total 125 fell into the Mesoamerican group, whereas the remaining 47 into the Andean group. This classification was based on posterior assignment probabilities p>0.5. This split was generally maintained from K=2 to 5, with the exception of K=4 and 5 (Fig. 21; Table 26). The analysis for K=2 populations showed individual accessions distributed between the two gene pools, which was congruent with neighbor-joining and PCoA that clearly separated the Mesoamerican and Andean gene pools. At K=3, looking jointly into the bar-graphs produced and membership coefficient values, the Mesoamerican gene poo,l genotypes further separated into two sub-groups but no meaningful interpretation of population structure could be made, while the Andean gene pool genotypes did not show any separation. At K=4, the Mesoamerican accessions further subdivided into two groups with a mild level of admixture, but no meaningful interpretation of population structure could be made. At K=5, the Andean accessions further subdivided into three groups with some admixture level, whereas the Mesoamerican ones did not show any more level of subdivision (Fig.21; Table 26). A more or less similar pattern of subdivision was also reported in Asfaw et al. (2009).

At K=5, the following five cluster groups were identified: Andean Cluster 1 (K4); Andean Cluster 2 (K5); the cluster containing the Andean control (K1); Mesoamerican Cluster 1 (K2) and the cluster containing Mesoamerican control (K3) (Fig. 21; Table 26). Considering Fst values recorded for each cluster group (Table 23), Andean groups had higher average values (0.381), compared to their Mesoamerican counterparts (0.1995), showing higher population differentiation in the former. This result is in agreement with that reported by Asfaw et al. (2009). On average, F_{st} values for Andean

populations (K1, K4, and K5) were lower (0.213) compared to those of Mesoamerican populations (K2, and K3) (0.451) (Table 23). The population admixture for each accession was also quantified (Fig. 24; Table 26). The Andean gene pool had a higher proportion of non-hybrid accessions than the Mesoamerican gene pool with 50.85 and 34.85% at the 0.8 cutoff, respectively (Table 22). The proportion of non-hybrid accessions in each K group ranged from 28% (Mesoamerican Controls K3) to 53.85% (Andean Cluster 2 K5) at the cutoff value of 0.8 (Table 22). A similar population structure was uncovered with the NJ tree. The majority of hybrid accessions had an ancestry involving the groups in the Mesoamerican gene pool. The same result was reported by Asfaw et al. (2009) and Kwak and Gepts (2009).

Table 22: Proportion of non-hybrid accessions in K = 5 groups identified by STRUCTURE

Groups	**Total Number of Accessions**	**0.8 Cutoff**	
		Number of Accessions	% from total
Total	125	53	42.4
Mesoamerican	66	23	34.85
Mesoamerican Cluster1 (K2)	41	16	39
Mesoamerican control (K3)	25	7	28
Andean	59	30	50.85
Andean Cluster1 (K4)	27	11	40.7
Andean Cluster2 (K5)	26	14	53.85
Andean Control (K1)	6	5	83.33

Table 23: F_{ST} values among five populations identified by STRUCTURE

K	**Andean Cluster 1 (K4)**	**Andean Cluster 2 (K5)**	**Andean Control (K1)**	**Mesoamerican Cluster 1 (K2)**	**Mesoamerican Control (K3)**
5	0.239	0.356	0.547	0.135	0.264

5.5.2 Genetic diversity within and among accessions and cluster groups

The proportions of polymorphic loci were 100% in the Andean Cluster 1 (K4) genotypes; 94.12% in the Andean cluster 2 (K5), the cluster containing the Andean control (K1), and the Mesoamerican cluster 1 (K2); 76.47% in the cluster containing the Mesoamerican control (K3) (Table 27). On average, the Andean groups had higher

number of alleles (Na); number of effective alleles (Ne); Shannon Index (I); observed heterozygosity; expected heterozygosity; fixation index; percent of polymorphic loci; genetic distance; and number of private alleles. On the other hand, the Mesoamerican groups had higher out-crossing rates than the Andean groups. The out-crossing rates were much higher than the ones reported in other related studies (Blair et al., 2010b; Okil et al., 2014a, b).

This might be explained by the fact that most of the accessions (>90%) were acquired from the National Gene Bank, which had been collected from subsistence farmers with a culture of keeping mixed seeds for consumption and subsequent planting seasons. The highest number of alleles; genetic distance (GD); observed heterozygosity (H_o); out-crossing rate (t) and percent of polymorphic loci were recorded for the Andean cluster 1 (K5). The cluster containing the Andean control (K1) had the highest Shannon index (I); fixation index (F); and number of private alleles (N_{pa}); number of effective alleles (N_e). Asfaw et al. (2009) and Burle et al. (2011) reported a contradictory result in that Mesoamerican groups had higher observed heterozygosity than the Andean counterparts among the common bean accessions in their study (Table 27).

5.5.3 Analysis of Molecular Variance

Results of AMOVA showed that 50% of allelic diversity was attributed to individuals within each of the gene pools ($P<0.001$); 31% among individuals in the total population; and the rest 19% was attributed to the diversity among populations. A highly significant genetic differentiation among subpopulations (0.186, $P<0.01$) was observed.

Some lower level of gene flow between different cluster of accessions was also reported (i.e., 1.095), with higher values among accessions from different Andean gene pool clusters (i.e. 1.573) compared to values observed among different Mesoamerican clusters (i.e. 0.251). Average Nei‘s unbiased genetic distance was higher within each gene pool (0.771) but lower between the Andean and Mesoamerican gene pools (0.689). Within gene pool, the Mesoamerican representatives presented lower genetic distances

(0.725) than the Andean gene pool representatives (0.817) (Fig. 22 and Table 24 and 25).

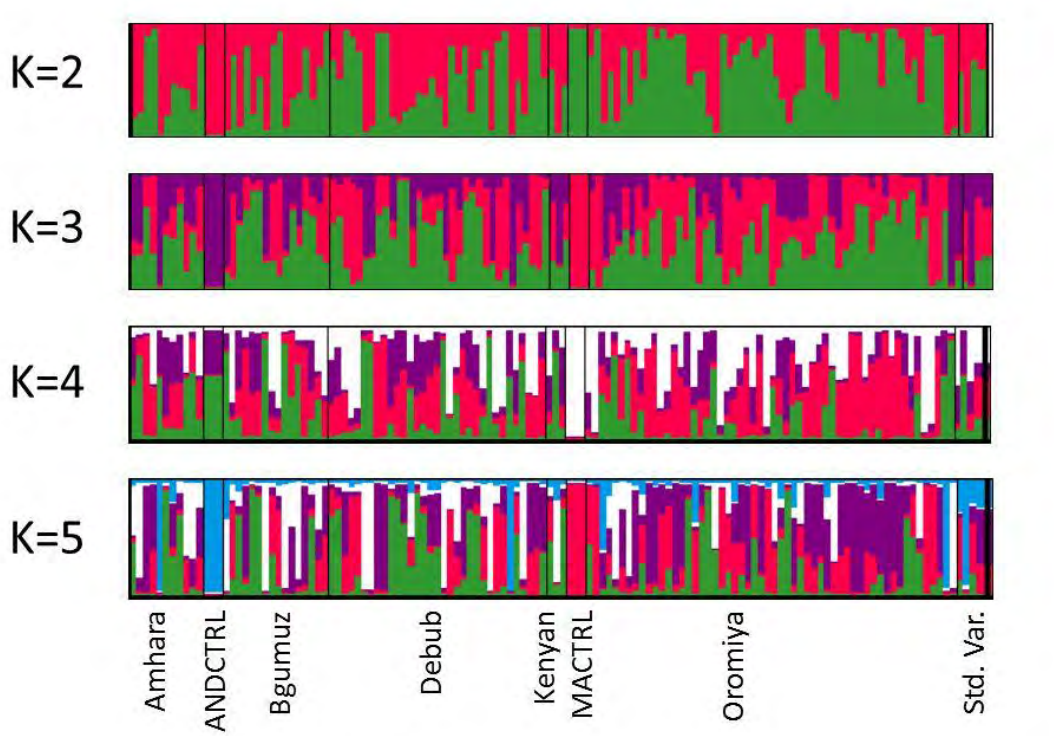


Figure 21: Population structure for 120 common bean accessions from different growing regions of Ethiopia and 3 Kenyan cultivars compared to Andean and Mesoamerican control genotypes at K = 2 to K = 5.

Predetermined group names indicated below figure are: Amhara = Genotypes from Amhara Regional State; andectrl = Andean control genotypes; Bgumuz = Genotypes from Benishangul Regional State; Debub = Genotypes from Southern Nations and Nationalities Regional State; Kenyan = Kenyan accessions; MACTRL = Mesoamerican control genotypes; Oromiya = Genotypes from Oromiya Regional State; and Standard Varieties with similar meaning.

Table 24: Values of AMOVA among populations and individuals; and within individuals in all populations in 125 Ethiopian/Kenyan common bean genotypes evaluated with 17 fluorescent SSR markers

Source	df	SS	MS	Est. Var.	%
Among Pops	4	116.473	29.118	1.100	19%
Within Pops	48	320.546	6.678	1.858	31%
Within Indiv	53	157.000	2.962	2.962	50%

Df=Degrees of Freedom; SS=Sum of Squares; MS=Mean Square; Est. Var.=Estimated Variability

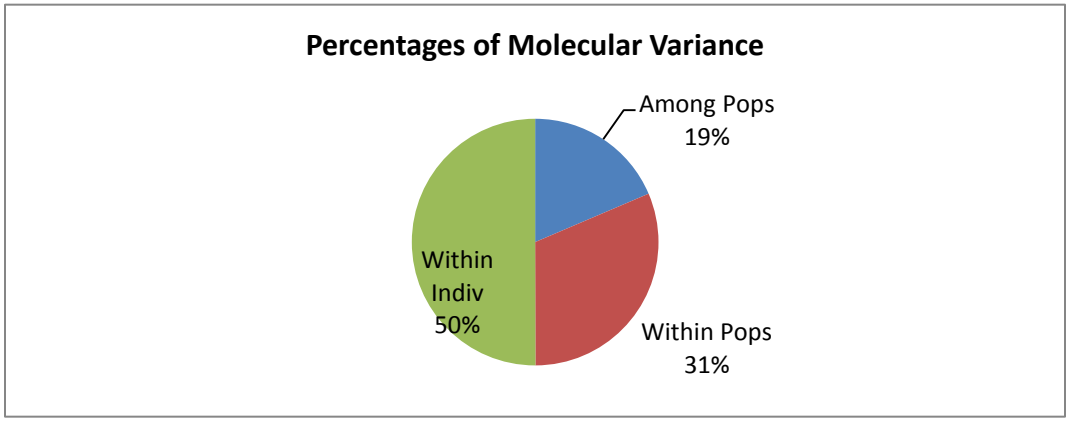


Figure 22: AMOVA pie-chart for the percentage of variation explained among individuals in a population; among populations; and within individuals in all the populations
Pops=Populations; Indiv=Individuals

Table 25: Pairwise population matrix of Nei unbiased genetic distance

	Andean Cluster1 K4	**Andean Cluster2 K5**	**Andean Control K1**	**MA Cluster1 K2**	**MA Control K3**
Andean Cluster1 K4	0.000				
Andean Cluster2 K5	0.327	0.000			
Andean Control K1	0.525	0.435	0.000		
MA Cluster1 K2	0.182	0.259	0.384	0.000	
MA Control K3	0.488	0.451	0.517	0.321	0.000

MA=Mesoamerican

Table 26: membership coefficients and posterior probability values for K values from 1-5

No.	Accession		Membership coefficient	Posterior probability values
1	211269	(5)	1 : 0.090 0.132 0.082 0.696	(0.000,0.301) (0.000,0.389) (0.000,0.263) (0.393,0.976)
2	241807	(17)	1 : 0.060 0.032 0.043 0.866	(0.000,0.206) (0.000,0.106) (0.000,0.145) (0.666,0.997)
3	211266	(0)	1 : 0.853 0.017 0.066 0.064	(0.648,0.996) (0.000,0.052) (0.000,0.216) (0.000,0.210)
4	211267	(0)	1 : 0.316 0.014 0.628 0.042	(0.069,0.553) (0.000,0.046) (0.406,0.838) (0.000,0.145)
5	241814	(29)	1 : 0.012 0.964 0.011 0.013	(0.000,0.038) (0.902,1.000) (0.000,0.034) (0.000,0.039)
6	211389	(0)	1 : 0.242 0.425 0.114 0.218	(0.000,0.606) (0.004,0.833) (0.000,0.331) (0.000,0.605)
7	211551	(0)	1 : 0.565 0.301 0.107 0.027	(0.270,0.859) (0.033,0.552) (0.000,0.325) (0.000,0.086)
8	215719	(5)	1 : 0.765 0.022 0.049 0.164	(0.545,0.968) (0.000,0.068) (0.000,0.166) (0.001,0.341)
9	211382	(5)	1 : 0.017 0.016 0.400 0.568	(0.000,0.052) (0.000,0.050) (0.191,0.601) (0.370,0.774)
10	211386	(5)	1 : 0.190 0.020 0.024 0.766	(0.000,0.422) (0.000,0.061) (0.000,0.080) (0.528,0.985)
11	211387	(0)	1 : 0.252 0.016 0.498 0.234	(0.000,0.562) (0.000,0.051) (0.237,0.742) (0.040,0.452)
12	ANDEAN Ctrl	(0)	2 : 0.013 0.963 0.008 0.016	(0.000,0.040) (0.899,1.000) (0.000,0.026) (0.000,0.050)
13	ANDEAN Ctrl	(0)	2 : 0.013 0.962 0.009 0.016	(0.000,0.040) (0.897,1.000) (0.000,0.027) (0.000,0.052)
14	ANDEAN Ctrl	(0)	2 : 0.012 0.964 0.008 0.015	(0.000,0.038) (0.901,1.000) (0.000,0.027) (0.000,0.050)
15	207934	(11)	3 : 0.015 0.341 0.027 0.617	(0.000,0.047) (0.174,0.519) (0.000,0.089) (0.430,0.796)
16	207938	(23)	3 : 0.035 0.053 0.745 0.167	(0.000,0.116) (0.000,0.177) (0.540,0.935) (0.002,0.361)
17	207933	(11)	3 : 0.544 0.291 0.018 0.146	(0.237,0.814) (0.001,0.603) (0.000,0.057) (0.000,0.387)
18	211349	(29)	3 : 0.876 0.025 0.070 0.029	(0.655,0.999) (0.000,0.075) (0.000,0.238) (0.000,0.088)
19	211345	(23)	3 : 0.881 0.022 0.022 0.075	(0.673,0.999) (0.000,0.066) (0.000,0.070) (0.000,0.249)
20	240512	(0)	3 : 0.879 0.057 0.035 0.029	(0.717,0.996) (0.000,0.167) (0.000,0.117) (0.000,0.095)
21	211347	(17)	3 : 0.017 0.015 0.019 0.949	(0.000,0.056) (0.000,0.046) (0.000,0.061) (0.860,1.000)
22	211349	(0)	3 : 0.148 0.044 0.668 0.140	(0.000,0.384) (0.000,0.150) (0.444,0.894) (0.000,0.374)
23	211361	(0)	3 : 0.063 0.038 0.826 0.073	(0.000,0.217) (0.000,0.124) (0.608,0.994) (0.000,0.245)
24	211362	(17)	3 : 0.044 0.032 0.014 0.910	(0.000,0.141) (0.000,0.100) (0.000,0.046) (0.781,0.998)
25	211348	(5)	3 : 0.442 0.016 0.018 0.524	(0.057,0.736) (0.000,0.050) (0.000,0.055) (0.237,0.881)
26	211356	(5)	3 : 0.063 0.012 0.046 0.879	(0.000,0.208) (0.000,0.039) (0.000,0.156) (0.689,0.998)
27	211344	(0)	3 : 0.829 0.021 0.116 0.035	(0.624,0.991) (0.000,0.067) (0.000,0.288) (0.000,0.111)

Table 26 (cont'd)

No.	Accession		Membership coefficient	Posterior probability values
28	211350	(5)	3 : 0.448 0.074 0.135 0.343	(0.009,0.855) (0.000,0.264) (0.000,0.319) (0.000,0.794)
29	211349	(23)	3 : 0.130 0.016 0.421 0.434	(0.000,0.406) (0.000,0.050) (0.164,0.646) (0.234,0.634)
30	240522	(5)	3 : 0.214 0.028 0.736 0.022	(0.000,0.467) (0.000,0.092) (0.486,0.972) (0.000,0.068)
31	241756	(11)	4 : 0.690 0.026 0.256 0.028	(0.406,0.967) (0.000,0.084) (0.001,0.524) (0.000,0.089)
32	241757	(17)	4 : 0.697 0.041 0.194 0.069	(0.324,0.988) (0.000,0.137) (0.000,0.509) (0.000,0.232)
33	213046	(11)	4 : 0.098 0.017 0.805 0.079	(0.000,0.333) (0.000,0.054) (0.531,0.996) (0.000,0.266)
34	235692	(0)	4 : 0.027 0.069 0.871 0.033	(0.000,0.086) (0.000,0.178) (0.733,0.986) (0.000,0.109)
35	211483	(0)	4 : 0.021 0.072 0.809 0.099	(0.000,0.067) (0.000,0.216) (0.636,0.965) (0.000,0.273)
36	211481	(23)	4 : 0.023 0.018 0.012 0.947	(0.000,0.075) (0.000,0.058) (0.000,0.038) (0.853,1.000)
37	211292	(5)	4 : 0.028 0.022 0.015 0.934	(0.000,0.093) (0.000,0.073) (0.000,0.047) (0.822,1.000)
38	211290	(11)	4 : 0.814 0.014 0.148 0.024	(0.603,0.990) (0.000,0.044) (0.000,0.348) (0.000,0.074)
39	211291	(5)	4 : 0.876 0.013 0.061 0.050	(0.684,0.998) (0.000,0.042) (0.000,0.206) (0.000,0.166)
40	241752	(23)	4 : 0.330 0.304 0.130 0.237	(0.000,0.802) (0.000,0.739) (0.000,0.372) (0.000,0.751)
41	241753	(11)	4 : 0.923 0.032 0.012 0.032	(0.796,0.999) (0.000,0.109) (0.000,0.038) (0.000,0.105)
42	241755	(29)	4 : 0.955 0.014 0.011 0.020	(0.876,1.000) (0.000,0.046) (0.000,0.033) (0.000,0.063)
43	211294	(5)	4 : 0.044 0.189 0.346 0.422	(0.000,0.144) (0.000,0.446) (0.108,0.577) (0.130,0.719)
44	211293	(5)	4 : 0.698 0.098 0.047 0.157	(0.413,0.966) (0.000,0.336) (0.000,0.160) (0.000,0.410)
45	211552	(5)	4 : 0.498 0.095 0.154 0.253	(0.223,0.751) (0.000,0.302) (0.000,0.392) (0.000,0.550)
46	237993	(17)	4 : 0.655 0.148 0.129 0.068	(0.313,0.942) (0.000,0.356) (0.000,0.399) (0.000,0.222)
47	212978	(5)	4 : 0.797 0.139 0.028 0.036	(0.514,0.995) (0.000,0.409) (0.000,0.093) (0.000,0.119)
48	211294	(0)	4 : 0.027 0.018 0.026 0.929	(0.000,0.090) (0.000,0.057) (0.000,0.086) (0.810,0.999)
49	241738	(0)	4 : 0.013 0.011 0.742 0.234	(0.000,0.040) (0.000,0.034) (0.582,0.885) (0.095,0.391)
50	241739	(11)	4 : 0.182 0.014 0.292 0.513	(0.000,0.458) (0.000,0.044) (0.002,0.554) (0.299,0.733)
51	211546	(5)	4 : 0.885 0.011 0.043 0.061	(0.720,0.996) (0.000,0.033) (0.000,0.148) (0.000,0.175)
52	241750	(5)	4 : 0.760 0.022 0.019 0.200	(0.506,0.988) (0.000,0.069) (0.000,0.061) (0.000,0.449)
53	241736	(17)	4 : 0.815 0.021 0.136 0.028	(0.574,0.994) (0.000,0.065) (0.000,0.366) (0.000,0.091)
54	244805	(29)	4 : 0.082 0.030 0.609 0.279	(0.000,0.283) (0.000,0.099) (0.196,0.957) (0.000,0.617)
55	241737	(23)	4 : 0.016 0.026 0.014 0.943	(0.000,0.052) (0.000,0.087) (0.000,0.044) (0.847,1.000)
56	241748	(0)	4 : 0.036 0.049 0.762 0.153	(0.000,0.119) (0.000,0.131) (0.601,0.909) (0.012,0.308)

Table 26 (cont'd)

No.	Accession		Membership coefficient	Posterior probability values
57	241733	(11)	4 : 0.106 0.045 0.835 0.014	(0.000,0.306) (0.000,0.146) (0.641,0.988) (0.000,0.043)
58	211286	(0)	4 : 0.014 0.959 0.013 0.014	(0.000,0.044) (0.890,1.000) (0.000,0.042) (0.000,0.044)
59	211394	(5)	4 : 0.165 0.208 0.430 0.197	(0.000,0.414) (0.000,0.523) (0.225,0.636) (0.000,0.490)
60	211278	(5)	4 : 0.084 0.012 0.032 0.872	(0.000,0.281) (0.000,0.038) (0.000,0.106) (0.657,0.999)
61	211277	(0)	4 : 0.124 0.011 0.842 0.023	(0.000,0.386) (0.000,0.035) (0.580,0.998) (0.000,0.074)
62	211279	(0)	4 : 0.065 0.011 0.908 0.016	(0.000,0.223) (0.000,0.035) (0.734,0.999) (0.000,0.050)
63	212860	(0)	4 : 0.923 0.032 0.025 0.020	(0.802,0.999) (0.000,0.103) (0.000,0.082) (0.000,0.063)
64	MWITEMA	(5)	5 : 0.107 0.159 0.565 0.169	(0.000,0.349) (0.000,0.416) (0.296,0.811) (0.000,0.439)
65	E7	(5)	5 : 0.026 0.253 0.014 0.707	(0.000,0.083) (0.003,0.501) (0.000,0.046) (0.456,0.962)
66	WANJIRU	(29)	5 : 0.362 0.194 0.269 0.176	(0.009,0.701) (0.000,0.500) (0.000,0.598) (0.000,0.508)
67	MA Ctrl	(11)	6 : 0.011 0.012 0.967 0.010	(0.000,0.034) (0.000,0.038) (0.911,1.000) (0.000,0.031)
68	MA Ctrl	(11)	6 : 0.010 0.011 0.968 0.010	(0.000,0.032) (0.000,0.036) (0.914,1.000) (0.000,0.031)
69	MA Ctrl	(11)	6 : 0.011 0.012 0.967 0.010	(0.000,0.033) (0.000,0.038) (0.911,1.000) (0.000,0.032)
70	241730	(0)	7 : 0.104 0.149 0.691 0.055	(0.000,0.310) (0.000,0.352) (0.487,0.887) (0.000,0.192)
71	212861	(5)	7 : 0.014 0.021 0.956 0.010	(0.000,0.042) (0.000,0.068) (0.882,1.000) (0.000,0.031)
72	230779	(23)	7 : 0.271 0.677 0.021 0.031	(0.026,0.489) (0.468,0.895) (0.000,0.068) (0.000,0.101)
73	235697	(11)	7 : 0.204 0.189 0.262 0.346	(0.000,0.521) (0.001,0.399) (0.000,0.547) (0.006,0.692)
74	211379	(23)	7 : 0.053 0.014 0.018 0.916	(0.000,0.180) (0.000,0.042) (0.000,0.056) (0.770,0.999)
75	237078	(11)	7 : 0.596 0.014 0.121 0.269	(0.337,0.828) (0.000,0.043) (0.000,0.368) (0.093,0.468)
76	211378	(5)	7 : 0.067 0.016 0.064 0.854	(0.000,0.227) (0.000,0.050) (0.000,0.210) (0.646,0.997)
77	211377	(0)	7 : 0.581 0.061 0.286 0.073	(0.259,0.880) (0.000,0.191) (0.029,0.558) (0.000,0.234)
78	216819	(0)	7 : 0.211 0.183 0.396 0.211	(0.000,0.483) (0.000,0.416) (0.194,0.599) (0.024,0.420)
79	211319	(0)	7 : 0.790 0.009 0.181 0.019	(0.574,0.984) (0.000,0.027) (0.000,0.397) (0.000,0.063)
80	211320	(5)	7 : 0.877 0.059 0.049 0.015	(0.706,0.996) (0.000,0.173) (0.000,0.168) (0.000,0.047)
81	211322	(0)	7 : 0.150 0.018 0.808 0.025	(0.000,0.340) (0.000,0.059) (0.624,0.969) (0.000,0.082)
82	211323	(0)	7 : 0.342 0.054 0.584 0.020	(0.134,0.565) (0.000,0.159) (0.368,0.791) (0.000,0.061)
83	211332	(0)	7 : 0.805 0.013 0.169 0.013	(0.594,0.980) (0.000,0.042) (0.002,0.372) (0.000,0.041)
84	211295	(5)	7 : 0.490 0.048 0.440 0.021	(0.218,0.760) (0.000,0.161) (0.188,0.700) (0.000,0.069)

Table 26 (cont'd)

No.	Accession		Membership coefficient	Posterior probability values
85	211315	(5)	7 : 0.937 0.024 0.020 0.019	(0.828,1.000) (0.000,0.079) (0.000,0.061) (0.000,0.058)
86	211317	(11)	7 : 0.032 0.354 0.568 0.046	(0.000,0.104) (0.188,0.526) (0.386,0.740) (0.000,0.160)
87	211318	(0)	7 : 0.536 0.206 0.203 0.056	(0.206,0.880) (0.000,0.447) (0.000,0.441) (0.000,0.196)
88	211331	(23)	7 : 0.373 0.145 0.041 0.441	(0.001,0.756) (0.000,0.500) (0.000,0.136) (0.008,0.900)
89	208647	(11)	7 : 0.116 0.118 0.013 0.753	(0.000,0.333) (0.000,0.346) (0.000,0.039) (0.528,0.976)
90	219234	(0)	7 : 0.016 0.016 0.909 0.059	(0.000,0.053) (0.000,0.052) (0.771,0.999) (0.000,0.182)
91	219235	(5)	7 : 0.060 0.028 0.647 0.265	(0.000,0.204) (0.000,0.086) (0.323,0.947) (0.000,0.584)
92	208646	(0)	7 : 0.102 0.230 0.644 0.024	(0.000,0.303) (0.093,0.385) (0.440,0.826) (0.000,0.078)
93	216819	(11)	7 : 0.566 0.017 0.397 0.020	(0.340,0.787) (0.000,0.055) (0.182,0.618) (0.000,0.062)
94	216820	(0)	7 : 0.703 0.028 0.245 0.023	(0.456,0.938) (0.000,0.093) (0.025,0.477) (0.000,0.071)
95	240173	(0)	7 : 0.219 0.053 0.643 0.085	(0.000,0.486) (0.000,0.188) (0.399,0.922) (0.000,0.273)
96	207949	(17)	7 : 0.923 0.021 0.035 0.021	(0.793,0.999) (0.000,0.069) (0.000,0.117) (0.000,0.064)
97	201066	(0)	7 : 0.021 0.062 0.900 0.016	(0.000,0.069) (0.000,0.164) (0.778,0.995) (0.000,0.053)
98	240190	(29)	7 : 0.057 0.017 0.423 0.504	(0.000,0.183) (0.000,0.052) (0.228,0.620) (0.311,0.699)
99	211340	(29)	7 : 0.620 0.231 0.064 0.085	(0.303,0.913) (0.000,0.479) (0.000,0.220) (0.000,0.274)
100	211341	(35)	7 : 0.274 0.517 0.141 0.068	(0.000,0.806) (0.029,0.900) (0.000,0.407) (0.000,0.215)
101	208705	(11)	7 : 0.373 0.229 0.122 0.277	(0.000,0.733) (0.001,0.494) (0.000,0.345) (0.000,0.747)
102	211271	(0)	7 : 0.077 0.035 0.357 0.532	(0.000,0.250) (0.000,0.116) (0.176,0.543) (0.333,0.727)
103	208695	(5)	7 : 0.282 0.012 0.684 0.022	(0.000,0.591) (0.000,0.036) (0.380,0.977) (0.000,0.073)
104	208698	(0)	7 : 0.607 0.010 0.324 0.060	(0.256,0.966) (0.000,0.031) (0.000,0.675) (0.000,0.209)
105	208702	(0)	7 : 0.766 0.021 0.195 0.018	(0.545,0.973) (0.000,0.069) (0.000,0.415) (0.000,0.056)
106	208703	(5)	7 : 0.496 0.185 0.054 0.265	(0.060,0.812) (0.001,0.389) (0.000,0.185) (0.000,0.632)
107	211337	(17)	7 : 0.019 0.024 0.281 0.677	(0.000,0.061) (0.000,0.076) (0.067,0.492) (0.462,0.892)
108	211338	(11)	7 : 0.378 0.026 0.567 0.030	(0.082,0.661) (0.000,0.086) (0.296,0.837) (0.000,0.093)
109	211339	(5)	7 : 0.493 0.012 0.481 0.014	(0.279,0.711) (0.000,0.037) (0.265,0.694) (0.000,0.044)
110	211342	(0)	7 : 0.930 0.013 0.036 0.021	(0.809,1.000) (0.000,0.039) (0.000,0.121) (0.000,0.065)
111	211388	(0)	7 : 0.915 0.038 0.028 0.019	(0.788,0.998) (0.000,0.118) (0.000,0.093) (0.000,0.059)

Table 26 (cont'd)

No.	Accession		Membership coefficient	Posterior probability values
112	211337	(5)	7 : 0.383 0.086 0.482 0.050	(0.082,0.654) (0.000,0.207) (0.254,0.706) (0.000,0.175)
113	211298	(0)	7 : 0.775 0.014 0.196 0.015	(0.548,0.975) (0.000,0.044) (0.003,0.418) (0.000,0.047)
114	211299	(5)	7 : 0.787 0.171 0.021 0.020	(0.615,0.945) (0.025,0.329) (0.000,0.071) (0.000,0.066)
115	211300	(0)	7 : 0.962 0.009 0.017 0.012	(0.895,1.000) (0.000,0.028) (0.000,0.053) (0.000,0.038)
116	211305	(5)	7 : 0.727 0.011 0.248 0.015	(0.506,0.943) (0.000,0.033) (0.033,0.465) (0.000,0.047)
117	211325	(0)	7 : 0.931 0.020 0.016 0.032	(0.831,0.999) (0.000,0.066) (0.000,0.051) (0.000,0.106)
120	211301	(35)	7 : 0.245 0.030 0.058 0.667	(0.000,0.688) (0.000,0.099) (0.000,0.202) (0.235,0.985)
121	208638	(11)	7 : 0.018 0.012 0.960 0.010	(0.000,0.057) (0.000,0.037) (0.892,1.000) (0.000,0.030)
122	219233	(17)	7 : 0.074 0.025 0.883 0.018	(0.000,0.213) (0.000,0.085) (0.732,0.995) (0.000,0.057)
123	201293	(0)	7 : 0.875 0.019 0.083 0.023	(0.661,0.998) (0.000,0.060) (0.000,0.282) (0.000,0.075)
124	201294	(5)	7 : 0.012 0.945 0.016 0.026	(0.000,0.037) (0.854,1.000) (0.000,0.052) (0.000,0.087)
125	211304	(0)	7 : 0.027 0.018 0.026 0.929	(0.000,0.087) (0.000,0.058) (0.000,0.087) (0.810,0.999)
126	CHERCHER	(0)	8 : 0.039 0.366 0.580 0.015	(0.000,0.128) (0.188,0.547) (0.388,0.766) (0.000,0.046)
127	GOBERASHA	(0)	8 : 0.014 0.925 0.025 0.036	(0.000,0.045) (0.805,0.999) (0.000,0.083) (0.000,0.125)
128	NASER	(0)	8 : 0.242 0.272 0.469 0.018	(0.008,0.473) (0.099,0.459) (0.272,0.658) (0.000,0.057)
129	Mexican-142	(5)	8 : 0.418 0.237 0.311 0.035	(0.185,0.648) (0.053,0.428) (0.128,0.501) (0.000,0.112)

Table 27: Mean SSR diversity for 17 microsatellite loci in Ethiopian common bean genotypes

	N	Na	Ne	I	Ho	GD	F	P(%)	N_{pa}	t
Andean Cluster 1 (K4)	11	4.118	2.598	1.032	0.325	0.304	0.380	100.00	0.154	0.449
Andean Cluster 2 (K5)	14	4.000	2.562	0.990	0.495	0.286	0.034	94.12	0.211	0.934
Andean Control (K1)	5	3.765	3.007	1.103	0.382	0.372	0.383	94.12	0.277	0.446
Mean		**3.961**	**2.722**	**1.042**	**0.401**	**0.321**	**0.266**	**96.1**	**0.214**	**0.610**
Mesoamerican Cluster 1 (K2)	16	3.647	2.077	0.819	0.363	0.229	0.209	94.12	0.174	0.654
Mesoamerican Control (K3)	7	2.412	1.606	0.524	0.272	0.356	0.067	76.47	0.106	0.874
Mean		**3.030**	**1.842**	**0.672**	**0.318**	**0.293**	**0.138**	**85.3**	**0.140**	**0.764**
Mean		**3.588**	**2.370**	**0.894**	**0.367**		**0.223**	**91.76**	**0.185**	**0.687**

N number of genotypes, N_A number of different alleles, N_E effective number of alleles, N_{PA} number of private alleles, GD gene diversity according to Nei (1978), H_O observed heterozygosity, I Shannon's information index, F fixation index, t = (1-F)/(1 + F) out-crossing rate, P (%) percent polymorphic loci.

5.5.4 Genetic associations among accessions

Genetic associations among accessions from different populations in Ethiopia with respect to Andean and Mesoamerican control genotypes were identified using variation for fluorescent microsatellite markers (Figs. 23 and 24). Both the PCoA and Neighbor-Joining graphs (Figs. 23 and 24) indicated the clustering of the bean genotypes into either of the Andean or Mesoamerican control genotypes.

The Principal Coordinate Analysis (PCoA) was done using 5 populations identified by STRUCTURE. The overall variation explained by the PCoA was 64.47% with dimensions 1, 2 and 3 explaining 25.70, 21.47 and 18.52%, respectively. PCoA separated the bean genotypes into their corresponding centers of domestication (Andean/Mesoamerican) (Fig. 23). Exceptions were Andean Cluster 4 genotypes in the second quadrant (four in number) and one genotype of the cluster containing the Andean Control (quadrant III), which showed mixed cluster membership with the Mesoamerican Cluster.

The mixed membership of Andean Cluster 1 (K4) concurred with the STRUCTURE and neighbor-joining analysis results. However, the mixed clustering of accessions from the cluster containing the Andean Control (K1) with the Mesoamerican groups was only exhibited in the PCoA and neighbor-joining tree. Such mixed membership was previously reported with the group of wild, presumably ancestral beans (Kwak and Gepts, 2009).

Likewise, the neighbor-joining dendrogram classified bean genotypes into the Andean/Mesoamerican gene pools (Fig. 24). The same populations identified by STRUCTURE at K5 were used for this analysis. The NJ tree results were in agreement with the results of STRUCTURE, in that they delineated genotypes between the two bean gene pools and enabled further differentiation of each gene pool into ecogeographic races.

STRUCTURE analysis yielded the differentiation of the common bean accessions into the Andean and Mesoamerican gene pools. Similar results were reported by several other authors (Gepts et al., 1986; Singh et al., 1991a, b, c; Becerra and Gepts, 1994; Islam et al., 2002; Blair et al., 2006, 2009; Kwak and Gepts, 2009). Such conservation of the primary centers of diversity in regions outside the centers of origin has been previously reported (Martin and Adams, 1987; Rodiňo et al., 2003; Blair et al., 2006; Zhang et al., 2008; Asfaw et al., 2009). The population structure depicted in the present study agrees with the already noted hierarchical scheme of classification in the common bean gene pools (Asfaw et al., 2009; Kwak and Gepts, 2009; Blair et al., 2010b; Blair et al., 2011).

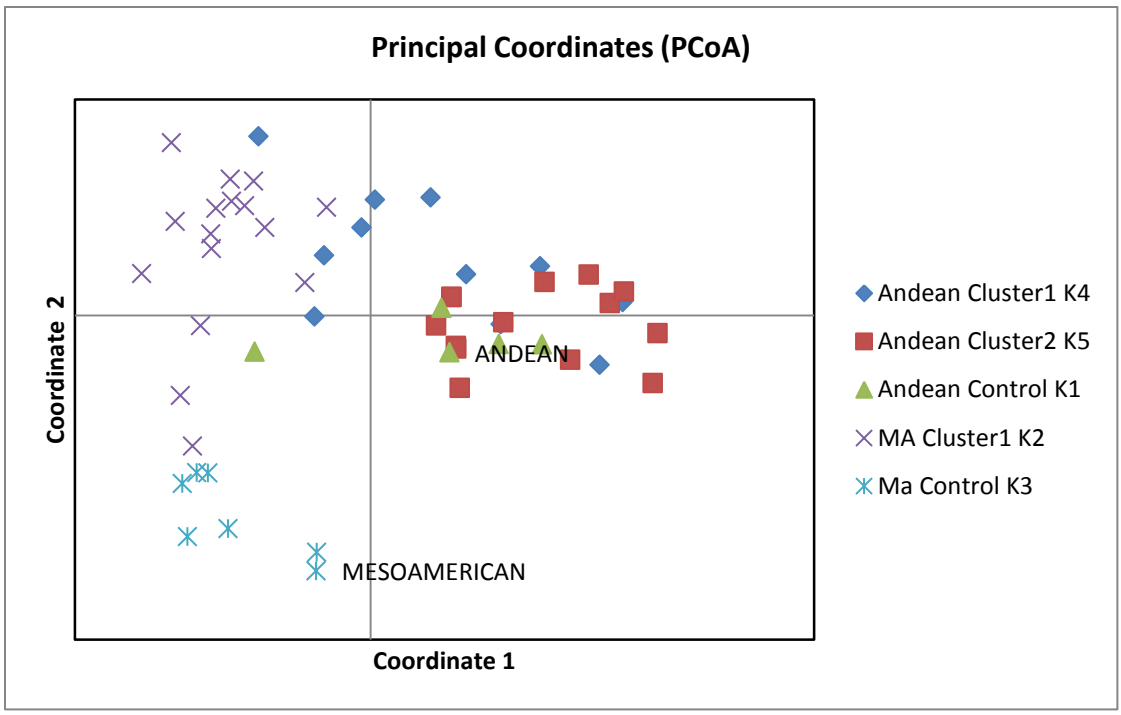


Figure 23: PCoA graph for the 53 accessions from different growing populations in Ethiopia

Furthermore, five groups from both the Andean and Mesoamerican gene pools were identified in the present study, Singh et al. (1991a); and Kwak and Gepts (2009) also reported the presence of five subgroups, belonging to the Mesoamerican and Andean gene pools. Though the groups were identified, the present racial structure of each cluster in a gene pool could not be clearly discerned, which may be corroborated by the argument of Kwak and Gepts (2009) that racial clustering patterns would not be well differentiated at the molecular level, but rather with the use of plant and seed morphological traits. To this end, these results from molecular marker data were integrated with morphological, and ecogeographic data (which shall be discussed in chapter 5.6). The occurrence of mixed membership of the Andean cluster 1 (K4) in the STRUCTURE analysis; and Andean Clusters 4 (K4) and the cluster containing the Andean control (K1) with the neighbor-joining and PCoA analyses concurs with the results of Kwak and Gepts (2009).

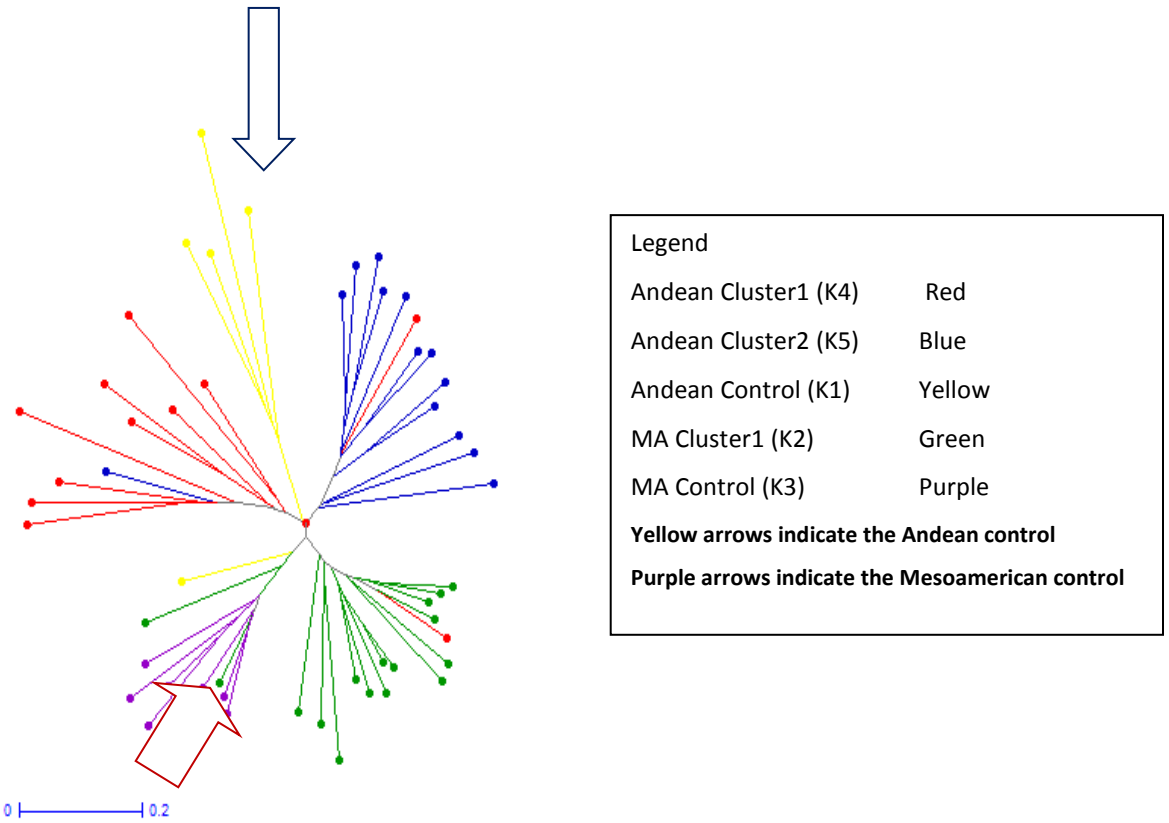


Figure 24: Neighbor-joining dendrogram depicting genetic relationship between common bean accessions from different bean growing populations in Ethiopia with respect to Andean and Mesoamerican control genotypes.

The PCoA showed all the Mesoamerican cluster groups and one of the Andean cluster groups clustered in closer proximity with each other. The same phenomenon was observed with the neighbor-joining tree. Such a pattern of clustering together may indicate the Andean cluster K5 may be more homogenous than the remaining two Andean clusters (Andean cluster 1, K4; and Andean control, K1). The presence of higher level of gene flow within each gene pool than that found between genepools observed in our study agrees with the result of Asfaw et al. (2009). This may be explained, in part, due to the lack of flowering synchronization, which could abate inter-gene pool gene flow. A larger proportion of the accessions (i.e. 57.6%) were introgressions, which contradicts with the reports of Asfaw et al. (2009) about the lower level of introgression with Ethiopian and Kenyan bean landraces/cultivars. This, in turn, negates the assumption of the aforementioned authors implying the genetic divergence in Ethiopian bean germplasm could be mainly due to the original differences in introduced germplasm from the primary centers of origin. Rather, the presence of a higher number of introgressions may be partially explained by the fact that the accessions were gene bank collections from farmers‘ fields often characterized by a higher level of mixtures. The common practice of subsistence farmers in the country who cultivate for consumption and save the segregant genotypes, resulting from any natural hybridization, as planting materials for subsequent generations could result in such type of introgressions (Blair et al., 2010b).

A final worth-noting remark may be the fact that inter-gene pool introgressions are often endowed with useful combination of traits, including enhanced adaptation to environmental stresses; higher resistance to diseases and pests; and higher nutritional quality, the introgressions identified in this study are of considerable importance in future bean breeding and conservation endeavors in Ethiopia. These merits of hybrids was evidenced in Islam et al. (2004); and Blair et al. (2010b), who reported that introgressions had higher mineral compositions than their respective non-hybrid parents. Consequently, it may be essential to tap into the useful genetic diversity found in such types of inter-gene pool introgressions, to be harnessed in further common bean breeding; improvement; and genetic conservation programs Ethiopia.

5.6 Integration of phenotypic and molecular markers in Ethiopian collection of common bean (*Phaseolus vulgaris* L.) landraces

5.6.1 Principal component analysis of variation for morphological traits

Figure 25 shows the first two principal components of diversity for morphological variables in common bean (accessions were labeled according to the groups or populations identified by Structure software simulations with preset K=2). The first principal component (PC1; 20.4% of total variation) separated the Mesoamerican and Andean groups. The eigen values formed the basis for identifying component axes in the PCA analysis (Panthee et al., 2006; Okii et al., 2014a), with scores, cut-off level arbitrarily set above 0.2 to show traits (Okii et al., 2014a), which explained most of the variation found in the studied accessions. In line with this, considering only PCA eigen values in PC score 1, most genotypes to the left of the axis were determinate and indeterminate climbing growth habits and various seed colors, whereas non-climbing accessions with short internodes and different seed colors were located to the right of this axis. The accessions with positive scores for the second principal component (PC2: 13.6% of total variation) were late flowering with various flower/seed/flower wing colors (in freshly-opened flowers), and larger seed weight. The separation of accessions of common bean in to the Mesoamerican and Andean gene pools in one of the principal component axes has also been reported in previous studies (Sing et al., 1991b; Asfaw et al., 2009; Burle et al., 2011). Regarding the placement of accessions with respect to the Andean and Mesoamerican control genotypes, overall the PCA depicted the separation of the landrace accessions into either of the gene pools of origin. Nonetheless, many of the accessions occupied intermediate positions between the two gene pools and the control genotypes for the two gene pools, probably due to introgression and/or shared morphological markers, such as seed color and growth habit (Fig. 25). Asfaw et al. (2009) reported a similar finding in which intermediate positioning of many accessions happened in common bean accessions from Ethiopia and Kenya.

In view of the overall morphological diversity present, both in the Mesoamerican and Andean gene pool groups, the Andean group had equal to nearly higher diversity than that of their Mesoamerican counterparts (Fig. 25). This is regardless of the smaller size of

the Andean group when compared to the Mesoamerican category (N_A=47 Vs N_{MA}=78). A total of 72 accessions (member coefficients less than 0.80 for each group) had been labeled as _hybrids' between the two groups by comparing the posterior probabilities of membership calculated on the basis of the molecular marker information (refer to section 5.5).

5.6.2 Agro-morphological traits distinguishing the Andean and Mesoamerican gene pools

A stepwise discriminant analysis was carried out on the following 14 variables for a model that discriminated the Andean and Mesoamerican groups in the order of their entrance into the model: seed coat color, plant growth habit, days to flowering, color of flower (standard), seed shape, 100-seed weight, seed color, seed brilliance, mean seed diameter, number of pods per plant, plant height, number of branches per plant, pod color, and color of flower wings (in freshly-opened flowers). Canonical correlations corresponding to the first two axes of the canonical discriminant analysis to differentiate the two major gene pools were significantly different from zero ($r= 0.98$ and $r=0.68$, $p < 0.001$) (Fig. 26).

The first canonical variable accounted for 88.9% of the variation. Fig. 26 shows the results of the canonical correlation analysis done with the aforementioned variables. Clustering along this canonical axis followed the pattern that while some of the accessions clustered with either of the Mesoamerican or Andean control genotypes, others clustered farther away from either. The latter may correspond to the introgressions, which were also identified in the PCA graph (Fig. 25). On the other hand, the second canonical correlation axis (Can2, which accounted for 3.8% of the variation) clearly separated the accessions into either of the two gene pools (Fig. 26).

The variables with larger effects on the first canonical variable were in descending order: days to flowering, seed shape, seed brilliance, 100 seed weight, color of seed coat, and plant height. On the other hand, the following variables had larger effects on the second

canonical variable: seed shape; seed brilliance; plant height; color of seed coat; 100 seed weight; and days to flowering.

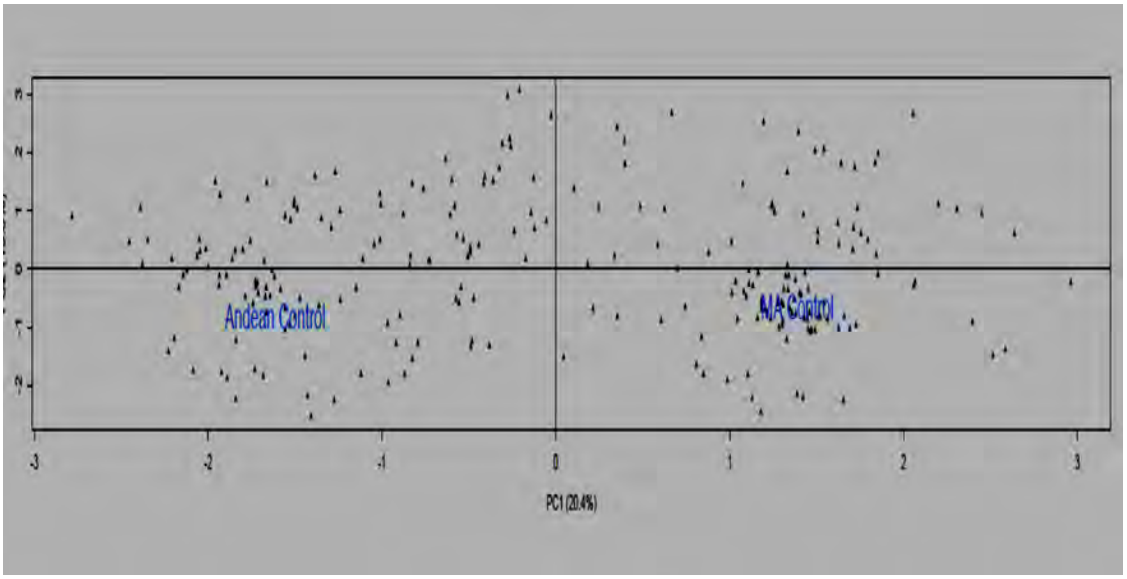


Figure 25: First two principal components of diversity (PrinComp1 and PrinComp2) for 12 morphological variables in Ethiopian common bean (*Phaseolus vulgaris* L.) accessions with respect to the Mesoamerican and Andean control gene pool genotypes.

5.6.3 Five groups based on molecular, morphological, and ecogeographic information

Canonical discriminant analysis, performed with agro-morphological data on accessions identified in K=5 of the structure analysis in the molecular analysis, identified combinations of agro-morphological traits that were able to discriminate among these groups of accessions. The stepwise discriminant and canonical correlation analyses were carried out with the aim of discriminating the three Andean and two Mesoamerican subgroups identified with Structure software simulations with preset K=5. A discriminant model identified the following variables, in descending order of impact: pod color, days to flowering, seed brilliance, and color of flower wings (in freshly-opened flowers). Overall, the level of separation of the Andean/Mesoamerican subgroups based on the morphological descriptors was fair, even though a considerable overlap was present (Fig. 27). To this end, Burle et al. (2011) noted that the blurred limits with regard to the limits of individual races may have happened in studies like this where race memberships for each group are an approximation due to the omission of control genotypes representative of each race and possible hybridization among races.

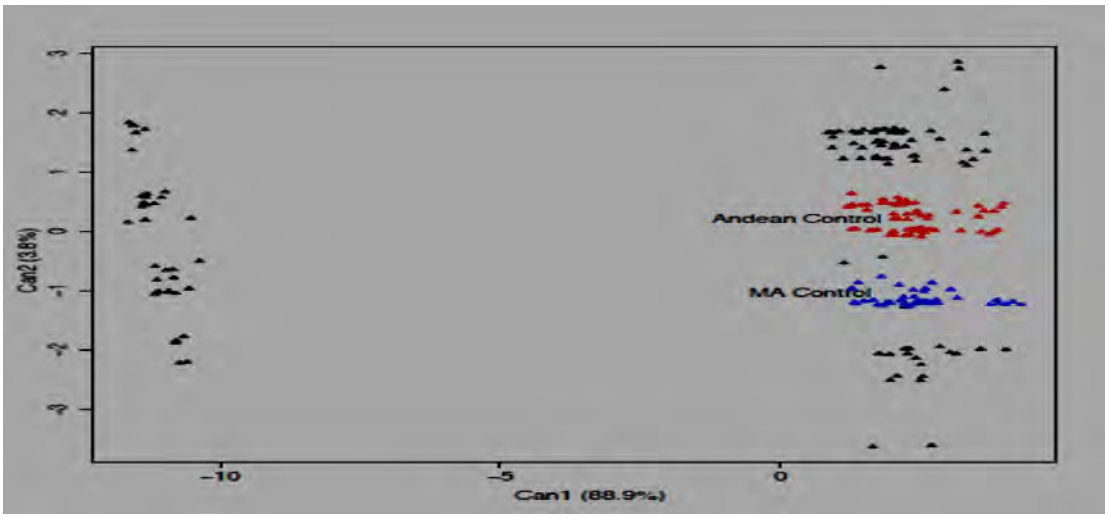


Figure 26: Canonical variables from the canonical discriminant analysis (Can1 and Can2) for morphological traits discriminating Andean (yellow) and Mesoamerican (brown) gene pools of common bean (*Phaseolus vulgaris* L.) in the Ethiopian germplasm collection.
Blue symbols: accession in the Mesoamerican control cluster; and Red symbols: accessions in the Andean control clutser. Gene pools were identified in Chapter 5.5 on the basis of molecular analysis (17 markers; Structure preset to K = 2)

Considering the canonical discriminant analyses of the agro-morphological data suggested that five groups would be the highest level of subdivision in the studied accessions, the main agro-morphological characteristics, and possible races observed in each of the five populations identified by Structure on the basis of molecular analyses were described in section 5.5 are presented in Table 28.

The following morphological characteristics were identified in the two Mesoamerican and three Andean groups identified when Structure was preset to K=5, Group 1 (Fig 27: red symbols) was an t Andean cluster, based on the presenceof the Andean control (K1). This group consisted of medium to large-seeded accessions having various different shapes (round, oval, and kidney) and colors (white, cream and red) (Table 28). Accessions had Type-II (indeterminate bush) and Type-I (determinate bush) plant types. Flowers were white, purple, and white with carmine stripes, whereas standards were white and dark lilac (Table 28, and Fig. 27).

Group 2 was a Mesoamerican group (Fig 27: orange symbols) comprising accessions, characterized predominantly by white seeds, and some red or cream or black seeds. Majority of them were Type-II (indeterminate bush) plant types, though Type-I (determinate bush) and Type-IV (indeterminate climbing) also occurred at small frequencies. Seeds in this group were of small sizes (100-seed weight=25-40 g and >40 g, respectively). Shape-wise, seeds assumed various forms (round, oval, cuboid, kidney, and truncated), standards were white, dark lilac, and white with lilac edges. Finally, flower wing colors (in freshly-opened flowers) were white, purple, and white with carmine stripes (Table 28).

Another MA group (K3) (Fig 27: black symbols) was comprised seven accessions with Type-II (indeterminate bush) (dominantly), Type-I (determinate bush), and Type-IV (determinate climbing) growth habits. Seeds were red, white, and cream with smaller weights (<25 g). Oval (predominantly) and cuboid were the variants of seed shapes in the group. Mean while, standards were with white, white with lilac edges, and dark lilac in color, whereas flower wings were dominantly white and purple (Table 28).

Group 4 (K4) (Fig 27: blue symbols) was an Andean group with accessions dominantly from Type-II (indeterminate bush) growth habit, though some Type-I and IV plant types did also occur. Seeds had medium size (mean 100-seed weight=36.23 g), commonly with cuboid, oval, and round shapes. Color-wise, the accessions had white, red, and cream seeds, whilst having white and dark lilac flower standards (Table 28).

The final group of accessions, K5 (Fig 27: green symbols), was an Andean group having medium sized seeds (25-40 g) with white, red, or cream colors. With respect to plant types, Type-II (indeterminate bush) accessions predominated, with the rest had Type-I (determinate bush) and Type-IV (indeterminate climbing) growth habits. Mean while, flower wings had white, white with carmine stripes, and purple colors, whereas, white (dominantly) and purple were the prevalent standard colors (Table 28).

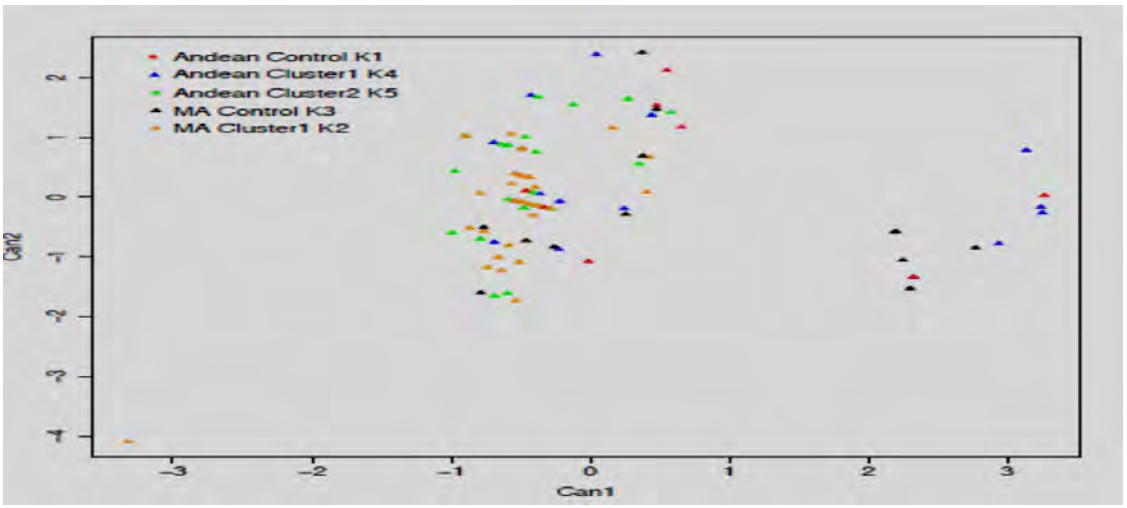


Figure 27: First two canonical variables for the canonical discriminant analysis (Can1 and Can2) for the five Mesoamerican/Andean groups identified on the basis of molecular data by presetting Structure to K = 5 and without potential hybrids

Group numbers and symbols: Group 1 (red) Groups 2 (Orange), 3 (black), 4 (Blue), and 5 (green), respectively, according to Structure for K = 5.

The identities of the different groups identified by Structure were further clarified with data on some racial and agro-morphological traits, which are characteristic of ecogeographic races. In view of this, the following interpretations as to the racial identity of each accession group were made.

Group 1 was a more heterogeneous group. Its core appeared to consist of landraces with Type-II, I, and IV growth habits, and medium sized seeds (25-40 g). Predominantly, accessions in the group had 80% round seeds, with the rest having oval and kidney-shaped seeds. Based on findings vis-à-vis the classification of ecogeographic races used in previous studies (Singh et al., 1991a, b, Burle et al., 2011), it was deduced that accessions in this cluster group with Type II and I growth habits and kidney-shaped seeds may belong to the race _Nueva Granada'. On the other hand, the Type-IV accessions of this group with round/oval seeds may probably belong to the Andean race, _Peru'. Similarly, existence of heterogeneous composition of accession groups from the Andean gene pool races, _Nueva Granada' and _Chile' has been reported in a previous study undertaken common bean (Singh et al., 1991a).

Group 2, a Mesoamerican group, had seeds of various colors, predominantly with Type-II growth habit (60%), with the remaining accessions being Type I and IV types. As per the shapes of seeds in accessions of this group, they had variability with round, oval, kidney, cuboid, and markedly-truncated seeds more prevalent. Moreover, accessions had small seeds (<25 g) (mean 100-seed weight=19.96 g) (Table 28). Consequently, the group, owing to the aforementioned features it had, may have belonged to race _Mesoamerica'. Singh et al. (1991a) and Burle et al. (2011) noted that race _Mesoamerica' is characterized by accessions from the growth habits: Type I, II, III, and IV having cylindrical, kidney, oval, cuboid, and truncated seeds. Furthermore, these authors reported that race _Mesoamerica' consists of small-seeded (<25 g) genotypes. These descriptions matched with the present findings. Nonetheless, the presence of round seeds in this race was not in agreement with the findings of the aforementioned authors.

Group 3 consisted of accessions with various color variants (red, white, and cream seeds) having dominantly Type-II, and some Type-I and IV plant types. Seeds were oval and cuboid, and of a small size (weight) (mean 100-seed weight=21.36 g) (Table 28). Hence, the group may be composed of accessions from the race _Mesoamerica'. Such identification of groups of collections from the Mesoamerica gene pool was reported previously (Singh et al., 1991a, Burle et al., 2011).

Accessions dominantly with Type-II, and, to some extent, Type-I and IV growth habits were in group 4 (K4 of the molecular structure analyses). The member accessions had cuboid, oval, and round seeds, while, predominantly, white/purple flowers (Table 28). Moreover, they had medium to large seeds. Owing to these common features accessions in this group had, it may be postulated that this Andean group, as with the preceding one (i.e., K1) was heterogeneous in composition. This means accessions of the group with Types-I and II growth habits and oval seeds may probably belong to the Andean race, _Nueva Granada' (Singh et al., 1991a, b). On the other hand, the rest of the members with strong climbing tendencies (of a Type-IV growth habit) having cuboid/round seeds may belong to another race in the Andean gene pool, _Peru'. Consequently, as race _Peru' has never been reported out of the Americas (and hence this is the first report in this regard),

the photoperiod sensitivity of the accessions deemed to be this ecogeographic race need to looked into (in detail), with respect to their respective photoperiod sensitivity (as this race is widely regarded as being highly photoperiod sensitive).

Finally, group 5, which had accessions with Types: II (dominantly), I, and IV growth habits may similarly constitute accessions from different races in the Andean gene pool. The first subgroup of accessions with Types I and II growth habits may belong to race _Nueva Granada', whereas, the second category, composed of accessions with Type-IV plant types and round/oval seeds may belong to race, _Peru' (Table 28). A similar group of accessions with memberships in to either of the Andean races: _Nueva Granada' and _Chile' was reported by Singh et al. (1991a).

Consequently, the aforementioned results of the present study can be summarized by the fact that the common bean landrace accessions in Ethiopia had a broad base in terms of diversity within the Andean gene pool (which contained two of the eco-geographic races in the Andean gene pool: _Nueva Granada' and _Peru'). On the other hand, the Mesoamerican groups had a relatively narrow genetic base, which only belonged to the race _Mesoamerica'. Asfaw et al. (2009) made a similar conclusive remark, which agrees with this study, with respect to the higher level of differentiation they observed in the Andean genotypes than Mesoamerican accessions. Nonetheless, their results contradicted with this one in that most of the Ethiopian common bean accessions were from the Mesoamerican gene pool. This is opposite to the present findings in that non-hybrid Mesoamerican accessions comprised only 18.4% of the total accession population studied (refer to chapter 5.5). Furthermore, the present study discovered the presence of only one of the three races of the Mesoamerican gene pool, i.e., race _Mesoamerica'. The differences in the results may be put down to the possible difference in the representation of major bean growing areas in Ethiopia, and the fact that integration of agro-morphological data with molecular marker information has not been employed by Asfaw et al. (2009). Furthermore, as the authors had not differentiated the groups identified at the optimum number of cluster (K=6 in their case), and based their conclusions on the classification they observed at STRUCTURE preset of K=2 (i.e. the dominance of

Mesoamerican germplasm accessions over the Andean groups). From this angle, this result of the authors agrees with the present findings, as 52.8% of the accessions identified at K=2 in the population structure based on molecular markers (refer to section 5.5) over 47.2% assigned for Andean genotypes.

Table 28: States, ranges, and means for some morphological descriptors of common bean (*Phaseolus vulgaris* L.) in the groups of accessions (subpopulations) identified at STRUCTURE preset, K=5

Sub-populations	**Seed Color**	**Growth Habit**	**Days to Flowering (50%)**	**Standard (flower) color**	**Seed Shape**	**Seed Weight**	**Seed brilliance**	**Color in freshly-opened flowers**
K1 (A) N=5	White Cream Red	II I	51.5-56 Days 54.5 days	White Dark Lilac White with red stripes	Round (80%) Oval Kidney	29.5-49.5g 37.8g	Shiny Medium	White Purple White with carmine Stripes
K2 (MA) N=16	White (53.33%) Red Cream Brown Dull/Black	II (60%) I IV	52.5-58 Days 55	White (73.33%) Dark Lilac White with Lilac Edges	Round (46.67%) Oval Cuboid Kidney-shaped Markedly-truncate	15.25-24.25g 19.96g	Shiny	White (66.67%) White with Carmine Stripes Purple
K3 (M) N=7	Red White Cream	II (71.5%) I V	51-56 Days 54.25 Days	White White with Lilac Edges Dark Lilac	Oval (85.7%) Cuboid	18.5-27 g 21.36 g	Shiny Others	White White with Carmine Stripes Purple
K4 (A) N=11	White (54.5%) Red Cream	II (63.6%) I IV V	52-58 Days 55.19	White (66.67%) Dark Lilac	Cuboid (36.36%) Oval (36.36%) Round	25-44.5g 36.23	Shiny (63.64%) Medium Others	White (90.91%) Purple
K5 (A) N=14	White (64.29%) Red Cream	II (64.28%) I IV	51-60.5 Days 54.86	White (84.62%) Dark Lilac	Cuboid Round Oval	24.5-62.5g 34.75g	Shiny	White Purple White with carmine Stripes

A=Andean; M=Mesoamerican

Consequently, the present study rendered unique implications in terms of determining the genetic diversity and structure of common bean landrace accessions in Ethiopia. This, in turn, can serve as baseline information source in future common bean breeding/conservation endeavors in Ethiopia.

6 Conclusions and Recommendations

The common bean plays an important role in the dietary and socio-economic aspects of the lives of urban and rural populations in Sub-Saharan Africa. Accurate assessment of the levels and patterns of genetic diversity has tremendous importance in the analysis of genetic variability in cultivars; identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection; and introgressing desirable genes from diverse germplasm into the available genetic base (Mohammadi and Prasana, 2003).

Though the East African highlands, including Ethiopia, are considered as a secondary center of diversity for the common bean, little has so far been done towards determining the genetic diversity and population structure of Ethiopian common bean landrace accessions. To this end, previous studies only concentrated on a smaller portion of the germplasm population with microsatellite markers.

In line with the aforementioned, the present study revealed that the common bean landrace accessions had significant variability with respect to qualitative and quantitative traits. These variables had wider ranges, both qualitatively and quantitatively. Moreover, the variations observed (both qualitatively and quantitatively) were fairly distributed across the regions/populations the accessions were collected from. These indicated potential genetic diversity prevalent in common bean landrace accessions, which can be harnessed future breeding and conservation endeavors in Ethiopia.

The present study also revealed that 100-seed weight and mean seed diameter are useful traits vis-à-vis being used jointly with seed yield for the indirect selection in breeding programs of common bean in Ethiopia. Moreover, comparison of eigen values from Principal Component Analysis (PCA) indicated that seed color; seed shape; 100-seed weight; seed brilliance; color of flower wings (in freshly-opened flowers); color of (flower) standard; and days to flowering were the most important traits in explaining the genetic variability explained in the first successive principal component axes. Hence, these traits, in conjunction with those identified through correlation and path coefficient

analyses, should be used to more accurately select for high yielding genotypes with maximized genetic variability in future common bean improvement programs in Ethiopia. Several other authors also reported supporting results, as to the importance of these traits in selecting for genetic variability in the common bean.

On the other hand, the presence of inter-gene pool introgressions was identified in both phenotypic and molecular-marker-based genetic diversity and population structure analyses. This presence of inter-gene pool introgressions in the present study concurs with results of various previous studies. In line with this, as inter-gene pool introgressions are often endowed with useful combination of traits, including enhanced adaptation to biotic stresses; higher disease and insect pest resistance; and enhanced nutritional quality, the ones identified in this study may be of paramount importance in prospective common bean breeding and genetic conservation schemes in Ethiopia. On another related subject, the proportion of hybrid/non-hybrid accessions, estimated with microsatellite markers, showed that the latter were more prevalent in the studied accessions. In view of the fact that, 92.8% (116 out of 125) of the accessions were gene bank collections, it may warrant revisiting of approaches and methods used in germplasm collection by the responsible institution and other stakeholders in order to ensure sustainable conservation of pure-line common bean accessions. Moreover, gene flow was also observed within each gene pool and within/between regions/collection sites. This was evident in cluster analyses (both at a molecular and morphological levels); PCA (at the morphological level); and PCoA (at the molecular level), where accessions from different regions/collection sites clustered together.

Furthermore, values, comparable to those recorded in related previous studies were recorded for allelic parameters like, number of alleles/effective alleles; Shanon‘s diversity index; average heterozygosity; genetic diversity; and Polymorphic Information Content (PIC), indicating the availability of adequate genetic diversity in the landrace accessions that can be exploited in the future. These variations in the allelic parameters were also pronounced across regions/populations/collection sites. Specifically, the geographic populations, ‗Amhara‘ and SNNP, had the highest number of effective

alleles; Shanon‘s diversity index; and expected/unbiased expected heterozygosity measures. This indicated that these regions possess higher importance towards through introgressing desirable genes into other common bean genotypes in Ethiopia. Nonetheless, additional samples of accessions from different common bean growing areas in Ethiopia should be examined, in order to further ascertain the present findings.

On the other hand, all the cluster analyses done: PCoA; PCA; and Bayesian structure analysis, unequivocally confirmed the presence of germplasm both from the Mesoamerican and Andean gene pools, which were evident through the separation of accessions along with the respective control genotypes in the primary gene pools (Mesoamerican and Andean). Such presence of genotypes from both gene pools, which is typical of primary centers of diversity, was also observed before in common bean accessions from East, Central, and Southern Africa. And, apparently, it is the hallmark of bean diversity of other secondary centers, outside the Americas, such as Southwest Europe (Rodiño et al., 2006) China (Zang et al., 2008); and East and Central Africa (Asfaw et al., 2009; Blair et al., 2010b).

Further grouping of the genotypes into five groups (of varying constitution) was also evident in all the cluster analyses done using both phenotypic and microsatellite markers. Similarly, structure analyses, using Bayesian models also identified K=5, as the optimum cluster number in the collection of common bean accessions studied. The similarity in the aforementioned results confirmed the presence of the five groups identified at both phenotypic and molecular levels. Structure-wise, accessions from the Andean gene pool had higher proportion of non-hybrid accessions, and higher allelic values than their Mesoamerican counterparts, though they were smaller in sample size than the same. These identified five groups were supposed to be racial groups, belonging to ecogeographic race(s) in either of the Mesoamerican or Andean gene pools. Another related occurrence was the mixed membership of Andean and Mesoamerican gene pool accessions in some of the groups (clusters) identified, which was evident both at phenotypic and molecular level. This unexplained-for happenstance was previously reported (Kwak and Gepts, 2009). To this end, further studies with the incorporation of

larger sample size, and relevant gene pool/racial control genotypes should be conducted to understand the possible reason behind the occurrence.

In view of ascertaining the same five groups identified in the molecular analysis were also evident at the morphological level, agro-morphological data were integrated with microsatellite marker information. In relation to this, stepwise discriminant analysis done using agro-morphological traits revealed that pod color; days to flowering; seed shape; 100-seed weight; seed coat color; and plant height had larger effects in explaining the separation of accessions into the Mesoamerican and Andean gene pools, identified in the 1st and 2nd canonical axes, respectively. Most of these variables, distinguishing accessions from the Mesoamerican and Andean gene pools, were also reported previously in related studies (Singh et al., 1991a,b; Burle et al., 2011), confirming the validity of the present findings.

On the other hand, results of PCA, stepwise discriminant analysis, canonical discriminant analysis, and various recorded values of important agro-morphological descriptors were jointly used to identify the groups identified in the molecular analysis at a STRUCTURE preset K=5. As a result, the three Andean groups (section 5.5) belonged to the Andean ecogeographic races, ‗Nueva Granada' and ‗Peru', whilst the remaining two Mesoamerican groups were predominated by accessions from the Mesoamerican race, ‗Mesoamerica'. Consequently, it was concluded that Andean groups had higher genetic diversity than their respective Mesoamerican counterparts in Ethiopian common bean germplasm. Nonetheless, future studies need to be done to further fine-tune the blurs/mix-ups, possibly brought about by gene flow between gene pools and ecogeographic races, and the omission of control genotypes for the respective Andean/Mesoamerican eco-geographic races.

Generally, the following recommendations can be given from the results of this study. Firstly, more extensive collection missions need to be carried out (in order to better represent the genetic diversity present in Ethiopian common bean germplasm). Moreover, genetic diversity and population structure should be studied using other powerful

molecular markers, like SNPs. With the same end objective, the population structure present in the present accession samples should be re-examined with the inclusion of additional accessions from different parts of the country by undertaking new collection missions and all relevant control genotypes for both the gene pools and respective eco-geographic races in each.

In conclusion, the present study generated unique results in terms of determining the phenotypic and molecular genetic diversity; population structure into gene pools; and the identity of sub-groups up to the racial level. It is considered to lay the ground work for future breeding and genetic resource conservation programs, vis-à-vis common bean germplasm in Ethiopia. Furthermore, as the occurrence of the Andean race, ‗Peru', is the first report out of the Americas, further evaluation of the photoperiod sensitivity of the so-labeled accessions to ascertain this finding, as this group is highly photoperiod-sensitive and is adapted to moderately wet and cool temperatures often requiting more than 250 days to maturity.

7 References

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8 Appendices

Appendix I: Passport data and other pertinent biological and geographical details of the common bean accessions used in the study

No.	Accession	Genus name	Species name	Region /State/Province	Zone	Woreda/District	Latitude	Longitude	Altitude	Locality
1	211269	*Phaseolus*	*vulgaris*	Amhara	West Gojam	Jabi Tehnan	10-41-00-N	37-16-00-E		Finote Selam town
2	241807	*Phaseolus*	*vulgaris*	Amhara	East Gojam	Bibugn	11-14-00-N	37-38-00-E	1860	44km East of Adet, the way to Mota town along Zema river
3	211266	*Phaseolus*	*vulgaris*	Amhara	West Gojam	Bure Wemberma				Bure Town
4	211267	*Phaseolus*	*vulgaris*	Amhara	West Gojam	Jabi Tehnan	10-41-00-N	37-16-00-E		Finote Selam town
5	241814	*Phaseolus*	*vulgaris*	Amhara	West Gojam	Adet	11-15-00-N	37-36-00-E	1890	40km East of Adet, the way to Mota town along Zema river
6	211389	*Phaseolus*	*vulgaris*	Amhara	North Shewa	Ankober	09-35-00-N	39-44-00-E		Ankober
7	211551	*Phaseolus*	*vulgaris*	Amhara	North Shewa	Ankober	09-35-00-N	39-44-00-E		Ankober
8	215719	Phaseolus	sp	Amhara	Oromiya	Bati	11-11-00-N	40-10-00-E		Bati area
9	211382	*Phaseolus*	*vulgaris*	Amhara	North Shewa	Ankober	09-35-00-N	39-44-00-E		Ankober Market
10	211386	*Phaseolus*	*vulgaris*	Amhara	North Shewa	Ankober	09-35-00-N	39-44-00-E		Ankober
11	211387	*Phaseolus*	*vulgaris*	Amhara	North Shewa	Ankober	09-35-00-N	39-44-00-E		Ankober
12	207934	Phaseolus	sp	Benishangul-Gumuz	Asosa	Asosa	10-04-00-N	34-38-00-E		Gari Kakei about 25Km away from Asosa on the way to Dabus

Appendix I:(cont'd)

No.	Accession	Genus name	Species name	Region /State/Province	Zone	Woreda /District	Latitude	Longitude	Altitude	Locality
13	207938	*Phaseolus*	*sp*	Benishangul-Gumuz	Asosa	Asosa	10-15-00-N	34-40-00-E	1400	Komesha 39Km away from Asosa on the way to Gizen
14	207933	*Phaseolus*	*sp*	Benishangul-Gumuz	Asosa	Asosa	10-04-00-N	34-38-00-E	1450	Gari Kakei about 25Km away from Asosa on the way to Dabus
15	211349	*Phaseolus*	*vulgaris*	Benishangul-Gumuz	MetekeL	Dangur				Mentaweha
16	211345	*Phaseolus*	*vulgaris*	Benishangul-Gumuz	MetekeL	Dangur				2km, W. of Mentaweha Village
17	211347	*Phaseolus*	*vulgaris*	Benishangul-Gumuz	MetekeL	Dangur				Mentaweha
18	240512	*Phaseolus*	*vulgaris*	Benishangul-Gumuz	MetekeL	Debate			1450	Dibate zuria
19	211351	*Phaseolus*	*vulgaris*	Benishangul-Gumuz	MetekeL	Dangur				Mentaweha
20	211361	*Phaseolus*	*vulgaris*	Benishangul-Gumuz	MetekeL	Dangur			1300	Manbuk
21	211362	*Phaseolus*	*vulgaris*	Benishangul-Gumuz	MetekeL	Dangur			1300	Manbuk
22	211348	*Phaseolus*	*vulgaris*	Benishangul-Gumuz	MetekeL	Dangur				Mentaweha

Appendix I:(cont'd)

No.	Accession	Genus name	Species name	Region /State/Province	Zone	Woreda /District	Latitude	Longitude	Altitude	Locality
23	211356	*Phaseolus*	*vulgaris*	Benishangul-Gumuz	MetekeL	Dibate			1250	10km, Dibati to Wenbera
24	211344	*Phaseolus*	*vulgaris*	Benishangul-Gumuz	MetekeL	Dangur			1660	9km, W. of Mentaweha
25	211350	*Phaseolus*	*vulgaris*	Benishangul & Gumuz	MetekeL	Dangur				Mentaweha
26	211352	*Phaseolus*	*vulgaris*	Benishangul & Gumuz	MetekeL	Dangur				Mentaweha
27	240522	*Phaseolus*	*vulgaris*	Benishangul & Gumuz	MetekeL	Mandura			1210	Edida
28	Mwitemania	*Phaseolus*	*vulgaris*	Kenyan						
29	E7	*Phaseolus*	*vulgaris*	Kenyan						
30	WANJIRU	*Phaseolus*	*vulgaris*	Kenyan						
31	241730	*Phaseolus*	*vulgaris*				06-57-08-N	38-17-08-E	1900	Shondololiyo,12 Km South to Erba on the way to Bilate
32	212861	*Phaseolus*	*vulgaris*	Oromiya	Bale	Nensebo				Werka sorenatoteabout 5km north of werka
33	230779	*Phaseolus*	*vulgaris*	Oromiya	Borena	Moyale	05-02-00-N	39-27-00-E	1220	Tuka-Ca 30Km, from Moyale to Mega

Appendix I:(cont'd)

No.	Accession	Genus name	Species name	Region /State/Province	Zone	Woreda /District	Latitude	Longitude	Altitude	Locality
34	235697	*Phaseolus*	*spp*	Oromiya	Borena	Yabelo	04-56-00-N	37-50-00-E	1480	Elewaye 80km. From Konso to Yabello
35	211379	*Phaseolus*	*vulgaris*	Oromiya	Bale	Ginir	07-08-00-N	40-42-00-E	1630	6km, Ginir to Goro
36	237078	*Phaseolus*	*spp*	Oromiya	Arssi	Gedeb	07-19-00-N	39-16-00-E	2325	Welkite 02, 15Km West of Gedeb
37	211378	*Phaseolus*	*vulgaris*	Oromiya	Bale	Ginir	07-08-00-N	40-42-00-E	1630	6km, Ginir to Goro
38	211377	*Phaseolus*	*vulgaris*	Oromiya	Bale	Agarfa				
39	216819	*Phaseolus*	*vulgaris*	Oromiya	East Harerghe	Meta	09-26-00-N	41-42-00-E	2400	Kulubi 23km. On kersa Kulibi Rd near Kulubi
40	211319	*Phaseolus*	*vulgaris*	Oromiya	East Harerghe	Goro Gutu				7km From Karamile to Harer
41	211320	*Phaseolus*	*vulgaris*	Oromiya	East Harerghe	Deder			2150	13km From Karanile to Harer
42	211322	*Phaseolus*	*vulgaris*	Oromiya	East Harerghe	Meta			2230	Chelenko 11km Kobo to Harer
43	211323	*Phaseolus*	*vulgaris*	Oromiya	East Harerghe	Meta			2430	Kulubi, 23km Kobo to Harer
44	211332	*Phaseolus*	*vulgaris*	Oromiya	East Harerghe	Deder				Furda
45	211295	*Phaseolus*	*vulgaris*	SNNP	East Harerghe	Damot Gale	06-58-00-N	37-52-00-E		Boditi
46	211315	*Phaseolus*	*vulgaris*	Oromiya	East Harerghe	Goro Gutu				7km From Karamile to Harer
47	211317	*Phaseolus*	*vulgaris*	Oromiya	East Harerghe	Goro Gutu				7km From Karamile to Harer

Appendix I:(cont'd)

No.	Accession	Genus name	Species name	Region /State/Province	Zone	Woreda /District	Latitude	Longitude	Altitude	Locality
48	211318	*Phaseolus*	*vulgaris*	Oromiya	East Harerghe	Goro Gutu				7km From Karamile to Harer
49	211331	*Phaseolus*	*vulgaris*	Somali	Shinile Zone	Afdem			1850	52km Mieso to SE of Afdem
50	208647	*Phaseolus*	*sp*	Oromiya	East Harerghe	Gursum	09-20-00-N	42-20-00-E		Dolis about 5Km S of Fugnan Bira market
51	219234	*Phaseolus*	*vulgaris*	Oromiya	East Harerghe		09-16-00-N	44-26-00-E	2200	Deru Dima 7Km away from Deder on the way to soka
52	219235	*Phaseolus*	*vulgaris*	Oromiya	East Harerghe		09-15-00-N	41-17-00-E	2060	Cheka Bokolla 19Km from Deder and 12 Km from Deder
53	208646	*Phaseolus*	*sp*	Oromiya	East Harerghe	Gursum	09-22-00-N	42-20-00-E		Lafto about 3Km NW of Fugna Bira market
54	216819	*Phaseolus*	*vulgaris*	Oromiya	East Harerghe	Meta	09-26-00-N	41-42-00-E	2400	Kulubi 23km. On kersa Kulibi Rd near Kulubi
55	216820	*Phaseolus*	*vulgaris*	Oromiya	East Harerghe	Meta	09-24-00-N	41-32-00-E	2260	3km. On chelenko to kobo road
56	240173	*Phaseolus*	*sp*	Oromiya	Illubabor	Metu			1540	Dizee 6km from Sore bridge on the way to Alge/ Sude
57	207949	*Phaseolus*	*sp*	Oromiya	Illubabor	Bure	08-14-00-N	36-06-00-E		Damocha, about 5Km NE of Sibu

Appendix I:(cont'd)

No.	Accession	Genus name	Species name	Region /State/Province	Zone	Woreda /District	Latitude	Longitude	Altitude	Locality
58	201066	*Phaseolus*	*vulgaris*	Oromiya	Jimma	Mana			2100	About 3kms from Yebu to Jimma
59	240190	*Phaseolus*	*vulgaris*	Oromiya	Jimma	Goma			1710	20km from Agaro to Jimma
60	211340	*Phaseolus*	*vulgaris*	Oromiya	West Wellega	Gawo Dale				Kebe
61	211341	*Phaseolus*	*vulgaris*	Oromiya	West Wellega	Gawo Dale				Kebe
62	208705	*Phaseolus*	*vulgaris*	Oromiya	West Wellega	Sayo			1900	Ado 8km W of Dembi Dolo
63	211271	*Phaseolus*	*vulgaris*	Oromiya	West Wellega	Gutin	09-28-00-N	36-31-00-E	1440	Guten Settlement, 80km From Nekemte
64	208695	*Phaseolus*	*vulgaris*	Oromiya	West Wellega	Sayo			1880	Ado michael mission 8Km W of Dembi Dolo
65	208698	*Phaseolus*	*vulgaris*	Oromiya	West Wellega	Sayo			1880	Ado Michael mission, 8Km W of Dembi Dolo
66	208702	*Phaseolus*	*vulgaris*	Oromiya	West Wellega	Sayo			1880	Ado Michael mission, 8Km W of Dembi Doloo
67	208703	*Phaseolus*	*vulgaris*	Oromiya	West Wellega	Sayo				Dembi Dolo
68	211337	*Phaseolus*	*vulgaris*	Oromiya	West Wellega	Gawo Dale				Kebe
69	211338	*Phaseolus*	*vulgaris*	Oromiya	West Wellega	Gawo Dale				Kebe
70	211339	*Phaseolus*	*vulgaris*	Oromiya	West Wellega	Gawo Dale				Kebe

Appendix I:(cont'd)

No.	Accession	Genus name	Species name	Region /State/Province	Zone	Woreda /District	Latitude	Longitude	Altitude	Locality
71	211342	*Phaseolus*	*vulgaris*	Oromiya	West Wellega	Gawo Dale				Kebe
72	211388	*Phaseolus*	*vulgaris*	Amara	North Shewa	Ankober	09-35-00-N	39-44-00-E		Ankober
73	211337	*Phaseolus*	*vulgaris*	Oromiya	West Harerghe	Gawo Dale				Kebe
74	211298	*Phaseolus*	*vulgaris*	Oromiya	West Harerghe	Chiro			2230	6km Asbe Teferi to Gelemso
75	211299	*Phaseolus*	*vulgaris*	Oromiya	West Harerghe	Tulo				Dabasu
76	211300	*Phaseolus*	*vulgaris*	Oromiya	West Harerghe	Tulo				Dabasu
77	211305	*Phaseolus*	*vulgaris*	Oromiya	West Harerghe	Tulo				Dabasu
78	211325	*Phaseolus*	*vulgaris*	Oromiya	West Harerghe	Tulo	09-15-00-N	41-08-00-E	2160	162km, Kombolcha to Addis
79	211327	*Phaseolus*	*vulgaris*	Oromiya	West Harerghe	Chiro	09-05-00-N	40-45-00-E	1850	9km, Asebe Teferi to Jifara
80	211329	*Phaseolus*	*vulgaris*	Oromiya	West Harerghe	Chiro	09-02-00-N	40-44-00-E	1870	20km Asebe Teferi to Jifara
81	211301	*Phaseolus*	*vulgaris*	Oromiya	West Harerghe	Tulo				Dabasu
82	208638	*Phaseolus*	*sp*	Oromiya	West Harerghe	Chiro	09-00-00-N	40-55-00-E		Segerya about 3Km NE of Kuni market

Appendix I:(cont'd)

No.	Accession	Genus name	Species name	Region /State/Province	Zone	Woreda /District	Latitude	Longitude	Altitude	Locality
83	219233	*Phaseolus*	*vulgaris*	Oromiya	West Harerghe		09-01-00-N	41-11-00-E	2070	Maberroobolesa 1Km away from tullo town
84	201293	*Phaseolus*	*vulgaris*	Oromiya	West Harerghe	Chiro			2250	Arbare Kate 339km from Addis Ababa
85	201294	*Phaseolus*	*vulgaris*	Oromiya	West Harerghe	Chiro			2250	Arbare Kate 339km from Addis Ababa
86	211304	*Phaseolus*	*vulgaris*	Oromiya	West Harerghe	Tulo				Dabasu
87	Melka Dima	*Phaseolus*	*vulgaris*	Released						
88	Chercher	*Phaseolus*	*vulgaris*	Released						
89	Goberasha	*Phaseolus*	*vulgaris*	Released						
90	Naser	*Phaseolus*	*vulgaris*	Released						
91	Mexican-142	*Phaseolus*	*vulgaris*	Released						
92	Awash-1	*Phaseolus*	*vulgaris*	Released						
93	241756	*Phaseolus*	*vulgaris*	SNNP	BENCH MAJI	Konso special	05-23-90-N	37-20-19-E	1460	Keser Gado,21 km S.west of Konso town

Appendix I:(cont'd)

No.	Accession	Genus name	Species name	Region /State/Province	Zone	Woreda /District	Latitude	Longitude	Altitude	Locality
94	241757	*Phaseolus*	*vulgaris*	SNNP	Bench Maji	Konso special	05-19-69-N	37-24-99-E	1350	Geldemi,2 Km South of Konso town (off the main Road}
95	213046	*Phaseolus*	*sp*	SNNP	Bench Maji	Konso special			1640	Kolmae 49km from Konso to Jinka
96	235692	*Phaseolus*	*sp*	SNNP	Bench Maji	Dirashe special	05-17-00-N	37-39-00-E	2300	Bore, 7km. W.of the town Gidolle
97	211483	*Phaseolus*	*vulgaris*	SNNP	Bench Maji	Konso special	05-20-00-N	37-25-00-E	1780	Gemolie, 3km Konso to Fasha
98	211481	*Phaseolus*	*vulgaris*	SNNP	Bench Maji	Konso special			1560	Durayie Konso Suburb
99	211292	*Phaseolus*	*vulgaris*	SNNP	Bench Maji	Dirashe special				Duro
100	211290	*Phaseolus*	*vulgaris*	SNNP	Bench Maji	Dirashe special				Duro
101	211291	*Phaseolus*	*vulgaris*	SNNP	Bench Maji	Dirashe special				Duro
102	241752	*Phaseolus*	*vulgaris*	SNNP	Bench Maji	Konso special	05-20-71-N	37-14-00-E	1410	Borkora , 31 Km S.west of Konso town
103	241753	*Phaseolus*	*vulgaris*	SNNP	Bench Maji	Konso special	05-24-90-N	37-14-06-E	1500	Getato 33 Km S.west of Konso town
104	211755	*Phaseolus*	*vulgaris*	SNNP	Bench Maji	Konso special	05-24-90-N	37-14-06-E	1500	Getato 33 Km S.west of Konso town
105	211294	*Phaseolus*	*vulgaris*	SNNP	North Omo	Damot Gale	06-58-00-N	37-52-00-E		Boditi

Appendix I:(cont'd)

No.	Accession	Genus name	Species name	Region /State/Province	Zone	Woreda /District	Latitude	Longitude	Altitude	Locality
106	211293	*Phaseolus*	*vulgaris*	SNNP	North Omo	Damot Gale	06-57-00-N	37-51-00-E		Boditi
107	211552	*Phaseolus*	*vulgaris*	SNNP	North Omo	Boreda Abaya	06-33-00-N	37-41-00-E	800	12km Boreda to Humbo
108	237993	*Phaseolus*	*sp*	SNNP	North Omo	Kindo Koysha			2880	Sorto 18km. from junction to Bele
109	212978	*Phaseolus*	*sp*	SNNP	North Omo	Gofa Zuria	36-52-00-N	06-19-00-E	1840	Menzewala 3Km from Sawla to Bulki
110	241294	*Phaseolus*	*vulgaris*	SNNP	North Omo	Damot Gale	06-58-00-N	37-52-00-E		Boditi
111	241738	*Phaseolus*	*vulgaris*	SNNP	North Omo	Sodo Zuria	06-52-59-N	37-44-76-E	1910	Damot Weja, 8km north of Sodo town the, road to Areka
112	241739	*Phaseolus*	*vulgaris*	SNNP	North Omo	Boloso Sore	06-52-59-N	37-47-56-E	1715	Foto, to east of Areka town , I.e east of the Road to Sodo
113	211546	*Phaseolus*	*vulgaris*	SNNP	North Omo	Damot Gale	06-57-00-N	37-55-00-E		Boditi
114	241750	*Phaseolus*	*vulgaris*	SNNP	North Omo	Arba minch zuria	06-02-21-N	37-30-18-E	2200	Genta Bonke 15 Km N.west of Arba minch town
115	241736	*Phaseolus*	*vulgaris*	SNNP	Hadiya	Badawacho	07-13-51-N	38-01-94-E	1740	Kerensa, 12 Km S. West of Alaba town

Appendix I:(cont'd)

No.	Accession	Genus name	Species name	Region /State/Province	Zone	Woreda /District	Latitude	Longitude	Altitude	Locality
116	244805	*Phaseolus*	*sp*	SNNP	Kembata Alabana Tembaro	Alaba			1766	Mulatumender, about 1km from kolito to Aje [Shashemene]
117	241737	*Phaseolus*	*vulgaris*	SNNP	Hadiya	Badawacho	07-31-51-N	38-01-94-E	1740	Kersnsa, 12 km S.west of Alaba town
118	241748	*Phaseolus*	*vulgaris*	SNNP	Kembata Alabana Tembaro	kacha bira	07-12-00-N	37-48-85-E	1960	Fundame 7 Km east of Shinshicho town on the way to Durame
119	241733	*Phaseolus*	*vulgaris*	SNNP	Sidama	Awasa	07-03-12-N	38-18-06-E	1800	Kejim tlumbulo 28Km west South of Awasa city of the Awasa L.
120	211286	*Phaseolus*	*vulgaris*	SNNP	South Omo	Bako Gazer				Jinka
121	211394	*Phaseolus*	*vulgaris*	SNNP	South Omo	Bako Gazer				Jinka
122	211278	*Phaseolus*	*vulgaris*	SNNP	South Omo	Bako Gazer				Jinka
123	211277	*Phaseolus*	*vulgaris*	SNNP	South Omo	Bako Gazer				Jinka
124	211279	*Phaseolus*	*vulgaris*	SNNP	South Omo	Bako Gazer				Jinka
125	212860	*Phaseolus*	*vulgaris*	Oromiya	Bale	NENSEBO				Chiri kumburta 5km.South of West

Appendix II: Climatic conditions prevailing for the growing period (June to November 2013) at Melkassa Research Station

No.	Month	Rainfall (mm)	Maximum T^0 (^{0}C)	Minimum T^0 (^{0}C)	Mean T^0 (^{0}C)	Relative Humidity (%)	Soil T^0 at 20cm (^{0}C)
1.	June	71.6	33.0	27.0	30.9	58.0	27.4
2.	July	399	29.5	22.0	26.1	73.452	22.1
3.	August	122.3	29.0	22.6	26.4	69.935	22.906
4.	September	139.1	31.0	25.0	28.3	64.9	23.8
5.	October	30.6	30.6	26.0	28.7	53.903	24.3
6.	November	14.9	30.6	22.5	28.0	52.1	24.08

Appendix III: Relative efficiency of lattice over RCBD calculated for the traits studied along with C.V (%) for both the designs

Character/Trait	RE (%) of lattice over RCBD	C.V (%) RCBD	C.V (%) Lattice
DTF	1.15	4.336	4.215
HSW	1.10	27.02	26.571
PLYLD	1.03	32.033	31.934
SH	1.06	28.251	27.681
SDIA	1.00	24.041	23.821
NoPP	1.13	38.615	38.278
PHT	1.08	23.33	23.181
NoSPPO	1.11	27.07	26.938
NBPT	1.06	29.36	28.57
NoSPPL	1.04	30.01	29.936

Appendix IV: A glimpse of the BIONEER Accupower premix used for PCR in the experiment

BIONEER
Innovation . Value . Discovery

AccuPower® Gold Multplex PCR PreMix

Bioneer Corporation
8-11, Munpyeongseoro
Daedeok-gu, Daejeon 306-220
South Korea
Tel. (Korea)1588 - 9788
(International) + 82 - 42 - 930 - 8777
Fax: + 82 - 42 - 930 - 8600
E-mail: sales@bioneer.com

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Toll free: + 1 - 877 - 264 - 4300
Fax: + 1 - 510 - 865 - 0350
E-mail: order.usa@bioneer.us.com

Bioneer Trade(Shanghai) Co., Ltd
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PuDong New District, Shanghai 201203 China
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Tel: + 82 - 2 - 598 - 1094
Fax: + 82 - 2 - 598 - 1096

Order
Domestic only: 1588-9788
E-mail: order@bioneer.co.kr
URL: www.bioneer.com

6. Maintain the reaction mixture at 4°C after amplification. The sample can be stored at -20°C until use.
7. Load 5 µl of the reaction mixture directly on agarose gel without adding a loading dye to analyze the PCR products.

X. Reaction Example

1. Reaction mixture

Component	Volume	Concentration
Template	1 µl	100 ng/µl
Primers	2 µl	1 pmole/µl each
D.W	17 µl	
Total	20 µl	

2. PCR cycling condition

Step	Temperature	Time	No. of Cycles
Pre-denaturation	95°C	5 min	1
Denaturation	95°C	30 sec	30
Annealing	57°C[1]/65°C[2]	30 sec	
Extension	72°C	1 min	
Final-Extension	72°C	5 min	1

Figure 1, Figure 2. (a)
Figure 2. (b)

XI. Experimental Data

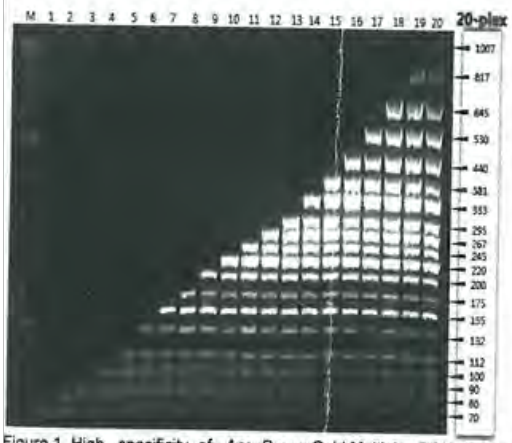
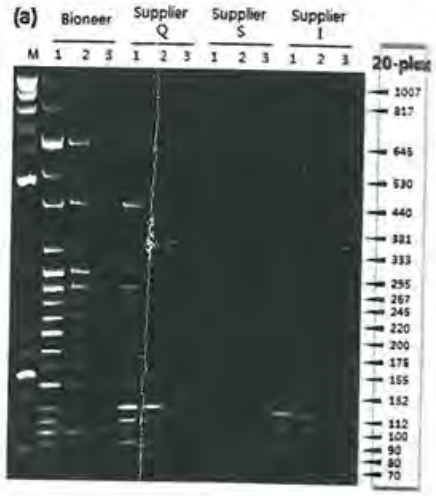


Figure 1. High specificity of *AccuPower* Gold Multiplex PCR PreMix. Each line from left to right represents the progressive number of primer sets up to 20 included in *AccuPower* Gold Multiplex PCR PreMix.



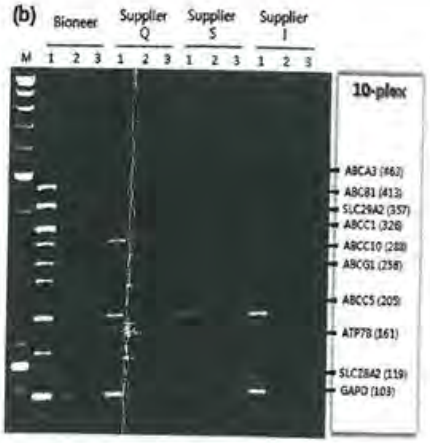


Figure 2. Comparison of amplification quality between *AccuPower* Gold Multiplex PCR PreMix and other supplier's Multiplex PCR kits.

Appendix V: Values recorded for important morpho-agronomic traits for clusters identified in Tocher and NJ clustering

No.	Accession	Region	Collection site	Seed Coat Color	Growth habit	seed shape	HSW
CLUSTER I							
1	201293	Oromiya	W.Hararghe	white	Type II: indeterminate bush	Kidney-shaped	39.5
2	207933	BG	Assosa	Cream	Type II: indeterminate bush)	Oval	47
3	208698	Oromiya	Wellega	white	Type I: determinate bush	Kidney-shaped	36.5
4	211266	Amhara	Gojam	Cream	Type IV: indeterminate climbing)	round	25
5	211267	Amhara	Gojam	white	Type I:determinate bush	Oval	50
6	211269	Amhara	Gojam	Red	Type II: indeterminate bush	Oval	44.5
7	211277	SNNP	S.Omo	white	Type II: indeterminate buh	round	44
8	211286	SNNP	S.Omo	white	Type II: indeterminate bush	round	48
9	211292	SNNP	Benchmaji	white	Type I: determinate bush	round	26
10	211301	Oromiya	W.Hararghe	red	Type I: determinate bush	Cuboid	27
11	211315	Oromiya	E.Hararghe	white	Type II: indeterminate bush	Oval	39.5
12	211332	Oromiya	E.Hararghe	cream	Type II: indeterminate bush	Kidney-shaped	21.5
13	211337	Oromiya	Wellega	yellow	Type II: indeterminate bush	Oval	43.5
14	211349	BG	Metekel	red	Type II: indeterminate bush	Oval	40
15	211382	Amhara	Shewa/Wello	yellow	Type III: indeterminate non-climbing	round	21.5
16	211389	Amhara	Shewa/Wello	Cream	Type IV: indeterminate climbing	Cuboid	40
17	211483	SNNP	Benchmaji	white	Type II: indeterminate buh	Cuboid	41
18	211550	Amhara	Shewa/Wello	Cream	Type IV: indeterminate climbing	Cuboid	41
19	212978	SNNP	N.Omo	white	Type III: indeterminat non-climbing	round	46
20	230779	Oromiya	Bale & Arsi	white	Type II: indeterminate buh	round	38.5
21	240512	BG	Metekel	Red	Type IV: indeterminate climbing	Oval	33.5
22	241739	SNNP	N.Omo	Cream	Type IV: indeterminate climbing	Cuboid	48
23	241752	SNNP	Benchmaji	Dull	Type II: indeterminate buh	Cuboid	43
24	241757	SNNP	Benchmaji	Cream	Type II: indeterminate bush	round	35.25
25	NASER	Released	Released	red	Type I: determinate bush	round	58.5

Appendix V: (Cont'd)

No.	Accession	Region	Collection site	Seed Coat Color	Growth habit	Seed shape	HSW
CLUSTER II							
1	211293	SNNP	N.Omo	white	Type II: indeterminate bush	round	52
2	211304	Oromiya	W.Hararghe	white	Type II: indeterminate bush	Oval	62.5
3	211305	Oromiya	W.Hararghe	red	Type II: indeterminate bush	Oval	48
4	211334	Oromiya	E.Haraghe	white	Type II: indeterminate bush	round	50.5
5	211348	BG	Metekel	white	Type IV: indeterminate climbing	Oval	61
6	241756	SNNP	Benchmaji	white	Type I: determinate bush	Oval	65.5
7	244805	SNNP	Sidama	cream/medium	Type II: indeterminate bush	Kidney-shaped	23
8	CHERCHER	Released	Released	red	Type IV: determinate climbing	Cuboid	70
CLUSTER III							
1	201666	Oromiya	Jimma&Illubabor	Cream	Type II: indeterminate bush	Oval	22
2	208638	Oromiya	W.Hararghe	cream/dull	Type II: indeterminate bush	round	21
3	208646	Oromiya	E.Haraghe	Dull/Brown	Type II: indeterminate bush	Oval	60.5
4	208647	Oromiya	E.Haraghe	White	Type II: indeterminate bush	round	21.5
5	208702	Oromiya	Wellega	Dull	Type II:indeterminate bush	round	38
6	208705	Oromiya	Wellega	Cream	Type IV: indeterminate climbing	Oval	36
7	211298	Oromiya	W.Hararghe	Brown	Type I: determinate bush	Oval	31
8	211319	Oromiya	E.Haraghe	White/yellow	Type II: indeterminate bush	Oval	22
9	211320	Oromiya	E.Haraghe	Brown	Type I: determinate bush	Cuboid	21.5
10	211338	Oromiya	Wellega	White	Type II: indeterminate bush	round	34.5
11	211342	Oromiya	Wellega	White	Type II: indeterminate bush	Oval	21
12	211344	BG	Metekel	White	Type I: determinate bush	round	20
13	211350	BG	Metekel	Red	Type II: indeterminate bush	round	22.25
14	211386	Amhara	Shewa/Wello	Brown	Type IV:indeterminate/determinate climbing	round	47
15	211387	Amhara	Shewa/Wello	Red	Type II: indeterminate bush	round	15.5

Appendix V: (Cont'd)

Cluster III (Cont'd)							
16	211388	Oromiya	Wellega	Brown	Type II: indeterminate bush	Oval	16
17	211552	SNNP	N.Omo	Red	Type II: indeterminate bush	Oval	21
18	212860	SNNP	S.Omo	dull/red	Type II: indeterminate bush	Oval	21
19	219233	Oromiya	W.Hararghe	Red	Type I: determinate bush	Oval	18.5
20	219234	Oromiya	E.Haraghe	white	Type I: determinate bush	Oval	19.5
21	219235	Oromiya	E.Haraghe	Red	Type I: determinate bush	Oval	19
22	235692	SNNP	Benchmaji	White	Type II: indeterminate bush	Cuboid	18
23	240187	Oromiya	Jimma&Illubabor	White	Type II: indeterminate bush	Cuboid	19
24	241738	SNNP	N.Omo	Red	Type IV: indeterminate climbing	Oval	19
25	241748	SNNP	Sidama	Red	Type II: indeterminate bush	Cuboid	22
26	241814	Amhara	Gojam	White/cream)	Type I: determinate bush	round	29.5
27	MA Control	Released	Released	Cream	Type II: indeterminate bush	Oval	23
Cluster IV							
1	207938	BG	Assosa	Cream	Type I: determinate bush	Oval	30.5
2	208695	Oromiya	Wellega	White	Type I: determinate bush	round	20.5
3	208703	Oromiya	Wellega	Red	Type II: indeterminate bush	round	19.5
4	211290	SNNP	Benchmaji	White/cream	Type I: determinate bush	Oval	24.25
5	211291	SNNP	Benchmaji	White/cream	Type I: determinate bush	round	24
6	211294	SNNP	N.Omo	White	Type II: indeterminate bush	round	31
7	211299	Oromiya	W.Hararghe	White	Type I: determinate bush	Markedly-truncated	24.25
8	211300	Oromiya	W.Hararghe	White/dull	Type II: indeterminate bush	round	23.5
9	211317	Oromiya	E.Haraghe	White	Type I: determinate bush	round	28.5
10	211318	Oromiya	E.Haraghe	White	Type II: indeterminate bush	round	29.5
11	211322	Oromiya	E.Haraghe	Red	Type II: indeterminate bush	round	27
12	211323	Oromiya	E.Haraghe	White	Type II: indeterminate bush	Oval	29
13	211331	Oromiya	E.Haraghe	White/dull	Type II: indeterminate bush	Oval	36

Appendix V: (Cont'd)

No.	Accession	Region	Collection site	Seed Coat Color	Growth habit	Seed shape	HSW
Cluster IV (cont'd)							
14	211339	Oromiya	Wellega	Brown	Type I: determinate bush	round	23.5
15	211347	BG	Metekel	Red	Type II: indeterminate bush	Cuboid	24.5
16	211356	BG	Metekel	White	Type II: indeterminate bush	Oval	46.5
17	211361	BG	Metekel	White	Type II: indeterminate bush	round	27
18	211362	BG	Metekel	White/brown	Type II: indeterminate bush	round	34
19	211377	Oromiya	Bale & Arsi	1&6 (white & red)	Type II: indeterminate bush	round	21.5
20	211379	Oromiya	Bale & Arsi	1=white	Type IV: indeterminate climbing	round	50.2
21	211481	SNNP	Benchmaji	1=white	Type II: indeterminate bush	Oval	26.4
22	213046	SNNP	Benchmaji	6=red	Type II: indeterminate bush	Oval	34.5
23	215719	Amhara	N.Shewa & S.Wello	6=red	Type I: determinate bush	Cuboid	32.25
24	216819	Oromiya	E.Haraghe	6=red	Type II: indeterminate bush	Cuboid	30.52
25	216820	Oromiya	E.Haraghe	6=red	Type II: indeterminate bush	Cuboid	15.5
26	218235	Oromiya	E.Haraghe	6=red	Type I: determinate bush	Cuboid	31
27	235697	Oromiya	Bale & Arsi	6=red	Type II: indeterminate bush	Cuboid	31.5
28	237993	SNNP	N.Omo	White	Type I: determinate bush	round	28
29	240173	Oromiya	Jimma&Illubabor	White	Type III: indeterminate non-climbing	Oval	30.5
30	240190	Oromiya	Jimma&Illubabor	White/cream	Type II: indeterminate bush	Oval	24
31	240522	BG	Metekel	Cream	Type IV: indeterminate climbing	Oval	27.5
32	241733	SNNP	Sidama	Cream	Type II: indeterminate bush	round	27
33	241737	SNNP	Sidama	White	Type I: determinate bush	Cuboid	25.5
34	241753	SNNP	Benchmaji	White	Type I: determinate bush	round	28.5
35	241765	SNNP	Benchmaji	White	Type II: indeterminate bush	round	35.5
36	241807	Amhara	Gojam	White	Type I: determinate bush	round	25
37	GOBERASHA	Released	Released	Cream/red)	Type II: indeterminate bush	round	49.5
38	MEX-142	Released	Released	White	Type I: determinate bush	round	29.5

Appendix V (cont'd):

CLUSTER V							
No.	**Accession**	**Region**	**Collection site**	**Seed Coat Color**	**Growth habit**	**Seed shape**	**HSW**
1	201294	Oromiya	W.Hararghe	Cream	Type II: indeterminate bush	round	31.5
2	207934	BG	Assosa	White	Type II: indeterminate bush	round	32.5
3	207949	Oromiya	Jimma&Illubabor	White	Type II: indeterminate bush	Oval	33
4	211271	Oromiya	Wellega	White	Type II: indeterminate bush	Oval	32.25
5	211278	SNNP	S.Omo	Cream	Type II: indeterminate bush	round	33.15
6	211279	SNNP	S.Omo	Dull	Type I: determinate bush	round	30
7	211325	Oromiya	W.Hararghe	White	Type II: indeterminate bush	Oval	31
8	211327	Oromiya	W.Hararghe	White	Type II: indeterminate bush	Oval	42.5
9	211329	Oromiya	W.Hararghe	Brown	Type II: indeterminate bush	round	43.5
10	211340	Oromiya	Wellega	Cream/dull	Type I: determinate bush	Oval	29
11	211341	Oromiya	Wellega	Red	Type II: indeterminate bush	Cuboid	34.5
12	211345	BG	Metekel	Dull	Type II: indeterminate bush	Cuboid	33.5
13	211378	Oromiya	Bale & Arsi	White	Type I: determinate bush	round	33.5
14	211394	SNNP	S.Omo	Cream	Type II: indeterminate bush	Oval	32
15	211546	SNNP	N.Omo	Red	Type II: indeterminate bush	round	35.1
16	211551	Amhara	N.Shewa & S.Wello	White	Type IV: indeterminate/determinate climbing	Cuboid	34.5
17	212861	Oromiya	Bale & Arsi	Red	Type II: indeterminate bush	Oval	23
18	216730	Gambella	Gambella	Red	Type I: determinate bush	Oval	32.25
19	237078	Oromiya	Bale & Arsi	Cream	Type II: indeterminate bush	Kidney-shaped	32.5
20	241730	Oromiya	Bale & Arsi	Red	Type II: indeterminate bush	round	46
21	241736	SNNP	Sidama	Red	Type II: indeterminate bush	Oval	32.5
22	241736	SNNP	N.Omo	Red	Type I: determinate bush	Cuboid	32.5
23	Andean Control	Released	Released	Red	Type II: indeterminate bush	Oval	30.5

9 Declaration

I declare that the thesis hereby submitted by me for the Degree of Doctor of Philosophy (PhD) in Biology (Applied Genetics) to the School of Graduate Studies of Addis Ababa University is my own independent work and has not previously been submitted by me or anybody else at another university. The materials obtained from other sources have been duly acknowledged in the thesis.

Signed on the --- of -----/2015, The School of Graduate Studies, Department of Microbial, Cellular, and Molecular Biology, Addis Ababa University, Arat Kilo.

PhD Candidate _____

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