GENETIC DIVERSITY STUDY OF ETHIOPIAN FABA BEAN (*VICIA FABA* L.) VARIETIES USING PHENOTYPIC TRAITS, ISSR AND SNP MARKERS

BY

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This is to certify that the thesis prepared by **Mr. Behailu Mulugeta**, entitled "**Genetic Diversity Study of Ethiopian Faba Bean (*Vicia faba* L.) Varieties Using Phenotypic Traits, ISSR and SNP Markers"** submitted in partial fulfillment of the requirement for the Degree of Master of Science in Biology (Applied Genetics) complies the regulation of the University and meets the accepted standards with respect to originality and quality.

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ABSTRACT

Faba bean (Vicia faba L.*) is one of the earliest food legumes of the world used as source of high protein for human and animal consumption. In Ethiopia, no studies were conducted on faba bean varieties genetic diversity assessment based on molecular markersand this study is designed to fill this gap. The study was designed to reveal the genetic diversity existing among Ethiopian faba bean varieties using phenotypic traits, ISSR and SNP markers. Field experiment was conducted at three locations (Sinana, Agarfa and Selka) for agro-morphological performance, while the molecular part was conducted at Addis Ababa University (ISSR) and UK, LGC genomics (KASP SNP genotyping). A total of 32 faba bean varieties were used for phenotypic and ISSR markers, whereas 16 additional breeding lines were included for SNP markers. A total of 23 phenotypic traits,11 ISSR and 37 SNP primers were used to assess genetic diversity and interrelationship among Ethiopian faba bean varieties. The genomic DNA extraction was made based on modified CTAB method and Oktopure extraction kit method from bulked young leaf sample for ISSR and SNP markers, respectively. The combined analysis of variance over the three locations showed highly significant ($p<0.01$) variations among the varieties for the majority of traits. Genotype by environment interaction showed highly significant difference ($p< 0.01$) among traits. UPGMA constructed based on phenotypic traits revealed five major clusters and the first six principal components (PCs) accounted for 85% of the entire diversity among the varieties for all the 23 traits and the first three PCA revealed 66% of the total variation. Eleven ISSR primers amplified 120 bands, of which 107 loci were polymorphic. The highest gene diversity (0.38) and Shannon index (0.56) were recorded by primer 860, while the least gene diversity (0.18) was revealed by primer 848 and 857. UPGMA cluster and PCO analysis grouped the cultivars into three major clusters based on Jaccard's similarity coefficient ranging from 0.41 to 0.77. Out of the 37 scoreable SNP markers, one was monomorphic across all the 48 genotypes and 36 SNP showed polymorphism with rate of 95.58%. Relatively high average Nei's genetic diversity (0.41) and PIC (0.32) were revealed by SNP markers. Based on obtained PIC value, 31 SNP primers were found to be reasonably informative. Genetic dissimilarity between different pairs of varieties varied from 0.15 (Dosha and Mesay) to a maximum of 0.80 (Tumsa and Kulumsa local varieties) with an average mean of 0.45. STRUCTURE, UPGMA and PCOA suggested that the varieties were divided into three main genetic groups. Therefore, the observed genetic variation in the study indicated the opportunity of using these materials in future faba bean breeding program via introgression with other germplasm resources for improvement of better genotypes.*

Key Words: Genetic distance, Genetic diversity, ISSR, Phenotypic traits, SNPs, *Vicia faba*

DEDICATION

This piece of work is dedicated to my mother **Aster Kebede Bulcha** who nurtured me with affection and love and has been waiting for my success, but passed away before celebrating the fate of her endeavors. You have successfully made me the person I am becoming. You will always be remembered.

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ACRONYM AND ABBREVAITIONS

AARC	Adet Agricultural Research Center
AAU	Addis Ababa University
AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analysis of Variance
AMOVA	Analysis of Molecular Variance
ARC	Agricultural Research Center
CSA	Central Statistical Authority
CTAB	Cetyltrimethyl Ammonium Bromide
DAP	Diammonium Phosphate
DbARC	Debre Birhan Agricultural Research Center
DzARC	Debrezeit Agricultural Research Center
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
HARC	Holetta Agricultural Research Center
ICARDA	International Center for Agricultural Research in Dry Areas
ISSR	Inter Simple Sequence Repeat
OARI	Oromia Agricultural Research Institute
PCA	Principal Component Analysis
PCoA	Principal Coordinated Analysis
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SARC	Sinana Agricultural Research Center

SAS	Statistical Analysis System
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SSR	Simple Sequence Repeat
UPGMA	Unweighted Pair Group Method with Arithmetic mean
WHO	World Health Organization

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1. INTRODUCTION

Faba bean (*Vicia faba* L., 2n = 2x = 12) is one of the earliest domesticated food legumes in the world. Vavilov (1936) discovered a primitive type of faba bean at the intersection of Himalaya and Hindu Kush, and proposed that faba bean was originated from Central Asia and then expanded westward along the mountains to Iran, Turkey, the Mediterranean and Spain. He considered areas along the coast of the Mediterranean Sea and Ethiopia as the secondary center of origin of faba bean. Cubero (1974) proposed that the center of origin for faba bean is in the Near East, Iraq and Iran, considering Afghanistan and Ethiopia as secondary centers of origin. Recently, archeological study strongly suggests that Neolithic people in Israel (the lower Galilee: Ahihud, Nahal Zippori and Yiftah'el) first domesticated faba beans 10,000 years ago, and they were eating as a staple food before grain began to be cultivated in the area (Caracuta *et al*., 2015).

Faba bean is the sixth most important pulse crop after common bean (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata* L.), chickpea (*Cicer arietinum* L.), field pea (*Pisum sativum*), and pigeon pea (*Cajanus cajan* L) at global level (FAOSTAT, 2008). Among the world faba bean producing countries, China is the largest producer which gives East Asia the largest share in world total area harvested (38%) and total production (42%) (Akibod and Maredia, 2011).

Faba bean is believed to be one of major grain legume crops grown in Ethiopia soon after domestication around the 5000 years B.C. (Bond, 1976). Currently, in Ethiopia it ranks first in terms of area (about 538,458.21 hectares) alotted to food legumes and volume of production

(991,700.28 tone), contributing 4.34% and 3.94% of total area and production of crops, respectively (CSA, 2014). Even though the ecological and economic contribution of faba bean in Ethiopia is high, the productivity is far below the potential because of several biophysical and socio-economic constraints including traditional production and inadequate technological interventions.

Faba bean is a grain legume crop grown for different purposes in Ethiopia. It is used as a source of food to the people, feed to animals, cash to the farmers and foreign currency earning to the country. It has high edible protein in seed used for human and animal consumption. The nutritional value and composition of faba bean is considered to be very similar in many ways to that of meat due to its high protein content, which ranges from 20-41%, despite the imbalance in Sulphur containing amino acids (Ali *et al.*, 1982; Larralde and Martínez, 1991; Crépona, *et al.*, 2010). Most of the proteins found in this crop are globulin (60%), albumin (20%), glutenin (15%) and prolamins (2-4%) (Larralde and Martínez, 1991). It is also a good source of sugars, minerals and vitamins. The chemical analysis of faba bean revealed that the seed contains 50-60% carbohydrate (mainly starch) and fiber (7.3-13%), while the proportion of lipids is relatively low (1-2.5%) of which oleic and linoleic acids representing about 75% (Larralde and Martínez, 1991; Duc *et al.*, 1999). Besides, faba bean provides major benefits in the cropping systems and the environment due to its ability to perform symbiotic nitrogen fixation (Boddey *et al.*, 2009; Kaur *et al.*, 2012), break disease cycles in the cereal-based rotations and control weeds (Schmidtke and Rauber, 2000). Erik *et al.* (2012) suggested that the substitution of larger proportion of industrial N fertilizer by leguminous N_2-fixation could minimize considerable amount of global CO_2 emissions.

Despite its importance, however, the productivity of faba bean in Ethiopia is affected by different factors which include biotic (diseases, weeds and insects) and abiotic (drought, water logging and marginal managment) and edaphic stresses like soil acidity (El-Fouly, 1982). One way of confronting this challenge is with sound crop breeding programs that improve the productivity of the crop through development of varieties resistant to major biotic and abiotic constraints, refinement of integrated pest management strategies, improving adaptation to changing environments, improving nutritional quality and adaptation under different agro-ecologies and launching an integrative approach leading to effective exploitation of the genetic and environmental manipulations.

The effectiveness of plant breeding depends upon the nature and magnitude of existing genetic variability and the degree of heritability or transmission of traits and genetic gain from selection. The presence of genetic variation is a basic requirement for long-term stability of crop in certain ecosystem, maintenance of diversity for their efficient utilization in breeding schemes and effective conservation. Welsh (1990) and Falconer and Mackay (1996) defined genetic variability as the occurrence of differences among individuals due to differences in their genetic composition. Genetic variation is portioned into component of genotypic and environmental effects. Singh (1990) reported genotypic variation as the component of variation, which is due to the genotypic differences among individuals within a population and argued as the main concern of plant breeders. Phenotypic variability is the total variability including both genotypic and environmental variation and hence changes under different environmental conditions and measured in terms of phenotypic variance. In attempting to develop improved varieties, the plant breeder bases his/her observation often on the measurement of the phenotype. For plant breeding to be effective, there must be phenotypic variation of the desired trait and some of the variation must be heritable from generation to generation (Stoskop *et al*., 1999).

Heritability is the other parameter that should be given attention in plant breeding and defined as the proportion of total variation caused by genotype ranging from one where all variation is due to genetic, to zero where all the variation results from the environment which means that heritability fall between these two extreme values. It is indicator of response to selection for particular traits and an index of their transmissibility (Johnson *et al*, 1955). Heritability indicates the effectiveness with which selection of phenotypes can be based on phenotypic performance. If heritability were 100%, then phenotypic performance would be perfect indication of genotypic value (Johnson *et al*., 1955). According to Mittal and Sethi (2004), heritability enables the plant breeder to recognize the genetic difference among genotypes and genetic variance indicates the potential for the improvement of a population. Rajput and Malik (1988) forwarded that the response to selection for qualitative characteristics is directly proportional to the function of its heritability and genetic variance. Low heritability is caused by the sensitivity of characters to environment and the nature of test population (Briggs and Knowels, 1987).

Genetic advance under selection plays an important role in improvement of the mean genetic value of the selected plants over the base population. It measures the expected genetic progress from selected best performing germplasms for a given trait. According to Allard (1960), genetic advance under selection depends on genetic variability, heritability or masking effect of non-genetic variability on the genetic variability and the selection intensity applied. Therefore, the utility of estimates of heritability is increased when they are used in conjunction with the selection differential, the amount that the mean of the selected lines exceeds the mean of the entire group (Johnson *et al*., 1955). According to Burton and DeVane (1953), genetic advance tells us the estimate of the expected gain for a particular character through selection. The estimation of heritability together with the genetic advance is more useful to predict the gain according to selection than the estimation of heritability alone.

Correlations among yield, yield components, and other economical traits are important for making selection in breeding program. Correlation coefficient analysis measures the mutual relationship between various plant characteristics and determines the component characters on which selection can be based for improvement in yield.

Considerable efforts at the molecular level have been undertaken worldwide toward the assessment of genetic diversity and relatedness in faba bean (Basheer-Salimia *et al.*, 2013). Molecular breeding strategies that integrate the latest innovations in genetics and genomics with the traditional breeding strategies have many potential applications for future faba bean cultivar development including improving the efficiency of selection, enhancing favorable gene action and expansion of useful genetic diversity for crop improvement (Gnanasambandam *et al*, 2012).

Currently, the number of faba bean varieties released in Ethiopia is increasing but it is difficult to differentiate them based on morpho-agronomical performance *per se* which are liable to genotype by environment interaction (Terzopoulosa and Bebeli, 2008). Starting from the early beginning of variety development in Ethiopia, molecular approaches have not been extensively used to evaluate the varieties of faba bean and others legume crop. Torres and Avilla (2011) reported that more than 80% of faba bean cultivation is in developing countries where the application of novel molecular breeding approaches are limited. Therefore, the application of genotypic marker along with phenotypic markers has a paramount importance in the varietal characterization and fingerprinting to reveal the diversity existing in Ethiopian faba bean varieties released so far over the years.

2. OBJECTIVES OF THE STUDY

2.1. General Objective

The general objective of the present research was to study the genetic diversity and relationships among Ethiopian faba bean varieties using phenotypic traits, ISSR and SNP markers.

2.2. Specific Objectives

The specific objectives of the study were: -

- To study the magnitude and pattern of genetic diversity of Ethiopian faba bean varieties released so far using phenotypic markers;
- To determine the magnitude of genetic variability, heritability and expected genetic gains from selection for morpho-agronomic traits;
- To assess the amount of genetic progress attained in grain yield through the past breeding efforts of faba bean in Ethiopia;
- To classify the test varieties into homogeneous groups and thereby examine if there is any definite relationship between ancestral derivatives and genetic diversity at both molecular and morpho-agronomic levels; and
- To compare the magnitude and pattern of genetic variations and similarity among the released faba bean varieties in Ethiopia using ISSR and SNP markers.

3. LITERATURE REVIEW

3.1. The Role of Agriculture

Agriculture has historically been the foundation of social and economic progress in the developed world and accepted as a key sector for reducing poverty and sustainable rural development especially in developing countries. Agriculture is an essential element of human life and provides the fundamental needs for human survival. It is also an important sector for economic and social development due to its contribution to Gross Domestic Product (GDP) and employment. It is the only source of income for the majority of the rural poor in some countries, particularly in Sub-Saharan Africa. Sixty-five percent (65%) of the world’s work force (86% of rural populations) is active in the agricultural system (World Bank, 2008), and agriculture will continue playing a major role in development. At the global level, agriculture share around 29% of total production in Gross Domestic Product, as in contrast to 2-4% in most of the industrialized countries (World Bank, 2008). In Ethiopia, the agricultural sector employees 85% of the total work force and accounts for 50% of the Gross Domestic Product and generates 90% of the national export earnings.

3.2. Taxonomical Classification of Faba Bean

Food legumes are grouped taxonomically into Leguminoeae family. Members of the Leguminoeae are morphologically diverse and include a number of trees, herbs, legume crops and some aquatic plants (Polhill and Raven, 1981). It is the third largest family of flowering plants behind orchids (Orchidaceae) and asters (Asteraceae) and consisting of approximately 650 genera and 18,000 species (Doyle and Luckow, 2003). The Leguminoeae have been divided into three major groups mainly on the basis of their morphological and floral differences; the Caesalpinioideae, Mimosoideae and Papilionoidaeae (70% of Luguminoeae)

(Doyle and Luckow, 2003). Faba bean belongs to the family *Fabaceae*, sub-family *Faboideae*, tribe *Fabeae,* and the genus *Vicia*. *Vicia* has a large genus of 140-190 species (Doyle and Luckow, 2003).

3.3. Origin and Geographical Distribution of Faba Bean

Vavilov (1936) discovered a primitive type of faba bean at the intersection of Himalaya and Hindu Kush, and proposed that faba bean originated from Central Asia and suggested a gradual increase in the faba bean's area expansion from Central Asia westward along the mountains to Iran, Turkey, the Mediterranean and Spain. He also concluded that areas along the coast of the Mediterranean Sea and Ethiopia could be the secondary origin of faba bean. Cubero (1974) also reported that the center of origin of faba bean is in the Near East, Iraq and Iran, later on Afghanistan and Ethiopia are considered as secondary center of origin. Among the world faba bean producing countries, China is the largest producer of faba beans which gives East Asia the largest share in world total area harvested (38%) and total production (42%) (Akibod and Maredia, 2011).

Faba bean *(Vicia faba* L.*)* has spread across the globe due to its wide adaptability. It grows well in the frosty northern areas of Europe as well as in the arid climates of the Middle East and Africa. It is chiefly produced in Europe, Asia and North America, extending to temperate South America and tropical East Africa (Allkin *et al*., 1986) and reached Ethiopia soon after domestication around the 5000 years B.C.

Faba bean is one of the major pulse crops occupying largest area coverage and volume of annual production of all pulses produced in the country and grown in the highlands (1800-3000 m.a.s.l) of Ethiopia (Gemechu *et al*., 2003). Ethiopia is now considered as one of the centers of secondary diversity for faba bean (Yohannes, 2000). Faba beans are extremely diverse crops in terms of cultivation methods, uses, and the range of environments to which they have been adapted.

3.4. Ecological Zones of Faba bean in Ethiopia

Faba bean is cultivated in Ethiopia in Weyndega zones (mid-altitudes 1800-2200 m.a.s. l, average annual rainfall of 740 mm, mean daily temperature of 18-22^{o}C) and Dega zones (high altitude, above 2200 m.a.s. l, average annual rainfall of 900 mm, mean daily temperature of 10-18 ^{o} C) (Asfaw *et al*, 1994). It is grown from June to December in rotation with cereals apart from its adaptation and distribution. Mostly subsistence farmers grow faba bean under rain-fed conditions.

3.5. Economic Importance and Use of Faba Bean

In terms of agricultural importance, legume crops come second to cereals as a source of human food and animal feed. Food legume crops represent an important component of agricultural food crops consumed in developing countries and are considered as a vital crop for achieving food and nutritional security for both poor producers and consumers. As a matter of fact, in dietary terms, food legumes complement cereal crops as a source of protein and minerals while in agricultural production; they serve as rotational crop with cereals and this helps to reduce soil pathogens and supply nitrogen to the cereal crop (Dyke and Prew, 1983). Food legumes also serve as a feed crop in many farming systems and fetch higher prices compared to cereals and are increasingly grown to supplement farmers’ incomes (Gowda *et al*., 1997). The important and diverse role played by food legumes in the farming systems and in diets of poor people, makes them ideal crops for reducing poverty and hunger, improving human health and nutrition, and enhancing ecosystem resilience. Their importance as food lies primarily in their high protein content. Legumes’ grain protein is the natural supplement to cereal grain protein.

They also provide fat and carbohydrates. Moreover, legumes are high in bone building minerals and vitamins essential for good health.

Faba beans are tightly coupled with every Ethiopian life. It is mainly used as an alternative with peas to prepare flour called 'shiro' which is used to make 'shiro wot' (a cook almost ever-present in Ethiopian dishes). Faba bean has several important attributes including high nutritional value, long storage times and relatively low cost in comparison to animal products. It makes an important contribution in protein, energy and micronutrient provision to populations in the developing world (Dilis and Trichopoulou, 2009).

Faba beans provide dietary protein that play essential role in human nutrition, especially in combination with other foods (Broughton *et al.*, 2003). Complementary foods can be prepared by mixing cereal grain products with locally available legume grain products to produce foods with adequate energy and protein levels. The recommendations from FAO/WHO/UNU to add a maximum of 40% legumes to cereal-based complementary food for young children has been made (FAO/WHO/UNU, 1985). However, the feasibility and acceptability of this recommendation deserves testing in Ethiopia, where beans are locally grown and relatively inexpensive (compared to meat). It also serves as income source for farmers, and also contributes as source of foreign currency earning. It reduces cost of fertilizer for the following cereal crop production for smallholder farmers by adding nitrogen. Faba bean N balance related to shoot biomass was reported as 19 kg/ton (Peoples *et al*., 2009) and 9 kg/ton (Vinther and Dahlmann-Hansen, 2005), compared to our value of kg/ton. Biological nitrogen fixation of faba bean attracts increasing interest because of the depletion of fossil fuel and environmental deterioration globally, in that it is renewable, clean and environment friendly compared with industrially produced nitrogen fertilizer (Giller, 2001; Jensen and Hauggaard-

Nielsen, 2003). An essential component of successful faba bean production is that it is a good weed control like other legumes, and the crop is very sensitive to competition for both broad-leaved and grassy weeds (Glasgow *et al.*, 1976; Lawson and Wiseman, 1978).

3.6. Faba Bean Production and Productivity in Ethiopia

Among the cultivated grain legume in Ethiopia, faba bean ranks first in terms of hectarages and total national production (CSA, 2014). However, the productivity per unit of land is still far below than the productive potential of the crop, though gradually yield improvement is maintaining from year to year. Eventhough, the contribution of this crop is high; still the farmer's variety is below the yield potential of improved varieties released by different agricultural research centers per unit of land area due to their susceptibility to biotic and abiotic factor. In the last decades, the total area and production under faba bean crop in Ethiopia has shown a considerable increasing trend. The contribution of this crop in 2013/2014 crop season in terms of area and production to the total national crop area under faba bean cultivation and production was about 4.34 % (about 538,458.21 hectares) and 3.94% (about 991,700.28 tone), respectively (CSA, 2014). The productivity of this crop at national level is increasing from time to time due the breeding efforts made at different national and regional agricultural research centers on improving the yield. Therefore, the production of faba bean currently reached about 1.8 ton/ha (CSA, 2014).

3.7. Faba Bean Production Constrains

Faba bean production in Ethiopia is affected by different biotic and abiotic factors which contribute to low yield and unstable productivity that affect yield stability. These includes climatic factors such as temperature and light (Asfaw *et al.*,1990), agronomic practices such as plant density, and unfavorable climatic condition like drought, salinity, excessive soil moisture (excessive water logging), inadequate supply of nutrients, distribution of assimilates and hormones imbalance (El-Fouly,1982).

In Ethiopia, the average yield of faba bean under small-holder farmers is not more than 1.8 ton/ ha (CSA, 2014), despite the availability of high yielding varieties which give more than 2 tons/ha (MoA, 2012). The low productivity of faba bean is attributed to susceptibility to biotic and abiotic stress (Mussa *et al*, 2008). Among the biotic category, diseases are important limiting factors that hinder the production of food-legume crops as a whole and specifically faba bean in Ethiopia (Berhanu *et al*, 2006). Most diseases are affecting faba bean, but only a few of them have either major or intermediate economic significance. Among these, fungi are the largest and perhaps the most important group affecting all parts of the plant at all stages of growth of faba bean (Nigussie *et al*., 2008). Diseases such as chocolate spot (*Botrytis fabae* Sard.), rust (Uromyces *Vicia fabae*), black root rot (*Fusarium solani*) and foot rot (*Fusarium avenaceum*) are among fungal groups that contributes to the low productivity of the crop (Berhanu *et al*, 2006). The low productivity may also be attributed to the inherently low yielding potential of the local varieties grown by the farmers.

According to the research results from Holeta and Debrezeyit ARC, chocolate spot and rust caused yield loss of 34.1% and 14-21%, respectively (Asfaw *et al.*, 1993). Hence, control measure is very important to minimize loss due to these diseases. Among many ways to control faba bean diseases, identification and utilization of resistant genotypes is believed to be most practical, economical and environmentally save control measure for farmers (Asfaw *et al*., 1994)

3.8. Faba Bean Breeding History in Ethiopia

A modest faba bean breeding program in Ethiopia was started in the 1960's with the establishment of Arsi Rural Development Unit (ARDU) followed by Debrezeit Agricultural Research Center, under Haramaya University the then Alemaya College of Agriculture and was designed with main objectives of improving the productivity of faba bean through developing and promoting improved cultivars with high and stable yield, resistant/tolerant to both biotic and abiotic stresses and suitable under different agro-ecologies and cropping systems of the country. Special focuses have been made to improve grain yield, diseases and waterlogging resistance/tolerance and increase seed size in response to the market demand (Asfaw *et. al.*,1994).

Even though the Institute of Agricultural Research was established in 1958, until mid 1980's research efforts in general and breeding works in particular on this crop were limited to a few research centres and some locations based on separate efforts. Strong and well organized breeding works were started in mid 1980's when research collaboration started among different research centres at different locations and the program was further strengthened by technical supports from ICARDA (Asfaw *et. al.*,1994). The commencement of hybridization of faba bean in Ethiopia dates back to the mid 1980's when the first crossing was started at Holetta Agricultural Research Center to transfer chocolate spot resistance from parental materials introduced from ICARDA into other adapted parents with other good agronomic traits and this center contributed a major role in releasing faba bean varieties involved in increasing production and productivity in the country.

3.9. Faba bean Germplasm Source in Ethiopia

Genetic variation is a pre-requisite for any improvement in crop and always the first step unless variations pre-existing (Sharma, 1994). Germplasm enhancement is achieved by contributors of genetic variation such as landrace collections, introduction of exotic materials and hybridization (Gemechu *et al.,* 2003). The desired changes in genotypes of crop species and the consequent benefit to farmers are brought about by a service of interrelated and largely inter dependent activities namely creation of genetic variation, selection, evaluation, multiplication and distribution (Sharma, 1994). Between 1994 and 2002, on average, more than 2500 germplasm accessions/lines and breeding materials of faba bean have been annually evaluated in Ethiopia (Mussa *et al.,* 2008). Out of these, 60% was from hybridization, 20% was from collection and 15% from introduction.

3.10. Faba Bean Genetic Diversity in the World and Ethiopia

Primary diversity for the genus is centered in the Near East and Middle East, with a large percentage of the species occurring in the Irano-Tauranian floristic region (Bellido, 1994) and has a wide morphological diversity. Bond (1976) considered Ethiopia as secondary centers of diversity for faba bean due to the existence of wide genetic variability. Gemechu *et al* (2003) indicated the existence of high genetic diversity which was not uniformly distributed among access and the regions in their study of the extent and pattern of genetic diversity among the 160 faba bean landraces collected from north and south Wello, North Gonder, North Shoa and Arsi zones. Report by Dawit *et al.* (1994) and Gemechu *et al.* (2005) also revealed the existence of a wealth of genetic variability among Ethiopian faba bean germplasms for most of the morpho-agronomic traits.

Different reports also indicated that modern plant breeding reduced crop genetic diversity (Vellve, 1993; Tripp,1996) and thereby causing to the vulnerability of the crop to biotic and abiotic stresses and failure of their ability to evolve and adapt to ever changing climates (Gemechu Keneni, *et al*., 2012). For instance, the outbreak of Irish potato blight in Iceland in 1940’s (Cox and Large, 1960), corn blight in U.S.A in 1970’s (Bauer, 1972) and new race development of stem rust Ug99 (Singh, *et al*.,2008) and yellow rust (Landuber *et al*., 2016) on wheat in East Africa are some the factors of intensification of uniform improved varieties to the farming community.

3.11. Determing Factors for Effective Faba Bean Breeding

3.11.1. Genetic variability

The existence of genetic diversity is a pre-condition in development of an effective plant breeding program. Allard (1960) and Falconer and Mackay (1996) defined genetic diversity as the occurrence of variability among the individuals due to difference in genetic composition. If the expression of traits for two individuals is measured in similar environment and a difference recorded, these variations would be caused from genetic control and named genetic variation (Falconer and Mackay, 1996). Genotypic variation is the component of variation caused by the genotypic differences among individuals within a population. Therefore, this type of variation among individuals is a core for plant breeders in crop species improvement and this can buffer against seasonal fluctuation (Sharma, 1998). Phenotypic variation is observable variation present in traits which include both genotypic and environmental components of variation and, as a result, its magnitude differs under different environmental conditions (Singh, 1977). Hence, phenotypic variation is the result of genotypic variation and environmental deviation (Falconer and Mackay, 1996).

Singh (2007) suggested that the presence of variability among individuals within a population can be estimated by using the following three ways: (1) simple measures of variability, such as range, mean, variance, standard deviation, coefficient of variability and standard error, (2) by estimating the various components of variance and (3) by measuring the genetic diversity (e.g. D^2 statistics). In faba bean high estimates of variability have been reported for a number of attributes by Sharif (2015), Bakhiet *et al*. (2015), Shelmary and Shivani (2015), Al Bari and Shtaya (2013), El-Badawy *et al.* (2012), Chaieb *et al.* (2011), Duc *et al.* (2010), Abdelmula and Abuanja (2007), and Gemechu Keneni and Musa Jarso (2008,2003).

Genotypic and phenotypic coefficients of variation were reported as the major tools used to measure the variability that exists in a given population (Burton and Devane, 1988). The larger environmental variance than genotypic variance signifies, the contribution of environment was greater in the phenotypic expression of that trait. Abdul-ariz *et al.* (2015) reported higher genotypic coefficient of variation with thousand seed weight and moderate with number of seeds per plant in faba bean. They also reported low genotypic coefficient of variation for number of pods per plant, number of seeds per pod and seed yield per plant. Tafere *et al.* (2013) also found high GCV for biomass and number of pods per plant in their study of genetic variability, heritability and correlation in some faba bean genotypes grown in Northwestern Ethiopia. Alghamdi (2007) also reported high genotypic coefficient of variation for days to flowering, number of pods per plant and thousand seed weight. Abdul-ariz *et al.* (2015) and Tafere *et al.* (2013) reported higher phenotypic coefficient of variation with number of pods per plant, thousand seed weight and moderate for number of seeds per pod and seed yield per plant. High phenotypic coefficient of variation was also reported for days to flowering, number of pods per plant and thousand seed weight by Alghamdi (2007).

3.11.2. Heritability

Heritability is the measure of the value of selection for specific traits and an index of their transmissibility (Phoelman and David, 1995; Johnson, 1955). Heritability in broad sense is the proportion of the total genetic variability to the total phenotypic variance (Allard, 1960), whereas heritability in narrow sense is the ratio of additive genetic variance to phenotypic variance (Falconer and Mackay, 1996). Heritability value ranges from zero where all the variation resulted from the environment to one where all variation is caused by genetic composition of individual's population. Practically, it is very difficult to determine the presence, amount or types of genetic variability in condition where phenotypic expressions are strongly influenced by the environment (Welsh, 1990). The greater the proportion of the total variability that is due to environment, the more difficult will it be to select for inherited differences.

Singh (1990) categorized heritability into very high heritability (>80%), moderate high heritability (50-80%), intermediate heritability (40-50%) and low heritability (<40%). Therefore, selection for characters with heritability more than 80% should be fairly efficient than for low heritability (<40%). This indicate that small environmental variability in relation to genotypic differences, because, there is a close correspondence between genotype and phenotype and relatively smaller contribution of environment to phenotype. For traits with low heritability, selection may be difficult or virtually impractical due to the masking effect of the environment on genotypic effects. Poehlmon and David (1995) suggested the influence of the environment on the trait with high heritability is very low and the increasing value of broad sense heritability could serve as an indicator of selection based on phenotype, particularly when it is accompanied by a relatively high variability and genetic advance. Hence, heritability indicates the effectiveness with which selection of genotypes can be based on phenotypic performance.

Sufficiently very high heritability values were reported by Sharifi (2015) for traits like pod length and pod width in genetic variation for seed yield and some of agro-morphological traits in faba bean (*Vicia faba* L.) genotypes. Abdul-ariz *et al*. (2015); Tafere *et al*. (2013); Mellion *et al.* (2012) and Alghamdi (2007) also reported moderately high heritability values for traits such as plant height, number of pod per plant, number of seed per plant, number of seed per pod, biomass weight per plot, thousand seed weight and seed yield. Abdel Aziz *et al*. (2015) found moderate heritability for seed yield per plant and thousand seed weight of faba bean genotypes and he also reported low heritability for traits: number of pods per plant, number of seeds per plant and numbers of seeds per pod. Alghamdi (2007) obtained the highest estimates of broad sense heritability for days to flowering, number of pods per plant, number of seeds per plant and days to maturity.

3.11.3. Expected genetic advance

According to Allard (1960), the improvement in the mean genotypic value of the selected plants over base population measures the expected genetic gain from selecting the best performing genotypes for a given character. Johnson *et al.* (1955) reported broad sense heritability along with genetic advances are usually more useful than heritability alone in predicting the resultant effect of selecting the best individuals. Bakhiet *et al.* (2015) reported high genetic advance with high heritability for traits: days to flowering, days to maturity, plant height, number of branches per plant, number of pods per plant, number of seeds per plant and seed yield per plot. Gemechu Keneni and Musa Jarso (2002) reported high genetic advance as percent of mean along with high broad sense heritability for thousand grain weight and seed yield per plot in comparison of faba genotypes. The report by Kalia and Sood (2004) found high heritability and high genetic advance for number of pod per plant

3.12. Importance of Correlation Studies

Different authors studied the significance of correlations in crop improvement programs. According to Gomez and Gomez (1984), correlation coefficient is the measure of the degree for linear association between two variables. Correlations between characters are frequent features of plant breeding and may arise from linkage or developmental genetic interaction, with or without purely phenotypic components (Bond, 1976). The ultimate expression of yield in crop plants is usually dependent upon the action and interaction of a number of important characters and these characteristics are generally interrelated or correlated and its value can be either negative or positive. Correlations of characters with yield are useful in crop breeding to identify criteria for indirect selection, to provide reliable information on the nature, extent and direction of selection (Falconer and Mackay, 1996; Sharma, 1998).

Correlations in quantitative genetics can be grouped into phenotypic, genotypic and environmental correlations. Phenotypic correlations (r_p) is the association between two characters that can be directly observed and measures the extent to which the two observed characters are linearly related. Genetic correlation (r_g) is the associations of breeding values (i.e additive genetic variance) of the two characters and measures the extent to which degree the same genes or closely linked genes cause co-variation (simultaneous variations) in two different characters. Genotypic correlation coefficient provides a measure of genetic association between traits in order to identify the important traits. Environmental correlations (re) is the correlation of environmental deviations together with non-additive genetic deviations (i.e. dominance and epistatic genetic deviations) (Singh and Chaudhary, 1977; Falconer and Mackay, 1996; Sharma, 1998).

Alghamdi (2007); Azarpou *et al*. (2012); Tafere *et al*., (2013); Sharif (2014) and Abdalla *et al*. (2015) reported positive and significant association between seed yield per plot and thousand seed weight. Tadele *et al. (*2011) found number of pods per plant, number of seeds per pod, thousand seeds weight had positive and significant association with seed yield per plot. Gemechu and Musa (2003) also observed positive and significant association between seed yield and number of pods per plant. Report by Alghamdi (2007) revealed positive and highly significant correlation between number of pod per plant and number of seed per plant and seed yield per plot. Ulukan (2003); Gemechu and Musa (2003); Abdelmula *et al*. (2007); Tadele *et al*. (2011); Azarpour *et al*., *(*2012); Sharifi (2014); and Abdella *et al*. (2015) were reported Positive and signification phenotypic relationship between number of pods per plant and traits: plant height, seed yield per plot, number of seed per plant and thousand seed weight. Abdelmula *et al*. (2007) and Oral *et al. (*2011) found positive and highly significant association between harvest index and seed yield per plot.

The report from different authors suggested days to flowering had positive and highly significant phenotypic association with plant height, seed yield (Alghamdi, 2007; Bakhiet *et al*, 2015 and Sharifi, 2015). Tafere *et al*. (2013) found positive and highly significant phenotypic relationship between days to flowering and days to maturity. Tafere *et al*., (2013) and Alghamdi (2007) reported positive and significant phenotypic association between days to maturity and number of branches per main stem, and thousand seed weight.

3.13. Estimation of Genetic Progress from Past Breeding

Understanding the magnitude of genetic progress in the past breeding efforts plays a key role for breeders in evaluating the amount of gain obtained via selecting for desirable traits and this could assist the breeder in setting criteria to be used in future faba bean breeding scheme particularly. The higher expected genetic progress from selection will be expected in the presence of higher level of genetic variability, heritability and selection intensity for a given trait in a given environment (Singh, 1990). Breeders may seek to know the level of genetic advance from a single cycle or a series of selection cycles made at a time. For this case, genetic progress under selection is simply measured as the difference between the mean genotypic values of the selected population over the mean genotypic values of the original population (Allard, 1960).

Different methods may be used to estimate genetic progress realized from a past cycles of selection from breeding efforts made over time. Even though accurate estimation of genetic progress made over time via breeding efforts is a difficult task, various procedures may be used for estimation of genetic gain. Cox *et al.* (1988) regressed the performance of cultivars in common environments over years of release as a continuous quantitative variable provided the most direct estimate of genetic progress from breeding and has widely been used in different crops.

Comparison of cultivars released in different periods can show an interesting light on the evolutionary trends of agro-morphological characteristics of faba beans grown in Ethiopia thereby provide the most direct estimates of breeding progresses. Different studies indicated direct estimation of genetic gain from yield and other yield related traits. Tamene *et al. (*2012) reported an annual rate of 8.74 $kgha^{-1}$ yr^{-1} (0.31%) and cumulative genetic progress of 288.4

Kg ha^{-1} in their study of genetic progress of faba bean genotypes in Ethiopia. The genetic progress was estimated on different crops like 0.83% relative gain in field pea (Teshome, 2011), 1.34% relative gain in barley (Wondimu, 2010) and 1.27% relative gain in soybean (Demisew, 2010) in Ethiopia. In other countries, the genetic gain report of 66 $kgha^{-1}yr^{-1}$ and 30 $kgha^{-1}yr^{-1}$ from maize breeding in USA (Duvick, 2005) and West Africa (Badu-apraku *et al*., 2014), respectively, and increased yield rate of 35.1 $kgha^{-1}yr^{-1}$ (0·88%) from wheat breeding in Spain (Sanchez-garcia *et al*., 2012).

3.14. Marker Systems and their Applications in Genetic Diversity Study

Different marker system such as morphological, biochemical and molecular markers are used in detection and analysis of genetic diversity. Therefore, each of these markers is detailed as follow: -

3.14.1. Morphological markers

Morphological marker systems are the earliest, simple and inexpensive genetic markers which lie on phenotypic appearance (Vos *et al*., 1995). It is the oldest method and considered as the first step in description and classification of germplasm (Hedrick, 2005). Its estimations are more dependent on environment and are more subjective than other measurements (Li *et al.*, 2009). However, morphological traits that exhibit continuous variation between individuals in a population often obscure the evaluation of genetic diversity. Thus, the very plastic nature of morphological traits in turn needs extra time and resources for evaluation in the field and greenhouse (Bretting and Widrlechner, 1995). Moreover, pleiotropism and a multi-factorial basis to morphological traits further conceal the characterization of the plant populations (Rahman *et al*., 2010).

3.14.2. Biochemical markers

Biochemical markers are protein based polymorphism markers. The most commonly used biochemical marker systems for study of genetic diversity and relationships in faba bean species are isozyme (Kaser and Steiner, 1983) and SDS PAGE (Sultana and Ghafoor, 2008). SDS-PAGE is useful tool for identifying genetic diversity of wild and cultivated plant species (Asghar *et al.,* 2004; Farshadfar and Farshadfar, 2008). SDS gel electrophoresis allows the separation of proteins in denatured condition. Migration of polypeptides is based on the molecular weight. Sodium dodecyl sulfate is an anionic detergent that denatures protein by wrapping around the polypeptide backbone. In doing so, SDS confers a negative charge to the polypeptide in proportion to its length. As a result, polypeptides become rod of negative charge with equal densities or charge per unit length.

Sultana and Ghafoor (2008) concluded for seed proteins, SDS-PAGE on 9.4% acrylamide in large gel based on seed protein could be used as a useful tool for intra-specific diversity study in crop species. SDS-PAGE based on seed storage proteins has proven to be a simple and effective method for distinguishing the cultivars of the largely cross-fertilized pasture grasses and legumes despite their high innate genetic variability (Gardiner and Forde, 1988; Sammour, 1988; Radovic and Vapa, 1996). Variation in number of bands, width and intensity of bands were observed in different peas (*Lathyrus sativus*) of same geographical origin (Przybylska *et al*., 1999; Dellagatta *et al*., 2002).

3.14.3. Molecular markers

DNA-based molecular markers have several advantages over the conventional phenotypic markers since their presence is not dependent on the growth stage of the crop and can be found in all tissues (Mondini *et al*., 2009). Molecular markers are selectively neutral because they are usually located in non-coding regions of DNA (Collard *et al.*, 2005). It is a sequence of DNA or a gene, which is situated on a chromosome (Schulmann, 2007). Bagali *et al.* (2010) suggested DNA sequence as the fragments carrying genes responsible for a trait. Beside; molecular techniques make it possible to analyze numerous characters, which are also independent, whereas morphological analysis provides fewer characters, often of dubious homology. Thus, the molecular approach is more objective than morphological approaches. Hence, molecular marker technologies are the most advanced and, possibly, the most effective means for understanding the basis of genetic diversity. They are efficient and accurate tools with which genetic variation can ultimately be identified and assessed in a rapid and thorough manner.

Several molecular marker systems have been developed and are applied to a range of crop species. These includes; Randomly Amplified Polymorphic DNA (RAPD) (El-Danasoury *et al*., 2008; Basheer-Salimia *et al.*, 2013), Amplified Fragment Length Polymorphism (AFLP) (Zeid *et al*., 2003; Zong *et al.,* 2009; Duc *et al*., 2010) and Simple Sequence Repeats (SSR) (Zeid *et al*., 2009; Kaur *et al*., 2012). The main limitations of these methods are low reproducibility of RAPD, high cost of AFLP analysis and the necessity to know the flanking sequences to develop species specific primers for SSR polymorphism (Jabbarzadeh *et al*., 2010). An alternative to these molecular markers is Inter-simple sequence repeat (ISSR) which is a method that overcomes most of technical limitations that the above-mentioned techniques have (Chen *et al*., 2008; Terzopoulos and Bebeli, 2008). ISSR is fast, simple and cost-efficient as well as it does not require any prior knowledge about the sequences to be amplified, and thus it is a tremendously useful molecular marker in genetic diversity, phylogeny, genomics and evolutionary studies (Aguilera *et al*., 2011). ISSR analysis has been successfully documented to determine genetic diversity and relationships in numerous economic legume species such as cowpea (Ajebade *et al*., 2000), common bean (Galvan *et al*., 2003; Gonzales *et al*., 2005, Sadeghi and Cheghamirza, 2012), lentil (Edossa *et al.*, 2007; Joshi, 2013), chickpea (Sudupak, 2004; Bhagyawant and Srivastava, 2008), mungbean (Singh *et al.*, 2013), in addition to faba bean (Terzopoulosa and Bebeli, 2008). ISSR markers also used to study genetic diversity of crops like coffee (Esayas *et al*., 2005; Solomon, 2007; Kassahun *et al*., 2014), linseed (Jana *et al*.,2012; Ziarovska *et al*., 2012), sesame (Parsaeian *et al*., 2011; Dagmawi *et al*., 2015), rice (Gezahagn *et al.*, 2010) and barley (Varshney *et al*., 2007). Sofalian *et al.* (2008) also reported that ISSR markers are efficient tools for estimating intra-specific genetic diversity in wheat and these molecular markers could differentiate the local varieties collected from different regions. Besides, this marker could be efficiently used to evaluate genetic variation in the wheat germplasm (Sofalian *et al.*, 2009). Hence, Song *et al.* (2002) and Fahmy *et al.* (2008) concluded that, ISSR markers could be used as highly informative markers for genome mapping and gene tagging because the evolutionary rate of change within microsatellites is considerably higher than many other types of DNA markers.

Single nucleotide polymorphism (SNPs) is the third-generation molecular markers and defined as 'single base pair positions in genomic DNA at which different sequence alternatives (alleles) exist in normal individuals in some population(s), wherein the rarest allele has frequency of 1% or greater' (Brookes, 1999). Based on suggested definition, SNPs can be bi-, tri- or tetra-allelic polymorphisms and excludes single base insertion/deletion variants (indels). Yong-Jin *et al*., (2009) reported the frequency of SNP occurrence in genome is one in every 100-3000bp. Single nucleotide polymorphisms (SNPs) represent the most common type of sequence polymorphism found in plant and animal genomes. They are distributed abundantly throughout plant genome and can be found in coding as well as non-coding region (Rafalski, 2002) and now become the most promising molecular markers for DNA fingerprinting and database construction in the genome studies.

SNPs have several advantages over others molecular markers. As pointed out by Kim *et al*. (2010), a wide application of SNPs includes marker assisted and genomic selection, association and QTL mapping, positional cloning, haplotype and pedigree analysis, seed purity testing, varietal identification and monitoring the combinations of alleles that perform well in target environments. Despite of these advantages, the study conducted by Schlötterer (2004) showed that SNPs genetic markers systems suffer a few limitation, for example; they are usually biallelic, so the information content of a single SNP is limited particularly if one of the two alleles occurs at a low frequency, the development of a set of SNP markers is time consuming and the cost is intensive; also SNPs might be located at the hyper mutable sites which violets the assumption that they are bi-allelic often made when analyzing SNPs for population genetics purposes

A variety of SNP genotyping platforms are available for studying of plant genomes. The high-throughput GoldenGate (Fan *et al*. 2003) and Infinium platforms (Steemers and Gunderson, 2007), TaqMan by Life Technologies (Livak *et al*. 1995), and the KASPar platform (KBiosciences' Competitive Allele-Specific PCR system) are the most important SNP genotyping platform. When a relatively small number (i.e. dozens) of SNP loci are to be assayed, a relatively flexible platform such as TaqMan, Sequenom, or KASPar is recommended; when more than 100 loci are available, the high-throughput GoldenGate or higher-throughput Infinium platform should be chosen (Deulvot *et al*., 2010). For DNA fingerprinting and database construction, platforms based on chip technology, such as

GoldenGate and Infinium, are appropriate choice. Here in the present study, the Kompetitive Allele Specific PCR (KASP) was utilized. KASP genotyping assays based on competitive allele-specific PCR and enable bi-allelic scoring of SNP and insertions and deletions (Indels) at specific loci. The Kompetitive Allele Specific PCR genotyping system (KASP) is a homogeneous, fluorescent, and endpoint genotyping technology. It offers the simplest, most cost-effective and flexible way to determine both SNP and insertion/deletion genotypes. The KASP genotyping system has been used successfully in a wide variety of organisms, achieving well over 90% SNP to assay conversion rate.

4. MATERIALS AND METHODS

4.1. Genetic Diversity Study of Ethiopian Faba Bean Varieties Using Phenotypic Markers

4.1.1. Description of the study area

The experiment was conducted under field conditions at three locations (Sinana, Agarfa and Selka) in South Eastern Ethiopia for one year during the main (*bona*) cropping season of 2014/2015 (Figure 1).

Sinana is located at 463 km away from Addis Ababa in South Eastern Ethiopia, 33 and 50 km from the nearby towns, Robe and Goba, respectively. It is geographically situated at 07^{o} 07' N latitude and 40^{o} 10’E longitude. The elevation of Sinana is 2400 meters above sea level (m.a.s.l) with topography of gentle slope to plain with a bimodal rainfall pattern receiving an average annual rainfall of 860 mm. The soil type of the testing site at Sinana is pellic vertisol with a slightly acidic soil (pH = 6.2) (SARC, 2005).

Agarfa is located 60 km from Sinana and 450 km from Addis Ababa in South Eastern Ethiopia at a geographical position of 07^{o}15’44’’N latitude and 039^{o}50’38’’E longitude. The elevation of the site is 2509 m.a.s.l with bimodal rainfall, receiving an average annual rainfall of 860 mm, and a soil type of pellic vertisol having a slight acidity (pH = 6.3) (SARC, 2005).

Selka is situated 7 km from Sinana and 456 km from Addis Ababa in South Eastern Ethiopia at a geographical location of 07^{o}04’28’’N latitude and 040^{o}12’18’’E longitude. The elevation of the site is 2455 m.a.s.l with bimodal rainfall, and a soil type of pellic vertisol having a slight acidity (pH = 6.5) (SARC, 2005).

Table1. Description of the test locations for geographical position and physico-chemical properties of the soils

	Location		
Parameter	Sinana	Agarfa	Selka
	Geographical position		
Latitude	07^{o} 07’ N	07^{o}15’44’’N	07^{o}04’28’’N
Longitude	40^{o} 10’00’’E	039^{o}50’38’’E	040^{o}12’18’’E
Altitude (m.a.s.l.)	2400	2509	2455
	Soil property		
pH	6.2	6.3	6.5
Texture	Clay	Clay loam	Clay
OMC (%)	3.9	3.4	3.2
Total N (%)	0.24	0.2	0.23
Pav(ppm)	30.4	32.41	41-80
K(mg/Kg)	240	572	475
CEC(meq/Kg)	64.4	71.5	35
Moisture Regime	SH2	SH2	SH2

Key: OMC = Organic matter content, N = Nitrogen, Pav = Phosphorus availability, K = Potasium, CEC = Cation exchange capacity, SH2 = Sub-humid tepid to cool sub-humid mid-highlands.

Source: SARC, 2005

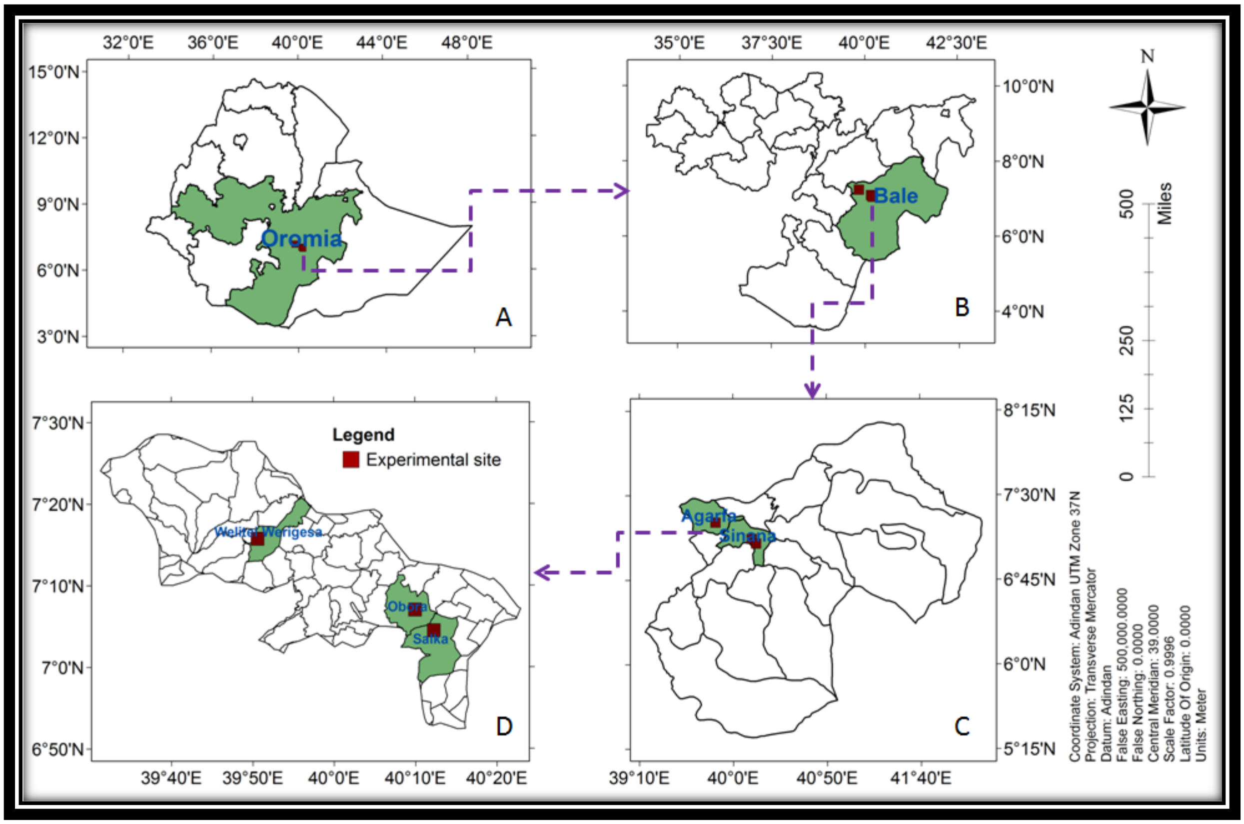


Figure 1: Map of Ethiopia showing the experimental sites.

A = Map of Ethiopia, B = Map of Oromia Region, C = Map of Bale Zone and D = Map of experimental site

4.1.2. Plant materials

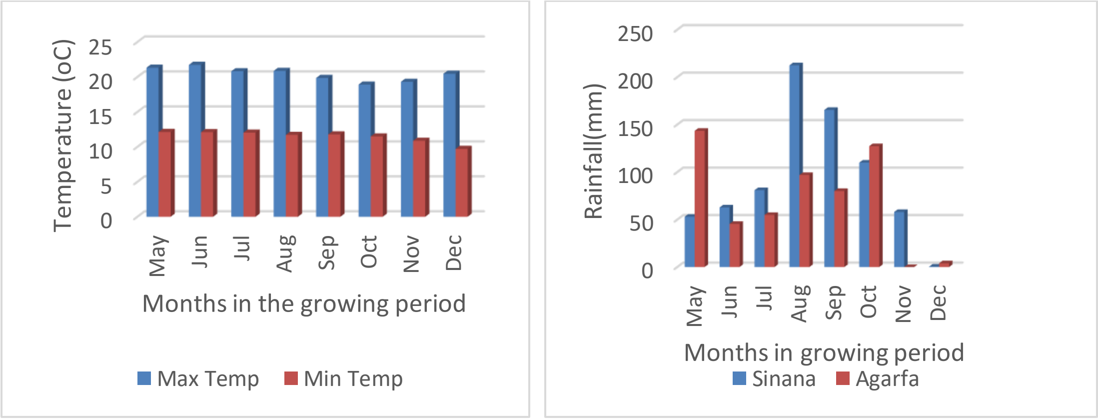
A total of 32 faba bean genotypes were evaluated over three locations during 2014/2015 *Bona* (*meher*) (August to December) main cropping season in three potential production areas of Bale (Sinana, Agarfa and Selka), Ethiopia (Table 2). These genotypes were collected from different national and regional research centers such as Holeta Agricultural Research Center (HARC), Adet Agricultural Research Center (AARC), Debre Birhan Agricultural Research Center (DzARC), Haramaya University (HU) and Sinana Agricultural Research Center (SARC).

Table 2. Description of faba bean genotypes in Ethiopia used in the present study

S/N	Genotype name	Pedigree	Year of Release	Maintaining Center	S/N	Genotype Name	Pedigree	Year of Release	Maintaining Center
1	Mosisa	EH99077	2013	SARC/OARI	17	Tesfa	75TA2626-1-2-1	1995/96	HARC/EIAR
2	Tumsa	EH99051-3	2010	HARC/EIAR	18	Mesay	74A12050x74TA236	1995/96	HARC /EIAR
3	Hachalu	EH00102-4-1	2010	HARC/EIAR	19	Bulga 70	Coll 111/77	1994/95	HARC/EIAR
4	Dosha	COLL 155/00-3	2009	HARC/EIAR	20	Kassa	-	1980	HARC/EIAR
5	Gachena	ETH91001-13-2	2008	HU	21	Gora	EH91026-8-2xBPL44-1	2013	KARC/EIAR
6	Walki	EH96049-2	2008	HARC/EIAR	22	CS-20-DK	CS-20-DK	1978	HARC/EIAR
7	Obse	EH95073-1	2007	HARC/EIAR	23	Kuse	Kuse 2-27-33	1979	HARC/EIAR
8	Moti	EH95078-6	2006	HARC/EIAR	24	Dida'a	ICB2717-1xR-878-3	2014	KARC/EIAR
9	Gabelcho	EH96009-1	2006	HARC/EIAR	25	Lalo	SELALE KASIM 89-4	2002	DARC/ARARI
10	Adet Hanna	PGRC/E25041-2-2	2005	ADARC/ARARI	26	Bako local	Local Variety	-	Bako
11	NC-58	SELALE KASIM 91-13	2002	HARC/EIAR	27	Debrebirhan local	Local Variety	-	Debrebirhan
12	Wayu	WAYU 89-5	2002	HARC/EIAR	28	Sinana local	Local Variety	-	Sinana
13	Degaga	R-878-3	2002	HARC/EIAR	29	Agarfa local	Local Variety	-	Agarfa
14	Dagm	GRARJARSO 89-8	2002	DARC/ARARI	30	Candidate variety	EKCSR-02006	-	SARC/OARI
15	Holetta-2	BP 1802-1-2	2002	DARC/ARARI	31	Adet local	Local Variety	-	Adet
16	Shalo	EH011-22-1	1999	SARC/OARI	32	Kulumsa local	Local variety	-	Kulumsa
Additional promising pipeline used for SNP marker Genotyping									
33	Pipeline	EH06070-3	-	HARC/EIAR	42	Pipeline	EK05001-1	-	HARC/EIAR
34	Pipeline	EH06031-3	-	HARC/EIAR	43	Pipeline	EK05023-1	-	HARC/EIAR
35	Pipeline	EK05006-3	-	HARC/EIAR	44	Pipeline	EH06027-2	-	HARC/EIAR
36	Pipeline	EH07015-7	-	HARC/EIAR	45	Pipeline	EK05037-5	-	HARC/EIAR
37	Pipeline	EH06084-6	-	HARC/EIAR	46	Pipeline	EH00126-1	-	HARC/EIAR
38	Pipeline	EH06006-2	-	HARC/EIAR	47	Pipeline	EKLS/CSR02022-1-1	-	SARC/OARI
39	Pipeline	EH05024-3	-	HARC/EIAR	48	Pipeline	EH00126-7	-	SARC/OARI
40	Pipeline	EK05001-1	-	HARC/EIAR					
41	Pipeline	EK05024-3	-	HARC/EIAR					

4.1.3. Climates conditions at experimental locations

The rainfall distribution and temperature data were collected for Sinana and Agarfa locations, but for Selka due to unavailability of meteorological station, these data were not collected. The average monthly and annual rainfall data for twenty-five (25) years (from 1990-2014) for Sinana and Agarfa are presented in Figure 3 and Appendix 1. During the growing season, the daily maximum and minimum temperatures ranged from 18.9 ^{o}C to 21.73 ^{o}C and 9.71 ^{o}C to 12.10 ^{o}C at Sinana, respectively (Figure 2 A). A total rainfall of 743.3 mm and 552.3 mm was received during the crop growing period at Sinana and Agarfa, respectively (Figure 2 B). The monthly average, minimum and maximum temperature values of Sinana for twenty-five years are given in the Appendix 2, 3, and 4, respectively.



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A. B.

Figure 2. A). Mean maximum and minimum monthly temperature at Sinana during crop growing period and B). Rainfall distribution at Agarfa and Sinana during the crop growing period.

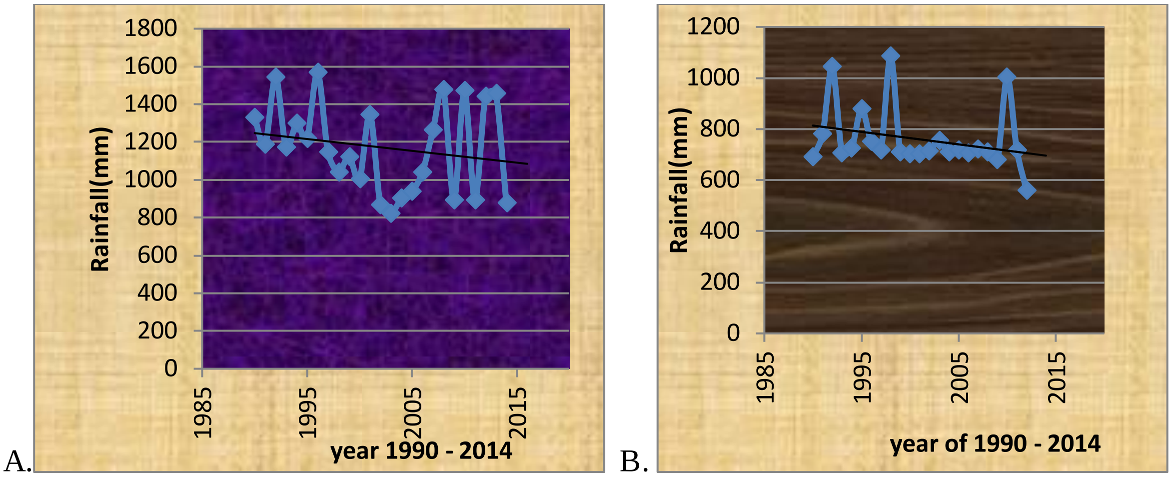


Figure 3: A). Rainfall distribution at SARC from 1990-2014 and B). Rainfall distribution at Agarfa from 1990-2014.

4.1.4. Experimental design and field management

The field experiment was laid out using Alpha design with two replications of four blocks having eight treatments in each block as suggested by Patterson and Williams (1976). The plot size was 3.2 m^2 (2 rows of 4m long) with a row to row spacing of 40 cm and plant to plant spacing of 10 cm. The genotypes were assigned to plots randomly within each replication. Fertilizer was appllied at the rate of 18/46 kg/ha N/P_2O_5 or 100 kg DAP at planting. All other crop management and protection practices were applied uniformly to all genotypes as recommended for each area. Data were collected either on plant basis for some characters (i.e. from five random plants) or plot basis according to descriptors of IBPGR and ICARDA (1985) as follows:

a) **Leaf length (LL) (cm):** Length from the base to the tip of the leaf. Three leaves from top, middle and bottom of the plant were taken and averaged.

b) **Leaf width (LW) (cm):** Width across the leaf at its widest point of the central leaflet of the trifoliolate leaf. Three leaves from top, middle and bottom of the plant were taken and averaged.

c) **Leaf area (LA) (cm^2):** This was calculated by leaf area estimation model as described by Peksen (2007) and three leaves per plant were measured and averaged

$$\mathrm{LA} = 0.919 + 0.682\mathrm{LW}$$

Where **L**= leaf length and **W** = leaf width

d) **Leaf area index** (LAI): This was calculated by the formula

$$\mathrm{LAI} = \frac{\text{Leaf Area x Number of leaf per plant}}{\text{Area covered by plant}}$$

e) **Number of pods per plant (NP/PL):** The average numbers of pods counted from samples of five plants taken randomly from each plot.

f) **Numbers of seeds per pod (NS/P):** The total number of seeds per plant divided by the total number of pods on the same plant and averaged over five plants taken randomly from each plot.

g) **Plant height (PH) (cm):** The average height of five plants taken randomly from each plot measured at physiological maturity.

h) **Height to the first poding node (HFPN) (cm):** The average height from ground to the first pod bearing nodes of five randomly selected plants from plots measured at physiological maturity.

i) **Pod length (PL):** Exterior distance of fully matured pod from the pod apex to the peduncle as measured in centimeters. Three pods from top, middle and bottom was taken and averaged.

j) **Pod width (PW)**: Three pods per plant of five randomly selected plants were measured at the center of pod using a caliper.

k) **Internode length (IL)**: Three internodes per plant of five randomly selected plants were measured and averaged.

l) **Number of branches per plant (NB/PL):** Numbers of branches per plant was counted from basal and mediated nodes.

Data collected on plot basis and their descriptions are indicated as follow: -

a) **Days to flowering (DTF**): Number of days from planting to 50% of plants bears flower

b) **Days to maturity (DTM**): The number of days from sowing to the stage when 90% of the plants in a plot have reached physiological maturity.

c) **Seed filling period (SFP):** The number of days from flowering to maturity (i.e. the number of days to maturity minus the number of days to flowering).

d) **Thousand seed weight (TSW)(g):** The weight of thousand seeds taken randomly from the harvest seed lots of each plot

e) **Seed yield per plot (SY/P):** Seed yield (g) from the specified harvestable plot area and adjusted to its recommended (10%) moisture content. This value was converted to Kg/ha and used for analysis.

f) **Seed production efficiency (SPE**): Seed filling duration divided by duration of vegetative period and then multiplied by grain yield

g) **Biomass weight per plot (BMPP in g/m^2):** Whole above ground plants parts on the plot where harvested, sun dried and weighted.

h) **Harvest index (HI):** Manfred (1993) and Rkmhay (1995) defined harvest index as the ratio of grain yield to above ground biological yield.

i) **Economic growth rate (EGR**): It is the ratio of seed weight per plant to seed filling duration and then multiplied by 100.

j) **Protein content (%)**. It is measured based on Micro-Kjeldahl technique (AOAC, 1970).

4.1.5. Data analysis

The data were subjected to statistical analysis using SAS version 9.1.3 (SAS Institute, 2003), Genstat (Rayne *et al*., 2012) and MINTAB version 14 computer packages (2003). Mean separation was made at 1% and 5% probability level using the LSD method.

4.1.5.1. Analysis of variance

Determination of the magnitude of reduction in experimental error by using alpha design over RCBD design was computed by employing relative efficiency estimation from both design and the computed relative efficiency indicated nearly similar precision (R. E = 1.02). Therefore, analysis of variance was done based on RCBD design linear additive model for the case of simpility as follow: -

$$\mathbf{P_{ij} = \mu + \pi_{(j)} + \tau_i + \xi_{ij}}$$

Where

$\mathbf{P_{ij}}$ = Phenotypic value of ith treatment under jth replication

$\boldsymbol{\mu}$ = Grand mean

$\boldsymbol{\pi_j}$ = The effect of replication j

$\boldsymbol{\tau_I}$ = The effect of ith treatments

$\boldsymbol{\xi}$ = experimental error (Pooled error)

Table 3. Analysis of variance model for RCBD design

A. For individual locations

Source of Variation	Degree of Freedom	Sum Square of Total	Mean Square	Expected Mean
Replication	r-1	SSr	MSr	$\sigma^2_e + g\sigma^2\ r$
Treatment	t-1	SSt	MSt	$\sigma^2_e + r\sigma^2_g$
Residual	(r-1) (t-1)	SSe	MSe	σ^2_e
Total	tr-1	-	-	-

Where r = number of replication; t = number of treatment; SSr = sum square of replication, SSt = sum square of treatment; SSe = sum square of error; MSr = mean square due to replication; MSt = mean square due to treatment;

σ^2_e = environmental variance; σ^2_g = genotypic variance

Before computing the combined analysis, error variance homogeneity test was verified using Hartley`s test (F-max test) (Gomez and Gomez, 1984). In the combined analysis of variance, locations were considered as random variable and genotypes were considered as fixed variable. The combined analysis of quantitative trait data in this experiment was done by using the following linear Additive model:

$$\mathbf{P_{ijs} = \mu + L_s + (\tau x l)_{is} + \pi_{j(s)} + \tau_i + \xi_{ijs}}$$

$\mathbf{P_{ijs}}$ = phenotypic value of i^th^ treatment variety under j^th^ replication at s^th^ location, $\boldsymbol{\mu}$ = grand mean; $\boldsymbol{\tau}_i$ = the effect of i^th^ treatment; $\boldsymbol{\pi}_{j(s)}$ = the effect of replication j within location s; $\mathbf{L}_s$ = the effect of location; $(\boldsymbol{\tau}\mathbf{xl})_{is}$ = the interaction effects between treatment and location; and ξ_{ijs} = pooled error.

B. Combined over location

Source of Variation	Degree of Freedom	Sum Square of Total	Mean Square	Expected Mean
Location	l-1	-	-	-
Replication within location	l(r-1)	SSrg	MSrl	$\sigma^2_e + gl\sigma^2\ r$
Genotype	t-1	SSg	MSt	$\sigma^2 e + r\sigma^2 gl + rl\sigma^2 g$
Genotype x Location	(l-1) (t-1)	SSgl	MStl	$\sigma^2_e + r\sigma^2_{gl}$
Residuals	l(t-1) (r-1)	SSE	MSe	σ^2_e
Total	tr$l$-1	SSt		

Where **r** = number of replication; **b** = number of blocks; **t** = number of treatment; **l** = location; **SSr** = sum square of replication, **SSt** = sum square of treatment; **SSe** = sum square of error; **MSr** = mean square due to replication; **MSt** = mean square due to treatment; **MStl** = mean square due to treatment by location; **MSe** = mean square due to error; $\boldsymbol{\sigma^2_e}$ = environmental variance; $\boldsymbol{\sigma^2_g}$ = genotypic variance; $\boldsymbol{\sigma^2_{gl}}$ = variance for treatment by location interaction;

4.1.5.2. Analysis of covariance

Covariance simply indicates whether two related characters tend to vary together (i.e. variation occurring simultaneously in two variables or characters). The analysis of covariance between all possible pairs followed the same form as the variance analysis (Table 4). Mean product expectation of the covariance analysis is analogous to the mean square expectation of the analysis of variances. Thus, estimate of genotypic and phenotypic covariance component between two traits (σg_{xy}, σp_{xy}) were derived in the same fashion as those for the corresponding variance components. Analysis of covariance (ANCOVA) was done by using SAS computer software (SAS, 2003).

Phenotypic and genotypic covariance analysis for the different traits was computed manually from ANCOVA table following the formula suggested by Singh (2007) as follows.

$$\boldsymbol{Covgxy = \frac{MSPgxy - MSPglxy}{lr}}$$

Where, Covgxy = genotypic covariance between traits x and y; MSPgxy = mean sum of product of genotype for traits x and y***;*** MSPglxy = mean sum of product of genotype by environment interaction for traits x and y***;*** r = number of replications and l = location

Table 4. Analysis of covariance model

Source of Variation	**Degree of Freedom**	**Mean Square Product**	**Expected Mean**
Location	l-1	-	-
Replication within location	l(r-1)	MSPrlxy	$\sigma^2_{exy} + gl\sigma^2\ rxy$
Genotype	t-1	MSPtxy	$\sigma^2 e\ xy + r\sigma^2 glxy + rl\sigma^2 gxy$
Genotype x Location	(*l*-1) (t-1)	MSPtlxy	$\sigma^2_{exy} + r\sigma^2_{glxy}$
Residuals	l(t-1) (r-1)	MSPexy	σ^2_{exy}

4.1.6. Estimation of phenotypic and genotypic variation

Estimation of environmental, genotypic and phenotypic variance components and their coefficients of variation were done based on the methods stated by Singh and Chaudhary (1977) and Sharma (1998).

i. For individual location

Environmental variance (σ^2e) = Mean square of error (MSE from Table 3)

Genetic variance (σ^2g) = $\boldsymbol{\frac{GMS-MSE}{r}}$

Phenotypic variance (σ^2p) = σ^2g + σ^2e

Where, GMS = mean square due to genotype, r = replication

ii. For combined location

$$\textbf{Genetic variance } (\sigma^2 g) = \frac{MSG - MSgl}{rl}$$

$$\textbf{Phenotypic variance } (\sigma^2 p) = \sigma^2 g + (\sigma^2 gl)/l + \sigma^2 e/rl$$

Where, $\sigma^2 gl$ = mean square due to genotype by environment interaction, **l** = location,

$\delta^2 e$ = combined error mean square

The phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV) were estimated as percentage of the corresponding phenotypic standard deviation (σ_p), genotypic standard deviation (σ_g) of the trait and dividing this value to μ (the grand populations mean for the trait). Hence,

$$\textbf{Phenotypic coefficient of variation (PCV)} = \frac{\sigma p \times 100}{\mu}$$

$$\textbf{Genotypic coefficient of variation (GCV)} = \frac{\sigma g \times 100}{\mu}$$

4.1.7. Broad sense heritability (Hb^2)

Heritability in broad sense for all traits was computed by adopting the formulae presented by Allard (1960).

$$\textbf{Heritability } (Hb^2) = \frac{\sigma 2g \times 100}{\sigma 2p}$$

4.1.8. Expected genetic advance from selection (GA)

Genetic advance for all characters was computed by adopting on the formulae presented by Allard (1960) and GA as percentage of the mean expected from selection of the best 5% of the genotypes were estimated as: -

$$\textbf{Expected genetic advance (GA)} = H^2 \times k \times \sigma p$$

$$\textbf{Expected genetic advance as percentage of mean} = \frac{(\mathrm{GA} \times 100)}{\mu}$$

Where, k is a constant value at selection intensity of 5% (k = 2.06), σ_p is the phenotypic standard deviation; H^2 is broad sense heritability ratio; and μ is the grand populations mean for the trait under considerations.

4.1.9. Estimation of phenotypic and genotypic correlation coefficient

Correlation coefficient measures the mutual relationship between various traits and determines the component character on which selection can be based for improvement in yield **(**Singh, 2007**).** The phenotypic and genotypic coefficients of correlation of the traits measured in the study were estimated using Pearson Simple Correlation which deals with association between any two traits. Phenotypic correlation is the observable correlation between two variables, which include both genotypic and environmental effects, and genotypic correlation is the inherent association between two variables.

Phenotypic and genotypic coefficients of correlation between two traits were determined by using the variance and covariance components (Singh, 2007; Sharma, 1998; Singh and Chaudhary, 1977)

$$r_{Pxy} = \frac{Covp(x,y)}{\sqrt{(\sigma 2px * \sigma 2py}}$$

Where

$Cov_{p\,(x,\,y)}$ **=** Phenotypic covariance between traits X and Y

r_{Pxy} = Phenotypic correlation coefficient between traits X and Y

$\sigma^2 p_x$ **=** Phenotypic variance of trait X

$\sigma^2 p_y$ **=** Phenotypic variance of trait Y

$$r\, g_{xy} = \frac{Covg(x,y)}{\sqrt{(\sigma 2gx * \sigma 2gy}}$$

$\mathrm{Cov}_{g\,(x,\,y)}$ = Genotypic covariance between traits X and Y

$r\mathbf{g}_{xy}$ = Genotypic correlation coefficient between traits X and Y

$\sigma^2 g_x$ **=** Genotypic variance of trait X

$\sigma^2 g_y$ **=** Genotypic variance of trait Y

Phenotypic and genotypic correlation coefficients were tested for their significance using the formula suggested by Robertson (1959), using the t- table at (g-2) degrees of at 5% and 1% level of significance; g is the number of genotypes used in the study.

$$t = \frac{\mathrm{rp}xy}{SEpxy} \text{ and } t = \frac{\mathrm{rg}xy}{SEgxy} \text{ respectively.}$$

Where $SEpxy$ and SE_{gxy} = Standard error for phenotypic and genotypic correlation respectively

$$SEpxy = \sqrt{\frac{(1-rpxy)2}{2Hx*Hy}} \text{ and } SE_{gxy} = \sqrt{\frac{(1-rgxy)2}{2Hx*Hy}}$$

Where H_x and H_y are Heritability estimate for trait x and y

4.1.10. Multivariate analysis

4.1.10.1. Cluster and distance analysis

Cluster analysis was made using SAS Software version 9.1.3 (SAS Institute, 2003). Principal component analysis (PCA) was made using the MINTAB statistical computer package, version 14.00 (MINITAB, 2003). According to Sneath and Sokal (1973) records for all traits were pre-standardized to means of zero and variance of unit before clustering and principal component analysis to avoid bias due to differences in measurement scales. Unweighted Pair Group Methods with Arithmetic-average (UPGMA) based on the generalized D^2 distances by average linkage method of agglomerative hierarchical clustering (the two closest clusters are merged into a single new cluster) built to group the faba bean genotypes into genetically distinct classes based on phenotypic traits. Appropriate number of clusters was determined by using points where local peaks of Pseudo F-statistics join with small values of the Pseudo t^2 statistics followed by a larger Pseudo t^2 for the next cluster fusion.

Genetic distance between pair of clusters was calculated using the generalized Mahalanobis's D^2 statistics based on the recommendation of Singh and Chaudhary (1996). The D^2 statistics measures the force of differentiation at intracluster and intercluster levels (Singh, 2007). Hence, genetic distance between cluster as standardized Mahalanobis's D^2 statistics (Mahalanobis, 1936) were calculated as follow: -

$$\mathbf{D\,^{2}ij = (X_{i} - X_{j})\,cov^{-1}\,(X_{i} - X_{j})}$$

Where, D^2_{ij} is distance between class i and j

X_i and X_j are the vector means of the traits for the i^{th} and j^{th} groups

$cov^{-1} =$ the inverse of pooled error variance and covariance matrix.

The D^2 value obtained for pairs of clusters was considered as the calculated value of Chi-squire (χ^2) and was tested for significance at 5% and 1% level of probability against the tabulated values of χ^2 for p degrees of freedom, where p is the number of characters considered.

4.1.11. Comparison of the performance of selected genotypes

In order to make comparison of 10 % of the best performed genotypes within the whole genotypes utilized in the present study, the materials were sorted and means were independently computed for each character. The absolute value of Student’s t test was calculated to compare genotypic values of the 10% best selected genotypes with the base population as:

$$t = \frac{\bar{x} - \mu}{\sigma/\sqrt{n}}$$

Where $\bar{x}$ is mean of selected genotypes, μ is mean of the base populations, σ is the standard deviation calculated for the base populations and n is the number of genotypes selected from the base population for better performance.

The significance of the difference between the population parameter (μ) and sample mean ($\bar{x}$) was tested using t table by comparing calculated value of t-test with that of tabulated t value, and considered as significant for greater value of calculated t-test than tabulated values of t-test (Singh, 2001).

4.1.12. Estimation of the magnitude of genetic progress

The combined mean data from all environments were used for estimating the magnitude of genetic progress by applying linear regression model (Cox *et al.*, 1988) as:

$$Y = bx + a$$

Where Y = mean value of the dependent variable, x = mean value of the independent variable, a = the constant, and b = the regression coefficient.

The relative gain achieved over about four decades in seed yield and thousand grain weight, was determined as the percentage ratio of annual genetic gain to the values of the oldest variety. Therefore, the variety released in 1978 was considered as an oldest reference base for the estimation of the sequential changes as a result of genetic progresses.

Annual rate of gain was calculated as follow: -

$$\textbf{The annual rate of gain} = \frac{\textbf{Covxy}}{\textbf{Varx}}$$

Where X = the year of variety release, Y = the mean value of each trait for each variety, Cov_{xy} = Covariance of the year of variety release and mean value of each trait for each variety and Var x = Variance of the year of variety release.

4.2.Genetic Diversity as Revealed by ISSR Marker

4.2.1. Plant materials

A total of 32 faba bean genotypes were considered in this study. The detailed description of test genotypes is given in Table 2. Healthy seeds with identical sizes were visually selected and planted in pots and kept in the Greenhouse at College of Natural Sciences, Addis Ababa University. Watering was done once a day until leaf samples from five representing plants were collected 30 days after planting. The leaves were cleaned using moist tissue paper to remove any dirt adhering to the leaf tissue and placed in plastic zipper bag containing silica gel.

4.2.2. Genomic DNA extraction

Genomic DNA was extracted following a method developed by Borsch *et al.* (2003) which involves a modified CTAB method employing triple extractions to yield optimal quantities of high-quality DNA. Fifty (50) mg of collected dried leaf samples from ten representative plant were bulked and filled in 2 ml labeled Eppendorf-caps containing two autoclaved beads with 5 mm diameter and grinded with Mixer Mill (Retsch GmbH, Germany) for three minutes at frequency of 30 Hz/sec. A total of 700 µl of CTAB solutions (Composed of 10ml (1M Tris-HCl), 40ml (5M NaCl), 4ml (0.5M EDTA), 2g CTAB and 1g PVP) was added to each grinded sample to break open plant cells, solubilize the contents and disrupt the cell, and incubated at 65ºC for 30 minutes in water bath and centrifuged at 13000 rpm for 7 minutes. After taking the supernatant, 600 µl chloroform was added and centrifuged at 13000 rpm for 7 minutes and this step was repeated for better cleanup of the genomic DNA. Cooled (4ºC) Iso-propanol (2/3 of volume) was added to solution and allowed to freeze for 2 hrs at -20ºC and then centrifuged at 13000 rpm for 15 minutes to pour off supernatant. The DNA pellet was washed with 200 µl of 70% ethanol, aspirated and then dried at room temperature for 15 minutes. Finally, the DNA pellet was suspended in 100 µl of TE (1x, p.a. grade), precipitated in salt solution (Ammonium acetate and Sodium acetate) and stored in refrigerator at -20^{o}C.

4.2.3. DNA quantification and quality test

DNA quantification and quality test was done by using gel electrophoresis and Nano-drop spectrophotometer methods.

An agarose gel (50 ml 1 x TBE, 0.5 g Agarose, and 1% ethidium bromide (10 mg/ml)) was prepared using casting tray. Stock solution of 10X Tris Borate EDTA (TBE) (108 g Tris base; 55 g Boric acid; 40 ml (0.5M) EDTA, pH 8.3 components per liter) was prepared and stored at room temperature. From the stock, working solution of 10X TBE prepared and then further diluted to 1x TBE and used to prepare the gel as well as to fill the electrophoresis tank (Bio-rad, Sub-cell®GT). Agarose gel with 1% (0.5 g) was prepared by boiling 1x TBE in 100 ml Erlenmeyer flask in micro oven for 2:30 minutes. After the agarose solution was cooled down at room temperature, 2 µl of ethidium bromide was added for better visualization of the gel. Then it was poured on to the gel tray with the inserted comb and the gel was left for 30 minutes. The comb was carefully removed and put the gel tray into the electrophoresis tank properly filled with electrophoresis buffer (1XTBE). The extracted DNA samples (4 µl), and 2 µl of 6x loading dye was loaded on the gel and electrophoresised at constant voltage of 80 v for 45 minutes. Gel picture was taken under UV light (Gel documentation system, Biosenc SC750) and digitally photographed. Finally, those with high band intensity and fewer smears were selected for PCR amplification out of the first two extraction fraction.

The quantity and qualities of extracted DNA were ao tested using Thermo Scientific NanoDrop spectrophotomer (NanoDrop 2000/2000c) by reading their absorbance at 260 nm wave length. Based on the reading obtained; the quantity and quality of first and second DNA extraction were evaluated, and the second extraction for the most of the samples was selected for PCR amplification based on the absorbance ratio at 260/280 reading.

4.2.4. Primer selection and optimization

A total of twenty-five ISSR primers obtained from the University of British Colombia (primer kit UBC 900) were used for assaying at Plant Genetics Research Laboratory Microbial, Cellular and Molecular Biology Department, Addis Ababa University, Addis Ababa, Ethiopia (Table 5). ISSR primer selection was made by referring published scientific articles by different authors on faba bean (Abdul-Razzak *et al.*, 2012; Merji *et al.*, 2012; Wang *et al.*, 2012; Salazar-Laureles *et al.*, 2014) and other related crops like chickpea (Bhagyawant and Srivastava, 2008), Lentil (Edossa *et al.*, 2007; Meenakshi *et al.*, 2013) and Mung bean (Singh *et al.*, 2013).

The selected primers were tested for their variability and reproducibility by using four genotypes from the whole genotypes used in this study. Dilution of genomic DNA to amount of 70 ng/µl was made for running of PCR to screen primers and optimize the reaction condition. Finally, 11 primers were selected based on their banding pattern, polymorphism and reproducibility for genotyping.

Table 5. Lists of primers, their sequence, repeat motif, Annealing temperature and Nucleotide

	Primer	Sequence from 5’-3’	Repeat motif	Annealing temp.(ºC)	Nucleotide types
1	808	5’AGAGAGAGAGAGAGAGC 3’	(AG)8C	48	Di-nucleotide
2	809	5’AGAGAGAGAGAGAGAGG 3’	(AG)8G	48	Di-nucleotide
3	811	5’GAGAGAGAGAGAGAGAC3’	(GA)8C	48	Di-nucleotide
4	812	5’GAGAGAGAGAGAGAGAG3’	(GA)8G	45	Di-nucleotide
5	813	5’CTCTCTCTCTCTCTCTT3’	(CT)8T	45	Di-nucleotide
6	814	5’CTCTCTCTCTCTCTCTA3’	(CT)8A	45	Di-nucleotide
7	818	5’CACACACACACACACAG3’	(CA)8G	48	Di-nucleotide
8	820	5’GTGTGTGTGTGTGTGTC3’	(GT)8C	48	Di-nucleotide
9	822	5’CTCTCTCTCTCTCTCTA3’	(CT)8A	45	Di-nucleotide
10	824	5’CTCTCTCTCTCTCTCTG3’	(CT)8G	48	Di-nucleotide
11	826	5’ACACACACACACACACC3’	(AC)8C	48	Di-nucleotide
12	835	5’AGAGAGAGAGAGAGAGYC 3’	(AG)8YC	48	Di-nucleotide
13	836	5’AGAGAGAGAGAGAGAGYA 3’	(AG)8YA	45	Di-nucleotide
14	841	5’GAGAGAGAGAGAGAGAG3’	(GA)8YC	53	Di-nucleotide
15	844	5’CTCTCTCTCTCTCTCTRC3’	(CT)8RC	48	Di-nucleotide
16	848	5’CACACACACACACACARG3’	(CA)8RG	48	Di-nucleotide
17	854	5’TCTCTCTCTCTCTCTCRG	(TC)8RG	48	Di-nucleotide
18	857	5’ACACACACACACACACYG3’	(AC)8YG	48	Di-nucleotide
19	860	5’TGTGTGTGTGTGTGTGRA3’	(TG)8RA	45	Di-nucleotide
20	864	5’ATG ATG ATG ATG ATG ATG3’	(ATG)6	45	Tri-nucleotide
21	872	5’GATAGATAGATAGATA3’	(GATA)4	38	Tetra-nucleotide
22	873	5’GACAGACAGACAGACA3’	(GACA)4	45	Tetra-nucleotide
23	878	5’GGAT GGAT GGAT GGAT3’	(GGAT)4	45	Tetra-nucleotide
24	880	5’GGAGA GGAGA GGAGA3’	(GGAGA)3	45	Penta-nucleotide
25	881	5’GGGTG GGGTG GGGTG3’	(GGGTG)3	53	Penta-nucleotide

Source: Primer kit 900 (UBC 900)

4.2.5. PCR amplification

Inter Simple Sequence Repeat (ISSR) PCR amplification was carried out in a total reaction volume of 25 µl containing 16.7 µl double-distilled water, 3.0 µl of $MgCl_2$ (25mM), 2.5 µl of 10x PCR buffer, 1.0 µl of dNTP (100 mM equimolar solution of each dATP, dCTP, dGTP and dTTP), 0.4 µl primer (20 mM), 0.4 µl (5U/ µl) of Taq polymerase (FIREPOL® DNA polymerase, Solis BioDyne, Estonia), and 1 µl of template DNA (70 ng/µl). PCR Amplification reactions were carried out in Biometra Tpersonal, a 48-well thermal cycler (Applied Biosystems, USA). PCR programming for amplifications were set for 40 cycles with an initial denaturing step of 4 minutes at 94 ºC, each cycle consisted of denaturation at 94 ^oC for 15 seconds, followed by primer-specific annealing temperature for 60 seconds and extension at 72 ^oC for 90 seconds. After 40 cycles, last extension step was set for 7 minutes at 72 ^oC and, finally, the PCR was adjusted to store the product for 24 hr at 4 ^oC.

4.2.6. PCR product and ISSR gel electrophoresis

Before loading ISSR gel, the PCR products were checked first on test gel (1% agarose gel (w/v), with 1xTBE) for the presence/absence of PCR products and later samples with ISSR -PCR products were separated on gel with 1.67% agarose gel. The amplified ISSR product of 7 µl was loaded onto ISSR gel with 3 µl loading dye (6x loading dye). DNA ladder (100 bp) was used to estimate fragment size and molecular weight of the amplified product. It was run at constant voltage of 100V for about 3 hours.

4.2.7. Gel staining and visualization

Before photographing the gel, the electrophoresis product was stained using a mixture of 50 µl ethidium bromide (10 mg/ml) and 450 ml distilled water for 30 minutes and de-stained (washed) for 30 minutes using distilled water. After completion of staining, the gels were visualized under UV (Gel Documentation System, Bioscens SC750) connected with camera on computer. The images were taken by camera in built with the gel doc.

4.2.8. Data scoring and analysis

The fragments generated from 11 primers with clearly distinguishable and reproducible bands were selected for scoring. Data scoring was performed as discrete statistics using a binary matrix “0” coded for absence, and “1” for presence of a band. Scored data were directly entered into computer for making analysis by using different software. Data generated were analyzed by using POPGENE version1.32 (Yeh *et al.*, 1999) to investigate and characterize genetic diversity in terms of number of polymorphic loci, percent polymorphism, means of Nei’s genetic diversity and Shannon index.

NTSYS- pc version 2.02 (Rohlf, 2000) and Free Tree 0.9.1.50 (Pavlicek *et al.*, 1999) softwares were used to compute Jaccard's similarity coefficient by using the following formula: -

$$Sij = \frac{a}{a + b + c}$$

Where; 'a' is the total number of bands shared between individuals i and j, 'b' is the total number of bands present in individual i but not in individual j and 'c' is the total number of bands present individual j but not in individual i

NTSYS- pc version 2.02 (Rohlf, 2000) was used to generate cluster analysis based on the Un-Weighted Pair Group Method with Arithmetic mean (UPGMA)) for analyzing and comparing of individual genotypes and standardized genetic distance analysis (Nei, 1972). Neighbor joining (NJ) clustering method was computed by using Free Tree 0.9.1.50 Software (Pavlicek *et al.,* 1999) to compare individual genotypes and evaluate patterns of genetic diversity. Principal coordinated analysis (PCoA) was figured using PAST version 1.18 (Hammer *et al.,* 2001) and STATISTICA version 5.5 (Statsoft Inc, 2001) softwares based on Jaccard's coefficient similarity for further examining of the patterns of variation among individual genotypes. Areliquin version 3.01 (Excoffier *et al., 1992*) was used for analysis of molecular variance (AMOVA) to partition the total variance into among and within populations.

4.3. Genetic Diversity Analysis Using SNP Marker

4.3.1. Plant materials

A total of 48 faba bean genotypes were considered in this study. The detailed description of test genotypes is given above in Table 2. Healthy seeds with identical sizes were visually selected and planted in pots in the Greenhouse, College of Natural Sciences, Addis Ababa University. Watering was done once a day. Young leaf samples from five plants representing individual genotypes were collected at 20 days after planting. The samples were collected according to the LGC genomics (http://www.kbioscience.co.uk/) and packed by plant sample collection kit.

4.3.2. Genomic DNA extraction

Genomic DNA extraction was done using Oktopure extraction kit method at LGC genomics DNA Extraction Department Laboratory, UK. The samples received in 96-well storage racks were centrifuged briefly to ensure the samples at the bottom of the tubes /wells. The seals were removed and a single ball bearing with a diameter of 4 mm was added to each sample. The samples were resealed using heat sealer; disrupted in the Genogrinder for 30 seconds at 1500 rpm and the samples were centrifuged briefly to ensure that all materials were settled at the bottom of the tube/well. The seal was removed and lysis buffer (Buffer L1) was added to each sample using multidrop and resealed again. The plate was vortexed and centrifuged briefly. The seal was removed and binding buffer (buffer C1) was added to each sample using multidrop, then resealed and vortexed to maintain the homogeneity of samples. The plates were incubated for 30 minutes at 55 ^{o}C; mixed gently at regular interval throughout the incubation period and then centrifuged briefly to ensure the lysate at the bottom of the tube/well. An incubated lysate was transferred to deep-well plate containing magbeads and care was taken to avoid transfer of cell debris and mixed until uniformly colored. The deep well plates were incubated for 15 minutes at approximately 55 °C and magnetized silica was pelleted using magnet, drawing all silica to the bottom of the wells. The plates attached to the magnet were shaken gently to release all pellet magbeads from the lid. The supernatant was removed carefully without disturbing. The buffer A1 was added to each sample, then plates were resealed and mixed carefully to maintain uniform colour. The plates were incubated at room temperature for 5 minutes. Magnetized silica was pelleted using magnet by drawing all silica at the bottom of the wells. The supernatant was removed without disturbing the pellet. The wash buffer (buffer W1) was added to each sample, the plated resealed and sample mixed until uniformly coloured. The magnetized silica was pelleted using magnet by drawing all silica to the bottom of the wells. The supernatant was removed without disturbing the pellet and tubes were dried approximately at 55 °C in the oven. The elution buffer (Buffer E1) was added to each tube, the plate resealed and vortexed for 2 minutes, then incubated for 30 minutes at 55 °C. The tubes shaked gently at regular interval; removed from the oven and briefly vortexed. The magnetized silica was pelleted using magnet by drawing all silica to the bottom of the wells. The plates were shaked gently while attached to the magnet to help pellet all magbeads from the lid. The plates were briefly centrifuged to ensure all liquid was removed from the lid. The eluted DNA was transferred into fresh plate, ensuring that the pellet was not disturbed. Finally, a barcoded plate was tracked to a -20 °C.

4.3.3. SNP genotyping

SNP genotyping was conducted at LGC genomics laboratory, UK by using a set of 40 SNPs adapted from previous work for genotyping of 48 faba bean genotypes (Table 2).

The SNPs were selected based on their polymorphic information content, distance in respecteive chromosome, covering the whole genome, quality and information revealed in previous work (Webb *et al.*, 2015) and were downloaded from a data base of validated KASPar SNP marker assay in NIAB vicia toolbox (http://www.viciatoolboc.org). In order to develop the KASPar assay, the primers were designed by KBioscience (http://www.kbioscience.co.uk/) from flanking sequences (50 bp each upstream and downstream) around the SNP position. Two allele-specific oligonucleotides and one common oligonucleotide were designed for each locus (Table 6). The SNPs were selected from all six chromosomes of faba bean so as to represent the genomic distribution with sufficient sequence information both in 5' and 3' to the target locus. The gene name, e-value, similarity of the locus with gene of its location, accession number, gene region with its location, total sequence of the gene, position of SNP on sequence and mutation type of the SNP locus used in the study was indicated in Appendix 7.

Table 6. Characteristic of 37 SNP primers for genotyping faba bean genotypes

S/N	**ID**[a]		SNP-Allele specific primer [b]	**Primer_Common**[c]	**AlleleX**	**AlleleY**
1	Vf_Mt1g014230_001	**AlleleX**	CAACATTTGAAGAAATTGGCTCATTGATA	CTTCCCTTGGTGATTTCCTTTACATCTAT	A	T
		AlleleY	CAACATTTGAAGAAATTGGCTCATTGATT			
2	Vf_Mt1g018320_001	**AlleleX**	GTTCTAGAAAATTCTCTAACTGCCGTT	CTGGCAGTAGGCGCGGATGTT	A	G
		AlleleY	CTAGAAAATTCTCTAACTGCCGTC			
3	Vf_Mt1g045800_001	**AlleleX**	CCTTGCGCCCCAAGAAAAAGGA	CAACGAAGTGAAAGATATCATTTAGCACAA	A	C
		AlleleY	CTTGCGCCCCAAGAAAAAGGC			
4	Vf_Mt1g072740_001	**AlleleX**	GATCATACTCTTGAGTTATCTGCCCT	GAGTGATAAGAAGATTGTTCAGTGGGATA	A	G
		AlleleY	ATCATACTCTTGAGTTATCTGCCCC			
5	Vf_Mt1g082210_001	**AlleleX**	TTGATGTGATTGTTGACAGGGAAACA	ACGGGACGATAAATTCGAGTAAGAAGATT	A	G
		AlleleY	GATGTGATTGTTGACAGGGAAACG			
6	Vf_Mt1g105040_001	**AlleleX**	AATGAAGCGGTGGTGCCTGATG	CAGATTCMCGAGGAGCAACGGTAT	G	T
		AlleleY	CAATGAAGCGGTGGTGCCTGATT			
7	Vf_Mt1g061800_001	**AlleleX**	AGACAAGGCAACTCCTTGAGCG	AAGGGGTTATATGTTCTTCTGCTGGAAAT	C	T
		AlleleY	CAGACAAGGCAACTCCTTGAGCA			
8	Vf_Mt2g010740_001	**AlleleX**	GACTTATAAGCATTCCACCTCCG	TACTAGCAGGAATATTAGGCGGTGTTTT	C	T
		AlleleY	GGACTTATAAGCATTCCACCTCCA			
9	Vf_Mt2g010970_001	**AlleleX**	TCTCATACTTCACAATACAAGGACTG	GATCAAAAGGGTGTTTTGGAAGTGAAGTT	C	T
		AlleleY	ATTCTCATACTTCACAATACAAGGACTA			
10	Vf_Mt2g097950_001	**AlleleX**	AGAAACTGGGGCCATCTCTGAC	TAGCTGCTGGAAACACTATCGTTCTAAA	G	T
		AlleleY	AAGAAACTGGGGCCATCTCTGAA			
11	Vf_Mt3g031280_001	**AlleleX**	GGGATGGTTAAAAGATGTTGATTCC	GCATTGYACCTTCAGGAACTGCCA	C	G
		AlleleY	GGGATGGTTAAAAGATGTTGATTCG			
12	Vf_Mt3g062540_001	**AlleleX**	ATCGCTCTGTGTAGGTAATCAGTGT	CGGCGCCAAGTCTCCAACCATT	A	G
		AlleleY	CGCTCTGTGTAGGTAATCAGTGC			
13	Vf_Mt3g077670_001	**AlleleX**	GACTGCTGTATCTTACATACCTGTC	CGGRATTCCCCTTGGGACTTTGTT	C	T
		AlleleY	GGACTGCTGTATCTTACATACCTGTT			
14	Vf_Mt3g084040_001	**AlleleX**	CATGGATGAAACAGGAGCATGGG	GAGGCAGGGTGCTACTCAGAGTA	C	G
		AlleleY	CATGGATGAAACAGGAGCATGGC			
15	Vf_Mt3g086980_001	**AlleleX**	CAAAAGAGTTTGCTGAGAAAGAAGGG	CTTCCAGTGCAGAGGTCTCTAAGAA	G	T
		AlleleY	CAAAAGAGTTTGCTGAGAAAGAAGGT			
16	Vf_Mt3g092810_001	**AlleleX**	GTTGGGCTTCATTTTCTCCGACAAT	TCGCATCAGGAATTGTCGGATGGTA	A	G
		AlleleY	GGGCTTCATTTTCTCCGACAAC			
17	Vf_Mt3g100500_001	**AlleleX**	CCTCATCGGTACTTGTGTCAAGG	GGAACACCAGGTCAACTTCTGTTCAT	C	T
		AlleleY	CCTCATCGGTACTTGTGTCAAGA			
18	Vf_Mt4g077610_001	**AlleleX**	TCGTCATTGTCTGTTACCGAAGT	GAGCAGCAATCTGATGGTGCACAAA	A	C
		AlleleY	CGTCATTGTCTGTTACCGAAGG			

Table 6. Contued…

S/N	ID[a]		SNP-Allele specific primer [b]	**Primer_Common[c]**	**AlleleX**	**AlleleY**
19	Vf_Mt4g113270_001	**AlleleX**	CTGACTGCTTATTCTTGGGCC	CTCAGTAGTAATCGTAACCAAATAAGCAAA	C	T
		AlleleY	ACTCTGACTGCTTATTCTTGGGCT			
20	Vf_Mt4g131830_001	**AlleleX**	GCCCATATGCAAAGAATCCAAAAAGAA	CAGTTACTCATTACTTCAACAAAGCCYTTT	A	G
		AlleleY	CCCATATGCAAAGAATCCAAAAAGAG			
21	Vf_Mt4g035200_001	**AlleleX**	CAAAGTGGATATTGATGCTGATGAAATAG	CGTGGCCGATCAAATTATCTTCATTCAAA	G	T
		AlleleY	ACAAAGTGGATATTGATGCTGATGAAATAT			
22	Vf_Mt5g007030_001	**AlleleX**	CAAAAGATGTTGCAAAGTTAGGAGTC	TTCTTGACAGCTCCCCACATTCCAT	C	T
		AlleleY	CCAAAAGATGTTGCAAAGTTAGGAGTT			
23	Vf_Mt5g009720_001	**AlleleX**	GTTGCTGTTTGAAGACTTTGGTTGTATTA	CGCTCACACAATCTCTGCAGTAAATTATA	A	G
		AlleleY	GCTGTTTGAAGACTTTGGTTGTATTG			
24	Vf_Mt5g016250_001	**AlleleX**	CCGGCGTCGGAGGTCAATCA	AGTTGCGACTGTTACCGGAGATGTT	A	T
		AlleleY	CCGGCGTCGGAGGTCAATCT			
25	Vf_Mt5g033220_001	**AlleleX**	GTTGAACACTCTTCCTCCATGATAC	TTCCTTTGAAGATAAGTGAACAAGAGGAAA	G	T
		AlleleY	GTTGAACACTCTTCCTCCATGATAA			
26	Vf_Mt5g078030_001	**AlleleX**	CCCAAAAAAAGTAGCATTATCGTACTTG	GGTCCAGCTCGACGGGTGTTT	C	G
		AlleleY	CCCAAAAAAAGTAGCATTATCGTACTTC			
27	Vf_Mt5g047260_001	**AlleleX**	CCGACAGAGTAGGACTATTATCAAAC	GTAACTGTGAGGCTATTCTCTCTAAAGAT	C	T
		AlleleY	ACCGACAGAGTAGGACTATTATCAAAT			
28	Vf_Mt7g080730_001	**AlleleX**	AAAACTGAACAGTATCACGGGATCG	GATGGTGAAAATGATCATCCAACGTCAAT	C	T
		AlleleY	AAAACTGAACAGTATCACGGGATCA			
29	Vf_Mt7g090890_001	**AlleleX**	CAGAAAAAATAAGAAACTATACCTCCCATTT	GTGTAGCCTGATGAGATAATGAAAAGGTT	A	T
		AlleleY	CAGAAAAAATAAGAAACTATACCTCCCATTA			
30	Vf_Mt7g100730_001	**AlleleX**	AGTTCTTGAAGTCGTGCTTCATCG	ATCAACAGTTTTCGGAACTTTCCGCTT	C	T
		AlleleY	CAGTTCTTGAAGTCGTGCTTCATCA			
31	Vf_Mt8g085850_001	**AlleleX**	GTCGTCTCCTCTATTTGGAGGC	CTCCTCCCTCTCTTTCCAACTAAGAT	C	T
		AlleleY	AGTCGTCTCCTCTATTTGGAGGT			
32	Vf_Mt8g102250_001	**AlleleX**	AGTATGGAGCTGGCTATCGTGC	TATCATGGGCTTAGTCTTTGGCACTTTA	C	T
		AlleleY	AAGTATGGAGCTGGCTATCGTGT			
33	Vf_Mt1g099390_002	**AlleleX**	AACAACCTTATCCCTCCGATTGGT	GATTCTACGTCGTTCACGCTTTGCAA	A	T
		AlleleY	AACAACCTTATCCCTCCGATTGGA			
34	Vf_Mt3g076660_001	**AlleleX**	GAGTTTCAGTCTCGTACTCAACG	GCTCGTGGAATTACAATTAACACTGCTA	C	T
		AlleleY	ACGAGTTTCAGTCTCGTACTCAACA			
35	Vf_Mt3g085280_001	**AlleleX**	CGTCGTGAAAAGACGGCTTATCG	GAGGAGTTATCCGCTGCTCTCATAA	C	T
		AlleleY	ATCGTCGTGAAAAGACGGCTTATCA			
36	Vf_Mt5g075540_001	**AlleleX**	TCAGCTCTCCGGCTCAAGTC	AGTGCATATATGACAGACAGCTGTTCAAA	C	G
		AlleleY	CAGCTCTCCGGCTCAAGTG			
37	Vf_Mt2g005900_001	**AlleleX**	AGCACATCCTARAATTGCAGACTCT	GGCTGTATTACCAAGMTCAATGCCATA	A	C
		AlleleY	GCACATCCTARAATTGCAGACTCG			

a = SNP Locus name, b = Allele X and Y forward primer, c = reverse prime

4.3.4. DNA normalization for PCR conditioning

Following a DNA extraction procedure, dilution of each DNA sample was done prior to using it in downstream applications to ensure optimal end result. The DNA was diluted at 1:20 dilutions for determination of sufficient quality and quantity of DNA concentration optimum for thermal cycling and KASP genotyping.

4.3.5. PCR conditioning

The KASPar assay was carried out in a total reaction volume of 10.14 µl with 96-well genotyping to combine KASP Master Mix and KASP assay mix. The reaction volume contains 5µl (70 ng/ µl) of template DNA, 2x Master Mix with volume of 5 µl and 0.14 µl allele specific forward and reverse common primers. PCR Amplification reactions were carried out in water based thermal cycler (hydrocycler). PCR conditions were programmed for 37 cycles with an initial denaturing step for 15 minutes at 94 ^{o}C one cycle, ten touchdown PCR cycle were programmed for 20 seconds’ denaturation at 94 ^{o}C and annealing from 61-55 ^{o}C by dropping of 0.6 ^{o}C per cycles for 60 seconds, the remaining 26 cycles were adjusted to denaturation temperature of 94 ^{o}C for 20 seconds and annealing temperature of 55 ^{o}C for 60 seconds.

4.3.6. SNP Data analysis

Data generated from KASP genotyping were directly entered into computer in order to prepare input file for making analysis by using different genetic analysis softwares. The key diversity parameters such as the total number of alleles (NA), allele frequency, major allele frequency (MAF) (i.e. allele with the highest frequency), gene diversity (GD), and polymorphism information content (PIC) at each locus were calculated by using Power

Marker version 3.25 software (Liu and Muse, 2005). Genalex version 6.5 software package (Peakall and Smouse, 2012) was used to analyze genetic parameters like number of effective alleles (Ne), Shannon information content (I), observed heterozygosity (Ho), expected heterozygosity (He), fixation index (F), and percent polymorphism (% P) of populations used in the study. These parameters were calculated as follows:

$$\textbf{No. of Effective Alleles (Ne)} = \frac{\mathbf{1}}{(\boldsymbol{\Sigma}\ \mathbf{pi2})}$$

Where pi^2 is the frequency of the i^{th} allele for the population and $\Sigma\ pi^2$ is the sum of the squared population allele frequencies.

$$\textbf{Shannon's Information Index (I)} = \mathbf{-1} * \boldsymbol{\Sigma}\ (\mathbf{pi} * \mathbf{Ln}\ (\mathbf{pi})),$$

Heterozygosity across all loci was used as a genetic variability indicator and was measured by determining the proportion of individuals that were homozygous or heterozygous for each particular SNP marker. For each locus, the observed heterozygosity (Ho) was calculated as:

$$\textbf{Observed Heterozygosity (Ho)} = \frac{\textbf{No. of Hets}}{\mathbf{N}}$$

The expected heterozygosity (He) for all SNP markers was calculated by relating the observed allele frequencies to the expected frequency of heterozygotes based on Hardy-Weinberg Equilibrium.

Genalex version 6.5 software package (Peakall, and Smouse, 2012) was used to carry out a chi-square (χ^2) test for each SNP marker to determine deviation from Hardy-Weinberg equilibrium (HWE) and the statistical significance was tested at $p \leq 0.05$. For a single locus with two alleles X and Y whose frequencies are p(X) and q(Y), where $p + q = 1$, the frequencies of the three possible genotypes are given by $p^2 + 2pq + q^2 = 1$. Therefore, the expected heterozygosity (He) was calculated as:

$$\textbf{Expected Heterozygosity (He)} = 1 - \Sigma\, \mathrm{pi2}$$

Value of PIC was calculated as:

$$\mathbf{PIC} = -\sum_{n=1}^{n} Pil2$$

Where *Pil* is the allele frequency of the i^{th} allele at l^{th} marker, and summed over *n* alleles.

$$\textbf{Fixation Index (F)} = \frac{(\mathrm{He} - \mathrm{Ho})}{\mathrm{He}} = 1 - \left(\frac{\mathrm{Ho}}{\mathrm{He}}\right)$$

Where Ho is observed heterozygosity, He is expected heterozygosity and N is effective size of population used in present study.

Analysis of genetic structure was implemented based on the Bayesian clustering model algorithm for inferring the population structure and assigning individuals to populations by using bi-allelic genotype data using STRUCTURE version 3.25 (Pritchard *et al.*, 2000). The length of the burn-in time and MCMC (Markov Chain Monte Carlo) iterations were set to 100,000 and 200,000 iterations, respecrively during the analysis. The optimum number of K was determined as suggested by Evanno *et al.* (2005), using Structure Harvester (Earl and VonHoldt, 2011). Of the 10 independent runs, the one with the highest ln Pr (X/K) value (log probability or log likelihood) was chosen and represented as bar plots.

Similarly, to see the corresponding result, the genetic relationships between the faba bean genotypes were also examined by using Neighbor Joining algorithm implemented in Darwin version 6 (Perrier and Jacquemoud-Collet, 2006) based on their genetic dissimilarity matrix. Dissimilarity was calculated using simple matching coefficient (Perrier *et al.*, 2003) as follows:

$$Dij = 1 - \frac{1}{L}\sum_{i=1}^{L}\frac{Mi}{\pi}$$

Where D ij is dissimilarity between units i and j, L is the number of loci, π is ploidy and Mi is the number of matching alleles for locus l (Perrier *et al.*, 2003).

Analysis of Molecular Variance (AMOVA) and Principal Coordinate Analysis (PCoA) were conducted using software GenAlEx V 6.5 (Peakall, and Smouse, 2012) to partition the total variation into among- and within-population variance and for further examining the patterns of variation among the individual genotypes. Bi-allelic data obtained from genotyping (the bases) were numerically coded as follows: A = 1, C = 2, G = 3, T = 4 and missing data was coded as 0 as suggested in GenAlEx V6.5 users manual (Peakall and Smouse, 2012).

4.3.7. KASP reaction component and running

KASP genotyping was carried out using the 96-well plate formats. Three components, namely the template DNA with the SNP of interest, KASP assay mix containing two different allele specific forward primers with unique tail sequences and one common reverse primer, the KASP master mix containing FAM™ and HEX™ specific FRET (fluorescence resonant energy transfer) cassette for distinguishing varieties plus KASpTaq polymerase, salts, dNTPS and an optimized buffer solution were used. The passive reference dye (ROX) was added to normalize the data by dividing FAM™ and HEX™ according to sample cluster which leads to lighter clustering while viewing using SNP viewer. The fluor labeled part of the FRET cassette is complementary to new tail sequences and binds, releasing the fluor from the quencher to generate a fluorescent signal. Two NTC (No Template Control) were included in genotyping plate to ensure validity/reliability of the genotyping result. Finally, end point fluorescent read was accomplished to see genotyping result.

4.3.8. Graphical viewing of genotyping data using cluster plot

Bi-allelic discrimination was achieved through the competitive binding of two allele-specific forward primers, each with a unique tail sequence that corresponds with two universal FRET cassettes; one labeled with FAM™ dye and the other with HEX™ dye by plotting on the *x*- and *y*-axes, respectively. Automatic allele calls provided by KlusterCaller software were visually checked with two-dimensional plot representations using SNPViewer software (KBioscience Ltd / http://www.lgcgenomics.com/snpviewer). Genotyped samples marked red are homozygous for the allele reported with HEX, those marked blue are homozygous for the FAM allele and those marked greens are heterozygous (KBioscience Ltd. / http://www.lgcgenomics.com/snpviewer).

5. RESULTS

5.1. Genetic Diversity Analysis based on Phenotypic Traits

5.1.1. Performance analysis of Ethiopian faba bean varieties

In this study, the morphological data of twenty-three quantitative traits were measured to determine phenotypic diversity of Ethiopian faba bean varieties.

5.1.1.1. Range of varieties over locations

Estimated range for each location and combined over locations are presented in Table 11. At Sinana, a wide range were recorded for grain yield per plot, plant height to first pod, thousand seeds weight, leaf area, leaf area index, numbers of pods per plant, numbers of seeds per plant, biomass per plot, seed production efficiency and economic growth rate.

At Agarfa, wide ranges were observed for leaf area index, plant height to first pod, plant height, numbers of pods per plant, numbers of seeds per plant, biomass per plot, grain yield per plot, harvest index, thousand seed weight, seed production efficiency, economic growth rate and crude protein content.

At Selka, ranges of measured traits were recorded for leaf width, leaf area, leaf area index, plant height to first pod, number of pods per plant, number of seeds per plant, biomass per plot, seed yield per plot, seed production efficiency and economic growth rate.

In combined locations, the range varies with the highest gaps for leaf area, leaf area index, internod length, plant height to first pod, plant height, number of branches per plant, number of pods per plant, number of seed per plant, biomass per plot, seed yield per plot, harvest index, thousand seed weight, seed production efficiency and economic growth rate. The minimum and maximum average seed yield combined over locations showed seed yield ranged from 2955.59 kgha^{-1} to 5631.46 kgha^{-1} (Table 11).

5.1.1.2. Mean performance of varieties over locations

The mean performance of thirty-two (32) Ethiopian faba bean varieties at Sinana, Agarfa, and Selka are presented in Table 8, 9, and 10, respectively. The combined mean performances over three locations for all varieties are presented in a Table 11. The mean performance of seed yield of the 32 faba bean varieties in this study were 5293 $kgha^{-1}$ at Sinana (Table 8), 4310 $kgha^{-1}$ at Agarfa (Table 9), 3880 $kgha^{-1}$ at Selka (Table 10) and 4494 $kgha^{-1}$ over combined locations (Table 11). The lower mean value of seed yield was observed over all varieties at Selka as compared to the other two locations (Agarfa and Sinana).

Table 7. Minimum, Mean, and Maximum values of 23 traits of thirty-two faba bean varieties for Sinana, Agarfa, Selka and combined over three locations

Parameter	**Sinana**			**Agarfa**			**Selka**			**Combined**		
	Min	**Mean**	**Max**	**Min**	**Mean**	**Max**	**Min**	**Mean**	**Max**	**Min**	**Mean**	**Max**
Leaf length(cm)	8.50	9.78	11.19	6.98	8.59	10.04	7.84	9.52	11.18	6.98	9.30	11.19
Leaf widthW(cm)	3.72	4.86	5.73	3.23	4.09	5.01	3.13	4.53	6.69	3.13	4.49	6.69
Leaf area(cm^2)	22.48	33.43	43.62	16.68	25.24	33.77	17.67	30.51	42.43	16.68	29.72	43.62
Leaf area index	176.90	290.20	492.80	92.19	170.80	310.30	116.70	208.60	344.80	92.19	223.20	492.80
Pod length(cm)	5.45	7.40	10.72	5.03	7.15	10.12	5.26	7.29	10.45	5.03	7.28	10.72
Pod width(cm)	0.88	1.39	2.01	0.83	1.35	1.78	0.83	1.27	2.49	0.83	1.34	2.49
Internod length(cm)	4.29	6.67	8.59	4.79	5.71	7.00	3.99	5.41	7.15	3.99	5.93	8.59
Plant height to first pod(cm)	30.20	42.97	77.60	34.60	52.46	81.00	24.60	35.94	45.00	24.60	43.79	81.00
Plant Height(cm)	137.20	154.40	171.20	119.00	147.20	172.00	122.80	145.50	168.80	119.00	149.00	172.00
Numbers of branches per plant	0.80	1.92	3.80	0.40	1.37	2.60	0.60	1.56	2.60	0.40	1.62	3.80
Number of pod per plant	19.20	41.83	68.20	15.20	29.64	50.20	13.40	29.99	57.80	13.40	33.82	68.20
Number of seed per plant	43.60	110.40	191.80	41.20	77.75	129.80	37.60	77.30	166.40	37.60	88.50	191.80
Number of seed per pod	2.16	2.66	3.44	2.02	2.64	3.37	1.99	2.58	3.14	1.99	2.63	3.44
Biomass per plot (g/plot)	2700.00	3619.00	5200.00	1700.00	3116.00	4700.00	1600.00	3373.00	4800.00	1600.00	3369.00	5200.00
Seed yield per plot (g/plot)	1063.00	1694.00	2263.00	677.80	1379.00	1969.00	780.60	1242.00	1735.00	677.80	1438.00	2263.00
Harvest index (%)	37.01	47.18	67.01	25.70	45.25	77.98	22.68	37.74	58.06	22.68	43.39	77.98
Thusound seed weight (g)	275.70	505.70	941.90	240.90	498.20	781.90	286.70	465.80	740.50	240.90	489.90	941.90
Days to flowering	48.00	51.86	57.00	50.00	53.45	58.00	55.00	58.00	62.00	48.00	54.44	62.00
Days to flowering	135.00	143.10	156.00	132.00	141.40	152.00	138.00	146.80	159.00	132.00	143.80	159.00
Seed filling period	83.00	91.25	105.00	78.00	87.95	100.00	78.00	88.78	102.00	78.00	89.33	105.00
Seed production efficiency	2061.00	3645.00	5115.00	1164.00	2771.00	4282.00	1455.00	2269.00	3326.00	1164.00	2895.00	5115.00
Economic growth rate	13.30	35.90	76.10	17.87	42.56	106.30	27.98	46.78	63.29	13.30	41.75	106.30
Crude protein content (%)	20.42	22.66	24.50	18.08	22.27	24.65	18.40	21.46	23.21	18.08	22.13	24.65

At Sinana, the highest seed yield was obtained from Mosisa (6553.68 kgha^{-1}), and the lowest were obtained from Lalo (3559.36 Kgha^{-1}) (Table 8). Similarly, at Agarfa the highest and lowest seed yield was recorded from Walki (5870.62 Kgha^{-1}) and Bako local (2224.12 Kgha^{-1}), respectively (Table 9). At Selka, the highest and lowest seed yield was obtained from Kuse (5118.14 Kgha^{-1}) and Kulumsa local (2549.48 Kgha^{-1}), respectively (Table 10).

The mean performance along with least significant difference and coefficient of variation for combined data for all locations are presented in Table 11. The highest combined mean value was recorded for Walki (5391.84 Kgha^{-1}), whereas the lowest mean value was recorded from Bako local variety (2955.59 Kgha^{-1}). The second higher yielding variety was EKCSR-02006 (5305.16 Kgha^{-1}) which is candidate variety at SARC followed by Degaga (5081.81 Kgha^{-1}). Similarly, the bottom lower yielders were Dagm (3551.45 Kgha^{-1}), Debrebirhan local (3791.04 Kgha^{-1}) and Kulumsa local (3847.39Kgha^{-1}), respectively.

The maximum and minimum crude protein content was recorded at Agarfa with value of 24.65% and 18.08 %, respectively (Table 9). The crude protein content ranged from 20.42 to 24.50 % at Sinana (Table 8), 18.08 to 24.65 % at Agarfa (Table 9) and 18.4 to 23.21% at Selka (Table 10). The highest mean value of crude protein content was recorded at Sinana with value of 22.66% and the lowest was observed at Selka with record of 21.6%.

At Agarfa, the highest mean performance of the crude protein content was recorded from recently released variety Gora (24.65 %) and followed by Shalo (23.92%) and Kulumsa local (23.55%). The lowest mean performance of crude protein content was observed from Holeta-2 (19.03%), Gachena (19.91) and Moti (20.65%) (Table 9). At Sinana, the highest mean performance of crude protein content was revealed from the variety Mosisa (24.57%) followed by Agarfa local (24.28%), Dagm (24.21%) and Shalo (23.7%). On the other hand, the lowest mean performance of crude protein content at Sinana was recorded from Kulumsa local (20.71%), Hachalu (21.36%), and Tumsa (21.44%) (Table 8). At Selka, the highest mean performance of crude protein content was scored from Tumsa (23.11%), Debrebirhan local (22.89) and Tesfa (22.60), respectively, while the least mean performance was measured from Kulumsa local (18.59%), Agarfa local (19.69%) and Gebelcho (20.13%) (Table 10).

In combined location, the highest mean performance of crude protein content was obtained from Tesfa (22.92%), Dagm (22.92%) and Mosisa (22.90%) and the lowest mean performance was recorded from Holeta-2 (20.59%), Kulumsa local (20.95%) and Hachalu (20.98%) correspondingly (Table 11). Therefore, in this study the crude protein content studied in Ethiopian faba bean varieties ranged from 18.08% to 24.65% while considering individual location, but varied from 20.59 to 22.92 % over three locations.

Table 8. Mean performance analysis of 23 traits of thirty-two Ethiopian faba bean varieties tested at Sinana during 2014 main cropping season

S/N	Variety	LL	LW	LA	LAI	PL	PW	IL	PHFP	PH	NBPPL	NPPL	NSPL	NSPPod
1	Mosisa	9.88ab	5.02	34.76	253.12	7.13	1.19	6.26	40.90	163.30	1.20	45.70	116.20	2.52
2	Tumsa	10.44	5.49	40.02	318.90	8.22	1.62	6.77	60.40	158.90	1.50	24.90	65.90	2.58
3	Hachalu	9.80	4.78	32.99	278.84	8.42	1.50	6.75	47.30	164.60	1.90	34.30	100.70	2.93
4	Dosha	9.78	5.13	35.12	302.31	7.92	1.46	5.02	41.00	145.60	2.10	39.40	104.70	2.67
5	Gachena	10.26	5.15	37.27	275.69	9.04	1.64	5.76	46.20	154.90	2.10	27.10	72.30	2.68
6	Walki	10.18	5.58	39.70	390.08	6.77	1.26	6.72	40.30	159.70	2.10	50.90	123.10	2.45
7	Obse	9.91	4.97	34.55	333.48	10.14	1.46	7.64	41.70	158.20	2.10	23.80	78.10	3.30
8	Moti	9.24	4.55	29.71	303.16	7.83	1.37	6.15	37.80	150.10	2.60	35.90	96.90	2.73
9	Gabelcho	10.02	4.64	32.75	387.38	7.89	1.69	7.29	46.10	152.80	2.80	37.60	93.70	2.50
10	Adet Hanna	10.03	5.02	35.27	293.21	7.07	1.24	7.41	42.20	153.20	1.60	49.90	123.00	2.47
11	NC-58	9.91	4.76	33.16	255.02	6.50	1.28	7.81	38.50	151.50	1.70	52.30	136.30	2.62
12	Wayu	9.29	4.53	29.66	202.84	5.66	1.31	7.03	47.20	141.40	1.40	45.20	114.60	2.53
13	Degaga	9.88	4.96	34.34	286.47	7.31	1.46	6.95	42.20	165.50	1.80	50.10	140.50	2.78
14	Dagm	9.02	3.99	25.57	273.27	6.00	0.97	6.23	44.00	143.00	3.00	47.60	130.00	2.75
15	Holetta-2	9.78	5.21	35.71	256.14	7.45	1.37	5.73	43.90	159.20	1.60	37.40	107.20	2.86
16	Shalo	10.01	4.91	34.52	358.58	7.65	1.75	7.25	55.70	160.30	2.00	37.60	92.70	2.49
17	Tesfa	9.67	4.81	32.68	268.27	7.28	1.30	6.67	42.00	155.60	1.70	39.40	105.30	2.68
18	Mesay	9.63	4.93	33.29	240.89	7.36	1.46	7.35	40.30	163.20	1.30	43.10	116.70	2.73
19	Bulga 70	8.91	4.37	27.50	229.71	6.06	1.13	6.96	40.50	151.10	2.10	45.70	124.10	2.70
20	Kassa	9.85	4.96	34.44	228.89	6.44	1.20	7.35	39.30	152.60	1.40	48.50	117.00	2.42
21	Gora	10.74	5.22	39.21	384.83	9.94	1.63	5.82	39.90	155.10	1.80	30.80	93.30	3.03
22	CS-20-DK	9.59	4.49	30.29	365.12	7.06	1.21	6.75	46.20	157.50	2.40	39.40	112.40	2.85
23	Kuse	9.52	4.57	30.58	256.65	7.04	1.47	6.70	41.40	154.20	1.60	38.10	100.50	2.63
24	Dida’a	10.16	4.88	34.72	299.77	8.45	1.57	5.67	34.20	154.90	2.00	32.50	80.90	2.49
25	Lalo	9.79	4.47	30.77	219.43	6.56	1.15	5.65	47.90	147.00	1.60	39.00	101.10	2.58
26	Bako local	9.06	4.52	28.88	270.50	6.42	1.17	6.86	45.60	148.80	1.90	52.00	144.00	2.77
27	Debrebirhan local(Eniwari)	9.59	4.05	27.48	325.27	6.02	1.11	7.43	39.90	144.30	3.20	62.20	160.00	2.58
28	Sinana local	9.48	4.95	32.96	264.98	7.14	1.57	6.54	38.50	147.30	1.50	45.90	115.60	2.52
29	Agarfa local	9.93	5.51	38.20	337.81	7.10	1.46	6.28	39.30	163.10	1.60	44.80	123.80	2.76
30	EKCSR-02006(Candidate)	9.69	4.99	33.94	253.52	7.41	1.50	6.53	38.50	153.80	1.90	42.10	104.20	2.47
31	Adet local	9.54	4.66	31.21	232.46	8.12	1.44	6.66	45.60	155.10	1.70	40.30	110.60	2.74
32	Kulumsa local	10.29	5.35	38.51	338.50	7.37	1.59	7.39	40.40	153.80	2.10	55.10	128.50	2.37
	Mean	**9.78**	**4.86**	**33.43**	**290.16**	**7.40**	**1.39**	**6.67**	**42.97**	**154.36**	**1.92**	**41.83**	**110.43**	**2.66**
	CV	**3.5**	**5.53**	**8.05**	**20.23**	**7.2**	**9.7**	**10.4**	**18**	**3.75**	**28.17**	**20.56**	**20.1**	**6.03**
	LSD	******	*******	******	**ns**	*******	*******	**ns**	**ns**	*****	**ns**	*****	**ns**	******

*** = Highly significant(p<0.001), ** = Highly significant(p<0.01),* = Significant (p<0.05), CV = Coefficient of variation, LSD = Least significant difference, LL = Leaf length, LW = Leaf width, LA = Leaf area, LAI = Leaf area index, Pl = pod length, PW = Pod width, IL = Internodes’ length, HFPN = Height to first poding node, PH = Plant height, NBPPL = Number of branches per plant, NPPL= Number of pods per plant, NSPL= Number of seed per plant, NSPPod = Number of seed per pod

Table 8. cont….

S/N	Variety	BMPP	SYPP	HI	TSW	DF	DM	SFP	SPE	EGR	CP
1	Mosisa	3600.00	2097.18	47.28	491.95	52.00	140.00	88.00	3437.13	59.32	23.84
2	Tumsa	4150.00	1793.97	43.48	622.21	49.00	145.50	96.50	4437.87	41.68	21.44
3	Hachalu	4000.00	1764.55	44.28	653.39	52.00	145.50	93.50	3928.76	33.23	21.36
4	Dosha	2950.00	1680.08	56.93	596.67	54.50	151.00	96.50	3562.39	30.05	23.26
5	Gachena	4100.00	1931.14	47.41	622.55	52.50	146.00	93.50	4104.02	32.19	22.02
6	Walki	3950.00	1823.45	46.26	511.70	50.00	139.50	89.50	4032.59	32.67	21.73
7	Obse	3650.00	1673.37	45.83	684.95	49.00	140.50	91.50	3928.06	29.11	22.02
8	Moti	3600.00	1923.11	37.77	496.93	48.00	149.50	101.50	3583.88	18.25	22.46
9	Gabelcho	3850.00	1731.44	45.14	695.50	55.50	145.50	90.00	3351.10	23.60	23.11
10	Adet Hanna	3550.00	1778.90	50.08	452.44	53.00	141.00	88.00	3560.01	41.38	22.68
11	NC-58	3000.00	1800.50	60.45	367.49	50.50	140.50	90.00	3924.25	44.00	22.97
12	Wayu	3450.00	1487.77	43.17	301.67	54.50	144.50	90.00	2952.00	42.50	23.19
13	Degaga	3600.00	1837.28	52.05	506.08	51.50	139.50	88.00	3802.52	42.70	22.90
14	Dagm	2950.00	1128.20	38.23	307.56	55.00	142.50	87.50	2168.72	14.34	24.21
15	Holetta-2	3875.00	1809.04	47.30	571.64	53.00	143.00	90.00	3707.04	43.20	22.09
16	Shalo	3900.00	1694.89	43.47	567.93	53.00	144.00	91.00	3465.95	42.08	23.70
17	Tesfa	3550.00	1752.92	44.30	459.66	51.00	140.50	89.50	3398.66	36.52	22.53
18	Mesay	3850.00	1828.02	47.57	447.02	53.50	140.00	86.50	3561.06	55.64	22.24
19	Bulga 70	3050.00	1527.66	50.14	404.89	49.50	139.50	90.00	3403.21	27.04	22.60
20	Kassa	4100.00	1944.87	48.61	402.49	50.50	144.50	94.00	4314.80	53.68	23.70
21	Gora	3550.00	1828.02	51.95	882.19	54.50	149.00	94.50	3756.19	35.47	21.73
22	CS-20-DK	3625.00	1612.59	44.65	492.97	53.00	143.50	90.50	3316.13	26.59	22.97
23	Kuse	3750.00	1707.58	45.81	416.97	51.00	139.50	88.50	3685.12	43.41	22.60
24	Dida’a	3800.00	1960.04	52.53	650.61	51.00	155.50	104.50	4772.34	31.25	22.82
25	Lalo	2900.00	1106.99	38.17	338.33	51.00	143.50	92.50	2441.19	25.86	22.46
26	Bako local	2700.00	1129.81	41.84	357.17	54.00	140.50	86.50	2218.80	22.10	21.89
27	Debrebirhan local	2850.00	1425.64	50.28	293.07	51.50	135.50	84.00	2889.48	18.10	22.73
28	Sinana local	3050.00	1573.45	57.68	487.74	53.50	140.00	86.50	3406.38	45.98	23.26
29	Agarfa local	4050.00	1359.57	47.47	504.58	50.50	140.50	90.00	4256.73	45.88	24.28
30	EKCSR-02006	4100.00	1989.05	49.34	520.33	50.50	141.50	91.00	4425.36	38.30	22.81
31	Adet local	4100.00	1804.87	44.33	501.85	49.50	143.00	93.50	4269.96	38.15	22.75
32	Kulumsa local	4600.00	1699.00	45.90	572.06	52.00	145.00	93.00	4594.01	34.60	20.71
	Mean	**3618.75**	**1693.91**	**47.18**	**505.71**	**51.86**	**143.11**	**91.25**	**3645.49**	**35.90**	**22.66**
	CV	**13.83**	**5.86**	**9.91**	**6.37**	**2.85**	**1.32**	**2.24**	**8.89**	**35.29**	**3.31**
	LSD	**ns**	*******	*****	*******	******	*******	*******	*******	**ns**	*****

*** = Highly significant (p<0.001), ** = Highly significant (p<0.01), * = Sgnificant (p<0.05), CV = Coefficient of variation, LSD = Least significant difference, SYPP = Seed yield per plot, HI = Harvest Index, TSW = Thousand Seed weight, DF = Days to flowering, DM = Days to maturity, SFP = Seed filling period, SPE = Seed production Efficiency, Economic growth rate, CP = Crude protein content.

Table 9. Mean performance analysis of 23 traits of thirty-two Ethiopian faba bean varieties tested at Agarfa during 2014 main cropping season

S/N	Variety name	LL	LW	LA	LAI	PL	PW	IL	PHFP	PH	NBPPL	NPPL	NSPL	NSPPod
1	Mosisa	8.37	4.13	24.77	131.20	7.65	1.28	5.96	49.20	144.10	1.00	20.20	54.60	2.70
2	Tumsa	8.10	3.68	21.59	161.46	6.83	1.42	5.80	68.50	151.20	1.20	23.70	58.80	2.48
3	Hachalu	8.45	4.33	26.22	179.14	8.56	1.67	5.40	58.20	152.90	1.30	23.70	65.30	2.78
4	Dosha	8.76	4.25	26.67	280.63	8.11	1.53	5.33	43.30	143.80	2.60	33.60	89.00	2.61
5	Gachena	8.74	4.31	27.00	194.31	9.44	1.56	5.81	57.30	153.30	1.10	19.90	56.90	2.81
6	Walki	9.08	4.56	29.64	194.59	6.57	1.26	5.72	48.10	146.80	1.40	36.70	90.20	2.49
7	Obse	7.93	3.67	21.29	127.44	8.81	1.65	6.23	57.60	151.50	1.40	18.20	47.60	2.63
8	Moti	7.94	3.90	22.28	147.64	8.32	1.37	4.99	60.30	150.80	1.80	19.10	56.50	2.96
9	Gabelcho	8.74	4.11	25.61	147.27	7.43	1.43	5.61	58.00	147.50	1.10	18.20	48.80	2.67
10	Adet Hanna	8.89	4.10	26.10	146.63	6.76	1.31	5.47	52.30	146.70	1.00	28.70	71.30	2.46
11	NC-58	8.21	3.81	22.54	138.60	5.87	1.17	6.15	45.10	139.80	1.30	39.70	103.90	2.60
12	Wayu	7.93	3.59	20.53	138.99	5.72	1.06	6.49	56.60	147.10	1.10	32.40	78.70	2.42
13	Degaga	8.63	4.21	25.73	175.75	6.13	1.26	6.02	48.40	153.80	1.20	34.60	91.70	2.67
14	Dagm	8.06	3.74	21.68	161.73	6.13	1.09	5.23	51.70	139.60	1.20	37.40	100.70	2.69
15	Holetta-2	8.80	3.98	24.87	158.78	7.23	1.41	5.54	62.30	149.10	1.90	39.10	98.70	2.52
16	Shalo	9.08	4.23	27.40	170.79	6.99	1.34	5.33	55.40	149.30	1.10	27.30	66.00	2.41
17	Tesfa	8.94	4.06	25.96	149.77	6.67	1.24	6.11	46.90	143.20	1.10	36.20	99.10	2.80
18	Mesay	8.65	3.98	24.71	156.76	6.37	1.25	6.06	45.40	153.20	1.00	25.20	77.20	3.08
19	Bulga 70	8.80	4.51	28.15	213.52	6.68	1.28	5.34	45.70	147.90	1.50	35.70	103.70	2.91
20	Kassa	8.68	3.98	25.00	169.94	6.57	1.24	5.32	51.10	155.50	1.60	36.00	77.50	2.15
21	Gora	9.92	4.73	33.11	242.28	9.64	1.67	5.41	53.70	151.20	1.20	20.90	62.00	2.92
22	CS-20-DK	8.45	3.94	23.85	157.39	6.69	1.24	5.52	48.30	141.10	1.60	25.30	71.20	2.83
23	Kuse	8.43	3.95	23.97	139.92	6.38	1.28	5.19	47.00	144.20	0.90	33.40	91.50	2.74
24	Dida'a	9.34	4.51	29.70	207.52	9.23	1.68	5.66	57.30	156.10	1.40	24.00	70.40	2.90
25	Lalo	8.44	3.59	21.71	162.07	5.80	1.08	5.23	56.70	142.80	1.60	39.90	106.90	2.68
26	Bako local	7.46	3.41	18.44	147.10	6.76	1.23	6.61	46.10	132.80	1.60	23.80	60.30	2.55
27	Debrebirhan local	8.06	3.43	19.96	162.41	5.19	1.14	5.73	45.20	132.20	1.50	44.90	120.10	2.69
28	Sinana local	8.83	4.47	28.34	159.09	6.87	1.34	5.85	46.00	159.80	0.90	28.50	74.70	2.62
29	Agarfa local	8.72	4.58	28.59	168.42	7.07	1.41	6.23	40.90	146.40	1.10	31.40	75.80	2.42
30	EKCSR-02006	8.66	4.07	25.01	162.61	6.69	1.33	5.95	45.70	139.20	1.50	30.90	68.60	2.30
31	Adet local	8.33	4.41	26.22	216.56	7.36	1.44	5.77	65.30	149.30	2.10	33.40	82.10	2.55
32	Kulumsa local	9.43	4.61	30.91	194.77	8.41	1.56	5.69	65.10	148.00	1.60	26.60	68.30	2.55
	Mean	**8.59**	**4.09**	**25.24**	**170.78**	**7.15**	**1.35**	**5.71**	**52.46**	**147.19**	**1.37**	**29.64**	**77.75**	**2.64**
	CV	6.62	8.57	14.07	21.91	4.80	7.54	5.60	14.55	4.00	30.09	20.10	19.50	9.67
	LSD	ns	*	ns	ns	***	***	ns	ns	ns	ns	**	**	ns

*** = highly significant(p<0.001), ** = highly significant(p<0.01), * = significant (p<0.05), CV = coefficient of variation, LSD = least significant difference, LL= leaf length, LW = leaf width, LA = leaf area, LAI = leaf area index, Pl = pod length, PW = pod width, IL = internodes' length, HFPN = height to first poding node, PH = plant height, NBPPL= number of branches per plant, NPPL = number of pods per plant, NSPL = number of seed per plant, NSPPod = number of seed per pod

Table 9. cont...,

S/N	Variety name	BMPP	SYPP	HI	TSW	DF	DM	SFP	SPE	EGR	CP
1	Mosisa	2350.00	1365.62	58.06	595.49	53.50	140.00	86.50	2716.68	52.65	22.39
2	Tumsa	3600.00	1416.84	39.70	662.58	56.00	146.50	90.50	2789.07	44.85	22.97
3	Hachalu	3350.00	1321.12	31.43	516.79	55.00	147.00	92.00	2095.08	29.86	21.15
4	Dosha	2800.00	1433.92	51.21	550.68	55.00	144.00	89.00	2774.32	20.66	21.88
5	Gachena	3300.00	1424.16	43.68	489.09	51.50	146.50	95.00	3214.11	47.91	19.91
6	Walki	3700.00	1878.60	53.34	689.99	51.00	140.00	89.00	4033.43	50.34	22.68
7	Obse	3100.00	1294.85	41.85	531.36	51.50	141.50	90.00	2807.94	34.23	21.51
8	Moti	3450.00	1397.19	40.65	666.27	51.50	151.00	99.50	3311.08	26.00	20.65
9	Gabelcho	2800.00	1772.37	36.56	278.55	54.50	139.50	85.00	1954.68	36.70	22.90
10	Adet Hanna	2100.00	882.47	42.01	376.37	53.00	133.00	80.00	1610.56	44.29	23.26
11	NC-58	3250.00	1637.92	52.49	456.81	53.00	135.00	82.00	3156.38	51.28	22.09
12	Wayu	3300.00	1307.89	39.63	721.64	56.50	148.50	92.00	2569.70	47.43	22.31
13	Degaga	3100.00	1546.94	53.36	431.29	53.00	132.00	79.00	2786.77	55.35	22.90
14	Dagm	3400.00	1132.28	33.84	468.87	53.50	140.00	86.50	2226.20	37.48	22.68
15	Holetta-2	3150.00	1439.21	49.40	436.09	54.50	136.50	82.00	2658.45	30.91	19.03
16	Shalo	3500.00	1708.60	49.15	463.01	53.00	141.00	88.00	3341.51	59.35	22.31
17	Tesfa	2900.00	1402.48	49.52	389.02	55.50	135.50	80.00	2386.35	53.38	23.92
18	Mesay	2700.00	1002.84	37.14	348.17	53.00	140.00	87.00	1989.39	39.79	22.68
19	Bulga 70	2850.00	1289.59	45.63	457.74	56.00	140.50	84.50	2342.03	34.17	23.70
20	Kassa	3100.00	1321.51	42.96	463.96	52.00	142.00	90.00	2762.98	30.81	22.53
21	Gora	3050.00	1821.25	35.16	467.38	53.00	146.00	93.00	2172.60	34.37	24.57
22	CS-20-DK	3150.00	1315.09	41.72	580.06	54.50	145.50	91.00	2598.28	30.88	21.00
23	Kuse	2900.00	1363.92	48.07	577.01	53.50	139.00	85.50	2681.71	65.28	20.78
24	Dida'a	3350.00	1662.44	50.55	602.22	53.00	145.50	92.50	3508.19	46.92	23.11
25	Lalo	3650.00	1668.97	46.09	480.89	57.50	146.00	88.50	3004.20	39.59	22.75
26	Bako local	2250.00	711.72	31.86	254.55	56.50	137.50	81.00	1226.69	18.30	22.99
27	Debrebirhan local	2000.00	1192.36	62.01	491.21	52.00	138.00	86.00	2394.02	62.71	22.77
28	Sinana local	2650.00	1039.73	49.95	287.81	50.50	137.00	86.50	2829.61	57.62	23.19
29	Agarfa local	4150.00	1029.59	42.69	589.86	50.50	141.50	91.00	3797.70	64.62	22.46
30	EKCSR-02006	3400.00	1829.20	53.73	500.19	52.50	145.50	93.00	3860.02	43.70	21.36
31	Adet local	3550.00	1519.80	44.38	587.82	54.00	142.00	88.00	2976.55	27.80	20.71
32	Kulumsa local	3800.00	1010.90	50.31	528.64	50.50	141.50	91.00	4091.79	42.65	23.55
	Mean	**3115.63**	**1379.23**	**45.25**	**498.17**	**53.45**	**141.41**	**87.95**	**2770.88**	**42.56**	**22.27**
	CV	**18.94**	**8.67**	**15.94**	**22.72**	**2.00**	**1.70**	**3.13**	**9.82**	**38.55**	**3.30**
	LSD	**ns**	*******	******	*****	*******	*******	*******	*******	**ns**	*******

*** = Highly significant(p<0.001), ** = Highly significant(p<0.01), * = Significant (p<0.05), CV = Coefficient of variation, LSD = Least significant difference, SYPP = Seed yield per plot, HI = Harvest Index, TSW =Thousand Seed weight, DF = Days to flowering, DM = Days to maturity, SFP = Seed filling period, SPE = Seed production efficiency, CP = Crude protein content.

Table 10. Mean performance analysis of 23 traits of thirty-two Ethiopian faba bean varieties tested at Selka during 2014 main cropping season

S/N	Variety name	LL	LW	LA	LAI	PL	PW	IL	HFPN	PH	NBPPL	NPPL	NSPL	NSPPod	BMPP
1	Mosisa	9.11	4.20	27.23	184.61	7.02	1.52	5.07	32.40	141.90	2.00	30.50	69.80	2.28	2950.00
2	Tumsa	9.30	4.56	29.88	236.54	6.96	1.08	5.29	42.60	151.10	1.50	26.40	60.90	2.30	3400.00
3	Hachalu	9.75	5.06	34.56	224.57	8.33	1.39	5.79	40.60	149.90	1.60	17.50	46.20	2.64	3400.00
4	Dosha	9.91	4.58	32.06	262.71	7.58	1.19	5.78	38.10	137.50	2.00	26.60	69.80	2.62	2300.00
5	Gachena	9.74	4.75	32.71	215.47	8.16	1.99	5.89	40.60	151.80	1.20	25.80	62.30	2.44	4150.00
6	Walki	9.33	4.78	31.42	159.19	7.37	1.33	5.19	31.60	148.20	0.90	22.30	57.80	2.61	3800.00
7	Obse	9.07	4.29	27.46	152.08	9.57	1.52	6.15	38.30	155.70	1.30	16.90	45.20	2.72	3300.00
8	Moti	9.24	4.30	28.15	210.68	8.24	1.30	5.85	36.20	143.50	1.40	24.00	73.40	3.03	3200.00
9	Gabelcho	9.69	4.30	29.39	214.30	7.98	1.49	5.51	37.40	152.50	1.70	24.60	62.90	2.54	4100.00
10	Adet Hanna	10.47	5.10	37.49	197.28	7.75	1.20	6.34	37.80	151.50	0.80	29.50	78.40	2.67	3700.00
11	NC-58	9.42	4.42	29.33	169.12	6.37	1.07	5.27	34.00	152.00	1.20	31.20	82.50	2.65	2750.00
12	Wayu	9.58	4.09	27.68	203.11	5.77	0.92	5.67	35.40	140.10	1.80	35.00	89.10	2.55	3150.00
13	Degaga	9.34	4.72	30.97	239.68	7.58	1.29	4.79	37.60	149.50	2.30	50.00	130.20	2.55	3500.00
14	Dagm	8.99	3.88	24.68	176.65	5.82	1.06	5.47	40.00	133.70	1.70	36.90	98.50	2.68	3150.00
15	Holetta-2	10.00	4.74	33.58	265.70	7.31	1.36	5.23	34.30	148.30	1.70	34.60	92.20	2.64	3700.00
16	Shalo	9.71	5.12	34.81	233.98	7.48	1.32	4.92	44.80	153.00	1.70	30.30	71.00	2.36	3850.00
17	Tesfa	9.55	5.56	36.75	220.97	6.83	1.30	5.56	35.80	147.40	1.10	28.10	75.30	2.71	3100.00
18	Mesay	9.43	4.15	27.62	185.79	7.05	1.26	5.21	25.70	145.80	1.20	30.40	86.20	2.84	3300.00
19	Bulga 70	9.51	4.46	29.84	210.60	6.23	1.00	5.67	30.30	143.00	1.90	34.60	99.00	2.87	2800.00
20	Kassa	10.18	4.69	33.47	213.29	6.35	1.17	6.50	38.00	139.20	2.00	45.90	120.40	2.61	2850.00
21	Gora	10.05[a]	4.79	33.80	222.95	10.02	1.46	4.96	38.50	148.90	1.60	21.90	47.70	2.19	3900.00
22	CS-20-DK	8.96	3.87	24.61	163.35	6.78	1.19	4.53	38.80	143.90	1.70	28.10	77.70	2.74	3400.00
23	Kuse	9.06	4.13	26.63	198.59	6.42	1.10	4.87	32.50	136.90	2.10	34.60	95.40	2.76	3450.00
24	Dida’a	10.59	4.74	35.08	219.04	8.89	1.50	5.25	41.40	155.70	1.20	22.00	56.70	2.54	3700.00
25	Lalo	9.79	4.05	27.95	162.65	6.30	1.29	5.15	35.30	137.20	1.40	23.60	63.50	2.68	3100.00
26	Bako local	9.36	4.41	29.12	281.93	6.38	0.99	5.59	37.20	142.30	2.00	35.80	92.70	2.61	2900.00
27	Debrebirhan local	8.54	3.63	22.29	153.59	5.50	0.90	4.88	30.10	130.30	2.00	35.70	89.50	2.51	2900.00
28	Sinana local	9.24	4.32	28.27	213.63	7.11	1.06	5.87	35.30	144.50	1.40	30.70	76.90	2.48	3450.00
29	Agarfa local	8.56	4.59	27.78	172.70	7.07	1.49	4.26	31.20	138.80	1.30	31.00	77.40	2.51	3150.00
30	EKCSR-02006	8.73	4.25	26.20	177.29	7.86	1.34	5.18	31.90	138.50	1.20	31.50	70.60	2.23	3800.00
31	Adet local	9.92	4.98	34.59	304.01	7.42	1.32	6.26	33.70	151.10	1.60	31.20	76.90	2.46	3550.00
32	Kulumsa local	10.54	5.55	40.79	230.10	7.67	1.26	5.25	32.80	151.70	1.30	32.40	77.50	2.40	4200.00
	Mean	**9.52**	**4.53**	**30.51**	**208.63**	**7.29**	**1.27**	**5.41**	**35.94**	**145.48**	**1.56**	**29.99**	**77.30**	**2.58**	**3373.44**
	CV	**5.36**	**10.5**	**12.42**	**19.81**	**5.88**	**12.9**	**9.15**	**9.41**	**5.26**	**18.36**	**19.53**	**8.6**	**8.6**	**17.23**
	LSD	**ns**	**ns**	*****	**ns**	*******	******	**ns**	******	**ns**	*******	******	**ns**	**ns**	**ns**

*** = highly significant ($p<0.001$), ** = highly significant ($p<0.01$), * = significant ($p<0.05$), CV = coefficient of variation, LSD = least significant difference, LL = leaf length, LW = leaf width, LA = leaf area, LN = leaf number, LAI = leaf area index, Pl = pod length, PW = pod width, IL= internodes’ length, HFPN = height to first poding node, PH = plant height, NBPPL = number of branches per plant, NPPL= number of pods per plant, NSPL = number of seed per plant, NSPPod = number of seed per pod, BMPP = biomass per plot

Table 10. Cont….,

S/N	Variety name	SYPP	HI	TSW	DF	DM	SFP	SPE	EGR	CP
1	Mosisa	1326.35	47.37	469.12	56.50	145.00	88.50	2521.96	49.97	22.46
2	Tumsa	1487.78	26.53	576.62	62.00	151.50	89.50	1483.85	32.73	23.11
3	Hachalu	1334.63	43.33	557.20	59.00	151.00	92.00	2484.16	48.34	20.42
4	Dosha	1350.69	38.58	523.30	57.00	151.00	94.00	1581.22	28.92	21.07
5	Gachena	1317.17	31.88	574.75	57.00	146.50	89.50	2408.02	49.05	22.17
6	Walki	1474.12	31.82	472.59	58.00	151.50	93.50	2326.91	43.01	22.31
7	Obse	1252.25	38.37	635.14	59.00	151.00	92.00	2324.02	45.42	20.34
8	Moti	1391.32	46.95	588.57	56.00	141.50	85.50	2557.33	54.22	21.22
9	Gabelcho	1359.94	33.31	599.91	58.00	14q7.50	89.50	2435.03	50.64	20.13
10	Adet Hanna	1421.63	38.46	436.66	56.50	145.50	89.00	2664.27	53.24	21.22
11	NC-58	1227.07	44.67	378.71	57.00	141.00	84.00	2192.21	48.69	21.81
12	Wayu	1179.02	38.41	308.99	61.00	143.00	82.00	1861.93	47.97	20.34
13	Degaga	1494.33	42.70	425.70	58.00	141.00	83.00	2531.21	60.01	21.88
14	Dagm	1148.91	36.63	311.09	60.50	150.00	89.50	2017.04	42.77	21.88
15	Holetta-2	1195.78	32.31	457.23	58.00	158.50	100.50	2451.27	39.69	20.64
16	Shalo	1313.15	34.16	490.02	57.50	138.50	81.00	2202.99	54.00	21.95
17	Tesfa	1190.35	38.45	385.50	59.50	139.00	79.50	1894.29	49.93	22.31
18	Mesay	1386.59	42.02	423.78	57.50	142.50	85.00	2481.32	54.37	22.60
19	Bulga 70	1294.50	47.60	375.78	58.00	145.50	87.50	2358.95	49.33	20.71
20	Kassa	1224.55	42.97	370.80	56.50	141.00	84.50	2178.00	48.29	21.22
21	Gora	1108.96	28.73	720.61	57.00	145.00	88.00	2053.45	42.01	22.31
22	CS-20-DK	1362.75	40.08	440.40	59.00	147.00	88.00	2447.39	51.62	21.88
23	Kuse	1637.80	47.92	418.99	56.50	148.50	92.00	3170.53	59.34	22.02
24	Dida’a	1039.19	28.50	536.32	57.50	139.50	82.00	1775.28	42.24	21.95
25	Lalo	917.53	29.73	322.08	56.50	150.00	93.50	1846.80	32.70	21.15
26	Bako local	995.83	34.46	388.96	59.00	143.00	84.00	1703.32	39.61	22.10
27	Debrebirhan local	1021.41	35.19	292.12	60.00	149.00	89.00	1788.12	38.22	22.89
28	Sinana local	1206.59	42.99	444.98	56.00	143.50	87.50	2745.73	56.16	21.07
29	Agarfa local	815.83	42.87	463.40	56.00	145.00	89.00	2584.02	50.56	19.69
30	EKCSR-02006	1274.70	33.54	507.25	59.00	154.00	95.00	2470.63	44.73	21.80
31	Adet local	1099.15	31.19	471.48	58.50	156.00	97.50	2173.05	37.59	21.58
32	Kulumsa local	878.86	36.07	536.24	58.50	154.50	96.00	2901.63	51.68	18.59
Grand mean		**1241.52**	**37.74**	**465.76**	**58.00**	**146.78**	**88.78**	**2269.25**	**46.78**	**21.46**
CV (%)		**6.67**	**16.1**	**7.6**	**1.52**	**1.12**	**2.02**	**8.5**	**6.41**	**2.6**
LSD		***	*	***	***	***	**	***	***	***

*** = Highly significant (p<0.001), ** = highly significant (p<0.01), * = Significant (p<0.05), CV = Coefficient of variation, LSD = Least significant difference, SYPP = Seed yield per plot, HI = Harvest index, TSW =Thousand seed weight, DF = Days to flowering, DM = Days to maturity, SFP = Seed filling period, SPE = Seed production efficiency, EGR = Economic growth rate, CP = Crude protein content

Table 11. Combined mean performance analysis of 23 traits of thirty-two Ethiopian faba bean varieties tested at Sinana, Agarfa and Selka during main growing season of 2014/15

Variety name	LL	LW	LA	LAI	PL	PW	IL	PHFP	PH	NBPPL	NPPL	NSPL	NSPPod
Mosisa	9.12	4.45	28.92	189.64	7.27	1.33	5.77	40.83	149.77	1.40	32.13	80.20	2.50
Tumsa	9.28	4.58	30.50	238.97	7.34	1.37	5.96	57.17	153.73	1.40	25.00	61.87	2.45
Hachalu	9.33	4.73	31.26	227.51	8.44	1.52	5.98	48.70	155.80	1.60	25.17	70.73	2.78
Dosha	9.48	4.65	31.28	281.89	7.87	1.40	5.38	40.80	142.30	2.23	33.20	87.83	2.63
Gachena	9.58	4.74	32.33	228.49	8.88	1.73	5.82	48.03	153.33	1.47	24.27	63.83	2.64
Walki	9.53	4.97	33.58	247.96	6.90	1.28	5.88	40.00	151.57	1.47	36.63	90.37	2.52
Obse	8.97	4.31	27.77	204.34	9.51	1.54	6.67	45.87	155.13	1.60	19.63	56.97	2.88
Moti	8.80	4.25	26.71	220.49	8.13	1.35	5.66	44.77	148.13	1.93	26.33	75.60	2.91
Gabelcho	9.48	4.35	29.25	249.65	7.77	1.54	6.14	47.17	150.93	1.87	26.80	68.47	2.57
Adet Hanna	9.80	4.74	32.95	212.38	7.19	1.25	6.41	44.10	150.47	1.13	36.03	90.90	2.53
NC-58	9.18	4.33	28.34	187.58	6.25	1.17	6.41	39.20	147.77	1.40	41.07	107.57	2.62
Wayu	8.94	4.07	25.95	181.65	5.72	1.10	6.39	46.40	142.87	1.43	37.53	94.13	2.50
Degaga	9.28	4.63	30.35	233.97	7.01	1.34	5.92	42.73	156.27	1.77	44.90	120.80	2.67
Dagm	8.69	3.87	23.98	203.88	5.98	1.04	5.65	45.23	138.77	1.97	40.63	109.73	2.71
Holetta-2	9.53	4.64	31.39	226.88	7.33	1.38	5.50	46.83	152.20	1.73	37.03	99.37	2.67
Shalo	9.60	4.75	32.24	254.45	7.38	1.47	5.83	51.97	154.20	1.60	31.73	76.57	2.42
Tesfa	9.39	4.81	31.80	213.00	6.93	1.28	6.11	41.57	148.73	1.30	34.57	93.23	2.73
Mesay	9.24	4.35	28.54	194.48	6.92	1.32	6.21	37.13	154.07	1.17	32.90	93.37	2.89
Bulga 70	9.08	4.44	28.49	217.94	6.33	1.14	5.99	38.83	147.33	1.83	38.67	108.93	2.83
Kassa	9.57	4.54	30.97	204.04	6.45	1.20	6.39	42.80	149.10	1.67	43.47	104.97	2.39
Gora	10.24	4.92	35.37	283.35	9.87	1.58	5.40	44.03	151.73	1.53	24.53	67.67	2.71
CS-20-DK	9.00	4.10	26.25	228.62	6.84	1.21	5.60	44.43	147.50	1.90	30.93	87.10	2.81
Kuse	9.00	4.22	27.06	198.39	6.61	1.28	5.59	40.30	145.10	1.53	35.37	95.80	2.71
Dida'a	10.03	4.71	33.17	242.11	8.86	1.58	5.53	44.30	155.57	1.53	26.17	69.33	2.64
Lalo	9.34	4.04	26.81	181.38	6.22	1.17	5.34	46.63	142.33	1.53	34.17	90.50	2.65
Bako local	8.63	4.11	25.48	233.18	6.52	1.13	6.35	42.97	141.30	1.83	37.20	99.00	2.64
Debrebirhan local	8.73	3.70	23.24	213.76	5.57	1.05	6.01	38.40	135.60	2.23	47.60	123.20	2.59
Sinana local	9.18	4.58	29.86	212.57	7.04	1.32	6.09	39.93	150.53	1.27	35.03	89.07	2.54
Agarfa local	9.07	4.89	31.52	226.31	7.08	1.45	5.59	37.13	149.43	1.33	35.73	92.33	2.56
EKCSR-02006	9.03	4.44	28.38	197.81	7.32	1.39	5.88	38.70	143.83	1.53	34.83	81.13	2.33
Adet local	9.26	4.68	30.67	251.01	7.63	1.40	6.23	48.20	151.83	1.80	34.97	89.87	2.58
Kulumsa local	10.09	5.17	36.74	254.46	7.81	1.47	6.11	46.10	151.17	1.67	38.03	91.43	2.44
Grand mean	9.30	4.49	29.72	223.19	7.28	1.34	5.93	43.79	149.01	1.61	33.82	88.50	2.63
CV	6.10	9.13	13.38	23.11	6.75	12.35	11.42	14.70	5.40	25.54	20.30	21.04	8.24
LSD	0.35	0.34	4.01	42.45	0.59	0.20	0.64	5.68	4.65	1.31	8.34	20.40	0.15
5%									ns				
P-Value **1%**	**	**	**	**	**	**	**	**	**	**	**	**	

** = highly significant(p<0.01), * = significant (p<0.05), CV = Coefficient of Variation, LSD = least significant difference, SYPP = Seed yield per plot, HI = Harvest Index, TSW =Thousand Seed weight, DF = Days to flowering, DM = Days to maturity, SFP =Seed filling period, SPE = Seed production Efficiency, EGR = Economic growth rate, CP = Crude Protein content.

Table 11. Contued…,

Variety name	BMPP	SYPP	HI	TSW	DF	DM	SFP	SPE	EGR	CP
Mosisa	2966.67	4573.92	50.91	518.85	54.00	141.67	87.67	2891.92	53.98	22.90
Tumsa	3716.67	4260.07	36.57	620.47	55.67	147.83	92.17	2903.59	39.75	22.51
Hachalu	3583.33	4311.36	39.68	575.79	55.33	147.83	92.50	2836.00	37.14	20.98
Dosha	2683.33	4093.57	48.90	556.88	55.50	148.67	93.17	2639.31	26.54	22.07
Gachena	3850.00	4867.15	40.99	562.13	53.67	146.33	92.67	3242.05	43.05	21.36
Walki	3816.67	5113.17	43.81	558.09	53.00	143.67	90.67	3464.31	42.01	22.24
Obse	3350.00	4396.33	42.02	617.15	53.17	144.33	91.17	3020.01	36.25	21.29
Moti	3416.67	4320.91	41.79	583.93	51.83	147.33	95.50	3150.76	32.82	21.44
Gabelcho	3583.33	4286.42	38.34	524.65	56.00	144.17	88.17	2580.27	36.98	22.05
Adet Hanna	3116.67	4253.14	43.52	421.82	54.17	139.83	85.67	2611.61	46.30	22.38
NC-58	3000.00	4859.89	52.54	401.00	53.50	138.83	85.33	3090.95	47.99	22.29
Wayu	3300.00	4140.30	40.40	444.10	57.33	145.33	88.00	2461.21	45.97	21.95
Degaga	3400.00	5081.81	49.37	454.36	54.17	137.50	83.33	3040.16	52.69	22.56
Dagm	3166.67	3551.45	36.24	362.51	56.33	144.17	87.83	2137.32	31.53	22.92
Holetta-2	3575.00	4629.20	43.00	488.32	55.17	146.00	90.83	2938.92	37.93	20.59
Shalo	3750.00	4913.17	42.26	506.99	54.50	141.17	86.67	3003.48	51.81	22.65
Tesfa	3183.33	4339.87	44.09	411.39	55.33	138.33	83.00	2559.77	46.61	22.92
Mesay	3283.33	4393.18	42.24	406.32	54.67	140.83	86.17	2677.26	49.93	22.51
Bulga 70	2900.00	4283.07	47.79	412.81	54.50	141.83	87.33	2701.40	36.85	22.34
Kassa	3350.00	4678.05	44.85	412.42	53.00	142.50	89.50	3085.26	44.26	22.48
Gora	3500.00	4112.37	38.62	690.06	54.83	146.67	91.83	2660.75	37.28	22.87
CS-20-DK	3391.67	4469.20	42.15	504.47	55.50	145.33	89.83	2787.27	36.36	21.95
Kuse	3366.67	4905.53	47.27	470.99	53.67	142.33	88.67	3179.12	56.01	21.80
Dida’a	3616.67	4855.91	43.86	596.38	53.83	146.83	93.00	3351.94	40.14	22.63
Lalo	3216.67	3847.39	37.99	380.43	55.00	146.50	91.50	2430.73	32.72	22.12
Bako local	2616.67	2955.59	36.06	333.56	56.50	140.33	83.83	1716.27	26.67	22.33
Debrebirhan local	2583.33	3791.04	49.16	358.80	54.50	140.83	86.33	2357.21	39.68	22.79
Sinana local	3050.00	4737.69	50.21	406.84	53.33	140.17	86.83	2993.91	53.26	22.51
Agarfa local	3783.33	5256.44	44.34	519.28	52.33	142.33	90.00	3546.15	53.69	22.14
EKCSR-02006	3766.67	5305.15	45.53	509.26	54.00	147.00	93.00	3585.34	42.24	21.99
Adet local	3733.33	4608.15	39.97	520.38	54.00	147.00	93.00	3139.85	34.52	21.68
Kulumsa local	4200.00	5631.46	44.09	545.65	53.67	147.00	93.33	3862.48	42.98	20.95
Mean	3369.27	4494.44	43.39	489.88	54.44	143.77	89.33	2895.21	41.75	22.13
CV	17.45	7.20	15.84	14.09	2.18	1.45	2.50	9.16	28.02	3.29
LSD	360.95	**123.94**	8.19	80.19	1.33	2.32	2.40	302.35	8.5	0.88
P-value 5%										
1%	**	**	**	**	**	**	**	**	**	**

** = highly significant(p<0.01), * = significant (p<0.05), CV = Coefficient of Variation, LSD = least significant difference, SYPP = Seed yield per plot, HI = Harvest Index, TSW =Thousand Seed weight, DF = Days to flowering, DM = Days to maturity, SFP = Seed filling period, EGR = Economic growth rate, CP = Crude Protein content

5.1.1.3. Estimation of analysis of variance

Mean squares of the 23 characters from analysis of variance (ANOVA) at individual locations (Sinana, Agarfa and Selka) are presented in Table 12, 13 and 14, respectively and mean square for combined over the three locations is presented in Table 15.

At Agarfa, significant differences among varieties ($p<0.05$) were observed for traits: leaf width, and thousand seed weight. Number of pods per plant, number of seeds per plant and harvest index revealed significant difference ($p<0.01$) and pod length, pod width, seed yield per plot, days to flowering, days to maturity, seed filling period, seed production efficiency, crude protein content showed highly significant difference ($p<0.001$). Contrary, traits such as leaf length, leaf area, leaf area index, pod width, plant height to first pod, plant height, internod's length, number of seeds per pod, biomass weight per plot and economic growth rate showed non-significant difference (Table 13).

At Selka, significant differences among varieties ($p<0.05$) were observed for traits like leaf area, and harvest index. Pod width, plant height to first pod, number of pods per plant, biomass weight per plot, and seed filling period revealed highly significant difference ($p<0.01$) and numbers of branches per plant, pod length, number of seeds per plant, seed yield per plot, thousand seeds weight, days to flowering, days to maturity, seed production efficiency, economic growth rate and crude protein showed highly significant difference ($p<0.001$). On the other hand, traits such as leaf length, leaf width, leaf area index, internod's length, plant height, number of seed per pod, number of seed per plant, biomass, and biomass production rate showed non-significant difference (Table 14).

At Sinana, significant differences among varieties ($p<0.05$) were observed for traits like plant height, number of pods per plant, harvest index and crude protein content. Leaf length, leaf area, number of seeds per pod and days to flowering revealed highly significant difference ($p<0.01$) and leaf width, pod length, pod width, seed yield per plot, thousand seeds weight, days to maturity, seed filling period and seed production efficiency showed highly significant difference at probability level of 0.1% ($p<0.001$). Contrary, traits such as numbers of branches per plant, leaf number per plant, leaf area index, internod's length, plant height to first pod, number of seeds per plant, biomass weight per plot and economic growth rate showed non-significant difference between the tested varieties (Table 12).

Table 12. Analysis of Variance for 14 traits of thirty-two faba bean varieties tested using Alpha design at Sinana during meher (main season) 2014/15

Traits	Rep (1)	Variety (31)	Rep x Block (6)	Pooled error (25)	Mean	CV (%)	R^2
LA	59.56**	23.94**	49.08**	7.24	33.44	8.05	0.92
PL	0.10ns	2.02**	1.22**	0.28	7.40	7.21	0.91
PW	0.20**	0.07**	0.08**	0.02	1.40	9.65	0.86
NPPL	13.32ns	149.25**	137.86ns	73.99	42.13	20.56	0.98
NSPL	29.40ns	855.60**	892.50ns	492.90	110.43	20.10	0.99
NSPPod	0.012ns	0.07**	0.07*	0.03	2.70	6.02	0.80
SYPP	769ns	32373**	13857**	1037	505.70	6.37	0.98
HI	5.64ns	6.87**	3.37ns	2.20	51.86	2.86	0.81
TSW	17.02ns	20.90**	65.02**	3.60	143.12	1.32	0.92
DF	3.06ns	26.78**	54.03**	4.18	91.18	2.24	0.92
DM	85522ns	777502**	229196ns	10514	3645.49	8.89	0.91
SFP	5.23ns	11.9**	12.9**	1.84	18.54	7.32	0.91
SPE	1.66ns	5.20**	3.66**	0.56	11.76	6.34	0.93
SFR	0.20ns	1.40**	0.77ns	0.56	22.66	3.31	0.77

*** = Highly significant($p<0.001$), ** = Highly significant($p<0.01$),* = Significant ($p<0.05$), ns = Non significant, CV = Coefficient of variation LA = Leaf area, PL= Pod length, PW = Pod width, NPPL = Number of pods per plant, NSPL= Number of seed per plant, NSP Pod = Number of seed per pod, SYPP = Seed yield per plot, HI = Harvest index, TSW = Thousand seed weight, DF = Days to flowering, DM = Days to maturity, SFP = Seed filling period, SPE = Seed production efficiency , CP = Crude protein content.

Table 13. Analysis of variance for 14 traits of thirty-two faba bean varieties tested using Alpha design at Agarfa during meher (main season) 2014/15

	Mean square						
Traits	Rep (1)	Variety (31)	Rep x Block (6)	Pooled error (25)	Mean	CV (%)	R^2
LA	0.67ns	20.58**	12.10**	0.92	24.89	3.81	0.85
PL	0.21ns	2.18**	2.58**	0.12	9.09	4.78	0.61
PW	0.00002ns	0.07**	0.09**	0.01	1.45	7.58	0.62
NPPL	1.69ns	115.88	29.43ns	35.51	29.27	20.10	0.80
NSPL	45.2ns	729.40**	281.30ns	229.9	77.08	19.50	0.81
NSPPod	0.008ns	0.08**	0.05**	0.01	3.66	4.08	0.60
SYPP	11424ns	137984**	141948**	14296	1395.14	8.67	0.90
HI	72.36ns	130.22*	100.10ns	52.05	64.39	15.94	0.62
TSW	207.00ns	26918*	7280ns	12814	489.50	22.72	0.66
DF	3.52ns	6.75***	3.25*	1.10	62.17	1.96	0.60
DM	4.00ns	34.28***	45.12**	5.84	140.05	1.71	0.68
SFP	15.02ns	38.27***	39.10**	7.56	88.79	3.13	0.69
SPE	2585ns	764510***	730001**	74019.00	2732.67	9.82	0.86
CP	0.88ns	2.60***	2.00**	0.54	22.23	3.29	0.89

*** = Highly significant ($p<0.001$), ** = Highly significant ($p<0.01$),* = Significant ($p<0.05$), ns = Non significant, CV = Coefficient of variation Pl = Pod length, PW = Pod width, NPPL = Number of pods per plant, NSPL= Number of seed per plant, NSP Pod = Number of seed per pod, SYPP = Seed yield per plot, HI = Harvest index, TSW =Thousand seed weight, DF = Days to flowering, DM = Days to maturity, SFP = Seed filling period, SPE = Seed production Efficiency , CP = Crude protein content.

Table 14. Analysis of variance for 14 traits of thirty-two faba bean varieties tested using Alpha design at Selka during meher (main season) 2014/15

Mean square							
Traits	Rep (1)	Variety (31)	Rep x Block (6)	Pooled error (25)	Mean	CV (%)	R^2
LA	92.78*	27.87*	64.24**	14.34	30.51	12.42	0.79
PL	1.08*	1.93***	1.47***	0.18	7.30	5.88	0.94
PW	0.02ns	0.07***	0.20***	0.03	1.30	12.90	0.84
NPPL	380.25***	102.27***	46.65ns	23.43	29.98	16.14	0.87
NSPL	52.56ns	608.65***	166.27***	28.99	77.10	6.98	0.96
NSPPod	0.00ns	0.06***	0.06***	0.01	2.60	4.44	0.87
SYPP	5251.00ns	65115.00***	43682***	6854	1241.52	6.67	0.93
HI	429.14**	64.60ns	72.04ns	36.91	37.74	16.10	0.76
TSW	665.00ns	19641.00**	6057.00**	1254	465.76	7.61	0.95
DF	0.56ns	3.57***	8.57***	0.77	58.00	1.52	0.89
DM	7.56ns	45.72***	49.15**	2.68	146.78	1.12	0.96
SFP	12.25ns	43.92***	34.86**	3.21	88.78	2.02	0.95
SPE	27164ns	287203**	98753*	37071.00	2269.25	8.48	0.91
CP	0.05ns	1.70***	2.36***	0.31	21.46	2.59	0.89

*** = Highly significant ($p<0.001$), ** = Highly significant($p<0.01$),* = Significant ($p<0.05$), ns = Non significant, CV = Coefficient of variation LA = Leaf area, Pl = pod length, PW = Pod width, PH = Plant height, NPPL= Number of pods per plant, NSPL= Number of seed per plant, NSP Pod = Number of seed per pod, SYPP = Seed yield per plot, HI = Harvest index, TSW = Thousand seed weight, DF = Days to flowering, DM = Days to maturity, SFP = Seed filling period, SPE = Seed production efficiency , CP = Crude protein content.

5.1.2. Estimation of variation components

5.1.2.1. Genotypic and phenotypic coefficient of variation

The combined estimates of genotypic (**σ^2g**), environmental (**σ^2e**) and phenotypic (**σ^2p**) variances and phenotypic (**PCV**) and genotypic coefficients of variation (**GCV**) of characters studied at Agarfa, Selka and Sinana are presented in Table 16. The amount of genotypic and phenotypic variability existing in a species is the most important point to be considered in crop selection criteria.

Among all characters, higher GCV (>10%) were observed for pod length (13.71%), pod width (11.28%|), number of pods per plant (17.14%), number of seeds per plant (16.12%), thousand seeds weight (14.22%), seed production efficiency (12.14%) and economic growth rate (15.09%) (Table 16). While intermediate genotypic coefficients of variability (>5% and <10%) were observed for leaf length (6.59%), leaf area (9.41%), leaf area index (6.64%), plant height to first pod (8.48%), number of branches per plant (8.81%), biomass weight per plot (9.64%), seed yield per plot (8.54%,) harvest index (6.98%) and grain filling period (5.52%).

Table 15. Combined analysis of variance for 23 traits of thirty-two Ethiopian faba bean varieties tested at Sinana, Agarfa and Selka during meher (main season) of 2014/15

Traits	Loc	Rep(loc)	Variety	Variety x loc	Pooled error	Mean	CV (%)	R^2
	(df=2)	(df=3)	(df=31)	(df=62)	(df=93)			
Leaf length(cm)	22.37***	0.22	0.91***	0.26***	0.09	9.32	3.26	0.91
Leaf widthW(cm)	9.52***	0.40**	0.70**	0.14**	0.08	4.49	6.40	0.87
Leaf area(cm2)	1108.08***	51.06**	59.38***	12.341***	10.21	29.72	10.75	0.84
Leaf area index	235538.98***	1329.23	4385.03***	3065.35***	1326.59	223.19	16.32	0.87
Pod length(cm)	0.96*	0.46	7.28***	0.3ns	0.24	7.28	6.78	0.91
Pod width(cm)	0.24***	0.07	0.17***	0.03ns	0.03	1.34	12.65	0.75
Internod length(cm)	27.54***	1.32	0.73***	0.51**	0.3	5.93	9.22	0.80
Plant height to first pod(cm)	4396.84***	24.60	118.54***	35.81*	22.78	43.79	10.90	0.87
Plant Height(cm)	1380.68***	23.14	163.21***	42.12***	15.56	149.07	2.64	0.88
Numbers of branches per plant	2.48ns	1.08	1.57ns	1.44ns	1.20	1.69	25.68	0.74
Number of pod per plant	3081.89***	131.75	265.52***	53.14ns	51.61.00	33.82	21.24	0.79
Number of seed per plant	23249.83**	42.41	1547.71***	328.94ns	284.56	88.49	19.08	0.81
Number of seed per pod	0.13**	0.01	0.12***	0.05***	0.02	2.63	5.10	0.81
Biomass per plant	31717.27***	26.73	1572.73***	758.59***	346.70	95.04	19.59	0.83
Seed yield per plot (g/plot)	3441447.89***	23325.12	179331.08***	888862.18***	10620.27	1438.22	7.17	0.95
Harvest index (%)	1590.82***	171.10	117.53***	62.55ns	44.90	43.39	15.45	0.73
Thusound seed weight (g)	288830.59**	547.00	46671.63***	17542.47***	4873.54	489.88	14.25	0.85
Days to flowering	649.83**	3.24	9.10***	5.05***	1.40	54.44	2.17	0.94
Days to flowering	482.92***	9.53	60.92***	33.95***	4.21	143.77	1.40	0.93
Seed filling period	188.26***	10.11	65.44***	32.92***	4.84	89.32	2.46	0.91
Seed production efficiency	310466638.86***	38423.81	1161147.27***	419447.58***	69442.9	2895.21	9.10	0.95
Economic growth rate	1926.35***	7.92	38227***	144.09***	53.89	41.75	17.58	0.83
Crude protein content (%)	23.81***	0.37	2.23***	1.98***	0.53	22.13	3.30	0.83

Number in bracket indicate degree of freedom, *** = highly significant(p<0.001), ** = highly significant(p<0.01), * = significant (p<0.05), Nns = nonsignificant, CV = Coefficient of Variation

The highest PCV (>10%) were observed for traits like leaf area (14.72%), leaf area index (22.02%), pod length (15.49%), pod width (17.22%), numbers of branches per plant (68.57%), internod's length (11.20%), plant height to first pod (14.99%), number of pods per plant (27.42%), number of seeds per plant (27.22%), biomass weight per plot (15.35%), seed yield per plot (17.70%), harvest index (18.28%), thousand seed weight (25.87%), seed production efficiency (20.95%) and economic growth rate (28.20%) (Table 16). In difference, the lower phenotypic coefficient of variations was observed for plant height (4.7%), days to flowering (3.63%) and days to maturity (3.38%).

5.1.2.2. ***Broad sense heritability and expected gains from selection***

Estimates of heritability in broad sense for tested varieties for the characters considered over combined locations are presented in Table 16. In this study, heritability value ranged from 8.45% for number of branches per plant to 95.2% for pod length. In connection to this range; the heritability values were sufficiently very high for traits like pod length (95.2%) and pod width (81.03%). The moderately high heritability values were observed for characters such as leaf length (71.88%), leaf width (78.30%), leaf area (79.09%), plant height to first pods (69.78%), plant height (74.19), number of pods per plant (79.15%), number of seeds per plant (78.75%), number of seeds per pod (60.86%), biomass weight per plot (72.79%), thousand seeds weight (62.41%), seed production efficiency (63.88%), and economic growth rate (62.31%).

Table 16. Mean, Genotypic variance, Phenotypic variance, Environmental variance, Genotypic coefficient of variation, Phenotypic coefficient of variation, Heritability in broad-sense, Genetic advance and Genetic advance as percent in mean of Ethiopian faba bean varieties

Traits	**Mean**	**σ^2e**	**σ^2g**	**σ^2g*l***	**σ^2p**	**GCV (%)**	**PCV (%)**	**Hb(%)**	**GA**	**GAM %**
Leaf length(cm)	9.32	0.09	0.11	0.08	0.28	3.54	5.69	71.88	0.79	8.43
Leaf widthW(cm)	4.49	0.08	0.09	0.03	0.20	6.59	9.99	78.30	0.72	16.11
Leaf area(cm2)	29.72	10.21	7.83	1.10	19.14	9.41	14.72	79.09	7.13	23.99
Leaf area index	223.20	1326.59	219.63	870.33	2416.55	6.64	22.02	30.05	30.43	13.63
Pod length(cm)	7.28	0.25	1.00	0.03	1.27	13.71	15.49	95-.20	2.21	30.37
Pod width(cm)	1.34	0.03	0.02	0.00	0.05	11.28	17.22	81.03	0.38	28.75
Internod length(cm)	5.93	0.30	0.04	0.11	0.44	3.21	11.20	29.81	0.41	6.88
Plant height to first pod(cm)	43.79	22.78	13.79	6.52	43.09	8.48	14.99	69.78	9.44	21.55
Plant Height(cm)	149.07	15.56	20.18	13.28	49.02	3.01	4.70	74.19	10.70	7.18
Numbers of branches per plant	1.69	1.20	0.02	0.12	1.34	8.81	68.57	8.45	0.20	11.94
Number of pod per plant	33.82	51.61	33.62	0.77	86.00	17.14	27.42	79.15	15.12	44.71
Number of seed per plant	88.43	284.56	203.13	22.19	509.88	16.12	25.54	78.75	36.63	41.42
Number of seed per pod	2.63	0.02	0.01	0.02	0.05	4.34	8.25	60.86	0.27	10.34
Biomass per plant	95.04	346.67	135.69	205.97	688.32	12.26	27.60	51.77	27.98	29.44
Seed yield per plot (g/plot)	1438.22	10620.27	15078.15	39120.96	64819.38	8.54	17.70	50.45	264.58	18.40
Harvest index (%)	43.39	44.90	9.16	8.83	62.89	6.98	18.28	46.78	7.64	17.61
Thusound seed weight (g)	489.88	4873.54	4854.86	6334.47	16062.86	14.22	25.87	62.41	162.95	33.26
Days to flowering	54.44	1.40	0.68	1.83	3.90	1.51	3.63	44.49	1.81	3.33
Days to flowering	143.77	4.21	4.49	14.87	23.58	1.47	3.38	44.26	4.43	3.08
Seed filling period	89.33	4.84	5.42	14.04	24.30	2.61	5.52	49.70	5.05	5.65
Seed production efficiency	2895.21	69442.90	123619.45	174993.84	368056.19	12.14	20.95	63.88	798.32	27.57
Economic growth rate	41.75	53.89	39.70	45.10	138.68	15.09	28.20	62.31	15.12	36.20
Crude protein content (%)	22.13	0.53	0.04	0.73	1.30	0.92	5.15	11.23	0.26	1.19

σ^2e = Environmental variance, **σ^2g** = Genotypic variance, σ^2gl = Genotype by environment interaction variance, σ^2p = Phenotypic variance, GCV = Genotypic coefficient of variation, PCV = Phenotypic coefficient of variation, Hb = Heretibility, GA = Genetic advance.

In the combined location, genetic advance as a percent mean ranged from 1.19% for crude protein content to 44.71% for number of pods per plant (Table 16). Within this range, a relatively high genetic advance as percent of mean was observed for leaf area (23.99%), pod length (30.37%), pod width (28.75%), number of pods per plant (44.71%), number of seeds per plant (41.42%), biomass weight per plot (23.02%), thousand seeds weight (33.26%), seed production efficiency (27.57%) and economic growth rate (36.20%).

5.1.3. Association studies between characters

Phenotypic (r_p) and genotypic (r_g) correlation estimates between all possible pairs of the 23 traits considered in studying of 32 Ethiopian faba bean varieties are presented in Table 17. The associations of different traits are presented as follow; -

5.1.3.1. Correlation of yield and yield related traits with other traits

In the present study, seed yield signified strong and highly significant positive genotypic correlation with leaf width ($p<0.01$), leaf area ($p<0.01$), pod width ($p<0.01$), plant height ($p<0.01$), harvest index ($p<0.01$), economic growth rate ($p<0.01$) and showed almost perfect highly significant genotypic relationship with seed production efficiency (r= 0.95, $p<0.01$). Seed yield also demonstrated significant ($p<0.05$) genotypic relationship with thousand seeds weight and leaf length. Additionally, seed yield showed positive genotypic association with leaf area index, pod length, number of pods per plant, and seed filling period. Differently, seed yield revealed negative and non-significant genotypic correlation internod length, pod length, number of branches per plant, number of pods per plant, number of seeds per plant, number of seeds per pod and crude protein content. Similarly, seed yield noted negative and highly significant ($p<0.01$) genotypic correlation coefficient with days to maturity (Table 17).

Seed yield showed positive and highly significant ($p<0.01$) phenotypic correlation coefficient with leaf length, leaf width, leaf area, leaf area index, pod width, internod length, plant height, number of pods per plant, number of seeds per plant, biomass per plot, harvest index, thousand seeds weight and seed filling period. Seed yield showed highly significant and strong perfect positive phenotypic association with seed production efficiency (r = 0.94). Some traits such as number of pods per plant, plant height to first pod, economic growth rate and crude protein content demonstrated positive and non-significant phenotypic correlation coefficient with seed yield in combined location. In opposite way, seed yield showed negative and non-significant phenotypic association with number of seeds per pod (Table 17).

There were positive non-significant genotypic association between number of branches per plant and characters such as leaf area index, internod length, number of pods per plant, number of seeds per plant, harvest index, days to maturity, seed filling period and crude protein content. In contrast to this, number of branches per plant signified negative and non-significant association with traits such as leaf length, leaf width, leaf area, pod length, pod width, plant height to first pod, plant height, number of seeds per pod, biomass weight per plot, seed yield per plot, thousand seeds weight, days to flowering, seed production efficiency and economic growth rate.

Number of branches per plant showed highly significant positive association with leaf area index and number of pods per plant and it revealed positive non-significant phenotypic relationship with leaf length, internod length, plant height, number of seeds per plant, seed yield per plot, harvest index, days to maturity, seed filling period, seed production efficiency and crude protein content. On the contrary, number of branches per plant demonstrated negative and highly significant phenotypic association with economic growth rate. In the same way, there were negative significant phenotypic interrelationship between number of branches per plant and characters such as number of seeds per pod and it also revealed negative non-significant phenotypic correlation coefficient with leaf width, leaf area, pod length, pod width, plant height to first pod, biomass weight per plot, thousand seeds weight and days to flowering.

Number of pods per plant signified strong and positive highly significant genotypic correlation coefficient with number of seeds per plant (r = 0.96) and also showed positive significant association with harvest index per plot. Number of pods per plant showed positive non-significant genotypic relationship with seed yield per plot, days to flowering, and economic growth rate. On the other hand, number of pods per plant revealed negative highly significant genotypic correlation with thousand seeds weight, days to maturity, seed filling period and showed negative significant genotypic association with biomass weight per plot. There was also negative non-significant genotypic relationship between number of pods per plant and traits such as number of seeds per pod and seed production efficiency.

Number of pods per plant revealed positive highly significant phenotypic correlation coefficient with leaf width, leaf area, leaf area index, internod length, number of branches per plant, number of seeds per plant (r = 0.94, strong association), seed yield per plot, harvest index per plot, seed production efficiency, crude protein content and significant association with biomass weight per plant. Similarly, number of pods per plant demonstrated positive non-significant phenotypic association with leaf length, plant height and biomass weight per plot. In contras to this, number of pods per plant signified negative highly significant phenotypic correlation with pod length, pod width, thousand seeds weight, days to flowering and days to maturity. In similar way, it showed negative significant phenotypic association with plant height to first pod, but number of pods per plant also showed negative non -significant phenotypic interrelationship with number of seeds per pod, seed filling period and economic growth rate.

5.1.3.2. Association between physiological parameters and other traits

Correlation between leaf length and other traits

In this study, strong and positive highly significant trends of genotypic correlation coefficient were observed between leaf length and characters such as leaf width (r = 0.74), leaf area (r = 0.88), pod length (r = 0.6), pod width (r = 0.62), plant height (r = 0.61), biomass weight per plot (r = 0.50) and thousand seeds weight (r = 0.50). Correspondingly, leaf length showed positive significant ($p<0.05$) genotypic association with seed production efficiency and also signified positive non-significant association with plant height to first pod, days to maturity and economic growth rate. On the contrary; leaf length demonstrated negative highly significant genotypic correlation coefficient with number of seeds per plant. Similarly; it showed negative non-significant genotypic relationship with internod length, number of branches per plant, number of pods per plant, number of seeds per pod, harvest index per plot, days to flowering and crude protein contents.

Leaf length showed strong positive highly significant phenotypic correlation with leaf width (r = 0.78), leaf area (r = 0.86), leaf area index (r = 0.64), pod length, pod width, internod length, plant height, biomass weight per plot, days to maturity, seed filling period, seed production efficiency. It also signified positive significant trends of phenotypic relationship with thousand seeds weight. In opposite to this, leaf length showed negative phenotypic relationship with plant height to first pod ($p<0.01$) and also revealed negative non-significant phenotypic relationship with harvest index per plot, days to flowering and crude protein contents.

Correlation between leaf width and other traits

In the present study, leaf width demonstrated strong positive significant genotypic correlation with leaf length (r = 0.74), leaf area (r = 0.97 which is almost perfect association), leaf area index (r = 0.57), pod length (r = 0.54), pod width (r = 0.64), plant height (r = 0.70), biomass weight per plot (r = 0.61), seed yield per plot (r = 0.62), thousand seeds weight (r = 0.53), seed production efficiency (r = 0.61). In same way, leaf width showed positive non-significant genotypic association with plant height to first pod, harvest index, days to maturity, seed filling period and economic growth rate. Differently, days to flowering showed negative significant genotypic association with leaf width. Accordingly, internod length, number of branches per plant, number of pods per plant, number of seeds per plant, number of seeds per pod and crude protein content signified negative non-significant genotypic relationship with leaf width (Table 17).

There were strong positive highly significant phenotypic association between leaf width and characters such as leaf length (r = 0.78), leaf area (r = 0.91), leaf area index (r = 0.63), plant height (r = 0.55). Likewise, leaf width showed positive highly significant phenotypic relationship with pod length, pod width, internod length, number of pods per plant, seed yield per plot, thousand seeds weight and seed filling period and also revealed significant association with number of seeds per pod. Oppositely, leaf width signified negative phenotypic correlation with days to flowering ($p<0.05$). Leaf width also demonstrated negative non-significant phenotypic correlation with plant height to first pod, number of branches per plant, number of seeds per pod and crude protein content.

Correlation between leaf area and other recorded traits

In studying, the genotypic correlation between leaf area and other recorded characters, strong positive highly significant association were observed among leaf area and other traits: leaf area index (r = 0.61), biomass weight per plot (r = 0.61), pod length (r = 0.57), pod width (r = 0.66), plant height (r = 0.67), seed yield per plot (r = 0.57), thousand seeds weight (r = 0.55), and seed production efficiency (r = 0.58).

There was strong highly significant phenotypic correlation between leaf area and parameters: leaf length (r = 0.86), leaf width (r = 0.91), leaf area index (r = 0.65), plant height (r = 0.52), and biomass weight (r = 0.52). Likewise, positive highly significant phenotypic correlation was observed between leaf area and traits: pod length, pod width, internod length, number of pods per plant, seed yield per plot, thousand seeds weight, seed filling period and seed production efficiency (Table 17) and significant association were resulted between leaf area and days to maturity

Correlation between leaf area index and other parameters

In this study, leaf area index demonstrated strong positive highly significant genotypic association with pod length, pod width, thousand seeds weight, and days to maturity. Plant height to first pod, plant height, number of branches per plant, biomass weight per plot, seed yield per plot, days to flowering, seed filling period and seed production efficiency signified positive and non-significant interrelationship with leaf area index and others scored parameters revealed negative non-significant association with leaf area index (Table 17).

At phenotypic level, leaf length (r = 0.64), leaf width (r = 0.63) and leaf area (r = 0.65) showed strong and positive highly significant phenotypic association with leaf area index. Similarly, majority of the traits noted positive highly significant association with leaf area index except that days to flowering and economic growth rate which were negative highly significant relationship.

Correlation between harvest index and other traits

There were positive highly significant association at genotypic level between harvest index and parameters: seed yield per plot (r = 0.46) and economic growth rate (r = 0.53) (Table 17). Positive significant association was also signified between harvest index and number of pods per plant, harvest index and number of seeds per plant, harvest index and seed production efficiency. However, leaf width, leaf area, internod length, number of branches per plant and crude protein content showed positive non-significant association with harvest index. Harvest index also showed negative highly significant association with plant height to first pod, days to flowering and days to maturity. Similarly, there was negative non-significant genotypic relationship between harvest index and traits: leaf length, leaf area index, pod length, pod width, plant height, number of seeds per pod, biomass weight per plot, thousand seeds weight and seed filling period. Harvest index indicated positive highly significant phenotypic association with internod length, number of pods per plant, number of seeds per plant, seed yield per plot and seed production efficiency. Generally other physiological parameters: biomass weight per plant, grain production efficiency and economic growth rates showed positive relationship among themselves in at both genotypic and phenotypic association (Table 17).

5.1.3.3. Correlation between phenological traits and other traits

Correlation of days to flowering with other characters

Days to flowering showed negative highly significant ($p<0.01$) genotypic correlation coefficient with harvest index, seed yield per plot and seed production efficiency. Seed yield indicated negative significant genotypic association with leaf width and economic growth rate. Similarly, days to flowering showed negative non-significant genotypic association with leaf length, leaf area, pod length, pod width, plant height, number of branches per plant, biomass weight per plot, and seed filling period. Oppositely, days to flowering showed positive non-significant genotypic correlation with leaf area index, plant height to first pod, number of pods per plant, number of seeds per plant, number of seeds per pod, days to maturity and crude protein content

Days to flowering showed positive highly significant ($p<0.01$) phenotypic correlation with days to maturity. Similarly, days to flowering revealed positive significant ($p<0.05$) phenotypic association with economic growth rate. Differently, days to flowering showed negative highly significant ($p<0.01$) phenotypic correlation with leaf area index, pod width, plant height to first pod, plant height, internod length, number of pods per plant, number of seeds per plant, seed yield per plot, harvest index per plot, seed filling efficiency and crude protein content. Similarly; days to flowering showed negative significant ($p<0.05$) phenotypic correlation coefficient with leaf width, biomass weight per plot and number of seeds per pod. On the other hand, days to flowering indicated negative non-significant phenotypic association with leaf length, leaf area, pod length, number of branches per plant, and thousand seeds weight.

Correlation of days' maturity and other traits

There were strong and highly significant ($p<0.01$) positive genotypic correlation between days to maturity and seed filling period (r = 0.93), days to maturity and thousand seeds weight (r = 0.64). Days to maturity also showed positive highly significant ($p<0.01$) genotypic association with plant height to first pod, pod length and leaf area index. In the same way, days to maturity also demonstrated significant ($p<0.05$) genotypic relationship with pod width and biomass weight per plot (Table 17). Days to maturity revealed positive non-significant genotypic correlation with leaf length, leaf width, leaf area, plant height, number of pods per plant, seed yield per plot, days to flowering and seed production efficiency. Differently, days to maturity revealed negative highly significant($p<0.01$) genotypic correlation with number of pods per plant, number of seeds per plant, harvest index per plot, economic growth rate and crude protein content. Negative significant genotypic interrelation was also observed between days to maturity and other traits such as internod length. Similarly; days to maturity revealed negative non-significant genotypic correlation coefficient with number of seeds per pod.

Days to maturity showed positive highly significant ($p<0.01$) phenotypic correlation coefficient with leaf length, pod length, biomass weight per plant, thousand seeds weight, days to maturity and also showed strong highly significant phenotypic correlation with seed filling period (r = 0.81). Furthermore, days to maturity revealed positive significant ($p<0.05$) phenotypic correlation coefficient with leaf area, but demonstrated positive non-significant phenotypic association with leaf width, leaf area index, pod width and number of branches per plant. In opposite way, days to maturity indicated negative highly significant ($p<0.01$) phenotypic correlation coefficient with internod length, plant height to first pod, number of pods per plant, number of seeds per plant, harvest index per plot, seed production efficiency, crude protein content and economic growth rate ($p<0.05$). Days to maturity revealed negative non-significant phenotypic association with plant height, number of seeds per pod, and seed yield per plot.

Correlation of seed filling period and other traits

Seed filling period had positive highly significant genotypic interrelationship with seed production efficiency, biomass weight per plot, pod length, and pod width. Seed filling period showed strong positive highly significant genotypic correlation with days to maturity (r = 0.93) and harvest index per plot (r = 0.71). Similarly, seed filling period showed positive non-significant association with leaf length, leaf width, leaf area, leaf area index, plant height to first pod, plant height, number branches per plant and seed yield per plot.

At phenotypic level, seed filling period showed positive highly significant phenotypic association with leaf length, leaf width, leaf area, pod length, pod width, biomass weight per plot, seed yield per plot, thousand seeds weight, days to maturity (r = 0.80) and seed production efficiency. Moreover, seed filling period revealed positive significant phenotypic relationship with plant height.

Table 17. Estimates of genotypic (Below diagonal) and phenotypic (Above diagonal) correlation coefficient of combined means of 23 traits studied for 32 Ethiopian faba bean varieties tested at Agarfa, Selka and Sinana during 2014/15 main cropping season.

Variable	LL	LW	LA	LAI	PL	PW	IL	PHFP	PH	NBPPL	NPPL	NSPL	NSPPod
LL	1	0.74***	0.88***	0.51**	0.59***	0.62***	-0.10	0.21	0.61***	-0.08	-0.32	-0.39**	-0.17
LW	0.78***	1	0.98**	0.57***	0.54***	0.64***	-0.06	0.12	0.70***	-0.19	-0.24	-0.34	-0.26
LA	0.86***	0.90***	1	0.60***	0.57***	0.66***	-0.10	0.18	0.68***	-0.14	-0.26	-0.37*	-0.29
LAI	0.64***	0.62***	0.65***	1	0.54**	0.52**	-0.30	0.30	0.29	0.09	-0.26	-0.30	-0.07
PL	0.36***	0.37***	0.39***	0.27***	1	0.89***	-0.15	0.31	0.63***	-0.16	-0.81***	-0.81***	0.22
PW	0.22**	0.28***	0.25***	0.21**	0.64***	1	-0.17	0.33	0.72****	-0.22	-0.73***	-0.78***	0.01
IL	0.28***	0.26***	0.26***	0.43***	0.02	0.16**	1	0.00	0.12	0.07	0.14	0.08	-0.11
PHFP	-0.34**	-0.23	-0.25	-0.18	0.16	0.27	0.11	1	0.33	-0.01	-0.47**	-0.53**	-0.12
PH	0.46***	0.55***	0.52***	0.42***	0.43***	0.42***	0.39***	0.20**	1	-0.25	-0.51**	-0.53**	0.07
NBPPL	0.01	-0.07	-0.02	0.22**	-0.04	-0.07	0.04	-0.01	0.04	1	0.33	0.29	-0.15
NPPL	0.13	0.19**	0.19**	0.41***	-0.40***	-0.25***	0.37***	-0.17*	0.04	0.20**	1	0.96***	-0.30
NSPL	0.14	0.16*	0.15*	0.45***	-0.39***	-0.25***	0.39***	-0.20**	0.06	0.15	0.94***	1	-0.04
NSPPod	0.03	-0.03	-0.02	0.11	0.20	-0.00	0.07	0.03	0.11	-0.17*	-0.13	0.11	1
BMPP	0.44***	0.49***	0.50***	0.34***	0.34***	0.37***	0.22***	0.05	0.54***	-0.01	0.04	-0.01	-0.15
SYPP	0.28***	0.41***	0.39***	0.39***	0.15*	0.30***	0.40***	0.08	0.47***	0.07	0.35***	0.35***	-0.01
HI	-0.04	0.045	-0.02	0.12	-0.11	0.06	0.22***	0.04	0.05	0.03	0.33***	0.37***	0.10
TSW	0.14*	0.24**	0.23***	0.24***	0.54***	0.44***	0.00	0.19**	0.34**	0.00	-0.23**	-0.24***	0.03
DF	-0.05	-0.16*	-0.13	-0.25**	-0.12	-0.24***	-0.43***	-0.34***	-0.41***	-0.10	-0.24***	-0.29***	-0.16*
DM	0.19**	0.12	0.16*	0.11	0.27**	0.11	-0.25***	-0.22**	-0.09	0.02	-0.25***	-0.29***	-0.10
SFP	0.22**	0.23**	0.25***	0.28***	0.35***	0.27***	0.01	-0.02	0.17*	0.08	-0.11	-0.12	-0.01
SPE	0.28***	0.41***	0.39***	0.43***	0.22**	0.33***	0.43***	0.13	0.51***	0.09	0.32***	0.31***	0.03
EGR	0.02	0.06	0.06	-0.40***	-0.12	-0.01	-0.14*	-0.13	-0.01	-0.35***	-0.14	-0.18*	-0.11
CP	-0.04	-0.01	-0.04	0.15*	-0.16*	-0.01	0.17	0.08	0.06	0.05	0.20**	0.24**	0.02

*** = highly significant at probability level of $p<0.001$, ** = highly significant at probability level of $p<0.01$, * = Significant at probability level of $p<0.05$ and ns = Non-significant, LL=leaf length, LW = Leaf width, LA = Leaf area, , LAI = Leaf area index, Pl = Pod length, PW = Pod width, IL= Internodes' length, HFPN = Height to first poding node, PH = Plant height, NBPPL= Number of branches per plant, NPPL= Number of pods per plant, NSPL= Number of seed per plant, NSP Pod = Number of seed per pod, = Seed yield per plot, HI = Harvest Index, TSW = Thousand Seed weight, DF = Days to flowering, DM = Days to maturity, SFP = Seed filling period, SPE = Seed production efficiency, EMR = Economic growth rate, CP = Crude Protein content

Table 11. Contued...,

Variable	BMPP	SYPP	HI	TSW	DF	DM	SFP	SPE	EGR	CP
LL	0.5**	0.40*	-0.04	0.49**	-0.16	0.21	0.26	0.38*	0.12	-0.07
LW	0.61***	0.62***	0.09	0.53***	-0.36*	0.12	0.25	0.61***	0.26	-0.18
LA	0.61**	0.57***	0.04	0.55***	-0.30	0.18	0.28	0.56***	0.21	-0.15
LAI	0.34	0.10	-0.23	0.58***	0.04	0.37**	0.35	0.16	-0.34	-0.09
PL	0.45***	0.22	-0.21	0.85***	-0.30	0.47**	0.56***	0.36*	-0.17	-0.32
PW	0.63***	0.45**	-0.14	0.79***	-0.30	0.41*	0.51**	0.52**	0.07	-0.36*
IL	-0.12	-0.05	0.04	-0.28	0.01	-0.41*	-0.40*	-0.09	0.14	-0.03
PHFP	0.46**	-0.12	-0.64***	0.40*	0.34	0.47**	0.33	-0.05	-0.27	-0.28
PH	0.63***	0.53**	-0.04	0.57***	-0.35	0.03	0.16	0.51**	0.34	-0.24
NBPPL	-0.16	-0.13	0.03	-0.15	-0.06	0.09	0.11	-0.08	-0.29	0.02
NPPL	-0.37*	0.01	0.44*	-0.76***	0.04	-0.53***	-0.53**	-0.13	0.21	0.28
NSPL	-0.48**	-0.12	0.42*	-0.80***	0.07	-0.56***	-0.57***	-0.26	0.13	0.27
NSPPod	-0.24	-0.35	-0.12	0.04	0.01	-0.01	-0.01	-0.29	-0.29	-0.18
BMPP	1	0.67***	-0.34	0.60***	-0.28	0.44*	0.52**	0.71***	0.18	-0.42*
SYPP	0.61***	1	0.46**	0.36*	-0.62***	0.02	0.25	0.95***	0.61***	-0.27
HI	-0.20**	0.51***	1	-0.19	-0.46**	-0.46***	-0.27	0.35*	0.52**	0.16
TSW	0.41***	0.39***	0.08	1	-0.25	0.64***	0.71***	0.51**	-0.10	-0.33
DF	-0.15*	-0.53***	-0.43***	-0.12	1	0.09	-0.28	-0.74***	-0.37*	0.14
DM	0.25***	-0.08	-0.32***	0.29***	0.38***	1	0.93***	0.24	-0.56***	-0.52**
SFP	0.36***	0.25***	-0.06	0.39***	-0.25***	0.80***	1	0.50**	-0.40*	-0.55**
SPE	0.58***	0.94***	0.47***	0.40***	-0.71***	-0.01***	0.45***	1	0.42*	-0.37*
EGR	0.10	0.16	0.12	-0.01	0.18*	-0.14*	-0.27***	0.01	1	0.23
CP	-0.12	0.08	0.18*	-0.13	-0.26***	-0.28***	-0.13	0.11	-0.09	1

*** = highly significant at probability level of $p<0.001$, ** = highly significant at probability level of $p<0.01$, * = Significant at probability level of $p<0.05$ and ns = Non-significant, LL=leaf length, LW = Leaf width, LA = Leaf area, LAI = Leaf area index, Pl = Pod length, PW = Pod width, IL= Internodes’ length, HFPN = Height to first poding node, PH = Plant height, NBPPL= Number of branches per plant, NPPL= Number of pods per plant, NSPP = Number of seeds per pod, BMPP = Biomass weight per plot, SYPP = Seed yield per plot, HI = Harvest Index, TSW = Thousand Seeds weight, DF = Days to flowering, DM = Days to maturity, SFP = Seed filling period, SPE = Seed production Efficiency, EGR = Economic growth rate, CP = Crude Protein content

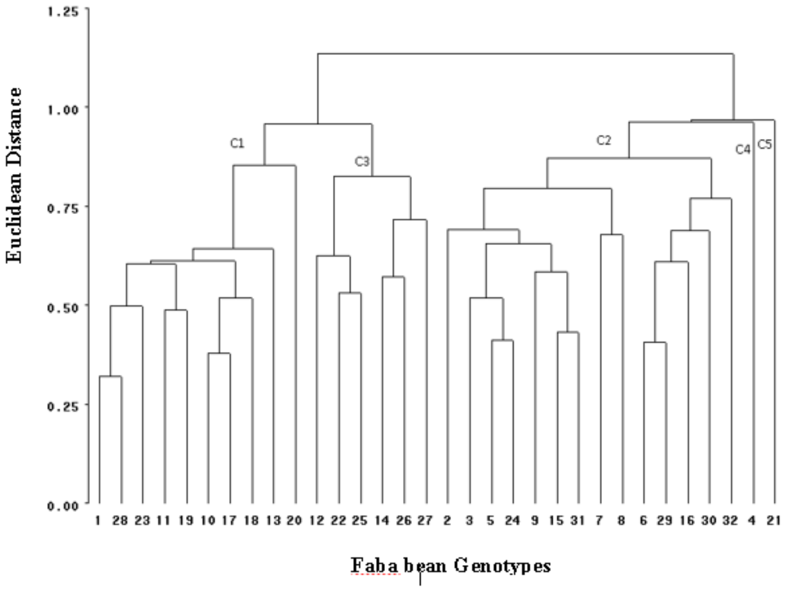
5.1.4. Cluster and principal component analysis

5.1.4.1. Cluster analysis

Cluster analysis using the whole genotypes revealed five distinct groups. The number of genotypes in each of five clusters ranged from one to fourtheen in the smallest and largest cluster, respectively (Table 18 & Figure 4). The first cluster was the second largest cluster containing 9 genotypes and one local variety (Table 18). This clusters contains genotypes derived from hybridization, introduced materials and selected from landraces and no clear grouping was observed with this cluster based on their genetic information. The second cluster was the largest cluster consisting 14 genotypes. The majority of genotypes derived via hybridization were grouped in this cluster. The third cluster contains six genotypes from landrace selection and local varieties. The 4th and 5th clusters contain each one variety called Dosha and Gora, respectively (Table 18).

Table 18. Lists of genotypes grouped under different clusters

Clusters	Number of Genotypes	Name of Varieties
1	10	Mosisa, Adet Hanna, Tesfa, Mesay, NC-58, Degaga, Kassa, Kuse, Bulga 70, Sinana Local
2	14	Tumsa, Gebelcho, Holetta-2, Hachalu, Obse, Gachena, Didae, Shallo, Walki, Moti, EKCSR-02006, Agarfa local, Adet local, Kulumsa local
3	6	Dagm, Wayu, CS-20-DK, Lalo, Bako local, Eniwari local
4	1	Dosha
5	1	Gora



Where 1 = Mosisa,2 = Tumsa,3 = Hachalu,4 = Dosha, 5 =Gachena,6 = Walki,7 = Obse, 8 = Moti, 9 = Gabelcho, 10 = Adet Hanna, 11 = NC-58, 12 = Wayu, 13 = Degaga, 14 = Dagm,15 = Holetta-2, 16 = Shalo, 17 = Tesfa, 18 = Mesay, 19 = Bulga 70, 20 = Kassa, 21 = Gora, 22 = CS-20-DK, 23 = Kuse, 24 = Dida'a, 25 = Lalo, 26 = Bako local, 27 = Eniwari local, 28 = Sinana local, 29 = Agarfa local, 30 = EKCSR-02006, 31 = Adet local, 32 = Kulumsa local

Figure 4. Dendrogram with UPGM and Euclidean distance showing similarity among 32 Ethiopian faba bean genotypes using 23 quantitative traits.

*5.1.4.2.**Genetic distance between clusters***

Pair wise generalized distances (D^2) among the five clusters are presented in Table 19. The distance analysis showed that all inter cluster distances were highly significant (*$p<0.01$*). The highly significant (*$p<0.01$*) and maximum distance ($D^2 = 554.60$) was detected between C1 & C5, while a minimum distance ($D^2 = 54.80$) was observed between C1 & C3. Similarly, the second and third most divergent clusters were C3 and C5 ($D^2 = 459.37$) and C1 and C4 ($D^2 = 411.55$), respectively.

Table 19. Distances between five clusters of 32 faba bean varieties grown in Ethiopia

Clusters	C1	C2	C3	C4	C5
C1	0	80.54**	54.80**	411.55**	554.60**
C2		0	89.76**	376.91**	358.07**
C3			0	388.79**	459.37**
C4				0	306.27**
C5					0

** = highly significant at probability level $p<0.01$ ($\chi^2_{22} = 40.29$)

5.1.4.3. Mean performance of genotypes in different clusters

Cluster mean performances showed the existence of significant variation among the different clusters for individual traits considered (Table 20). The first cluster (C_1) showed the highest cluster mean performance for internod length and economic growth rate. On the other hand, this cluster revealed the lowest mean performance for leaf area index, days to flowering and seed filling period.

The second cluster (C_2) showed the highest mean performance for plant height to first pod, plant height, biomass weight, seed yield per plot and seed production efficiency, Similarly, this cluster contains early flowering varieties (lower days to flowering) and lowest mean performance of crude protein content.

The third cluster (C_3) revealed the highest mean performance for numbers of branches per plant, numbers of pods per plant, number of seeds per plant and flowering. In contrary, this cluster showed the lowest mean performance for leaf width, leaf area, pod length, pod width, plant height, seed yield per plot, thousand seeds weight and seed production efficiency. The fourth cluster (C_4) showed the highest mean performance for numbers of branches per plant, harvest index, days to flowering and seed filling period, but this cluster revealed lowest internod length, biomas weight per plot and economic growth rate.

The fifth cluster showed the highest mean performance for leaf length, leaf width, leaf area, leaf area index, pod length, pod width, thousand seeds weight and crude protein content. In contrast, this cluster revealed the lowest mean performance for numbers of branches per plant, numbers of pods per plant, number of seeds per plant and harvest index.

Table 20. Cluster mean performance for traits of 32 Ethiopian faba bean varieties grown in Ethiopia

Traits	**C1**	**C2**	**C3**	**C4**	**C5**	**Mean**
Leaf length(cm)	9.32	9.42	8.89b	9.48	10.24a	9.47
Leaf widthW(cm)	4.51	4.66	3.98b	4.66	4.92a	4.54
Leaf area(cm2)	29.73	31.11	25.26b	31.28	35.37a	30.55
Leaf area index	205.27b	234.46	207.08	282.05	282.98a	242.37
Pod length(cm)	6.80	7.88	6.14b	7.87	9.87a	7.71
Pod width(cm)	1.26	1.46	1.12b	1.40	1.58a	1.36
Internod length(cm)	6.09a	5.91	5.89	5.38b	5.40	5.73
Plant height to first pod(cm)	40.74b	46.07a	44.01	40.80	44.03	43.13
Plant Height(cm)	149.95	152.03a	141.39b	142.30	151.73	147.48
Numbers of branches per plant	1.69	1.61	1.82	2.23a	1.53b	1.78
Number of pods per plant	37.41	30.17	38.01a	33.20	24.53b	32.66
Number of seeds per plant	98.03	77.86	100.65a	87.83	67.67b	86.41
Number of seeds per pod	2.64	2.60b	2.65	2.63	2.71a	2.65
Biomass weight per plot (g/plot)	3153.33	3685.12a	3047.22	2666.67b	3500.00	3210.47
Seed yield per plot (g/plot)	1475.40	1525.83a	1213.60b	1309.94	1315.96	1368.14
Harvest index per plot (%)	47.28	41.87	40.33	48.90a	38.62b	43.40
Thusound seeds weight (g)	431.68	552.03	397.31b	556.88	690.06a	525.59
Days to flowering	54.03	54.01b	55.86a	55.50	54.83	54.85
Days to flowering	140.38b	145.63	143.75	148.67a	146.67	145.02
Seed filling period	86.35b	91.62	87.89	93.17a	91.83	90.17
Seed production efficiency	2883.14	3187.51a	2315.00b	2639.31	2660.75	2737.14
Economic growth rate	48.79a	40.82	35.49	26.54b	37.28	37.78
Crude protein content (%)	22.47	21.75b	22.34	22.07	22.87a	22.30

A = highest value, b = lowest value

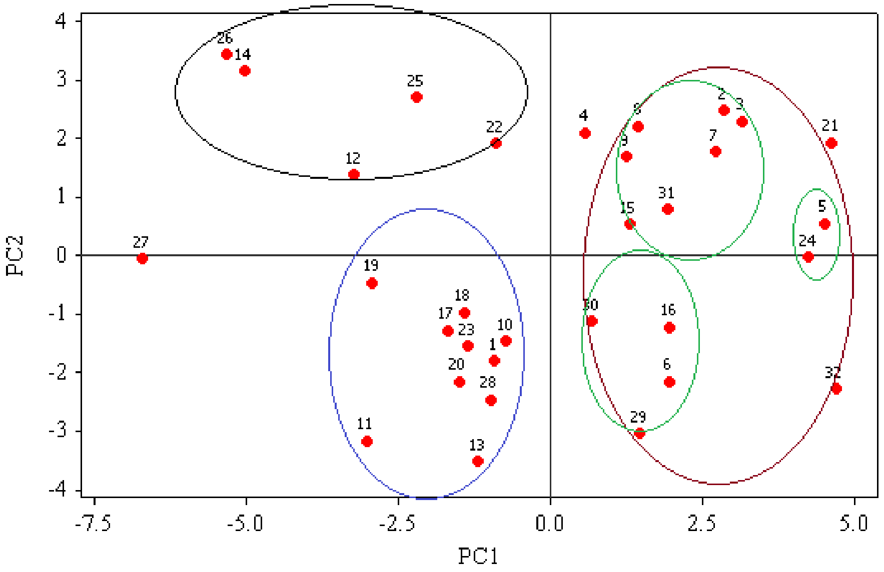
5.1.4.4. Principal component analysis (PCA)

The principal component analysis was applied to estimate the relative contribution of traits towards the variation in the 32 Ethiopian faba bean varieties. The first six principal components (PCs) with eigenvalue greater than one accounted for 85% of the entire diversity among tested varieties for all the 23 traits (Table 21). The PCA grouped the genotypes into three major distinict groups which is constistent with cluster analysis and two varieties separated solely from the groups supporting the cluster analysis.

Table 21. Eigenvalues, Variance percent, Cumulative percent and Eigenvectors of the first eight principal components of 23 quantitative traits of 32 Ethiopian Faba bean varieties

	Principal Components					
	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
Eigenvalue	8.78	4.24	2.06	1.83	1.50	1.10
Variance (%)	0.38	0.19	0.09	0.08	0.07	0.05
Cumulative variance (%)	0.38	0.57	0.66	0.74	0.80	0.85
Traits	**Eigenvectors**					
Leaf length(cm)	0.236	-0.104	-0.189	0.249	-0.167	0.080
Leaf widthW(cm)	0.257	-0.197	-0.131	0.223	-0.076	0.035
Leaf area(cm2)	0.262	-0.176	-0.137	0.275	-0.123	0.050
Leaf area index	0.195	0.084	-0.043	0.373	-0.343	0.075
Pod length(cm)	0.288	0.099	-0.112	-0.147	-0.217	0.158
Pod width(cm)	0.307	-0.001	-0.098	-0.097	-0.081	0.028
Internod length(cm)	-0.073	-0.095	-0.233	-0.057	0.388	0.599
Plant height to first pod(cm)	0.147	0.243	-0.134	0.202	0.407	-0.001
Plant Height(cm)	0.251	-0.139	-0.269	-0.111	0.088	0.195
Numbers of branches per plant	-0.066	0.037	0.317	0.306	-0.085	0.519
Number of pods per plant	-0.237	-0.227	0.171	0.295	0.007	0.053
Number of seeds per plant	-0.263	-0.177	0.146	0.192	-0.085	0.085
Number of seeds per pod	-0.024	0.175	-0.091	-0.456	-0.307	0.230
Biomass weight per plot (g/plot)	0.266	-0.070	0.073	0.091	0.339	-0.089
Seed yield per plot (g/plot)	0.184	-0.360	0.186	-0.041	0.129	-0.085
Harvest index per plot (%)	-0.066	-0.355	0.129	-0.137	-0.279	0.027
Thusound seeds weight (g)	0.301	0.086	0.036	-0.072	-0.110	-0.059
Days to flowering	-0.115	0.279	-0.251	0.263	0.123	-0.199
Days to maturity	0.195	0.276	0.334	0.056	0.047	-0.143
Seed filling period	0.230	0.174	0.418	-0.028	0.000	-0.048
Seed production efficiency	0.222	-0.282	0.290	-0.083	0.107	-0.032
Economic growth rate	0.005	-0.400	-0.159	-0.143	0.172	-0.248
Crude protein content (%)	-0.149	-0.090	-0.290	0.163	-0.260	-0.294

The PC_1 contributed 38% of total variation among Ethiopian faba bean genotypes and this variation were contributed by traits: leaf length, leaf width, leaf area, pod length, pod width, plant height, biomass weight per plot, thousand seeds weight and seed production efficiency. Similarly, PC_2 contributed 19 % of total variation and contributed by traits such as plant height to first pod, number of pods per plant, seed yield per plot, days to flowering, days to maturity and seed production efficiency. On the other hand, PC_3 contributed 9 % of total variation and traits that caused this variation were internod length, plant height, number of branches per plant, days to flowering, days to maturity, seed filling period, seed production efficiency and crude protein.



Where 1 = Mosisa,2 = Tumsa,3 = Hachalu,4 = Dosha, 5 =Gachena,6 = Walki,7 = Obse, 8 = Moti, 9 = Gabelcho, 10 = Adet Hanna, 11 = NC-58, 12 = Wayu, 13 = Degaga, 14 = Dagm,15 = Holetta-2, 16 = Shalo, 17 = Tesfa, 18 = Mesay, 19 = Bulga 70, 20 = Kassa, 21 = Gora, 22 = CS-20-DK, 23 = Kuse, 24 = Dida'a, 25 = Lalo, 26 = Bako local, 27 = Eniwari local, 28 = Sinana local, 29 = Agarfa local, 30 = EKCSR-02006, 31 = Adet local, 32 = Kulumsa local

Figure 5. Relative position of the 32 Ethiopian faba bean varieties in the first and second principal axis.

As it is visualized in Figure 5, the improved and local genotypes in this study were distributed in all four quadrants of the principal component axis. Eniwari local (27) was plotted far apart from the group. Genotypes number 26, 14, 27, 25, 19 and 12 highly contributed to PC1 in negative direction and genotype 32, 24, 5, and 29 contributed highly in positive direction for PC1. On the other hand, the traits of genotypes number 11, 13, 1, 20, and 28 contributed highly and negatively to the PC2, whereas genotype 21, 2, 3, 4 and 26 contributed highly and positively in this PC2.

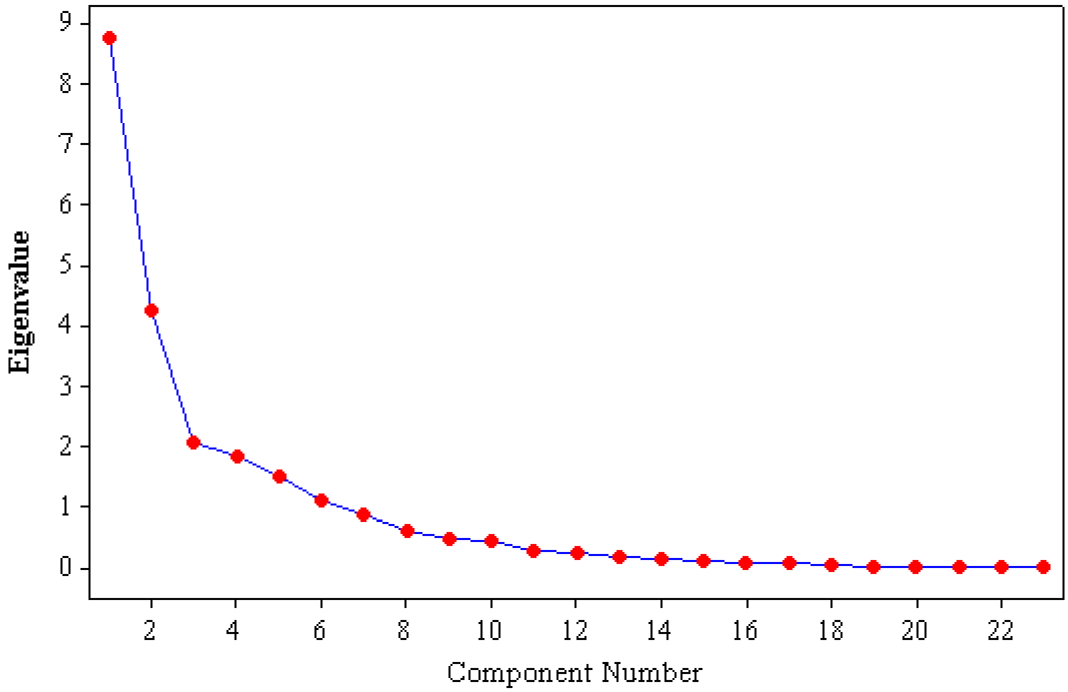


Figure 6. The relative magnitude of contribution of each component to the overall diversity in the 32 Ethiopian faba bean genotypes.

The relative contribution of the different principal components (PCs) were plotted and presented graphically in Figure 6. There was a sharp decline in contribution from PC1 to PC2 and then from PC2 to PC3 in that order, while the rate of decrease in contribution became gradually lower and lower for the remaining PCs. This shows that the first few principal components had the greatest contribution to the overall variation in the Ethiopian faba bean genotypes for the 23 measured traits.

5.1.5. Comparison of the performance of selected genotypes over all individuals

The comparison using t-test showed highly significant differences between means of the selected subsets of the top 10% best genotypes ($\bar{X}$) and the population parameters (μ) for pod length and showed significant difference for leaf length, leaf area, pod width, number of branches per plant, number of seeds per plant, seed yield per plant, thousand seeds weight and economic growth rate (Table 22). The least percentage of change with the recorded traits was observed for days to maturity (3.02%), crude protein contents (3.53%), days to flowering (4.2%) and seed filling period (5.23%) (Table 22). On the other hand, the highest percentage of change was observed for traits such as economic growth rate (30.67%), thousand seeds weight (31.17%) and number of branches per plant (68.15%).

Table 22. Comparison of the mean performances of selected subsets ($\bar{X}$) of the 10% best genotypes for 23 characters with the average performances of the whole population (μ) of 32 faba bean genotypes

Trait	Mean of selected sample ($\bar{X}$)	Mean of base population (μ)	Change via selection ($\bar{X}-\mu$)	change as % population parameter	t-value	P value
Leaf length	10.12	9.32	0.80	8.59	1.94	*
Leaf width	5.02	4.49	0.53	11.69	1.65	ns
Leaf area	35.23	29.72	5.51	18.55	1.71	*
Leaf area index	276.44	223.20	53.23	23.85	1.33	ns
Pod length	9.42	7.28	2.14	29.37	3.31	**
Pod width	1.63	1.34	0.29	22.04	2.17	*
Internode length	6.50	5.93	0.57	9.57	1.14	ns
Plant height to first pod	52.61	43.79	8.82	20.15	1.63	ns
Plant height	155.88	149.07	6.81	4.57	1.49	ns
Number of branches per plant	2.84	1.69	1.15	68.35	1.72	*
Number of pods per plant	45.32	33.82	11.50	34.01	1.83	*
Number of seeds per plant	115.97	88.43	27.54	31.14	1.75	*
Number of seeds per pod	2.89	2.63	0.27	10.09	2.11	*
Biomass weight per plot	3950.00	3361.72	588.28	17.50	1.85	*
Seed yield per plot	5397.68	4494.44	903.25	20.10	1.59	ns
Harvest index per plot	51.22	43.39	7.82	18.03	1.51	ns
Thousand seeds weight	642.56	489.88	152.68	31.17	2.09	*
Days to flowering	56.72	54.44	2.28	4.20	1.21	ns
Days to maturity	148.11	143.77	4.35	3.02	1.42	ns
Seed filling period	94.00	89.33	4.67	5.23	1.59	ns
Seed production efficiency	3664.65	2895.21	769.45	26.58	1.61	ns
Economic growth rate	54.56	41.75	12.81	30.67	1.78	*
Crude protein content	22.91	22.13	0.78	3.53	1.10	ns

5.1.6. Estimation of the magnitude of genetic progress

Regression analysis by using seed yield per hectare and thousand seeds weight showed changes over the year of variety release. Upon linear regression analysis; a positive association (r = 0.25) was observed between seed yield and years of variety release (Figure 7). The annual genetic gain in seed yield using the oldest cultivar (CS-20-DK) as base reference was 10.45 kgha^{-1} year^{-1} representing relative genetic gain of 0.23% annually and the cumulative genetic progress from past 36 years of faba bean breeding in Ethiopia was also 376.2 Kg ha^{-1} (8.4%). The trends of genetic gain obtained in the past faba bean breeding program from 1977 to 2014 was presented in Table 23.

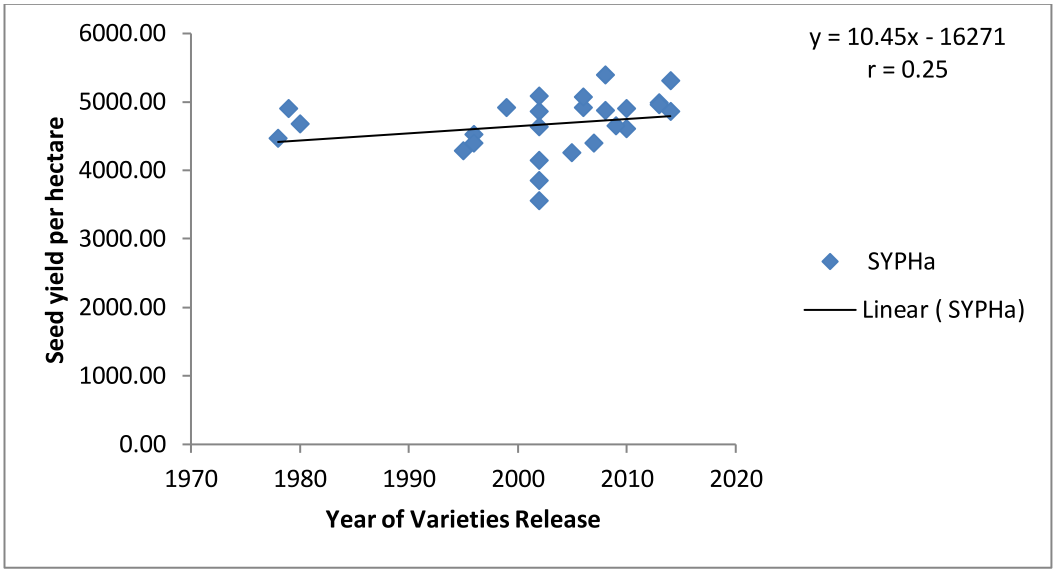


Figure 7. Bi-plot analysis of seed yield (kg ha^{-1}) against years of varieties release starting from 1977 to 2014.

The result presented in Table 23 shows that there is gradual increase in seed yield with the year of release. The average yields of varieties released in similar decades compared, the mean value of respective years indicated an increase in seed yield over CS-20-DK by 7.22%, 1.34%, 6.98% and 10.39%, respectively (Table 23).

In the present study, varieties derived from intra-specific hybridization, introduction and local collection produced an average seed yield of 4853 kgha^{-1}, 4786 kgha^{-1} and 4545 kg ha^{-1} with percentage of increment of 8.59%, 7.10% and 1.7%, respectively over the oldest variety CS-20-DK (Table 23).

Table 23. Trends in genetic progresses in grain yield from breeding faba bean released in 1980's, 1990's, 2000-2010, 2011-2014 and those promising in pipelines

variety	Year of release	Mean grain yield (Kg/ha)	Incerement in (Kg/ha) over CS-20-Dk	Incerement in (%) over CS-20-Dk
CS-20-DK	1977	4469.20		
Kuse Kassa	1980's	4791.9	322.70	7.22
Shalo Tesfa Mesay Bulga-70	1990's	4529.07	59.87	1.34
NC-58 Wayu Degaga Dagm Holeta-2 Obse Moti Gebelcho Tumsa Hachalu	2000-2010	4781.37	312.17	6.98
Mosisa Gora Didea	2011-2014	4933.7	464.50	10.39
EKCSR-02006	Promising pipeline	5305.15	835.95	18.70
Hybridization derived	-	4853.00	383.8	8.59
Introduction derived	-	4785.85	316.65	7.10
Local collection derived	-	4544.97	75.77	1.70

Linear regression analysis was also made for estimating genetic gain of thousand seeds weight by regressing over year of release by referencing the oldest variety and revealed positive association (r = 0.52) with year of variety release. Seed size revealed 4.41 g per thousand seeds weight (0.87% per 1000 seeds weight) of an annual rate of improvement in the past faba bean breeding in Ethiopia and the average genetic increment of 158.76 g per thousand seeds weight (31.5% per 1000 seeds weight) (Figure 8).

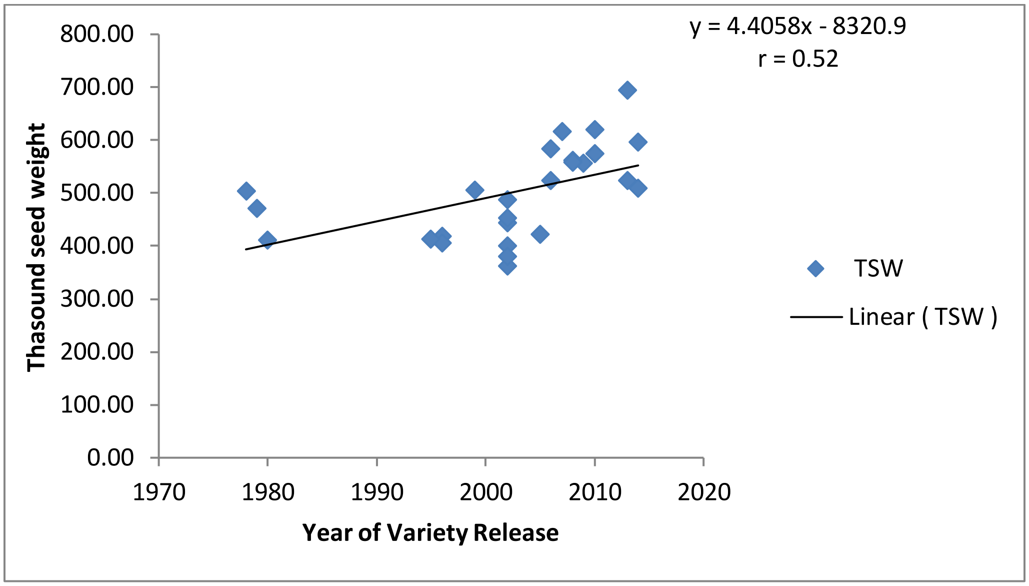


Figure 8. Bi-plot analysis of thousand seeds weight (g) against years of varieties release starting from 1977 to 2014.

5.2. Genetic Diversity Analysis based on ISSR Markers

5.2.1. ISSR-PCR amplification and banding pattern

A total of 25 ISSR primers were initially screened for molecular analysis to characterize genetic diversity among 32 Ethiopian faba bean genotypes. Among these, only 11 primers produced relatively clear amplification, sharp and reproducible bands. The names, their repeat motifs, amplification patterns and number of scored bands of the utilized primers are shown in Table 24. The size of all amplified bands ranged from about 200 to 3000 bp (Figure 9). A total of 120 bands were scored from eleven primers ranging from seven for 818 to seventeen for primer 811 with the average number of bands and polymorphic bands per primer of 11 and 10, respectively.

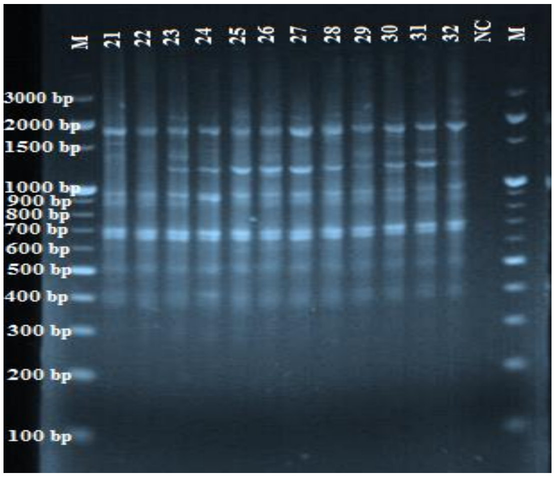
Table 24. Banding patterns generated using the eleven primers, their repeat motifs, amplification quality and number of bands scored

S/N	Primer	Repeat motif	No of scored bands
1	811	(GA)8C	17
2	812	(GA)8G	15
3	818	(CA)8G	7
4	848	(CA)8RG	9
5	854	(TC)8RG	10
6	857	(AC)8YG	10
7	860	(TG)8RA	9
8	864	(ATG)6	11
9	873	(GACA)4	13
10	880	(GGAGA)3	9
11	881	(GGGTG)3	10
	Total bands		**120**

Source: Primer kit 900 (UBC 900)

5.2.2. Polymorphic loci and percentage polymorphisms

A total of 107 polymorphic loci were found in 11 assayed primers ranging from five for 818 to 17 for 811 with an average of 10 polymorphic loci per primer with the total of 90% polymorphisms. The di-nucleotide primers revealed the highest polymorphic loci (65), while the tri-nucleotide primer (864), tetra-nucleotide primer (873) and penta-nucleotides primer (880 & 881) were observed to have lower polymorphic loci with10, 13 and 19, respectively. The primers 811, 860, 873 and 881 revealed 100% polymorphic bands (Figure 9 and Table 26).



Where M = DNA ladder, Samples number =21-32, NC = Negative control

Figure 9. ISSR fingerprint generated from 12 individuals of faba bean varieties using primer 873

5.2.3. Diversity indices of faba bean genotypes

Among cultivated Ethiopian faba bean genotypes considered in the present study, varieties derived from hybridization showed higher gene diversity and Shannon index with values of 0.27 and 0.41, respectively as compared to the other categories (Table 25). The least gene diversity and Shannon information index was demonstrated by farmer varieties with value of 0.12 and 0.18, respectively.

Table 25. Diversity parameters indicating the variability of four categories of faba bean genotypes in the present study.

Population Type	NPL	PP	na	ne	h	I
Hybridization	101	84.17	1.84+0.37	1.44+0.34	0.27+0.17	0.40+24
Local Selection	92	76.67	1.77+0.42	1.39+0.34	0.24+0.18	0.37+0.25
Introduction Sel.	56	44.67	1.44+0.5	1.30+0.35	0.17+0.20	0.26+0.28
Local Varieties	45	37.5	1.37+0.48	1.19+0.32	0.12+0.17	0.18+0.25
Over all genetic diversity	**107**	**90.00**	**1.90±0.30**	**1.43±0.34**	**0.27±0.16**	**0.41±0.23**

Where, NPL = number of polymorphic loci, PP = Percent polymorphisms, na = number of allele, ne = effective number of allele, h = gene diversity, I = Shannnon diversity index.

The highest gene diversity (0.38) and Shannon information index (0.56) was observed from primer 860 and followed by primer 873 with gene diversity (0.33) and Shannon information index (0.50). The least gene diversity (0.18) was obtained from primer 848 and 857. On the other hand, the lowest Shannon diversity (0.28) was obtained from primer 857 (Table 26).

Table 26. Diversity parameters used to indicate the diversity of Ethiopian faba bean varieties based on 11 ISSR primer

Primer	**NPL**	**PP**	**na**	**ne**	**h**	**I**
811	17	100.00	2.00±0.00	1.40±0.31	0.25±0.15	0.41±0.18
812	12	80.00	1.80±0.41	1.45±0.40	0.26±020	0.39±0.28
818	5	71.43	1.71± 0.49	1.38± 0.40	0.23±0.20	0.35±0.28
848	7	77.78	1.78±0.44	1.27±0.27	0.18±0.15	0.30±0.22
854	9	90.00	1.90±0.32	1.32±0.2	0.22±0.12	0.37±0.18
857	8	80.00	1.80±0.42	1.30±0.37	0.18±0.20	0.28±0.27
860	9	100.00	2.00±0.00	1.66±0.29	0.38±0.11	0.56±0.13
864	9	81.82	1.82±0.40	1.36±0.32	0.23±0.18	0.36±0.24
873	13	100.00	2.00±0.00	1.57±0.34	0.33±0.15	0.50±0.18
880	8	60.00	1.60±0.52	1.29±0.36	0.18±0.20	0.27±0.28
881	10	100.00	2.00±0.00	1.52±0.31	0.32±0.13	0.49±0.16
Total	**107**	**90.00**	**1.90±0.30**	**1.43±0.32**	**0.27±0.16**	**0.41±0.23**

Where, NPL = number of polymorphic loci, PP = Percent polymorphisms, na = number of allele, ne = effective number of allele, h = gene diversity, I = Shannnon diversity index.

5.2.4. Analysis of molecular variance (AMOVA)

Analysis of molecular variance was carried out on the over all ISSR data score of Ethiopian faba bean genotypes. AMOVA revealed higher percentage of variation (91%) is attributed to the within group variation, whereas the remaining variation is due to among group variation (9%) (Table 27).

Table 27. Percentage of molecular variance of four populations of faba bean varieties grown in Ethiopia

Source	df	SS	MS	Est. Var.	% var	Value	P-value	Fst
Among Pops	3	104.620	34.873	1.909	9%	0.085	0.017	0.085
Within Pops	28	574.380	20.514	20.514	91%			
Total	31	679.000		22.423	100%			

5.2.5. Genetic similarity

The genetic association between Ethiopian faba bean genotypes were obtained by using Jaccard's similarity coefficient. The matrix of genetic similarity showed an average range from 0.29 to 0.77 (Table 28).

Table 28. Genetic similarity matrix between 32 Ethiopian faba bean genotypes based on Jaccard’s similarity coefficient from 11 ISSR primer data fingerprinting

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20	F21	F22	F23	F24	F25	F26	F27	F28	F29
F1	1.00																												
F2	0.63	1.00																											
F3	0.67	0.58	1.00																										
F4	0.62	0.65	0.62	1.00																									
F5	0.53	0.70	0.62	0.71	1.00																								
F6	0.51	0.53	0.57	0.53	0.59	1.00																							
F7	0.57	0.55	0.63	0.61	0.59	0.57	1.00																						
F8	0.49	0.56	0.50	0.56	0.56	0.49	0.41	1.00																					
F9	0.58	0.55	0.56	0.64	0.54	0.52	0.47	0.66	1.00																				
F10	0.60	0.64	0.64	0.62	0.62	0.56	0.57	0.53	0.62	1.00																			
F11	0.45	0.50	0.49	0.49	0.53	0.54	0.43	0.43	0.49	0.44	1.00																		
F12	0.55	0.54	0.59	0.56	0.58	0.54	0.51	0.46	0.55	0.51	0.55	1.00																	
F13	0.51	0.49	0.53	0.57	0.52	0.45	0.47	0.38	0.43	0.40	0.58	0.58	1.00																
F14	0.50	0.47	0.47	0.54	0.49	0.49	0.42	0.44	0.52	0.41	0.56	0.63	0.67	1.00															
F15	0.49	0.42	0.43	0.45	0.46	0.44	0.42	0.39	0.43	0.36	0.58	0.58	0.74	0.73	1.00														
F16	0.51	0.54	0.47	0.54	0.52	0.45	0.45	0.49	0.47	0.49	0.50	0.55	0.68	0.65	0.68	1.00													
F17	0.44	0.43	0.43	0.41	0.35	0.36	0.35	0.39	0.39	0.34	0.50	0.51	0.49	0.56	0.53	0.55	1.00												
F18	0.41	0.45	0.41	0.38	0.40	0.37	0.32	0.41	0.36	0.38	0.50	0.54	0.48	0.54	0.57	0.57	0.74	1.00											
F19	0.49	0.53	0.46	0.46	0.47	0.46	0.38	0.46	0.45	0.41	0.58	0.63	0.57	0.69	0.61	0.64	0.70	0.77	1.00										
F20	0.50	0.50	0.47	0.48	0.47	0.42	0.44	0.38	0.41	0.39	0.52	0.57	0.59	0.66	0.58	0.60	0.61	0.62	0.75	1.00									
F21	0.41	0.47	0.39	0.46	0.46	0.40	0.37	0.45	0.41	0.36	0.56	0.40	0.56	0.55	0.54	0.66	0.54	0.53	0.59	0.56	1.00								
F22	0.40	0.41	0.40	0.45	0.44	0.39	0.37	0.42	0.43	0.35	0.49	0.42	0.55	0.55	0.59	0.56	0.49	0.51	0.59	0.52	0.74	1.00							
F23	0.44	0.43	0.45	0.48	0.47	0.45	0.41	0.46	0.45	0.42	0.54	0.48	0.62	0.59	0.63	0.58	0.45	0.47	0.55	0.51	0.66	0.66	1.00						
F24	0.37	0.35	0.38	0.34	0.32	0.35	0.29	0.40	0.36	0.31	0.38	0.44	0.49	0.43	0.57	0.49	0.53	0.51	0.51	0.42	0.59	0.62	0.63	1.00					
F25	0.36	0.38	0.36	0.39	0.37	0.38	0.33	0.46	0.46	0.35	0.48	0.42	0.49	0.49	0.53	0.51	0.43	0.44	0.55	0.42	0.64	0.70	0.75	0.73	1.00				
F26	0.39	0.41	0.36	0.40	0.41	0.35	0.34	0.38	0.45	0.39	0.44	0.43	0.49	0.42	0.52	0.46	0.48	0.48	0.51	0.48	0.55	0.55	0.59	0.58	0.59	1.00			
F27	0.41	0.45	0.42	0.41	0.46	0.38	0.35	0.40	0.42	0.34	0.46	0.44	0.48	0.49	0.51	0.52	0.50	0.45	0.50	0.55	0.60	0.59	0.59	0.59	0.55	0.63	1.00		
F28	0.42	0.42	0.41	0.43	0.43	0.39	0.33	0.46	0.48	0.36	0.42	0.48	0.48	0.58	0.54	0.60	0.50	0.49	0.60	0.52	0.67	0.60	0.67	0.63	0.67	0.59	0.74	1.00	
F29	0.41	0.38	0.43	0.47	0.46	0.38	0.35	0.51	0.50	0.39	0.46	0.45	0.49	0.53	0.52	0.52	0.39	0.41	0.51	0.46	0.61	0.60	0.70	0.66	0.73	0.55	0.67	0.76	1.00
F30	0.49	0.43	0.42	0.50	0.45	0.36	0.36	0.50	0.52	0.43	0.46	0.47	0.48	0.52	0.55	0.56	0.47	0.46	0.49	0.44	0.56	0.63	0.69	0.52	0.63	0.58	0.61	0.70	0.66
F31	0.51	0.50	0.44	0.49	0.45	0.37	0.40	0.47	0.53	0.44	0.47	0.51	0.49	0.53	0.52	0.56	0.45	0.49	0.55	0.49	0.61	0.64	0.61	0.65	0.67	0.59	0.63	0.68	0.71
F32	0.44	0.41	0.42	0.45	0.43	0.36	0.36	0.41	0.43	0.35	0.45	0.45	0.56	0.53	0.54	0.51	0.48	0.50	0.52	0.46	0.68	0.72	0.67	0.67	0.68	0.64	0.64	0.69	0.65

Key: F1 = Mosisa, F2 = Tumsa, F3 = Hachalu, F4 = Dosha, F5 = Gachena, F6 = Walki, F7 = Obse, F8 = Moti, F9 = Gabelcho, F10 = A/Hanna, F11 = NC-58, F12 = Wayu, F13 = Degaga, F14 = Dagm, F15 = Holetta-2, F16 = Shalo, F17 = Tesfa, F18 = Mesay, F19 = Bulga70, F20 = Kassa, F21 = Gora, F22 = CS-20-DK, F23 = Kuse, F24 = Didea, F25 = Lalo, F26 = B/local, F27 = D/local, F28 = S/local, F29 = Ag/local, F30 = EKCSR, F3 1= Adet local, F32 = Kulumsa local

Results from Jaccard's coefficients of similarity showed high similarity between genotypes derived from hybridization and local collection (61%), and followed by genotypes derived from introduction and local varieties with value of 50% similarity (Table 29) and (Figure 10). The least similarity (36.47%) was observed between genotypes derived from local collection and local varieties.

Table 29. Similarity matrix for Jaccard's coefficients for 4 categories of Ethiopian faba bean of genotypes populations based on the 120 bands obtained with eleven ISSR primers

Population Type	Hybridization	Local Selection	Introduction	Local variety
Hybridization	1.0000			
Local Selection	0.6073	1.0000		
Introduction	0.4339	0.4458	1.000	
Local variety	0.4120	0.3647	0.4885	1.000

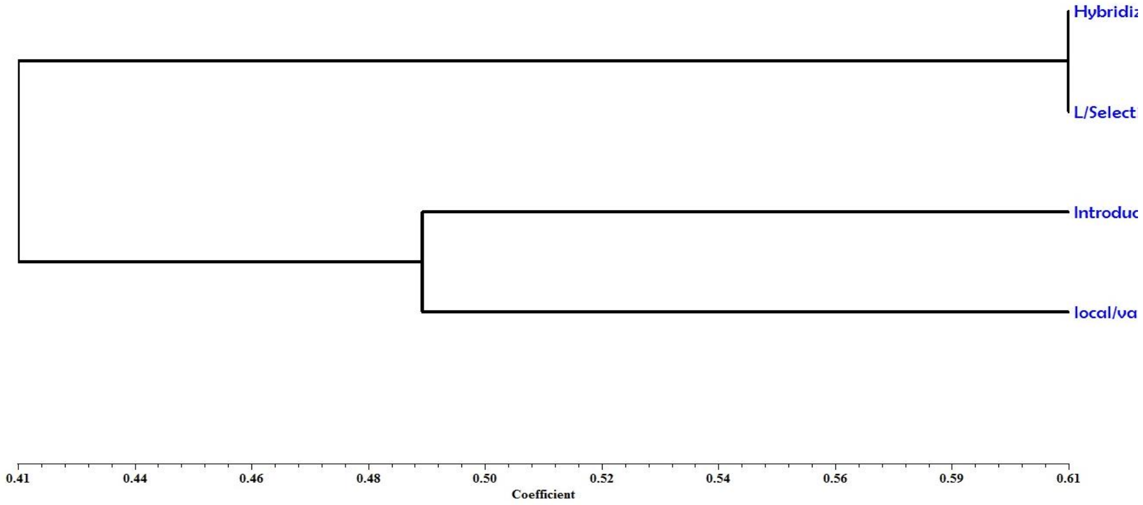


Figure 10. UPGMA based dendrogram for 4 groups of *Vicia faba* genotypes using 11 ISSR primers.

5.2.6. Cluster analysis

Clustering among 32 Ethiopian faba bean genotypes containing four groups were generated using UPGMA and NJ based on 120 amplified bands generated by 11 ISSR primers (Figure 13 and Figure 12). The genotypes were categorized based on their breeding information into four populations (hybridized genotypes, derived from introduced materials, derived from landraces, and local varieties).The position of cut-off line on dendrogram was determined by calculating the mean of the similarity matrix data (0.53) and the numbers of clades were determined by using obtained mean value to draw the line which helps to count how many individuals are included in each clade (Jamshidi, and Jamshidi, 2011). All of the genotypes were grouped into three clusters in a UPGMA-based dendrogram, with an average similarity coefficient of 0.53 as the cutoff value (Figure 20). The first major cluster mostly contained genotypes from hybridization which includes: Mosisa, Hachalu, Tumsa, Gachena, Obse, Walki, Moti and Gebelcho and two genotypes derived from local collection (Dosha and Adet Hanna). The second major cluster also comprised three genotypes from local collection (Wayu, Bulga-70 and Dagm), three genotypes from introduction (Degaga, Holeta-2 and Tesfa) and two genotypes from hybridization (Shalo and Mesay). All farmers' varieties (Eniwari local, Sinana local, Agarfa local, Adet local, Kulumsa local and bako local) assigned in the third major cluster with three genotypes from hybridization (Gora, Didea and EKCSR-02006), two genotypes from local selection (CS-20-DK and Lalo) and one from introduction (Kuse).

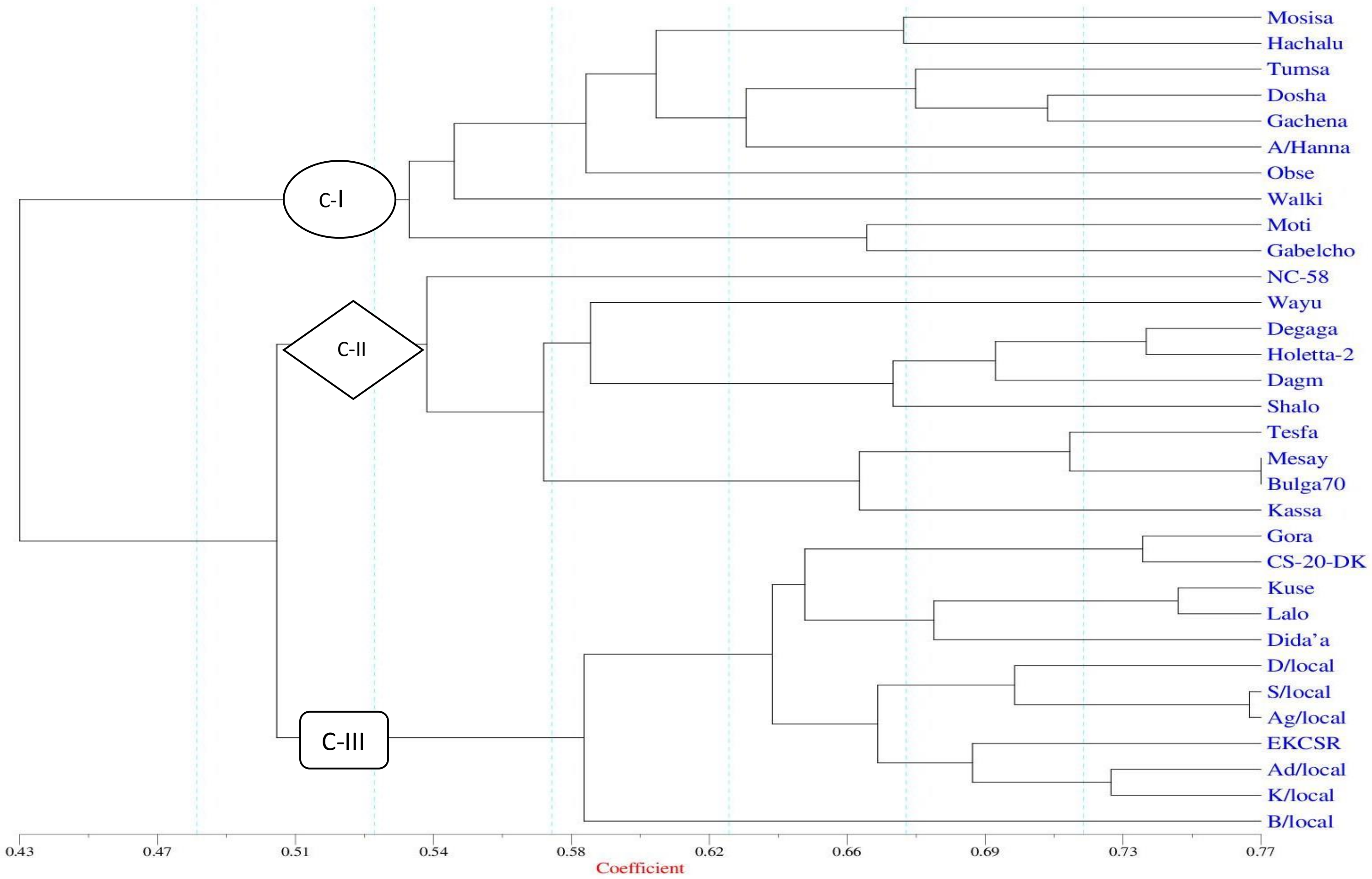


Figure 11. Dendrogram for 32 faba bean genotypes based on UPGMA analysis of amplified bands by eleven ISSR primers.

The UPGMA algorithm is based on Jaccard's similarity coefficients obtained after pair wise comparison of the presence-absence fingerprint.

The Neighbor-joining (NJ) analysis of the same dataset showed three distinct clusters (cluster I, cluster II and cluster-III) and sub-clusters within the first major cluster (cluster I-I and cluster I-II). Farmer variety (local variety) from Bako was found as outlier in NJ the denderogram. Major Cluster-I sub-cluster-II did not show a clear grouping because it was formed from varieties derived from local collection, introduction, and hybridization.

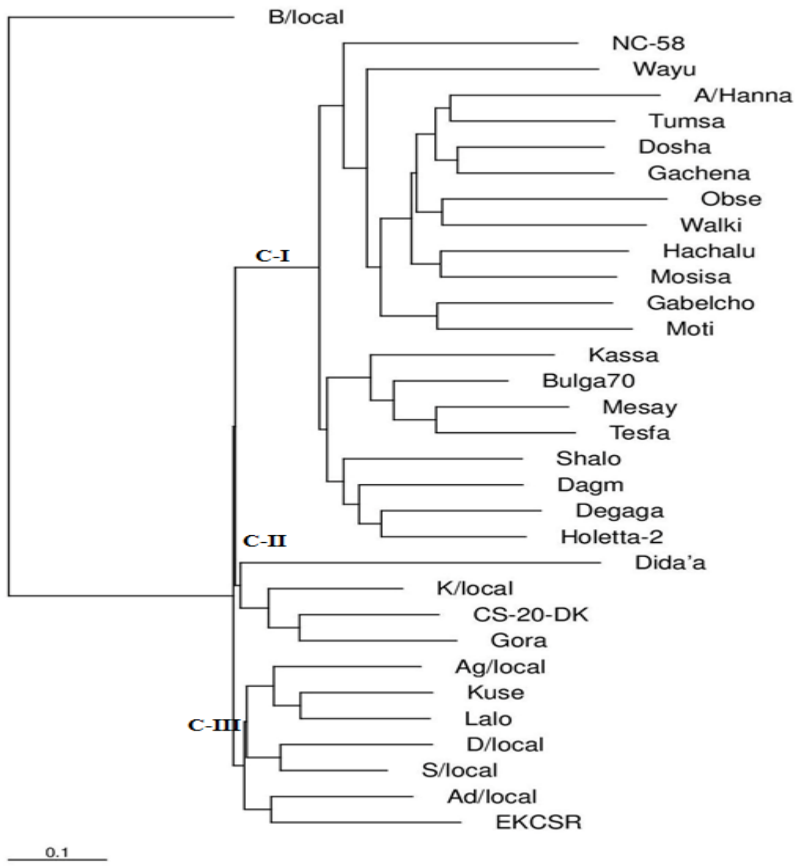


Figure 12. Neighbor joining clustering of 32 faba bean varieties grown in Ethiopia

5.2.7. PCoA analysis

PCO analysis were undertaken for all data obtained from 11 ISSR primer using Jaccard’s coefficients of similarity. PCoA analysis also categorized genotypes into three different groups without following their genetic information, but the result was congruent with the

UPGMA and NJ analysis. Individuals grouped in same cluster were grouped in similar cluster using PCoA. The first three PC1, PC2 and PC3 explained with total variation of 24.02 (11.48%, 7.276% and 5.26%, respectively). Therefore, the grouping of individuals using two and three coordinates is indicated in Figure 13 and Figure 14, respectively.

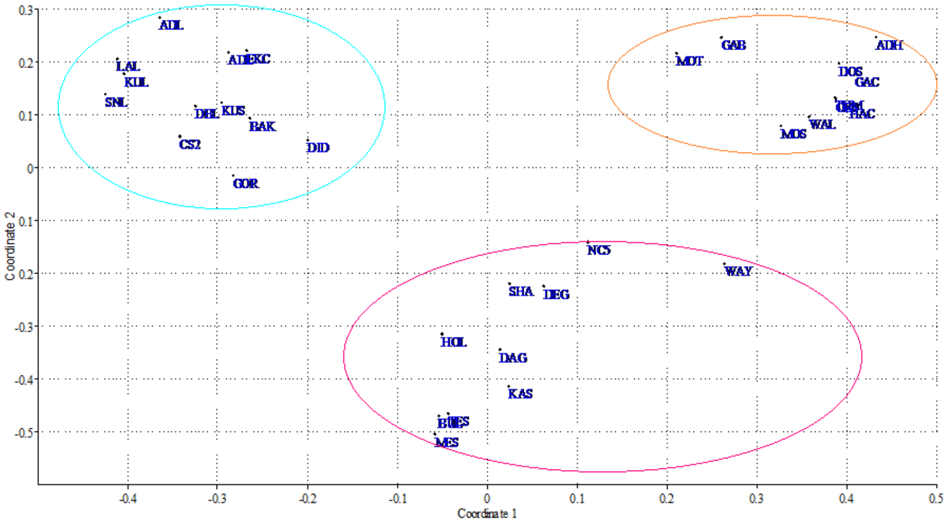


Figure 13. Two-dimensional representation of principal coordinate analysis of genetic relationships among 32 Ethiopian faba bean varieties using ISSR data.

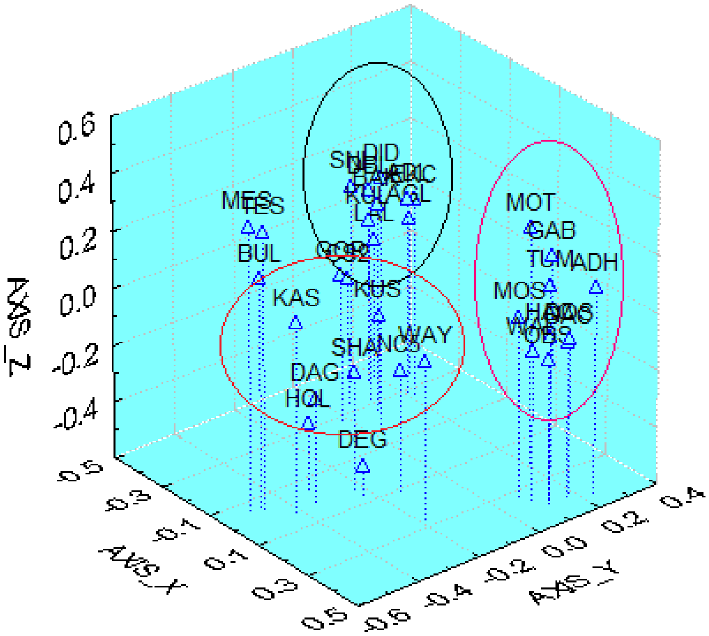


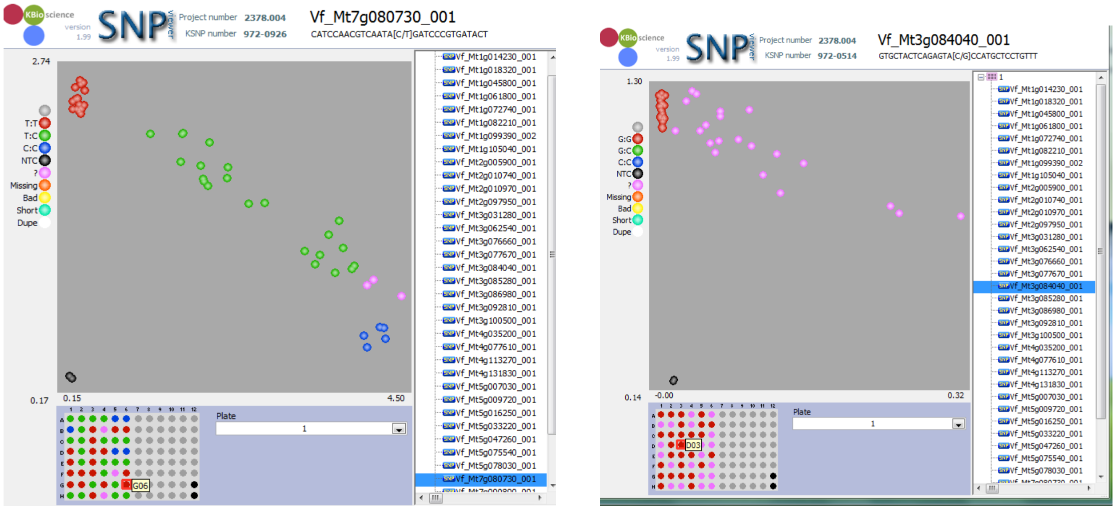
Figure 14. Three-dimensional representation of principal coordinate analysis of genetic relationships among 32 Ethiopian faba bean genotypes using ISSR data.

5.3. Genetic Diversity as revealed by SNP Markers

5.3.1. SNPs quality and their genomic distribution

A total of 1,776 data points was produced by genotyping of 48 faba bean genotypes with KASP genotyping assay. Out of 40 SNPs selected for detection of polymorphisms, 37 (92.5%) SNPs with high quality genotype calls were identified (Figure 15), while the remaining 3 (7.5%) failed to generate clear genotyping and these were excluded from genotyping. The KlusterCaller showed a group two opposites homozygous genotypes clustered near two axes and the cluster of heterozygous genotypes placed between the two homozygous genotypes clusters (Figure 15). Genotyped samples marked red are homozygous for the allele reported with HEX, those marked blue are homozygous for the FAM allele and those marked greens are heterozygous. Due to bi-allelic nature of KASP genotyping assay, all SNPs produced only two alleles (Table 32).

For the purpose of keeping even distribution of loci over the physical chromosome in genome of faba bean, six linkage groups viz., linkage group Vf_Chr_1B, linkage group Vf_Chr_2B, linkage group Vf_Chr_3B, linkage group Vf_Chr_4B, linkage group Vf_Chr_5B and linkage group Vf_Chr_6B; have been screened (Webb, *et. al*, 2015). Accordingly, 11 loci from linkage group 1, 11 loci from linkage group 2, 8 loci from linkage group 3, 2 loci from linkage group 4, 2 loci from linkage group 5 and 3 loci from linkage group 6 were taken and the number of loci were determined based on the size of chromosome. SNP distribution, Nei's gene diversity and PIC values estimated for each linkage group of the chromosome were listed in Table 30.



a) Polymorphic pattern **b) Monomorphic pattern**

Figure 15. Genotyping data plotted using Kluster Caller software for Vf_Mt7g080730_001 and Vf_Mt3g084040_001 assaying of all samples

Table 30. Distribution and diversity index of 37 SNP markers in a set of 48 Ethiopian faba bean accessions

Chromosome	No. of SNP marker	No. of polymorphic marker	Genetic Diversity	PIC
linkage group Vf_Chr_1B	11	11	0.415	0.325
linkage group Vf_Chr_2B	11	10	0.398	0.308
linkage group Vf_Chr_3B	8	8	0.393	0.308
linkage group Vf_Chr_4B	2	2	0.423	0.341
linkage group Vf_Chr_5B	2	2	0.456	0.351
linkage group Vf_Chr_6B	3	3	0.437	0.339
Total mean	37	36	0.420	0.329

Nei's gene diversity and PIC values across linkage groups on studied faba bean chromosome ranged from 0.393 to 0.456 and from 0.308 to 0.341, respectively. The chromosome linkage group Vf_Chr_5B had higher genetic diversity and PIC, whereas the linkage group Vf_Chr_2B and 3B had lower genetic diversity than the total average.

Table 31. Lists of allele and their frequencies produced by 37 SNP markers

S/N	Marker	Alleles produced		Count by each		Freq by each	
1	Vf_Mt1g014230_001	**A**	**T**	81	11	0.88	0.12
2	Vf_Mt1g018320_001	**A**	**G**	46	30	0.61	0.39
3	Vf_Mt1g045800_001	**A**	**C**	43	33	0.57	0.43
4	Vf_Mt1g072740_001	**A**	**G**	31	65	0.32	0.68
5	Vf_Mt1g082210_001	**A**	**G**	29	39	0.43	0.57
6	Vf_Mt1g105040_001	**G**	**T**	42	36	0.54	0.46
7	Vf_Mt1g061800_001	**C**	**T**	8	86	0.09	0.91
8	Vf_Mt2g010740_001	**C**	**T**	41	37	0.53	0.47
9	Vf_Mt2g010970_001	**C**	**T**	54	22	0.71	0.29
10	Vf_Mt2g097950_001	**G**	**T**	39	39	0.50	0.50
11	Vf_Mt3g031280_001	**C**	**G**	13	63	0.17	0.83
12	Vf_Mt3g062540_001	**A**	**G**	63	13	0.83	0.17
13	Vf_Mt3g077670_001	**C**	**T**	43	33	0.57	0.43
14	Vf_Mt3g084040_001	**G**	**-**	54	-	1.00	-
15	Vf_Mt3g086980_001	**G**	**T**	59	27	0.69	0.31
16	Vf_Mt3g092810_001	**A**	**G**	46	28	0.62	0.38
17	Vf_Mt3g100500_001	**C**	**T**	39	31	0.56	0.44
18	Vf_Mt4g077610_001	**A**	**C**	18	62	0.23	0.78
19	Vf_Mt4g113270_001	**C**	**T**	32	38	0.46	0.54
20	Vf_Mt4g131830_001	**A**	**G**	21	61	0.26	0.74
21	Vf_Mt4g035200_001	**G**	**T**	36	44	0.45	0.55
22	Vf_Mt5g007030_001	**C**	**T**	57	21	0.73	0.27
23	Vf_Mt5g009720_001	**A**	**G**	55	33	0.63	0.38
24	Vf_Mt5g016250_001	**A**	**T**	30	58	0.34	0.66
25	Vf_Mt5g033220_001	**G**	**T**	20	60	0.25	0.75
26	Vf_Mt5g078030_001	**C**	**G**	8	74	0.10	0.90
27	Vf_Mt5g047260_001	**C**	**T**	17	63	0.21	0.79
28	Vf_Mt7g080730_001	**C**	**T**	32	58	0.36	0.64
29	Vf_Mt7g090890_001	**A**	**T**	44	44	0.50	0.50
30	Vf_Mt7g100730_001	**C**	**T**	44	18	0.71	0.29
31	Vf_Mt8g085850_001	**C**	**T**	28	48	0.37	0.63
32	Vf_Mt8g102250_001	**C**	**T**	33	39	0.46	0.54
33	Vf_Mt1g099390_002	**A**	**T**	25	71	0.26	0.74
34	Vf_Mt3g076660_001	**C**	**T**	27	39	0.41	0.59
35	Vf_Mt3g085280_001	**C**	**T**	46	42	0.52	0.48
36	Vf_Mt5g075540_001	**C**	**G**	52	26	0.67	0.33
37	Vf_Mt2g005900_001	**A**	**C**	39	43	0.48	0.52

Where A = Adenine, G = Guinine, C = Cytosine, T = Taminine

5.3.2. SNP polymorphisms

In the present study, a total of 36 polymorphic SNPs was found in 37 SNPs assayed with an average of 95.58% polymorphisms. SNP polymorphism was measured in terms of the gene diversity value ranging from 0.00 for Vf_Mt3g084040_001 to 0.500 for Vf_Mt2g097950_001 and Vf_Mt7g090890_001 and PIC value varying from 0.00 for Vf_Mt3g084040_001 to 0.375 for Vf_Mt2g097950_001 and Vf_Mt7g090890_001, with averages of 0.4104 and 0.3195, respectively (Table 32). Major allele frequencies ranged from 0.500 (Vf_Mt2g097950_001) to 1.00 (Vf_Mt3g084040_001) with an average of 0.6681. The highest level of heterozygosity was detected by Vf_Mt2g005900_001 (0.9024) followed by Vf_Mt1g105040_001 (0.8205), and the lowest heterozygosity was obtained at locus Vf_Mt3g084040_001 (zero).

Table 32. Diversity measures utilized in signifying Ethiopian faba bean varieties diversity based on SNP marker data

S/N	Marker	MAF	GNO	SS	NOO	NA	GD	H	PIC	f
1	Vf_Mt1g014230_001	0.88	3.00	48.00	46.00	2.00	0.21	0.20	0.19	0.08
2	Vf_Mt1g018320_001	0.61	3.00	48.00	38.00	2.00	0.48	0.53	0.36	-0.09
3	Vf_Mt1g045800_001	0.57	3.00	48.00	38.00	2.00	0.49	0.66	0.37	-0.33
4	Vf_Mt1g072740_001	0.68	3.00	48.00	48.00	2.00	0.44	0.56	0.34	-0.28
5	Vf_Mt1g082210_001	0.57	3.00	48.00	34.00	2.00	0.49	0.56	0.37	-0.13
6	Vf_Mt1g105040_001	0.54	3.00	48.00	39.00	2.00	0.50	0.82	0.37	-0.64
7	Vf_Mt1g061800_001	0.91	3.00	48.00	47.00	2.00	0.16	0.13	0.14	0.19
8	Vf_Mt2g010740_001	0.53	3.00	48.00	39.00	2.00	0.50	0.59	0.37	-0.17
9	Vf_Mt2g010970_001	0.71	3.00	48.00	38.00	2.00	0.41	0.42	0.33	-0.01
10	Vf_Mt2g097950_001	0.50	3.00	48.00	39.00	2.00	0.50	0.59	0.38	-0.17
11	Vf_Mt3g031280_001	0.83	3.00	48.00	38.00	2.00	0.28	0.29	0.24	-0.01
12	Vf_Mt3g062540_001	0.83	3.00	48.00	38.00	2.00	0.28	0.29	0.24	-0.01
13	Vf_Mt3g077670_001	0.57	3.00	48.00	38.00	2.00	0.49	0.55	0.37	-0.11
14	Vf_Mt3g084040_001	1.00	1.00	48.00	27.00	1.00	0.00	0.00	0.00	-
15	Vf_Mt3g086980_001	0.69	3.00	48.00	43.00	2.00	0.43	0.44	0.34	-0.01
16	Vf_Mt3g092810_001	0.62	3.00	48.00	37.00	2.00	0.47	0.54	0.36	-0.14
17	Vf_Mt3g100500_001	0.56	3.00	48.00	35.00	2.00	0.49	0.54	0.37	-0.09
18	Vf_Mt4g077610_001	0.78	3.00	48.00	40.00	2.00	0.35	0.30	0.29	0.15
19	Vf_Mt4g113270_001	0.54	3.00	48.00	35.00	2.00	0.50	0.51	0.37	-0.02
20	Vf_Mt4g131830_001	0.74	3.00	48.00	41.00	2.00	0.38	0.41	0.31	-0.08
21	Vf_Mt4g035200_001	0.55	3.00	48.00	40.00	2.00	0.50	0.50	0.37	0.00
22	Vf_Mt5g007030_001	0.73	3.00	48.00	39.00	2.00	0.39	0.38	0.32	0.04
23	Vf_Mt5g009720_001	0.63	3.00	48.00	44.00	2.00	0.47	0.70	0.36	-0.49
24	Vf_Mt5g016250_001	0.66	3.00	48.00	44.00	2.00	0.45	0.59	0.35	-0.30
25	Vf_Mt5g033220_001	0.75	3.00	48.00	40.00	2.00	0.38	0.30	0.30	0.21
26	Vf_Mt5g078030_001	0.90	3.00	48.00	41.00	2.00	0.18	0.15	0.16	0.18
27	Vf_Mt5g047260_001	0.79	3.00	48.00	40.00	2.00	0.33	0.38	0.28	-0.11
28	Vf_Mt7g080730_001	0.64	3.00	48.00	45.00	2.00	0.46	0.49	0.35	-0.06
29	Vf_Mt7g090890_001	0.50	3.00	48.00	44.00	2.00	0.50	0.41	0.38	0.19
30	Vf_Mt7g100730_001	0.71	3.00	48.00	31.00	2.00	0.41	0.39	0.33	0.08
31	Vf_Mt8g085850_001	0.63	3.00	48.00	38.00	2.00	0.47	0.32	0.36	0.33
32	Vf_Mt8g102250_001	0.54	3.00	48.00	36.00	2.00	0.50	0.69	0.37	-0.39
33	Vf_Mt1g099390_002	0.74	3.00	48.00	48.00	2.00	0.39	0.35	0.31	0.09
34	Vf_Mt3g076660_001	0.59	3.00	48.00	33.00	2.00	0.48	0.58	0.37	-0.18
35	Vf_Mt3g085280_001	0.52	3.00	48.00	44.00	2.00	0.50	0.73	0.37	-0.45
36	Vf_Mt5g075540_001	0.67	3.00	48.00	39.00	2.00	0.44	0.46	0.35	-0.03
37	Vf_Mt2g005900_001	0.52	3.00	48.00	41.00	2.00	0.50	0.90	0.37	-0.80
	Mean	0.67	2.95	48.00	39.59	1.97	0.41	0.47	0.32	-0.12

NB = Major allele frequency (MAF), Genotype number (GNO), Sample size (SS), Numbers of observation (NOO), Numbers of alleles (NA), Genetic diversity (GD), heterozygosity (H), Polymorphisms information Content (PIC) and Inbreeding coefficient (f)

5.3.3. Diversity indices of faba bean genotypes as revealed by SNP marker

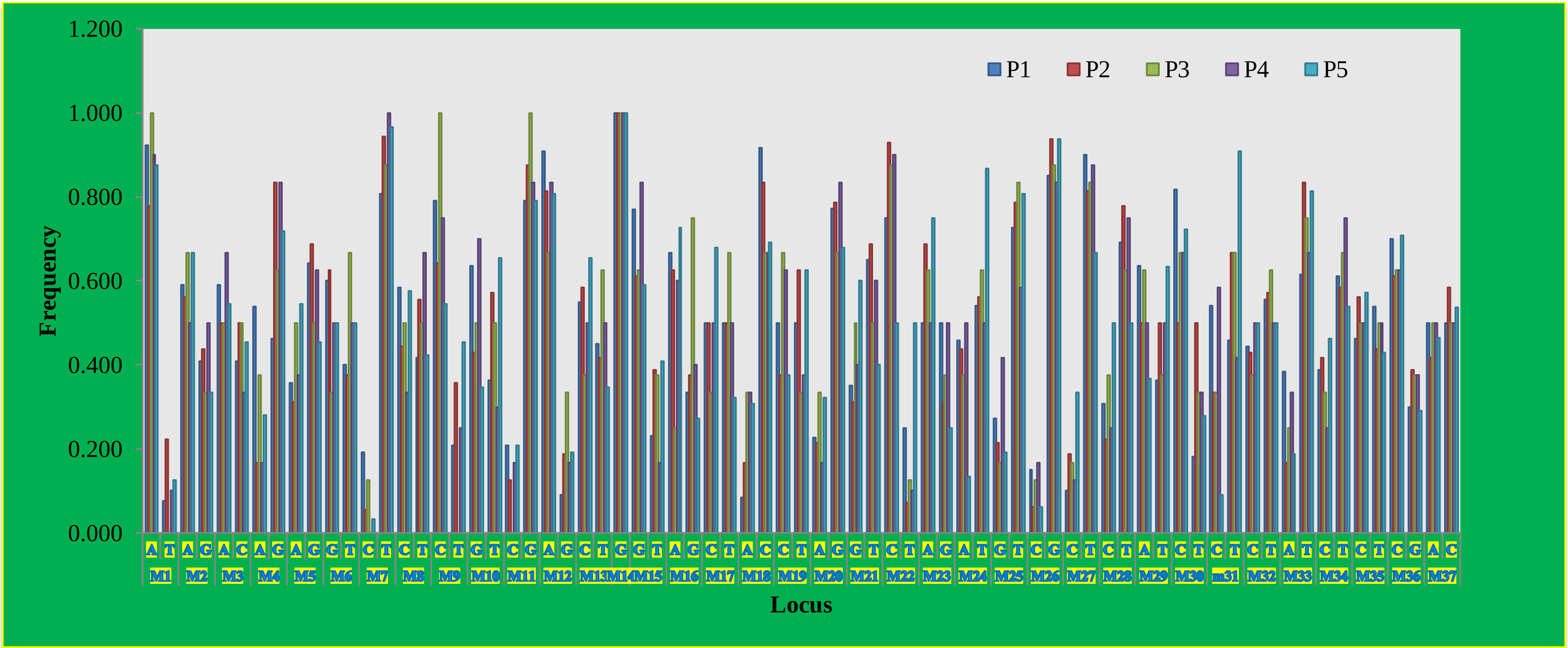
High-quality genotyping data obtained for all 36 polymorphic SNP markers were used to assess the diversity of faba bean cultivars and line from Ethiopia. The highest expected heterozygosity (He) and Shannon information content (I) was detected using SNP markers in the cultivars developed via hybridization with values of 0.40 and 0.57 (Table 33) and the least was observed from cultivars developed from introduced materials with values of 0.55 and 0.38, respectively. The mean observed heterozygosity across all population was 0.49, ranging from 0.40 to 0.57, while the mean expected heterozygosity was 0.39 ranging from 0.38 to 0.40 (Table 33). The highest polymorphic loci (36) and percent polymorphisms (97.30%) were revealed by hybridized cultivars followed by selection from landraces and breeding lines, while the least were signified by introduced (33 and 89.19%) and farmer cultivars (35 and 94.59%).

The locus Vf_Mt3g084040_001 was found monomorphic across the assaying in all genotypes, but in varieties developed from introduction; four loci (Vf_Mt1g014230_001, Vf_Mt2g010970_001, Vf_Mt3g031280_001 and Vf_Mt3g084040_001) were found monomorphic. The highest allele frequency in hybridized cultivars was generated from polymorphic locus Vf_Mt1g014230_001 with allele ‘A’ (0.923), while the least allele frequency within this group were obtained from polymorphic locus Vf_Mt5g078030_001 of allele ‘C’ (0.150). The highest allele frequency was obtained from locus Vf_Mt1g061800_001 with allele ‘T’ (0.944) in varieties derived from landrace collection and the least were from locus Vf_Mt5g007030_001 of allele ‘T’ (0.071). Similarly, the highest allele frequencies were obtained from polymorphic locus Vf_Mt1g014230_001 with allele ‘T’ (0.90) and locus Vf_Mt1g061800_001 with allele ‘T’ (0.967) in farmer varieties and breeding lines, respectively. On the other hand, least allele frequency was obtained from locus Vf_Mt1g014230_001 with allele ‘T’ (0.100) and locus Vf_Mt1g061800_001 with allele ‘C’ (0.033) in farmer varieties and breeding lines, respectively (Figure 16).

Table 33. Diversity parameters used in genetic diversity study of Ethiopian faba bean varieties

Pop	**N($\bar{x}$±SE)**	**Na($\bar{x}$±SE)**	**Ne($\bar{x}$±SE)**	**I($\bar{x}$±SE)**	**Ho($\bar{x}$±SE)**	**He($\bar{x}$±SE)**	**F($\bar{x}$±SE)**	PL	%P
P1	10.92±0.28	1.97±0.03	1.72±0.05	0.57±0.03	0.40±0.04	0.40±0.02	0.021±0.06	36	97.30
P2	7.54±0.19	1.97±0.03	1.70±0.05	0.57±0.03	0.54±0.05	0.39±0.02	-0.35±0.06	36	97.30
P3	3.43±0.10	1.90±0.05	1.70±0.05	0.55±0.04	0.53±0.06	0.38±0.03	-0.36±0.09	33	89.19
P4	4.65±0.20	1.95±0.04	1.70±0.05	0.56±0.03	0.57±0.05	0.39±0.02	-0.43±0.05	35	94.59
P5	13.05±0.35	1.97±0.03	1.71±0.05	0.57±0.03	0.42±0.03	0.40±0.02	-0.06±0.05	36	97.30
Mean	7.92±0.29	1.95±0.02	1.70±0.02	0.56±0.01	0.49±0.02	0.39±0.010	-0.23±0.03	36	95.58

NB: Numbers of different alleles (NA), Number of effective alleles (Ne), Shannon information content (I), Observed heterozygosity (Ho), Expected heterozygosity (He), Fixation index (F), polymorphic loci (PL) and percent polymorphism, P1= Hybridized cultivar, P2=Local selection cultivar, P3= Introduced cultivar, P4= Farmer cultivar, P5= Breeding lines

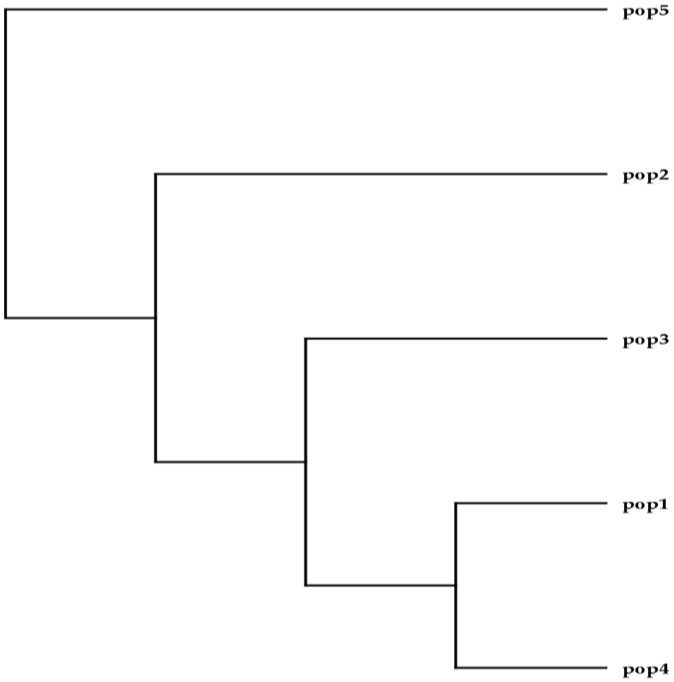


NB: P1 = Developed via Hybridization, P2 = developed from landrace selection, P3 = developed from introduced materials, P4 = local varieties, P5 = Breeding lines

Figure 16.Graph of allele frequency contributed by each locus (37 SNP) in all Ethiopian faba bean varieties groups based on their breeding history

5.3.4. Genetic distance and identity as revealed by SNP markers

Genetic distances (D) were calculated for all faba bean groups (depending on their breeding strategies and history) used in this study based on the shared-allele distance (Table 34). The highest genetic distance (0.066) was obtained between varieties derived from landraces collection and breeding lines, followed by varieties derived from introduction and breeding lines with value of 0.06 (Table 34) and (Figure 17). The least genetic distance (the most related groups) was observed between varieties derived from hybridization and local varieties. The highest genetic identity was recorded between hybridized varieties and local selection cultivars, while the least was recorded between local varieties (farmer varieties) and breeding lines.



NB: Pop1 = Developed via Hybridization, Pop2 = Developed from landrace selection, Pop3 = Developed from introduced materials, Pop4 = Farmer cultivars, Pop5 = Breeding lines

Figure 17. UPGMA based dendrogram for 5 groups of *Vicia faba* varieties using 37 SNP markers

Table 34. Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

	Hybridization	Local selection	Introduction	Landrace	Breeding lines
Hybridization		0.9701	0.9625	0.978	0.9465
Local selection	0.0303		0.961	0.9742	0.9631
Introduction	0.0382	0.0397		0.9638	0.9416
Landrace	0.0223	0.0261	0.0368		0.9362
Breeding lines	0.0549	0.0376	0.0602	0.0659	

5.3.5. Analysis of molecular variance (AMOVA)

Analysis of molecular variance indicated narrow genetic diversity among population (varietal groups) (1%) considered, whereas 99% genetic variation was observed in within population (Table 35).

Table 35. AMOVA among Ethiopia faba bean varieties as revealed by SNP markers

Source	**df**	**SS**	**MS**	**Estimated Variance**	**Percentage of variation**	**Value**	**P-value**	**Fst**
Among population	4	115.92	28.98	0.202	1%	0.005	0.282	0.011
Within population	43	1167.35	27.15	24.15	99%	0.311	0.001	
Total	47	1283.27		27.35	100%	0.314	0.001	

5.3.6. Genetic structure among faba bean varieties as detected by SNP marker

5.3.6.1. STRUCTURE analysis

A total of 36 polymorphic SNP markers were used based on the Bayesian clustering model (Markov Chain Monte Carlo (MCMC)) implemented in structure software to estimate the group membership of each individual. The optimum number of clusters was determined after 10 independent runs for each K value ranging from 1 to 10, using the admixture model and correlated allele frequencies. Of the 10 independent runs, the one with the highest ln Pr (X/K) value (log probability or log likelihood) was chosen and represented as bar plots in Fig 20. Finally, the data indicated that K = 2 was the most likely number of genetic clusters (Figure 18) and divided varieties into two genetically distinct group.

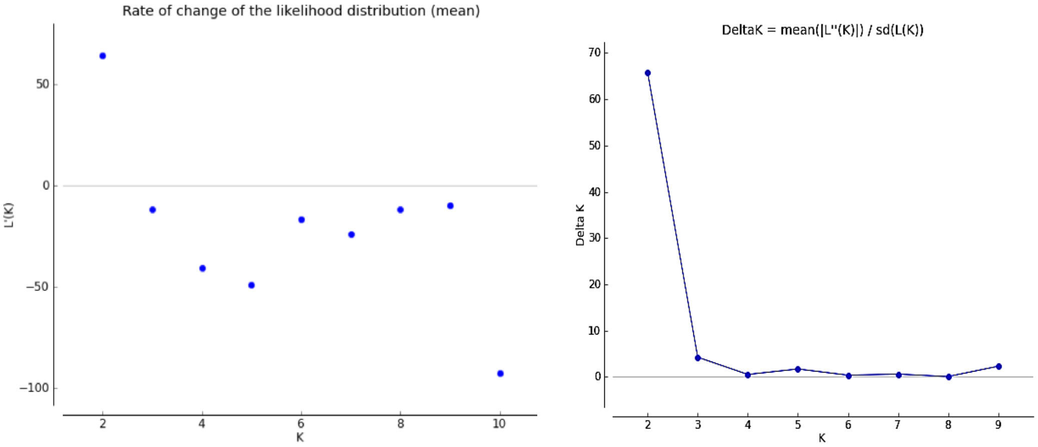
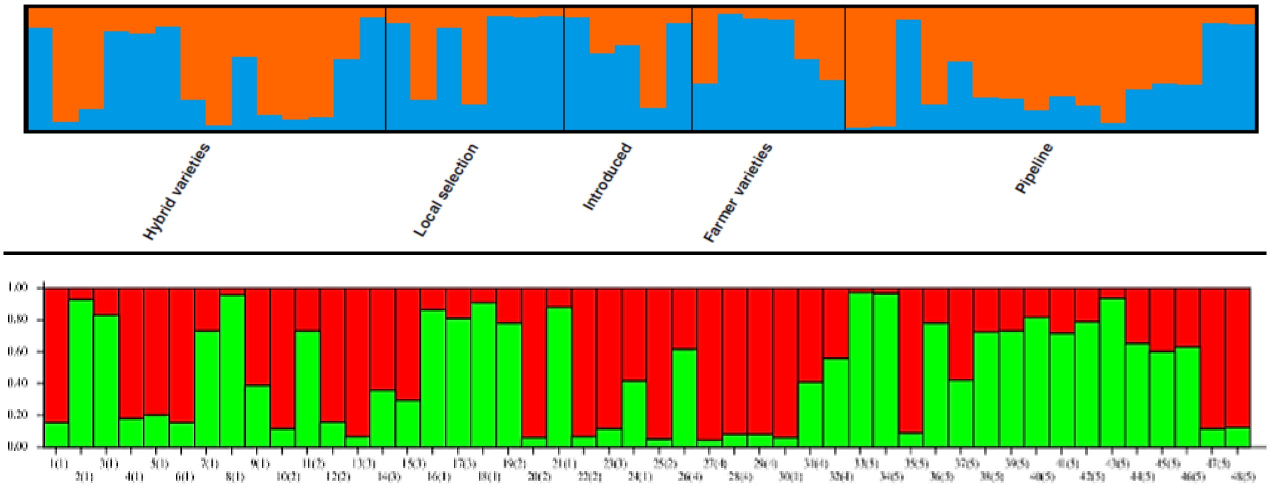


Figure 18. Changing trends of estimated Ln probability of data (LnP (D)) and Pritchard's K (ΔK) over ten repeats at each K value in the STRUCTURE analysis.



K=2

Figure 19. classification of the 48 Ethiopian *Vicia faba* varieties using Structure 2.3.3 into two distinct clusters by the model-based method at k = 2

5.3.6.2. Cluster analysis

The neighbor joining clustering pattern based on the genetic dissimilarity were constructed and categorized the genotypes into three major groups. The majority of the varieties/ lines formed their respective clusters, with admixing of genotyping regardless of their breeding history and the parental pedigree information (Figure 20).

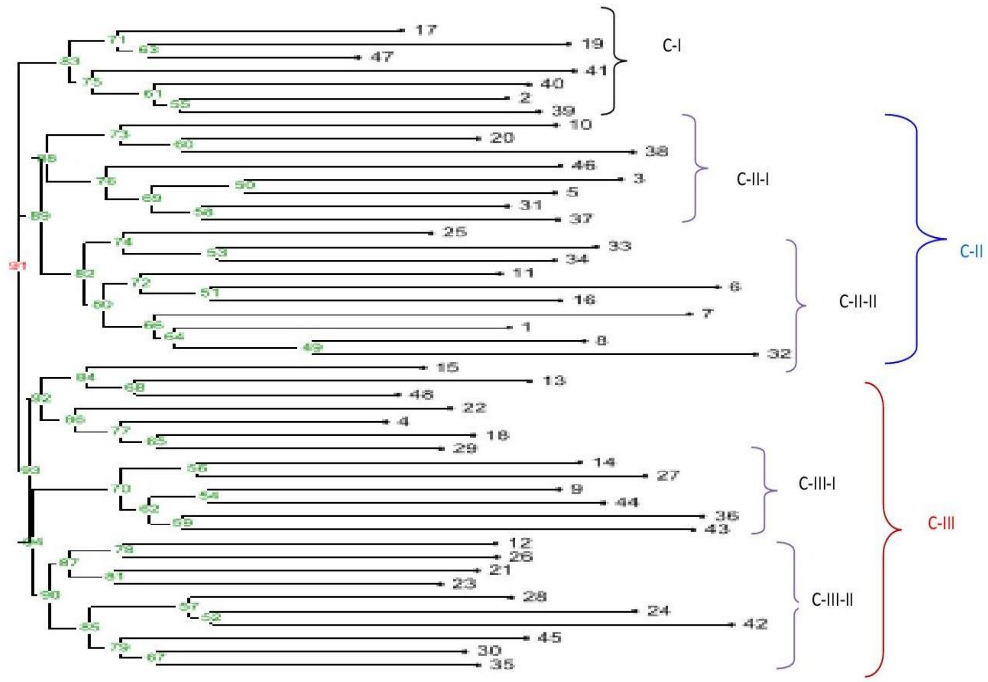


Figure 20. Neighbor joining cluster analysis of 48 faba bean varieties/pipelines based on data from 37 SNP markers.

Genetic dissimilarity between different pairs of genotype varied from 0.15 (Dosha and Mesay) to a maximum of 0.80 (Tumsa and Kulumsa local variety) with an average mean of 0.45.

5.3.6.3. Principal Coordinate Analysis (PCoA)

In order to gain further insight into genetic structure between faba bean genotypes, PCoA was alternatively implemented to study pattern of relationship among individuals. The first three PC1, PC2 and PC3 showed a total variation of 22.16%. Genotypes were clustered into three different groups and the result was consistent with the cluster analysis. The grouping of individuals using two and three coordinates are also indicated in Figure 29 and Figure 30, respectively.

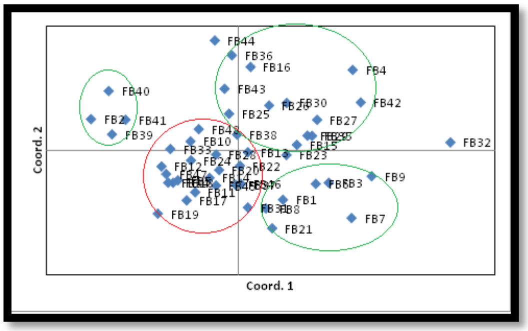


Figure 21. Two-dimensional representation of principal coordinate analysis of genetic relationships among 32 Ethiopian faba bean genotypes using SNP data

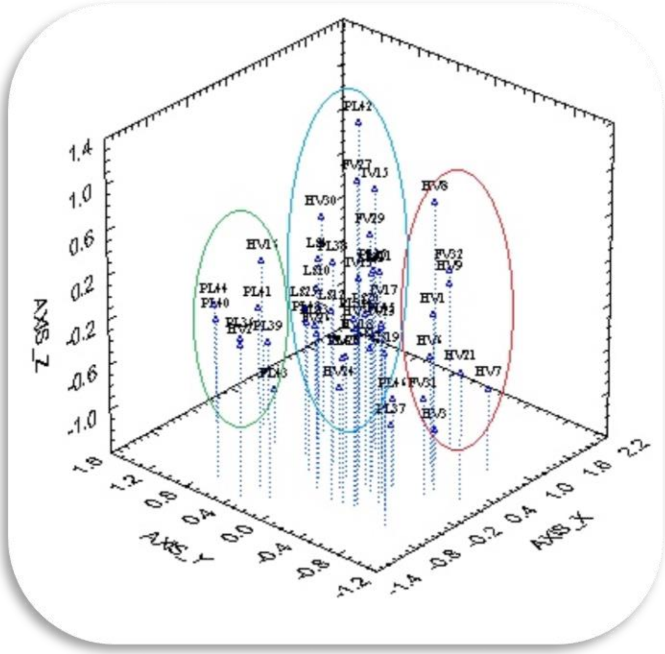


Figure 22. Three-dimensional representation of principal coordinate analysis of genetic relationships among 32 Ethiopian faba bean varieties using SNP data

6. DISCUSSIONS

6.1. Genetic Diversity based on Phenotypic Traits

6.1.1. Performance of Ethiopian faba bean varieties

In the present study, the range showed wide gap for most of the traits and this indicated the existence of high degree of genetic diversity among Ethiopian faba bean varieties regarding the measured traits and this implies that the availability of potential variation among the utilized faba bean materials.

The highest combined mean value for grain yield was recorded from variety Walki revealed that the variety has highly performed in all locations as compared to the others materials, whereas the lowest mean value was recorded from Bako local variety remarking that the local variety may not perform well in all locations than their specific areas. The second higher yielding variety was promising genotype (EKCSR-02006) which indicated that the line performed well in all locations than others materials and this line has the potential of adapting in different locations of similar agro-ecology. Similarly, the bottom lower yielder was Dagm, Debrebirhan local and Kulumsa local, respectively and these varieties had narrow adatabilty in different locations and may grow best in their specific growing area.

The crude protein content ranged from 18.08% to 24.65% at individual location and the combined result across locations revealed ranges from 20.59 to 22.92 % protein variation between varieties. The report by Griffiths & Lawes (1978) revealed a wide range of crude protein content variation between faba bean genotypes which ranges from 20 to 40% and observed smallest protein percent from Ethiopian collection. Chaven, *et al*. (1989) also reported wide ranges of crude protein content variation between faba bean genotypes ranging from 20 to 41%. Therefore, the observed ranges of crude protein content in the present study agrees with previous investigation except that the materials showed narrow range.

In contrary, Musallam *et al.* (2004) reported protein content varied significantly and ranged from 25.1 to 28.9% under rainfed conditions and 27.2 to 30.1% under irrigation conditions which was more than the present report. Bond *et al.* (1985) suggested protein concentration is influenced by both genetic and environmental factors and reported inheritance of this trait is additive with some partial dominance. Kelly (1973) reported differences in the crude protein contents of legumes to environmental conditions, genotypes, and agricultural practices.

The mean squares from the combined variance analysis over the three locations showed highly significant ($p< 0.01$) variations among the varieties for majority of recorded traits. This suggested adequate amount of genetic variability among varieties utilized in the present study for use of them in future faba bean improvement programs. Sharifi (2015) reported highly significant difference with days to maturity, pod length, number of seeds per plant and thousand seeds weight. Ammar *et al. (*2015) similarly measured highly significant difference between faba bean genotypes with traits: days to flowering and maturity, plant height, number of branches per plant, number of pods per plant and number of seeds per plant. Sharifi (2014) found highly significant difference between faba bean genotypes for seed yield per plot, number of pods per plant, number of seeds per pod, pod length and thousand seeds weight. Alghamdi (2007) also observed highly significant difference between faba bean genotypes on traits such as flowering date, maturity date, number of branches per plant, number of pods per plant, number of seeds per plant, seed yield, thousand seeds weight, and biomass weight. Gemechu Keneni *et al. (*2005) in extent and pattern of genetic diversity for morpho-agronomic traits in Ethiopian faba bean landraces; obtained highly significant difference between landraces over combined location for days to maturity, thousand seed weight and seed yield per plot. This investigation is also in agreement with the result reported by Hassan and Ishag (1972), Salim and Saxena (1992), Suso *et al. (*1993, 1996) and Terzopolas *et al*. (2003) on this crop.

6.1.1.1. Genotype by environment interaction effects

As indicated in Table 15, genotype by environment interaction was highly significant ($p < 0.01$) for the traits considered except for plant height to first pod ($p < 0.05$). This indicates that the performance of Ethiopian faba bean varieties could be affected significantly by environmental conditions and indicated that some varieties perform best to specific locations. Similarly, it comparable with that of Ammar *et al. (*2015) who reported highly significant difference between faba bean genotypes in genotype by environment interaction for traits: days to flowering and maturity, plant height, number of branches per plant, number of pods per plant and number of seeds per plant. Sharifi (2014) indicated highly significant mean performance between faba bean genotypes over different locations and genotype by environment interaction for seed yield per plot, number of pods per plant, number of seeds per pod, pod length and thousand seeds weight. In contrary, some traits: pod length, pod width, number of branches per plant, number of pods per plant, number of seeds per plant and harvest index revealed non-significant difference under three locations. This indicated that most of the varieties showed differential performance at the three locations.

6.1.2. Estimates of variation components

6.1.2.1. Genotypic and phenotypic coefficient of variation

As indicated in Table 16, higher GCV (>10%) were observed for pod length, pod width, number of pods per plant, thousand seeds weight, seed production efficiency and economic growth rate. For these traits, variations were observed due to the presence of genetic difference than environmental difference. Intermediate genotypic coefficients of variability (5% < x <10%) were observed for majority of the trait as presented in Table 16. In contrast, lowest GCV (< 5%) were observed for leaf length, internod length, plant height, days to flowering, days to maturity and crude protein content. Abdul-ariz *et al.* (2015) reported higher genotypic coefficient of variation with thousand seeds weight and moderate with number of seeds per plant. They also reported low genotypic coefficient of variation for number of pods per plant and number of seeds per pod. Tafere *et al.* (2013) also found high GCV for biomass weight and number of pods per plant in their study of genetic variability, heritability and correlation in some faba bean genotypes grown in Northwestern Ethiopia. Alghamdi (2007) also reported high genotypic coefficient of variation for days to flowering, number of pods per plant and thousand seed weight.

The highest PCV (>10%) were observed for traits like leaf area, leaf area index, pod length, pod width, numbers of branches per plant, internod's length, plant height to first pod, number of pods per plant, biomass weight per plot, seed yield per plot, harvest index, thousand seeds weight, seed production efficiency and economic growth rate. Abdul-ariz *et al.* (2015) and Tafere *et al.* (2013) reported higher phenotypic coefficient of variation with number of pods per plant, thousand seeds weight and moderate for number of seeds per pod. High phenotypic coefficient of variations was also reported for days to flowering, number of pods per plant and thousand seed weight (Alghamdi, 2007).

Genotypic and phenotypic coefficients of variation were reported as the major tools used to measure the variability that exists in a given population (Burton and Devane, 1988). The larger environmental variance than genotypic variance signifies, in the phenotypic expression of that trait with larger environmental variance the contribution of the environment was greater.

Generally, the GCV values were lower than that of PCV for all locations and followed similar pattern for all characters at tested location. Due to this reasons, the combined estimate of all variance components and coefficient of variability was used for indicating variability that exist between Ethiopian faba bean varieties. In this study, the PCV values were greater than GCV values across an environment which is in agreement with different scientist report (Alghamdi, 2007; Abdul-ariz *et al.*, 2015 and Tafere *et al.*, 2013). This indicated that the existence of environmental effect for the expression of most characters.

6.1.2.2. Heritability in broad sense and expect genetic gain from selection

In this study, heritability value ranged from 8.45% for number of branches per plant to 95.2% for pod length. In connection to this range; the heritability values were sufficiently very high for traits like pod length (95.2%) and pod width (81.03%) indicating the possibility of progress from selection. This result agrees with report by Sharifi (2015) and highlights that the effects of environmental factor were very small in influencing traits with high heritability. Therefore, selection based on mean would be crucial for the improvement of these traits in the faba bean varieties development program.

The moderately high heritability values were observed for characters such as leaf length, leaf width, leaf area, plant height to first pods, plant height, number of pods per plant, number of seeds per plant, number of seeds per pod, biomass weight per plot, thousand seeds weight, seed production efficiency and economic growth rate and indicates the possibility of improvement via selection for these traits. Abdul-ariz *et al.* (2015); Tafere *et al.* (2013); Mellion *et al.* (2012) and Alghamdi (2007) reported moderately high heritability for these traits. Hence, high heritability values for most of the characters could be attributed to the relatively favorable environment at combined locations. Moderately high heritability values were obtained for most of the trait including grain yields as reported by previous investigators (Abdul-ariz *et al.*, 2015, Mellion *et al*, 2012, Alghamdi, 2007).

Intermediate heritability values were recorded for harvest index, seed yield, seed filling period, days to flowering and days to maturity. Alghamdi (2007) obtained the highest heritability for days to flowering and maturity which is not similar to the result of this investigation. Conversely, low heritability values were recorded for leaf area index, internode length, number of branches per plant and crude protein content. Heritability estimates are expected to be lower in poor environments where heritability is concealed due to a greater genotype by environment interaction component.

Genetic advance as a percent mean ranged from 1.19% for crude protein content to 44.71% for number of pods per plant. Within this range, a relatively high genetic advance as percent of mean was observed for the majority of traits considered as in Table 16. Johnson *et al.* (1955) reported broad sense heritability along with genetic advances are usually more useful than heritability alone in predicting the resultant effect of selecting the best individuals. In addition to this, they also indicated that high heritability along with high genetic advance as percentage of mean implies the role of additive genes for the expression of the characters and thus it could be very effective in crop improvement program upon selection. In this study, high genetic advances as percent of mean with high heritability was observed on characters such as pod length, pod width, leaf area, number of pods per plant, number of seeds per plant, biomass weight per plot, thousand seeds weight and seed production efficiency.

Similarly, these characters also showed high to moderate genotypic and phenotypic coefficient of variation. Therefore, selection based on these traits with a relatively high genetic advance as a percent mean will result in the improvement of the performance of the genotypes for that trait. We can use this trait as selection criterion by considering the ultimate evaluation with target environment prior to using them. This could also help in understanding the mode of inheritance of quantitative traits. The report by Kalia and Sood (2004) found high heritability and high genetic advance for number of pods per plant which indicated high additive gene action and possibility of trait improvement through selection. The lowest genetic advance as percent of mean observed for crude protein content, days to flowering and maturity and seed filling period, internod length, plant height and leaf length arised from low estimate of phenotypic variance and heritability. Therefore, when we see with the present study, the trait considered are less affected by environmental factors and highly inherited traits. The difference observed could be due to high contribution of their genetic composition and differences.

6.1.3. Correlation studies between characters

In breeding action, the improvement of a target trait can be achieved via indirect selection of other characters. This needs knowledge of the associations of the different traits with the target characteristic and among the different characters. Hence; correlation coefficient measures the mutual relationship between various traits and determines the components on which selection can be based for improvement in crop yield (Singh, 2007). Therefore; correlation studies are helpful in formulating efficient breeding program for multiple trait selection.

In the present study, seed yield showed strong and highly significant positive genotypic correlation and phenotypic correlation with majority of the traits considered (Table 17) and therefore, breeding for improvement of these traits will improve the yield of faba bean and breeder can use these traits as selection criterion. In the contary, seed yield revealed negative and non-significant genotypic correlation with some traits and so selection based on these traits will not improve the yield of faba bean.

Alghamdi (2007); Azarpou *et al.* (2012); Tafere *et al*. (2013); Sharif (2014) and Abdalla *et al*. (2015) reported positive and significant association between seed yield per plot and thousand seed weight. Tadele *et al. (*2011) found number of pod per plant, number of seed per pod, thousand seed weight had positive and significant association with seed yield per plot. Gemechu and Musa (2002) also observed positive and significant association between seed yield and number of pods per plant.

As indicated in Table 17, the number of pods per plant signified strong and positive highly significant genotypic correlation coefficient with some traits and this shows us the propability of potential improvement for number of pods per plant via selection for these traits. On the other hand, number of pods per plant revealed negative highly significant genotypic correlation with thousand seeds weight, days to maturity, seed filling period and showed negative significant genotypic association with biomass weight per plot. This indicates a reverse association between number of pods per plant and these traits and improvement for these trait decreases the number of pods per plant.

Number of pods per plant revealed positive highly significant phenotypic correlation coefficient with number of seeds per plant (r = 0.94, strong association) and some other traits, hence, indirect selection based on this trait will improve number of pods per plant. Alghamdi (2007) also reported positive and highly significant correlation between number of pods per plant and number of seeds per plant and seed yield per plot. Ulukan (2003); Gemechu and Musa (2002); Abdelmula *et al*. (2007); Tadele *et al.* (2011); Ouji *et al*. (2011); Azarpour *et al*. (2012); Sharifi (2014) and Abdella *et al*. (2015) were reported positive and significant phenotypic relationship between number of pods per plant and traits: plant height, seed yield per plot, number of seeds per plant and thousand seeds weight. It implies that considering the correlation of this trait with other important parameters in faba bean improvement plays a crucial role in breeding strategies. These results suggested that improvement of grain yield in faba bean is linked to these traits and selection for these traits might have good impact on yield per plant. Abdelmula *et al*. (2007) and Oral *et al. (*2011) found positive and highly significant association between harvest index and seed yield per plot.

Days to flowering showed negative highly significant (p<0.01) genotypic correlation coefficient with harvest index, seed yield per plot and seed production efficiency. Seed yield indicated negative significant genotypic association with leaf width and economic growth rate. The report from different authors suggested days to flowering had positive and highly significant phenotypic association with plant height, seed yield (Alghamdi, 2007; Bakhiet *et al,* 2015 and Sharifi, 2015). Tafere *et al*. (2013) found positive and highly significant phenotypic relationship between days to flowering and days to maturity.

There were strong and highly significant positive genotypic correlation between days to maturity and seed filling period, days to maturity and thousand seed weight. Days to maturity also showed positive highly significant ($p<0.01$) genotypic association with plant height to first pod, pod length and leaf area index. Tafere *et al*., (2013) and Alghamdi (2007) reported positive and significant phenotypic association between days to maturity and number of branches per main stem, and thousand seed weight. The possibility for the existance of their strong relationship is that days to flowering, maturity and thousand seed weight highly dependent on each other

6.1.4. Cluster and principal component analysis

The multivariate analyses are a set of tools for analyzing multiple variables in an integrated and powerful way. It deals with the statistical analysis of the data collected on more than one (response) variable. It has proved useful for characterization and classification of plant genetic resources evaluated for several morphological and agronomic traits (Gemechu *et al.,* 2005).

Cluster analysis distinguished five different groups of faba bean genotypes (Table 18). The pair wise generalized squared distances (D^2) among the clusters showed the highly significant ($p<0.01$) and maximum distance (554.60) between clusters 1& 5. Similarly, the second and third most divergent clusters were C3 and C5 (459.37) and C1 and C4 (411.55), respectively. This high values of inter cluster distances may result due to the difference in genetic background of the materials, in which case some are derived from hybridization, the others via selection from introduced germplasm and landraces and others are still local varieties. Consequently, this may be a cause for their divergence and these materials can be suggested in breeding program for variety development. For better genetic recombination; hybridization of these materials with others faba bean lines may improve the adaptability and genetic constituent of faba bean germplasm in Ethiopia and also helps in selection of genetically divergent parents for exploitation in crossing programs. This finding is consistent with Million and Habtamu (2012) who used twenty-five elite faba bean genotypes for studying genetic variability on seed yield and related traits of elite faba bean genotypes.

Different scientists used cluster analysis in investigation of genetic diversity of faba bean germplasms and genotypes across the world. Polignano *et al*. (1993) used cluster analysis for classification of 106 faba bean accessions from Ethiopia and Afghanistan using eight morphological traits. Gemechu Keneni *et.al. (*2005) used cluster analysis to classify faba bean landraces in the study of the extent and pattern of generic existence of Ethiopian faba bean landraces using 160 accession and 11 phenotypic traits and the report revealed the existence of high genetic diversity in Ethiopian faba bean landraces. Another investigation also indicated that the hierarchical cluster analysis was used for classification of 14 faba bean genotypes according to some morphological traits (Chaieb *et al*. 2011). Similarly, Sarparast *et al*. (2011) and Chaubey *et al*. (2012) used cluster analysis to study genetic divergence of 70 faba bean accessions and to investigate genotypic structure of nine faba bean genotypes, respectively using morphological traits. Yahia *et al. (*2012) also clustered 42 faba bean populations based on phenotypic traits. Al-barri and Shtaya (2013) used cluster analysis to further investigate the interrelationships of Palestine faba bean germplasms using six agronomic traits.

The result from principal components (PCs) with eigenvalue greater than one contributed 85% of the entire diversity among the varieties and local varieties for all the 23 traits considered. Rencher (2002) reported that the first principal component is the linear combination with maximal variance; the second principal component is the linear combination with maximal variance in a direction orthogonal to the first principal component, and so on. Chahal and Gosal (2002) reported that characters with relatively larger absolute values of eigenvector weights in PC_1 had the largest contribution to the differentiation of varieties into cluster as it is normally assumed that characters with larger absolute values closer to unity within the PC_1 influence the clustering more than those with lower absolute values closer than those with lower absolute values closer to zero. Therefore, most of phenotypic traits contributed ±0.000 to 0.456 variation in PC_1 to PC_4 and differentiation of varieties into different cluster was indicated by the cumulative effects a number of characters. In the first two principal component traits: leaf length, leaf width, leaf area, pod length, pod width, plant height, number of pods per plant, biomass weight per plot, thousand seeds weight, seed production efficiency, seed yield per plot, days to flowering, and days to maturity were found an important character and had high contribution to the entire variation with the genotypes. This finding agrees with the results reported by Gemechu *et al.* (2005) and Yahia *et al. (*2012).

6.1.5. Comparison of performance of selected genotypes with the whole individuals

The comparison using t-test showed highly significant differences between means of the selected subsets of the top 10% best genotypes ($\bar{x}$) and the population parameters (μ) for pod length and showed significant difference for leaf length, leaf area, pod width, number of branches per plant, number of seeds per plant, thousand seeds weight and economic growth rate. Therefore, the mean comparison of best top 10% of the selected genotypes and the average performance of whole genotypes indicated the probabilities of improvements via selection for these parameters. On the other hand, some of the traits not showed significance difference among the mean performance of the best top 10 % of the genotypes and the whole base population indicating no significant improvement was made via selection for these parameters.

6.1.6. Estimation of the magnitude of genetic progress

Knowing the magnitude of genetic gain from past breeding activities plays an important role for breeders in evaluating the amount of gain obtained while selecting for desirable characters and this could assist the breeder in ascertaining criteria to be used in future faba bean breeding scheme particularly.The linear regression analysis and a positive association between seed yield and year of variety release mainly accompanied due to increased amount of other yield related traits and harvest index. The result obtained in this study was slightly greater than an annual rate of 8.74 kgha^{-1} yr^{-1} and cumulative genetic progress of 288.4 Kg ha^{-1} that reported by Tamene *et al. (*2012) in their study of genetic progress of faba bean varieties in Ethiopia. Contrary, the genetic progress estimated on faba bean breeding in Ethiopia was less than the reports on different crops like 0.83% relative gain in field pea (Teshome, 2011), 1.34% relative gain in barley (Wondimu, 2010) and1.27% relative gain in soybean (Demisew, 2010) in Ethiopia.

In other country, reports also showed higher genetic gain of 66 kgha^{-1}yr^{-1} and 30 kgha^{-1}yr^{-1} from maize breeding in USA (Duvick, 2005) and West Africa (Badu-apraku et al., 2014) respectively, and increased yield rate of 35·1 kgha^{-1}yr^{-1} (0·88%) from wheat breeding in Spain (Sanchez-garcia *et al.*, 2012). Based on their derivative ancestors (hybridization derived, introduction derived and local selection derived), the varieties revealed an increment on average yields and percentage over oldest variety indicating the genetic gain over decades in faba bean breeding in Ethiopia.

Linear regression analysis was also made for estimating genetic gain of thousand seed weight by regressing over year of release. Seed size revealed 4.41 g per thousand seed weight (0.87% per 1000 seeds weight) of an annual rate of improvement in the past faba bean breeding in Ethiopia and the average genetic increment of 158.76 g per thousand seeds weight (31.5% per 1000 seed weight). Breeding for seed size indicated better genetic improvement than improvements made for seed yield in the past faba bean breeding. The magnitude of genetic gain revealed by seed size in this study was less than the result suggested by Tamene *et al.* *(*2015), but followed similar patterns of high increment.

6.2. Genetic Diversity in Ethiopian Faba Bean Varieties as revealed by ISSR and SNP Markers

6.2.1. Magnitude of genetic diversity

Information on extent and amount of genetic diversity that exist in crop plants plays a significant role in development of breeding strategies and designing future conservation practices of agricultural crops. Maintained genetic diversity in crop plants also helps the crops populations to evolve and cope up themselves with current environmental change by maintaining the sustainability of crops species in agricultural production system. Amos and Harwood (1998) suggested genetic variability as the clay of evolution, providing the base material on which adaptation and speciation depends on. If there is little or no genetic diversity with cultivated crops, the probability of the crops to cope up to the changing environment and susceptibility to wide spread disease will aggravate. In line with these, efforts have been made by many scientists to investigate crop genetic diversity using different markers system and generated considerable amount of information about genetic diversity that existed in conserved or actively utilized germplasms (Rauf *et al*., 2010).

The present study was designed to see the genetic diversity existing between Ethiopian faba bean varieties released so far in Ethiopia. In this study, the extent and pattern of genetic variability among 32 Ethiopian faba bean varieties were estimated using 11 ISSR primers. Similarly, a total of 48 faba bean genotypes (31 varieties and 17 breeding lines) were genotyped using 37 SNP primers with KASP genotyping assay platform to investigate the extent and pattern of genetic diversity among Ethiopian faba bean. Both markers detected vast diversity among Ethiopian faba bean varieties and breeding lines.

The ISSR markers amplified a total of 120 loci from eleven ISSR primers and 107 were polymorphic loci with an average of 90 % polymorphism. High level of polymorphism was obtained with an average of 10 polymorphic bands per primer. Lower average percentage polymorphism than the present research was reported by using ISSR markers in the study of genetic diversity of faba bean germplasms by different investigator. Abdel-razzak *et. al*. (2012) detected a range of percent of polymorphisms which varied from 52.08% to 92.31% with an average 69.10% within 10 faba bean genotypes from Egypt using nine ISSR primers. Terzopoulosa and Bebeli (2008) also found percent of polymorphisms ranging from 37.5% to 84.62% with an average of 67.48% by using 11 ISSR primers in studying genetic diversity of Greek faba bean population. The present study detected higher percent polymorphism than both finding, which could be explained by divergent source that ranges from landraces to elite introduced and crossed materials. Moreover, Zeid *et al*. (2003) detected 74.3% polymorphisms using AFLP primer on cultivars from Germany. Bashirsaliamia *et al*. (2013) reported 62.76% polymorphisms using RAPD primers in Palestinian landraces. Abdul-razzak *et al*. (2012) also reported 53.57% polymorphisms using 18 RAPD primers in10 faba bean cultivar diversity analysis in Iraq. Hou et al. (2014) based on SDS-PAGE also considered polymorphism ranging from 30.56 to 58.33% with average 44.69% in diversity analysis of China faba bean genotypes. In the same way, lower average of percentage polymorphisms than the present study was reported in genetic diversity study of other legume crops: lentil (59.57% and 80.13%) by Edossa *et al*. (2007) and Meenakshi *et al*. (2013), respectively, chickpea (63.63%) by Bhagyawant, and Srivastava (2008) and Common bean (76.12%) by Sadeghi and Cheghamirza (2012).

On the other hand, higher percentage of average polymorphisms than our result was reported by some authors. Recently, study by Ammar *et al*. (2015) considered highest level of percent polymorphisms (100%) using 6 SRAP and 4 AFLP primers for faba bean genetic diversity assessment in Saudi Arabia. Merji *et al*. (2012) also described 97.3% of average polymorphism in analysis of effect of gamma radiation on various characters of 22 faba bean genotypes grown in Tunisia using 15 ISSR primers. Similarly, Wang *et al. (*2012) reported percentage of polymorphisms ranging from 91% to 100% with an average of 93% in the study of genetic diversity and relationship of global faba bean germplasms. Likewise, Salazar-Laureles *et al*. (2015) also found percent polymorphisms ranging from 71.4 to 100% with an average of 91.3% in analysis of genetic variability within Chilean faba bean accession using ISSR markers. Therefore, higher mean percent polymorphism (90%) observed in this study showed the capacity and use of ISSR markers for genetic diversity study and varietal identification and can be applied for future routine applications.

The degree of polymorphism among the groups/category of faba bean varieties ranged from 37.5% (45 loci) for the local varieties to 84.17% (101loci) for the varieties those derived from hybridization. The highest polymorphisms observed in the varieties derived from hybridization as compared to local varieties could also be explained by the broader spectrum initially acquired from subsequent genetic recombination and this could practically have broadened the genetic base of the national breeding programs via introduction of new allele to the varieties derived from hybridization.

Out of the 40 SNPs initially selected, the 37 scoreable SNP loci screened, one was found monomorphic across all the 48 genotypes and 36 SNP showed 95.58% average polymorphism. This result is greater than the one reported by Cottage *et al. (*2012) who reported 83.5% polymorphisms generated by 80 SNPs on 65 faba bean lines from ICARDA collection. Similarly, Kaur *et al*. (2014) also reported 85.4% successful polymorphisms in assessment of genetic diversity in faba bean genotypes using SNP markers. Therefore, the higher average percentage of polymorphisms reported in the present study using SNP markers indicated that there is high level of genetic diversity between Ethiopian faba bean varieties.

The comparative analysis of ISSR vs SNP marker in this study clearly indicated that, the polymorphisms revealed by the SNP marker was slightly greater than the polymorphisms signified using ISSR marker. The reason could be the accuracy of estimations of polymorphisms using dominant markers (ISSR) is usually reduced relative to codominant markers (SNP) as reported by Lynch and Milligan (1994). Another cause for high percentage of SNP polymorphisms could be high occurrence of SNP loci in the genome.

Using 11 ISSR primers, varieties developed via hybridization revealed the highest gene diversity and Shannon diversity index, whereas farmer (local) varieties showed the least gene diversity and Shannon diversity index. The average gene diversity and Shannon diversity index ranged from 0.18 to 0.38 and 0.27 to 0.56 with mean value of 0.27 and 0.41, respectively. This report is comparable with study by Wang *et al*. (2012) who signified gene diversity and Shannon diversity index ranging from 0.18 to 0.26 and 0.27 to 0.39, respectively using 11 ISSR primers. This also followed similar patterns of diversity as that of polymorphisms obtained among individual faba bean varieties in the study.

The Shannon diversity indices and expected heterozygosity based on SNP marker and pedigree information indicated that the hybridized cultivar and breeding lines had slightly higher Shannon diversity indices and expected heterozygosity (gene diversity) values than selection from local landraces, introduced cultivar and farmer varieties. This could be due to the presence of recombination with hybridized and breeding lines. Most of all, the reason why genetic diversity is larger in hybridized cultivars than in landraces may be due to breeding strategy and breeders' efforts made during variety development.

The average number of alleles per SNP locus was 2.00 (Table 31). Because SNP markers with KASP genotyping assays are mainly bi-allelic in nature. Thus, the bi-allelic nature of KASP SNP markers caused the value of gene diversity and PIC less than 0.5, but in multi-allelic markers (e.g. SSRs) the maximum can approach to a unit (1). Relatively high average Nei's genetic diversity (0.41) and PIC (0.32) were revealed by SNP markers in the present study and this indicates the high resolution of SNP used. We reported better results than previous study in faba bean and high level of genetic diversity observed in this study. For example, Kaur *et al*. (2014) reported lower genetic diversity (0.36) and PIC (0.28) than the present study in assessment of genetic diversity in Australian faba bean genotypes. On the other hand, Cottage *et. al. (*2012) studied genetic diversity of 65 faba bean germplasms and reported mean genetic diversity of 0.29. The level of heterozygosity ranged from 0.00 to 0.9024 across 48 faba bean genotypes. The high heterozygosity observed in most of the genotypes might be due to out-crossing character of the crop and this shows that the materials are segregating.

Generally, our results suggested that ISSR and SNP markers could be utilized to detect genetic variation and varietal identification. These genotypes and lines can be further used for development of segregant materials and both markers can be utilized to follow inheritance.

6.2.2. Relationship among faba bean cultivars and pattern of grouping

Cluster analysis (UPGMA and neighbor joining) grouped 32 faba bean varieties into three distinct clusters based on the data from 11 ISSR markers and showed relationships among Ethiopian faba bean varieties (Figure 13 and Figure 12). Most of the varieties were grouped based on their breeding information (derived from hybridization, derived from introduced materials, selected from landraces, and local varieties) while few of the cultivars tend to spread all over the dendrogram without forming strict grouping. Therefore, out-crossing floral biology of faba bean has its own impact on the intermixing of varieties from different genetic information into similar cluster.

The C1 mostly contained varieties derived from hybridization which includes: Mosisa, Hachalu, Tumsa, Gachena, Obse, Walki, Moti and Gebelcho and two varieties derived from local collection (Dosha and Adet Hanna). The possible reason for grouping of these varieties into the same cluster could be the breeding objectives designed by breeder in our country Ethiopia. The breeding objectives of faba bean was ultimately designed to improve faba bean genotypes for their yield, resistance for biotic and abiotic factors and recently for seed size (Asfaw *et al*., 1994; Tamene *et al*., 2012). Therefore, these common objectives could make the materials to carry similar gene responsible for yield, resistance to biotic and abiotic and seed size.

The present clustering concedes with Abdel-razzak *et al. (*2012) in their studies of genetic diversity in 10 faba bean genotypes from Egypt using ISSR grouped the individuals depending on their genetic similarity. Wang *et al.* (2012) also grouped 802 global faba bean accessions into four groups based on their genetic similarity in their studies of genetic diversity and relationship of global faba bean accession. Hence, these finding agrees with the recent result obtained in genetic diversity studies of Ethiopian faba bean varieties.

From the cluster analysis, the estimated of genetic similarity among faba bean varieties ranged from 0.29 to 0.77 (Table 28). Depending on an estimated genetic similarity matrix, the highest genetic similarity value was observed between Mesay and Bulga-70 (0.77). This trend also followed between Sinana local and Agarfa local (0.76) and between Kuse and Lalo (0.75). The causes for high similarity between Sinana and Agarfa local varieties, could be, both varieties are found in similar geographical location and the probability of seed exchange between farmers is high. Kuse and Lalo varieties also showed high genetic similarity with each other and these varieties were released for vertisol areas and they could carry similar gene for resisting waterlogging stress (Tamene *et al*., 2015). This similarity coefficient gives us evidence that these varieties are genetically more similar and hence the hybridization between these groups may not be sound useful in getting desirable segregating materials. The least similarity value was observed between variety Obse and

Didea (0.29), followed by association between Gachena and didea (0.32), Tumsa and Didea (0.35), Mosisa and Lalo (0.36), and these varieties are genetically the most distant while considering all varieties in the present study. Therefore, it is important to use these materials in hybridization program to widen ranges of genetic variability of Ethiopian faba bean varieties. It will provide scope of selecting desirable genotypes from F2 and subsequent segregating generations from hybridization program.

The Neighbor-joining (NJ) analysis based on 11 ISSR data of Ethiopian faba bean varieties also showed three distinct clusters and the major cluster one sub-cluster two did not show a clear grouping because it constituted varieties from three different sources (varieties derived from local collection, introduction and hybridization). Clustering from different groups into the same cluster without showing clear cut was also reported by different authors like Abdel-razzak *et al. (*2012), Wang *et al.* (2012) and Salazar-laureles *et al. (*2015).

Principal Coordinates analysis (PCO) based on ISSR data revealed a clear differentiation between Ethiopian faba bean varieties and yielded similar result with that of cluster analysis and predominantly separated faba bean varieties on the basis of their pedigree relationships. This result is in agreement with Wang *et al. (*2012) and Salazar-laureles *et al. (*2015) in their studies of faba bean genetic diversity using ISSR marker. In dendrogram and PCoA clustering, individuals from local varieties clearly separated from cultivars. Bako and Kulumsa local varieties separated solely from the group in PCoA and indicated genetic distinctness from the materials utilized in the present study.

The neighbor-joining tree based on the genetic dissimilarity also similarly revealed three major groups and in agreement with the clustering based on model-based population structure analysis. This dendrogram reveals that the majority of genotypes were grouped together from different genetic background/information (Hybridized cultivar, selection from landraces, selection from introduction, farmer's variety and breeding lines) which means the genotypes did not follow the pattern of their genetic background or parental information **(**Figure 20). In this grouping, cluster one (C1) and three (C3) signified sub-groups, which suggests that selection of parental lines from different sub-groups might be an effective way for making hybrid combinations.

Principal Coordinate Analysis (PCoA) (Figure 21) based on SNP data also revealed three clear groups of faba bean varieties /breeding lines which were in agreement with the genetic distance-based neighbor joining trees and model-based population structure analysis for SNP marker.

6.2.3. Partitioning the genetic diversity and population structure

The materials used in the present study showed a certain degree of admixture indicating the introduction of chromosomes of different ancestry and allele frequency. Therefore, the possible factors for such admixture could be differential selection, mutation effect and out crossing behavior of the crop. Furthermore, this could give a clue that faba bean varieties/pipelines in Ethiopia consider a significant genetic variation and this plays a vital role for development of new variety that can withstand the ever-changing environmental factors. Tracy and Chandler (2006) and Lu *et al*. (2009) reported the lines within a group or sub-group have a low level of genetic dissimilarity and crosses between genetically divergent lines selected from different groups or subgroups generally produce better-performing heterotic genotypes than the closely related parents.

Analysis of molecular variance based on ISSR and SNP data was utilized to see the level of genetic differentiation among Ethiopian faba bean varieties within and between groups. Data based on ISSR result attributed 91% of within group variation and 9% among group variation. The results revealed significant genetic differentiation between groups ($p < 0.018$, $Fst = 0.085$) indicating a significant genetic differentiation between groups and highly significant genetic differentiation was observed within groups ($p<0.001$). On the other hand, AMOVA analysis based on SNP data showed very high significant genetic variation within groups (99%) with the least variation among groups (1%).The genetic differentiation within the group was highly significant ($p<0.001$, $Fst = 0.011$), but showed non-significant genetic differentiation among groups ($p<0.282$). Therefore, this marker approved the presence of high genetic variation with Ethiopian faba bean varieties. Recent study by Oliviera *et al*. (2016) observed the highest proportion of 81% within genetic variation and 19% among genetic variation in genetic diversity and structure analysis using 26 nuclear SSRs.

The highest within genetic variation observed in the groups using both markers indicates that groups are constituted by genetically distinct individual. The partially allogamous nature of faba bean could also be the other possible factor for high genetic variation of within groups. Because, the pollen of faba bean can easily move by insect pollinators like bee and bee beetles causing outcrossing of the varieties (Ellwood *et al.*, 2008). Similarly, the alternate possibility may be found in breeding history of the cultivars with which primarily experienced artificial selection and secondarily natural selection for some desirable traits. Therefore, the result helps breeders to accelerate faba bean improvement by addressing the patterns of genetic variation within faba bean varieties and maximize the level of variation present in segregating populations by crossing cultivars with greater genetic distance.

6.2.4. Implication of study for faba bean improvement

The study of genetic diversity is an important practice for designing relevant breeding program. The present study indicated high genetic diversity among Ethiopian faba bean varieties based on the phenotypic and genotypic diversity assessed, especially with varieties derived via hybridization. Therefore, utilization of these materials in variety development scheme will provide a sound result for selection of individuals with different important characters. The diversity parameters like gene diversity and genetic distances observed in the present study showed high genetic variability with cultivated faba bean varieties and considering these diversity parameters in breeding program could be valuable approach.

Another point that should be given a due attention is, the impact of improved varieties on genetic diversity of Ethiopian faba bean germplasms. A number of faba bean varieties released nationally or regionally so far showed significant amount of genetic diversity, however, care should be taken while popularizing and pushing these varieties towards farming system known to have landraces with unique features. Replacement of landraces varieties by improved once could result in narrowing down the genetic bases faba bean in Ethiopia. Therefore, this problem can be solved by awaring farmers by giving them different option of varieties and Ethiopian Institute of Biodiversity should give a due attention for conserving the varieties to maintain the diversity among Ethiopian varieties.

7. CONCLUSION AND RECOMMENDATIONS

7.1. Conclusion

In Ethiopia, faba bean is produced under wide spectrum of altitude range (1800-3000 m.a.s.l). It plays significant economic and ecological role in socio-economic life of farming community: serving as food and feed, fixing nitrogen, break disease cycles in the cereal-based rotations, control weeds and could serve as climate conscious crop by mitigating considerable amount of global CO_2 emissions. Thus, documenting genetic information on this important crop is crucial for various purposes. Of these, genetic diversity study plays a paramount role in signifying genetic variability among and within species, developing selection criteria, selecting heterotic parents for hybridization, choosing effective breeding procedures and determining conservation strategies. Estimation of genetic variation among faba bean varieties in Ethiopia is, therefore, the main concern of breeders as the effectiveness of selection depends on the proportion of the heritable variation.

Genotypic coefficient of variation ranged from 1% (crude protein content) to 17.14% (number of pods per plant) and phenotypic coefficient of variation also ranged from 3.38% (days to maturity) to 68.57% (numbers of branches per plant). Generally, the phenotypic coefficient of variability was higher than recorded the genotypic coefficient of variability which indicated the existence of environmental effect for the expression of most characters. Heritability estimate ranged from 8.45% for number of branches per plant to 95.2% for pod length. The expected genetic advance expressed as a percent mean varied from 1.19% for crude protein content to 44.71% for number of pods per plant.

Seed yield signified almost highly significant genotypic and phenotypic correlation coeficient with seed production efficiency ($g_r = 0.95$, $p<0.01$ *and* (Pr = 0.94), respectively.

On the other hand; seed yield revealed negative and highly significant ($p<0.01$) genotypic correlation coefficient with days to maturity.

UPGMA clustering based on phenotypic traits grouped 32 faba bean varieties into five clusters. There was highly significant difference ($p<0.01$) among all inter cluster distances and maximum distance (554.60) was detected between clusters 1 & 5, while the minimum distance (54.80) was observed between cluster 1 & 3. Based on PCA analysis, the first three principal components accounted 66% of the total variation among faba bean varieties grown in Ethiopia.

The extent and pattern of genetic variability among faba bean varieties were also estimated using 11 ISSR primers and revealed a total of 120 amplified bands with an average of 11 bands per primer, of which 107 loci were found to be polymorphic with 90% polymorphism. The degree of polymorphisms per primer varied from 60% to 100%. A total of 37 SNP markers were used to estimate the extent and pattern of genetic diversity of 48 faba bean varieties/pipelines. Among 37 SNPs assayed, 36 SNPs were found polymorphic with an average of 95.58% polymorphisms. Relatively high average Nei's genetic diversity (0.41) and PIC (0.32) were revealed by SNP markers.

Overall, the three markers used in present study based on all the diversity parameters signified the presence of high genetic diversity among Ethiopian faba bean varieties, especially SNP marker clearly indicated the variation among individual faba bean varieties grown in Ethiopia. Therefore, the presence of genetic diversity within a given population plays an essential role for designing better faba bean breeding strategies for genetic improvement aimed at solving the ultimate needs of the producers and contributes to the well-being of present and future generations.

7.2. Recommendations

Based on the results of this study, the following recommendations are made for future consideration.

- This study revealed an immense amount of genetic variation among cultivated faba bean varieties in Ethiopia. Therefore, breeders should consider these materials in future faba bean breeding program for introgression with other germplasm resources.
- In the present study, sufficiently very high and moderaly high heritability values with high genetic advances were recorded for some traits and this indicated the possibility of progress from intensity of selection. Therefore, selection breeding based on mean would be crucial for the improvement of these traits in the faba bean varieties development program.
- The present and past studies indicated small ranges of crude protein contents in Ethiopian faba bean germplasms. Therefore, it is recommended that future study should consider more number of Ethiopian landraces to see the ranges of protein contents.
- In this study, eleven ISSR primers sucessfully estimated the extent and pattern of genetic variability among released Ethiopian faba bean varieties. Therefore, these primers could be used in studies of genetic diversity, genomics and evolutionary studies, genome mapping and gene tagging of more faba bean landraces.
- In the present study, relatively high genetic diversity and PIC revealed by SNP and thus, it is recommended to utilize these SNP primers in genetic characterization of faba bean germplasm and other related studies.

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9. APPENDICES

Appendix 1 Monthly and Annual Rainfall (mm) of Sinana Agricultural Research Center (1990-2014)

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual RF
1990	35.38	71.23	145.69	250.46	109.54	25.08	32.62	150.46	242.46	176.15	56.15	33.85	1329.07
1991	34.46	41.69	166.46	146.77	193.54	48.31	139.54	191.23	143.38	39.54	15.54	25.23	1185.69
1992	45.85	45.38	20.62	146.31	254.15	144.46	47.08	259.54	241.23	182.92	66.92	89.23	1543.69
1993	115.69	131.54	0.00	181.23	145.38	27.69	38.77	113.08	228.15	150.92	26.15	13.69	1172.29
1994	0.00	0.00	80.92	147.69	242.31	40.31	70.92	179.69	211.08	159.23	130.77	34.92	1297.84
1995	0.00	25.85	124.00	204.77	157.54	139.38	81.69	166.92	178.62	112.62	14.15	14.31	1219.85
1996	37.38	11.69	124.31	244.31	128.62	263.69	100.46	210.15	238.00	154.00	36.92	16.77	1566.30
1997	1.85	0.00	88.31	170.77	120.15	140.62	63.08	82.46	149.08	191.23	128.15	9.08	1144.78
1998	115.38	8.15	58.92	73.85	115.90	54.15	114.31	103.23	145.23	213.54	36.15	0.77	1039.58
1999	0.00	0.00	128.77	116.15	136.92	59.08	109.54	222.15	156.77	150.15	42.77	0.00	1122.30
2000	2.77	0.00	20.46	117.85	185.23	48.31	105.85	98.77	187.38	179.69	40.92	16.46	1003.69
2001	0.00	18.62	133.23	158.92	194.15	170.62	124.62	115.23	249.23	118.15	43.54	18.92	1345.23
2002	42.46	0.00	96.00	101.69	93.08	78.77	66.00	52.00	137.69	125.54	16.31	56.00	865.54
2003	2.62	0.00	68.15	108.77	62.46	35.54	70.62	80.31	198.92	64.15	58.46	72.92	822.92
2004	56.15	36.00	60.15	139.85	54.15	20.46	71.23	131.54	184.15	79.08	23.38	44.00	900.14
2005	35.85	17.38	56.00	162.46	193.23	60.15	62.00	43.08	206.46	66.92	36.92	0.00	940.45
2006	16.77	71.38	54.31	179.55	131.54	86.15	62.46	110.00	139.54	129.38	23.38	35.38	1039.84
2007	0.00	6.20	49.10	286.30	108.60	94.60	167.10	172.40	152.80	122.40	103.20	0.00	1262.70
2008	9.70	0.00	28.40	274.80	275.80	119.80	98.30	164.30	239.30	126.30	139.20	0.00	1475.90
2009	49.90	0.00	31.30	207.10	142.90	49.60	58.00	89.20	136.20	44.20	86.10	0.00	894.50
2010	0.60	18.20	133.60	196.20	250.90	40.30	190.40	222.10	320.00	97.10	0.00	0.00	1469.40
2011	0.00	0.00	123.00	136.00	141.60	69.60	95.60	87.50	68.90	98.40	74.30	0.00	894.90
2012	0.00	0.00	7.00	188.00	149.30	132.90	94.30	325.50	338.60	124.00	53.60	26.20	1439.40
2013	46.80	0.00	234.10	151.50	138.60	76.70	170.60	126.60	138.20	159.70	213.90	0.00	1456.70
2014	0.00	0.00	79.50	53.00	53.00	62.80	81.00	212.50	165.50	110.00	58.00	0.50	875.80

Appendix 2. Monthly and Annual Rainfall (mm) of Agarfa from 1990-2014

Year	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Annual RF
1990	22.70	11.50	56.20	125.40	95.00	17.40	58.40	154.90	89.60	41.50	19.60	0.00	692.2
1991	20.70	39.40	99.00	142.60	78.50	25.90	47.80	186.97	98.60	27.20	13.50	0.00	780.17
1992	29.10	39.30	94.00	198.64	50.30	40.20	145.00	224.50	148.60	56.50	14.00	2.40	1042.54
1993	0.00	7.00	95.00	116.20	23.40	30.10	118.30	142.60	71.90	48.10	49.20	2.50	704.3
1994	0.00	18.70	79.80	152.30	84.12	18.00	116.50	138.90	68.90	33.00	1.60	13.10	724.92
1995	13.20	13.20	73.00	125.80	87.20	101.00	102.20	215.68	89.60	31.80	20.00	6.10	878.78
1996	13.20	13.20	86.21	96.90	65.10	101.00	102.20	137.90	77.62	31.80	20.00	6.10	751.23
1997	13.70	0.00	91.60	131.60	56.20	47.10	78.90	126.80	75.90	35.40	56.70	2.00	715.9
1998	66.10	56.60	90.30	176.80	94.00	40.80	98.65	248.10	85.30	104.20	19.40	3.50	1083.75
1999	5.00	18.00	99.40	100.30	92.50	39.60	104.00	105.60	89.50	52.10	1.80	2.30	710.1
2000	0.00	12.00	89.40	142.80	79.20	29.00	98.50	134.80	91.60	16.90	0.00	7.60	701.8
2001	0.00	5.00	78.60	139.70	87.20	27.80	89.70	149.56	87.12	25.90	11.00	1.30	702.88
2002	12.40	0.00	74.00	111.50	76.80	19.10	95.62	182.60	112.60	14.60	4.00	10.90	714.12
2003	0.00	0.00	93.40	124.60	91.20	34.30	98.15	165.80	97.80	22.60	4.10	22.50	754.45
2004	21.40	7.10	97.20	118.90	68.10	19.60	97.60	167.50	88.60	13.50	8.40	0.00	707.9
2005	6.90	0.00	98.20	161.50	75.40	2.30	98.70	142.50	97.80	23.50	9.30	0.00	716.1
2006	6.10	25.00	84.30	124.70	81.20	25.80	44.30	152.10	98.40	38.90	17.40	6.50	704.7
2007	6.40	25.00	85.30	115.60	70.70	40.40	78.90	167.80	71.30	35.50	24.70	0.00	721.6
2008	4.60	0.50	97.60	114.30	66.80	17.60	93.00	166.40	66.50	28.10	54.30	0.60	710.3
2009	14.50	1.50	96.80	101.90	67.20	15.20	86.40	138.30	84.20	50.40	3.60	17.80	677.8
2010	2.70	135.00	80.00	110.30	95.70	37.00	112.30	214.35	164.90	46.10	4.00	0.00	1002.35
2011	1.60	4.00	13.40	151.80	70.00	45.80	124.00	156.40	111.60	6.90	33.10	0.00	718.6
2012	0.00	0.00	37.60	133.00	67.20	23.40	71.30	145.10	0.00	60.20	11.40	10.00	559.2
2013	0.00	0.00	0.00	86.40	98	55.2	4.7	179	133.1	185.00	26.30	0.00	767.7
2014	0.00	0.00	22	18.8	143.5	45.5	54.9	97	80.1	127.3	0.00	4.00	496.1

Appendix 3. Mean Monthly Maximum Temperature (^{o}C) of Sinana Agricultural Research Center (1990-2014)

Year	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec	Mean
1990	21.70	21.90	20.80	20.50	21.80	22.70	22.50	21.40	17.90	19.80	20.10	21.50	21.05
1991	22.90	23.30	22.00	20.90	20.60	21.50	20.40	20.50	20.80	21.70	20.50	20.60	21.31
1992	21.80	21.20	22.30	21.30	20.60	20.50	20.80	20.40	19.70	18.90	18.80	20.00	20.53
1993	19.90	20.40	22.10	19.40	20.20	21.30	20.80	20.70	20.10	18.40	19.30	21.40	20.33
1994	22.10	23.70	22.90	20.80	20.20	22.00	21.60	20.40	20.30	19.50	20.00	21.40	21.24
1995	23.20	23.60	20.90	20.80	21.40	22.30	21.40	21.20	19.80	18.70	19.70	21.10	21.18
1996	21.80	23.50	21.80	20.30	19.90	19.40	19.90	20.20	20.20	19.70	20.20	21.10	20.67
1997	22.80	23.40	22.90	19.90	20.20	20.40	20.80	21.30	20.60	19.00	19.50	20.20	20.92
1998	20.30	22.20	23.00	22.60	21.80	22.20	21.30	20.70	20.00	19.30	19.20	20.80	21.12
1999	22.50	23.10	20.80	21.50	20.70	21.00	20.30	20.20	19.90	18.70	19.50	20.80	20.75
2000	22.60	23.10	23.60	22.00	21.20	21.20	21.10	20.20	19.90	18.70	19.80	21.30	21.23
2001	22.90	23.50	22.40	22.00	22.20	20.30	20.80	20.80	20.20	19.70	19.60	21.80	21.35
2002	22.40	24.20	23.00	23.20	23.30	23.00	22.50	22.30	22.20	20.40	22.40	22.00	22.58
2003	24.20	27.60	23.50	22.00	22.40	21.40	20.90	20.40	20.50	19.90	21.00	20.10	21.99
2004	21.80	22.30	22.60	20.00	22.70	20.90	21.00	20.60	19.90	19.40	21.00	21.70	21.16
2005	22.30	23.40	22.10	21.10	20.20	20.80	20.80	21.40	20.60	19.20	19.80	21.30	21.08
2006	22.30	22.70	22.50	20.50	21.20	21.80	21.00	20.40	19.80	18.90	19.80	20.40	20.94
2007	22.10	22.70	22.80	10.20	20.30	21.10	20.00	19.90	19.00	19.30	19.40	20.90	19.81
2008	21.40	21.80	22.60	19.80	20.20	20.00	19.80	19.50	19.50	18.90	19.40	20.40	20.28
2009	20.20	21.70	21.80	19.30	19.80	21.10	20.80	20.20	19.70	20.30	20.80	19.50	20.43
2010	21.50	19.90	20.00	20.20	20.30	21.10	20.10	20.10	19.70	19.70	20.50	21.40	20.38
2011	22.00	21.60	21.48	22.26	18.94	20.06	20.20	20.20	19.40	20.20	19.50	21.60	20.62
2012	22.10	24.00	23.80	21.00	20.80	20.80	20.60	18.60	18.20	19.32	20.74	15.39	20.45
2013	21.55	22.68	20.23	19.73	20.23	21.00	21.23	20.06	20.20	19.61	20.06	21.48	20.67
2014	22.52	22.61	22.03	21.94	21.32	21.73	20.81	20.86	19.86	18.90	19.30	20.45	21.03
Mean	22.03	22.80	22.16	20.53	20.90	21.18	20.86	20.50	19.92	19.45	20.00	20.75	20.92

Appendix 4. Mean Monthly Minimum Temperature (° C) of Sinana Agricultural Research Center (1990 – 2014)

Year	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec	Mean Tmin
1990	7.30	10.30	9.90	10.30	10.10	9.70	9.70	9.10	10.40	8.70	8.20	7.10	9.23
1991	8.20	8.60	10.00	10.30	10.40	10.00	9.80	9.60	10.30	9.10	7.60	7.80	9.31
1992	7.90	9.40	9.80	10.80	10.10	9.30	9.10	9.10	9.60	9.10	7.90	8.40	9.21
1993	8.90	8.60	8.50	9.60	10.20	9.80	9.50	9.60	9.20	9.50	7.80	6.80	9.00
1994	6.90	8.20	9.60	10.30	9.90	10.20	9.80	9.70	9.70	8.70	7.90	6.60	8.96
1995	6.60	8.90	10.20	10.60	9.90	9.80	9.70	9.90	9.70	9.90	7.70	7.50	9.20
1996	8.50	8.90	10.30	11.10	10.40	10.10	9.40	9.00	10.00	8.60	7.70	6.60	9.22
1997	1.20	6.80	9.60	10.70	9.80	9.30	9.90	9.60	9.60	10.70	10.30	8.90	8.87
1998	10.10	10.60	11.70	11.80	11.90	10.90	10.80	11.10	10.50	11.40	8.10	6.30	10.43
1999	8.00	8.50	10.10	10.80	10.30	9.90	9.90	9.80	10.10	10.10	7.50	7.40	9.37
2000	7.00	8.00	9.50	10.20	10.80	10.30	10.50	10.20	9.90	10.50	8.20	7.60	9.39
2001	8.10	8.50	10.30	10.80	10.60	9.80	9.50	9.70	9.90	9.70	8.10	7.60	9.38
2002	8.30	7.90	10.10	10.50	10.70	9.80	9.90	9.90	9.60	10.30	8.30	10.20	9.63
2003	9.30	9.90	10.60	11.50	10.80	10.50	10.40	10.20	9.40	9.30	9.30	8.10	9.94
2004	9.70	9.30	10.00	10.70	10.80	10.40	9.30	9.40	10.00	9.20	8.00	8.30	9.59
2005	7.80	9.30	11.00	11.00	11.00	10.30	10.20	9.70	10.30	9.70	8.10	6.90	9.61
2006	7.80	9.50	10.10	10.70	10.70	10.30	10.00	10.30	10.30	10.00	8.80	8.60	9.76
2007	8.40	10.10	10.50	11.00	11.00	10.50	10.10	10.10	10.40	9.60	8.60	6.70	9.75
2008	8.00	8.20	8.80	10.60	10.60	10.00	10.40	10.50	10.90	10.70	8.70	7.30	9.56
2009	9.10	8.90	9.90	11.30	11.10	10.60	10.30	10.20	10.60	9.70	8.70	10.50	10.08
2010	9.30	10.90	10.80	11.20	11.60	10.60	10.80	10.30	10.80	10.80	8.50	7.60	10.27
2011	8.37	8.58	9.90	10.68	11.10	11.30	13.15	11.30	10.91	10.03	10.20	7.40	10.24
2012	7.10	8.40	9.10	10.90	10.90	10.70	10.40	10.80	11.03	9.98	10.42	8.13	9.82
2013	8.10	8.43	10.45	10.83	10.42	10.77	10.58	9.97	10.23	10.06	8.81	6.48	9.59
2014	7.58	9.95	10.26	11.19	12.13	12.10	12.03	11.71	11.78	11.48	10.87	9.71	10.90
Mean	7.90	8.99	10.04	10.78	10.69	10.28	10.21	10.03	10.21	9.87	8.57	7.78	9.61

Appendix 5. Mean Monthly Temperature (o C) of Sinana Agricultural Research Center (1990-2014)

Year	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec	Mean
1990	14.50	16.10	15.35	15.40	15.95	16.20	16.10	15.25	14.15	14.25	14.15	14.30	15.14
1991	15.55	15.95	16.00	15.60	15.50	15.75	15.10	15.05	15.55	15.40	14.05	14.20	15.31
1992	14.85	15.30	16.05	16.05	15.35	14.90	14.95	14.75	14.65	14.00	13.35	14.20	14.87
1993	14.40	14.50	15.30	14.50	15.20	15.55	15.15	15.15	14.65	13.95	13.55	14.10	14.67
1994	14.50	15.95	16.25	15.55	15.05	16.10	15.70	15.05	15.00	14.10	13.95	14.00	15.10
1995	14.90	16.25	15.55	15.70	15.65	16.05	15.55	15.55	14.75	14.30	13.70	14.30	15.19
1996	15.15	16.20	16.05	15.70	15.15	14.75	14.65	14.60	15.10	14.15	13.95	13.85	14.94
1997	12.00	15.10	16.25	15.30	15.00	14.85	15.35	15.45	15.10	14.85	14.90	14.55	14.89
1998	15.20	16.40	17.35	17.20	16.85	16.55	16.05	15.90	15.25	15.35	13.65	13.55	15.78
1999	15.25	15.80	15.45	16.15	15.50	15.45	15.10	15.00	15.00	14.40	13.50	14.10	15.06
2000	14.80	15.55	16.55	16.10	16.00	15.75	15.80	15.20	14.90	14.60	14.00	14.45	15.31
2001	15.50	16.00	16.35	16.40	16.40	15.05	15.15	15.25	15.05	14.70	13.85	14.70	15.37
2002	15.35	16.05	16.55	16.85	17.00	16.40	16.20	16.10	15.90	15.35	15.35	16.10	16.10
2003	16.75	18.75	17.05	16.75	16.60	15.95	15.65	15.30	14.95	14.60	15.15	14.10	15.97
2004	15.75	15.80	16.30	15.35	16.75	15.65	15.15	15.00	14.95	14.30	14.50	15.00	15.38
2005	15.05	16.35	16.55	16.05	15.60	15.55	15.50	15.55	15.45	14.45	13.95	14.10	15.35
2006	15.05	16.10	16.30	15.60	15.95	16.05	15.50	15.35	15.05	14.45	14.30	14.50	15.35
2007	15.25	16.40	16.65	10.60	15.65	15.80	15.05	15.00	14.70	14.45	14.00	13.80	14.78
2008	14.70	15.00	15.70	15.20	15.40	15.00	15.10	15.00	15.20	14.80	14.05	13.85	14.92
2009	14.65	15.30	15.85	15.30	15.45	15.85	15.55	15.20	15.15	15.00	14.75	15.00	15.25
2010	15.40	15.40	15.40	15.70	15.95	15.85	15.45	15.20	15.25	15.25	14.50	14.50	15.32
2011	15.19	15.09	15.69	16.47	15.02	15.68	16.68	15.75	15.16	15.12	14.85	14.50	15.43
2012	14.60	16.20	16.45	15.95	15.85	15.75	15.50	14.70	14.62	14.65	15.58	11.76	15.13
2013	14.82	15.55	15.34	15.28	15.32	15.88	15.90	15.02	15.22	14.84	14.44	13.98	15.13
2014	15.05	16.28	16.15	16.56	16.73	16.92	16.42	16.29	15.82	15.19	15.08	15.08	15.96
Mean	14.97	15.89	16.10	15.65	15.79	15.73	15.53	15.27	15.06	14.66	14.28	14.26	**15.27**

Appendix 6. Lists of 37 SNP Primers used for genotyping of Ethiopian faba bean varieties

S/N	ID	Sequence
1	Vf_Mt1g014230_001	TGCTTACTTGCCGTATGACCGAGAGTGGATTAAGCAACAGACCTTTCAACATTTGAAGAAATTGGCTCATTGAT[A/T]GAACCCCAAAGTTTTAGCAAATAGATGTAAAGGAAATCACCAAGGGAAGAACTTATTTGTTATGTGGGCTATCCTTTGATGGCTATGTTACTTAATATGA
2	Vf_Mt1g018320_001	GAAATTGAGCATGTGTTTTCTGCMATGGAAGCAAACTACGAAGCTTGGGTGAATGGTTTTGCKCCGCTGGCAGTAGGCGCGGATGTTCC[A/G]ACGGCAGTTAGAGAATTTTCTAGAACACTTTTCAACATGAGACCTGACATATCACTGTTTGTTTCAAGGACGGTTTTCAACAGTGATCTAAGAGGGATTT
3	Vf_Mt1g045800_001	GTGGTGGTGTTATTGTGATGGTTTCTGGATCTGTGCAAATTAAGGACAATCTGAGGAGGAAATTTATGCAGACGTTCTTCCTTGCGCCCCAAGAAAAAGG[A/C]NTTTTTGTGCTAAATGATATCTTTCACTTCGTTGAAGATGATCTTATTCATCACCATCACCACCAGGCAGTCTTGTTAGCTCAGAGCAATCTTGATTCAAA
4	Vf_Mt1g072740_001	AGATCCCTTATGTTGTGAAGCTTAACCCGGATGAGGATAAGCAGAATGTTTTGTTGGCTGGTATGAGTGATAAGAAGATTGTTCAGTGGGATATGAATAC[A/G]GGGCAGATAACTCAAGAGTATGATCAGCATTTAGGGGCTGTGAATACCATTACTTTTGTTGATAACAATAGGAGATTTGTTACTTCAAGTGATGACAAGT
5	Vf_Mt1g082210_001	TCAACCGTGACTTGGCGGAGTTTCTTGATCGGAAAGTTCCGGCGAATGCAAACCCTGTGGATGGAGTGTTTTCTTTTGATGTGATTGTTGACAGGGAAAC[A/G]AATCTTCTTACTCGAATTTATCGTCCCGTTGAGGGAGATGAAAGTGTGAACATTGTTGATCTTGAGAAGCCTGTTACCTCTGAGGTT
6	Vf_Mt1g105040_001	TGCAGTCCAGCTTTATGCCAAAGAGTGCAGTAAGCTCCTCCTAGATGTGCTTAAACGAGAACCTAATAAGAACAGTGACAATGAAGCGGTGGTGCCTGAT[G/T]ATACCGTTGCTCCTCGKGAATCTGTTTTTGATATATCAAAAGGCAAAAGGGCTTTCATTGAAGCAGAGGAAGCTCGTGAACTTTTGAG
7	Vf_Mt1g061800_001	GGTATTCTCATTTAAGCTCCGTGGAGCTTATAATATGATGGCAAAACTTCCGGAGGAAGTCTTGGAGAAAGGGGTTATATGTTCTTCTGCTGGAAATCA[C/T]GCTCAAGGAGTTGCCTTGTCTGCCAAGAGATTGAATTGTAAYGCTGTTATTGCTATGCCCGTTACCACGCCTGACATCAAG
8	Vf_Mt2g010740_001	AGGTACAATGTCAGAACAGAAATTGTCCATCAAATAAGCTTGTTTTTCCATTGATGGCACTACTAGCAGGAATATTAGGCGGTGTTTTCGGANNNNAT[C/T]GGAGGTGGAATGCTTATAAGTCCACTTCTTCTTCAAGTCGGAATAGCTCCTGAGGTAA
9	Vf_Mt2g010970_001	AAGAGGTTCTCCGGAGCCATGGTTTACCGGCAGGACTATTTCCGGAGAGTGTGAAATCGTATAATTTGGATCAAAAGGGTGTTTTGGAAGTGAAGTTGGA[C/T]AGTCCTTGTATTGTGAAGTATGAGAATAGAGTGTTTTTCGAAACTGTTGTTCGTGCTAACCTTAGTTTTGGTCAGCTTAAAGATTTGGAAGGTCTTTCTC
10	Vf_Mt2g097950_001	GGACTGTCCTTGGAGCCATTAATCGGAGCTGTAGCTGCTGGAAACACTATCGTTCTAAAACC[G/T]TCAGAGATGGCCCCAGTTTCTTCTTCTCTCCTAGCCACTGTTCTACCAAATTATTTGGACATTAATGGTATTAAGATTATTGAAGG
11	Vf_Mt3g031280_001	ATCCTACCTAAAGCAACTGCCATGCCAAATATTTGATCCAATCCCATTAGGCGAGGGTGCTGGTGTGGGATGGTTAAAAGATGTTGATTC[C/G]GTGGCAGTTCCTGAAGGTRCAATGCTRTATGAGCTGGTGAAGACTGGCATTGAACAAACACACGCAGCCGTTGGAGTAGTAGTTCGTTTGAGGAAAGAGT
12	Vf_Mt3g062540_001	TGGTGGACGGAGGAACTAGCTGTTCTCGACATCAAGTTCTTCGGGAGAGCCATCAGTGGTATGAAACAGCGCGGCGCCAAGTCTCCAACCATTGCAAGC[A/G]CACTGATTACCTACACAGAGCGATCTCTCCAAGAGCTAGTTCGGAATCACTCCGGCAATGGAATCCGATCTTCAGGTTACCACGATTCAGA
13	Vf_Mt3g077670_001	AGGTTGGCTACGAGGACTTATAGCTGATTACGGAGTCCCCTTAATGATTCTCGTATGGACTGCTGTATCTTACATACCTGT[C/T]AACAAAGTCCCAAGGGGAATYCCGAGGCGGCTTTTCAGTCCAAATCCATGGTCTCCTGGTGCATACTCAAATTGGACTGTTGTAAAGG
14	Vf_Mt3g084040_001	GAAGGCTTAGCTCAGTGTTCAATCAAAATGGCTTTCTGCAACAAGCTTGGAAACCTCTTGAGGCAGGGTGCTACTCAGAGTA[C/G]CCATGCTCCTGTTTCATCCATGCTTAACTATCTTCGCCACATGTCTTCAAGCAAGCTTTTCATTGGAGG
15	Vf_Mt3g086980_001	AAGAATATAGTCATCATTCTTATAGGAAACAAAAGTGATCTTGAGGACCAGCGGGCAGTACCGACAGAGGATGCAAAAGAGTTTGCTGAGAAAGAAGG[G/T]TTATTTTTCTTAGAGACCTCTGCACTGGAAGCAATTAACGTTGAGACATCCTTTATGACTGTTTTGACAGAAATATTTAACATTATGAATAAGAAGAACC
16	Vf_Mt3g092810_001	GTTCAGGTGGTTACGNTTAGGGTTATTGGAAAAGACGGAAAAGTCCTGGTTGAATCGCATCAGGAATTGTCGGATGGTAGAGTCAGGGAGCGGGGTAGACC[A/G]TTGTCGGAGAAAATGAAGCCCAACGAGGAACCGGAATCGGCTGCTGTTAGAGGGATTAAGGAAGAGCTTGGTTCTGT
17	Vf_Mt3g100500_001	GCTTCGGGCCAGGCTTTTAACATCGGCAACTACGTTGTCCGTGTCAAAATCGGAACACCAGGTCAACTTCTGTTCATGGT[C/T]CTTGACACAAGTACCGATGAGGCTTTTGTCCCTTCCTCCGGCTGCACCGGTTGTTCCGC
18	Vf_Mt4g077610_001	TATCCACAAGAACCAGTACAAGAGACTGATGCCATAGAGATGCCAAGTGAAAAGCAGCAGGAGCAGCAATCTGATGGTGCACAAAATGGCACCGAG[A/C]CTTCGGTAACAGACAATGACGAAGTAATGGTTGAGGCTGATACGGGTGACGATGTGACCATTGAAATGGAAACTTTTGAAGA

Appendix 6. Cont…,

S/N	ID	SEQUENCE
19	Vf_Mt4g113270_001	GTTGTTATTCTTGCTGGTGCTGTTGGTTACGTTGCTACCGATTCGGGTTTTACTCTGACTGCTTATTCTTGGGC[C/T]TTTGCTTATTTGGTTACGATTACTACTGAGATGGTTTATATTAAGCAYATGGTTATGAGTCTTGGCTTGAATACTTGGGGTTTTGTTCTTTATAATAATG
20	Vf_Mt4g131830_001	GAGATGATATCGCCGCTGAGAGTAAAACAGTGCAGTTGGATTCGTAAATGGGCCATGGAAGCCCATATGCAAAGAATCCAAAAAGA[A/G]GCCCAYGAAAARGGCTTTGTTGAAGTAATGAGTAACTGCAGACTCACCATTTGTAGCATTCTCATTTGTCTCTGTTTCGGTGCGAAGATAACAGAAGAAA
21	Vf_Mt4g035200_001	AAAATGGAAACAAGCATCTTTTCACAAAGTGGATATTGATGCTGATGAAATA[G/T]GTAATTTGAATGAAGATAATTTGATCGGCCACGGTGGTACGGGAAAGGTTTATCGAGTCGCGTTGAAGAAAANCGGAATGGTAGTGGCCGTGAAGCA
22	Vf_Mt5g007030_001	TGGAATCTCGCAAAGGTGATGGTCAGTTGTCTGCATGCGAGGTTACCCTTTTGCATCACGAAGACCTGGGAATTCCAAAAGATGTTGCAAAGTTAGGAGT[C/T]CGCCATGGAATGTGGGGAGCTGTCAAGAAATTGCACTCTGGTATGAGAGCATACCAGAATGCTAGGAAAACAGATACTTCTTTGTCAAGATGTGCATTGA
23	Vf_Mt5g009720_001	GATCCGCAAGGAGCTCTTTGGACAAGTTGCTGTTTGAAGACTTTGGTTGTATT[A/G]TTTAGTTTCACCTACAATAATATTTATAATTTACTGCAGAGATTGTGTGAGCGGAAGATATGTGGTGCCATTGCTCTTGTAAACAGARTATTATTGT
24	Vf_Mt5g016250_001	AAATGGATCGCAAAAACGTTCTCTCCATCGCATTGGTCTGCATTGTCTTCGCCGGCGTCGGAGGTCAATC[A/T]CCCTCCTCAGCTCCGACAACATCTCCGGTAACAGTCGCAACTCCCTCTGTTTCTCCMGTCGCAGCACCTTCCAAACCAAAATCACCAGCTCC
25	Vf_Mt5g033220_001	ATCAATTTAAGAACACCTTTGAATTTGAAGCCAATATACACAATTCCTTTGAAGATAAGTGAACAAGAGGAAA[G/T]TATCATGGAGGAAGAGTGTTCAACAACACCAAGAGGGGAAGGATCAAGAATTCCAACATGTTTGATATGTCCACCAGCTCCRAGGAAGCGAAAA
26	Vf_Mt5g078030_001	TGCCTGACTGCGCGTCCTGCGTGGCACGCGCAGTCACGCGGGCGGACGACATGTGTCGCAACACATGCGGAGGCACGGTCCAGCTCGACGGGTGTTTGGT[C/G]AAGTACGATAATGCTACTTTTTTGGGTGTGGAGGATAAGAATGTTTTGTTGAAAAAGTGCGGGCCTTCCGTGGGCTATAATCCGGAAGCTATGGGCTC
27	Vf_Mt5g047260_001	AGGGTTTGAAGCTAGAAATCTGCACCACCGACAGAGTAGGACTATTATCAAA[C/T]GTGACACGTATCTTTAGAGAGAATAGCCTCACAGTTACAAGAGCTGAAGTAACAACAAAAGGAGGCAAAGCTGTGAACACATTCTATGTTCGTGGAGCTT
28	Vf_Mt7g080730_001	AAAAAGATAATGCCAAGTGGCAAAANGGTCTGGTGCTAGGCAAGGCAGCTGAAGAAAGCGACATATTCAAGATGGTGAAAATGATCATCCAACGTCAATA[C/T]GATCCCGTGATACTGTTCAGTTTTAGCAAGAGGGAGTGTGAATTTCTTGCAATGCAG
29	Vf_Mt7g090890_001	TATACGTGCTTGTCTCTGGAGGAAACCCCCCACCTAAGAAATTGAAGCCATCTGCCAGTGTAGCCTGATGAGATAATGAAAAGGTTATGTTACTTAGA[A/T]AATGGGAGGTATAGTTTCTTATTTTTCTGTGAATCAAAATAAGGCTGTAAGCTAGTAATTTATCCAAGTCACTT
30	Vf_Mt7g100730_001	GAAAAGCTGAAGAGGAGATTGAAGAAGCAAAGAAGCAGATAGTATCCTTGTCAAAGAAGCTGGAGGAATCCCATCAACAGTTTTCGGAACTTTCCGCTTC[C/T]GATGAAGCACGACTTCAAGAACTGAGTAAAATATCTCAGGATCGAGATCGAGCATGGCAGTCTGAACTTGAGGCTGTCCAGAAGCAGCACTCAATGGATT
31	Vf_Mt8g085850_001	CTACTGGAAGAACTTCCGACAAAATTGTCAAAAATTTATCGTATGTTATCGAAGATGATGTTCCAAATGATAGTTCCGAGTCGTCTCCTCTATTTGGAGG[C/T]CATCTTAGTTGGAAAGAGAGGGAGGAGAGTTTCAAACTAAAGTCAAATATGAAGGT
32	Vf_Mt8g102250_001	CCACACTCACCTTCTGGTGACACAGTTCTCATAACCAAAGATGATCTTGAAGGCTATCATAGCACATCTGATACAAGTATGGAGCTGGCTATCGTG[C/T]CTAAAGTGCCAAAGACTAAGCCCATGATAGCACGACTATCTTGCCTTTTTGCATCCTTAAAAGTATCAGGTAGTAGTGTTCCAATGACAAG
33	Vf_Mt1g099390_002	CAAACACTATCTCTCTTCGTCCTTCGAAACCACACTCGTTTCAACTTTCTCTARATTGGAAGCTTCKGTTTTACGATTCTACGTCGTTCACGCTTTGCAA[A/T]CCAATCGGAGGGATAAGGTTGTTGAGTTTTTCACTGTTCATGGTCCTGATTTGTTGCAGAGTTCTCAGGAGGATTGGACTCAATGGTTTG
34	Vf_Mt3g076660_001	TGGCCCTCGCTTCCCTCGGTAACAGCGCCCCTAAAAAGTACGACGAAATCGACGCTGCTCCCGAAGAGCGCGCTCGTGGAATTACAATTAACACTGCTAC[C/T]GTTGAGTACGAGACTGAAACTCGTCACTACGCTCATGTTGATTGTCCAGGTCACGCTGATTACGTTAAGAATATGATTACCGGAGCYGCGCAAATGGACG
35	Vf_Mt3g085280_001	ATTTGAATCCGAATAAGCGAGGAGGATTTCGTTGCAGAACCATTAAGGCTCGTCCGTTTCACGGTGGCGGTGGAGGAGTTATCCGCTGCTCTCATAA[C/T]GATAAGCCGTCTTTTCACGACGATCAAGGTCCTCCTCAAGAAGCTGTGTTGAAGGCCATTTCAG
36	Vf_Mt5g075540_001	TTTGCCGATGGGTTTCCGAATATATTTATAAATAATGCAGARGAGCTCCGGGGTCAACATGTTGCCTTTTTGGCGTCGTTCAGCTCTCCGGCTCAAGT[C/G]TTTGAACAGCTGTCTGTCATATATGCACTCCCTCGTCTATTTGTTGCTTCCTTCACTTTGGTATTGCCTTTCTTCCCTACTGGATCATTTGA
37	Vf_Mt2g005900_001	GGAAAACCGGTATTCATGTATAGCATGGGAGGGCTTGCTGAATACTGTGTTGTGCCAGCAAATGCATTGGCTGTATTACCAAGMTCAATGCCATATAC[A/C]GAGTCTGCAATTYTAGGATGTGCTGTTTTTACTGCYTATGGTGCTATGGCTCATGCGGCTGAAGTGCGTCCGGGTGATTCTGTTGCTGTTATTGGAACTG

Appendix 7. Gene name, E-value, Similarity of locus sequence with the gene, Accession number, Gene region with its location, Total sequence of the gene, Position of SNP on sequence and Mutation type of the SNP locus used in the Study.

SN	Gene name	E-value	Similarity	Accession nuumber	Gene region	Region number	Total gene sequence	Position of SNP on Sequence	Mutation Type	
									Amino acid	Effects
1	Mt- WD repeat putative m(RNA)	3.00E-69	91%	XM_012714713.1	Exon	1	5984	2589	Ser to Thr	non-synonmous
2	Mt- root phototropisms like protein mRNA	6.00E-53	85%	XM_004500334.2	Exon	1	904	461	Ala to Thr	non-synonmous
3	Medicago truncatula boron transporter-like protein mRNA	9.00E-63	92%	XM_003601205.2	Exon	1	2092	781	Val to val	synonmous
4	Medicago truncatula hypothetical protein mRNA	2.00E-52	87%	XM_003602342.2	Exon	1	1704	911	pro to pro	synonmous
5	Medicago truncatula nucleotide-sugar transporter mRNA	7.00E-71	95%	XM_013602615.1	Exon	1	1508	805	Phe to Leu	non-synonmous
6	MT-transmembrane protein 14C mRNA	3.00E-43	85%	XM_003625049.2	Exon	1	1223	827	Ile to Phe	non-synonmous
7	Mt- Zn-dependent alcohol dehydrogenase family protein mRNA	2.00E-71	90%	XM_003630357.2	Exon	1	1848	815	Thr to Pro	non-synonmous
8	Mt- clone mth2-91o18, complete sequence	5.00E-60	89%	AC174301.24			140289	50838	NI	NI
9	Medicago truncatula LRR receptor-like kinase family protein mRNA	7.00E-44	87%	XM_003605588.2	STS	1	3417	2354	NI	NI
10	Medicago truncatula sulfite exporter TauE/SafE family protein, putative partial mRNA	3.00E-56	92%	XM_003593324.2	Exon	1	1401	1038	Ile to Ile	synonmous
11	Medicago truncatula cytochrome P450 family 87 protein partial mRNA	8.00E-58	87%	XM_003610354.1	Exon	1	1596	570	Glu to Glu	synonmous
12	Medicago truncatula four ACT domain ACT domain protein which protein mRNA	1.00E-54	92%	XM_003614218.2	STS	2	1994	1382	NI	NI
13	Medicago truncatula clone MTYFD_FE_FF_FG1G-O-23 unknown mRNA	9.00E-76	94%	BT052343.1	Exon	1	1223	303	Val to Val	synonmous
14	Medicago truncatula NAD-dependent aldehyde dehydrogenase family protein partial mRNA	9.00E-43	87%	XM_013609973.1	Exon	1	1416	465	pro to pro	synonmous
15	Medicago truncatula dual specificity phosphatase domain protein mRNA	4.00E-68	91%	XM_003630640.2	Exon		141084	66613	NI	NI
16	Medicago truncatula chromosome 5 clone mth2-44c15, COMPLETE SEQUENCE	1.00E-48	90%	CR962124.2	NI	NI	136068	19845	NI	NI
17	Medicago truncatula hypothetical protein mRNA	5.00E-59	91%	XM_003613090.2	Exon	1	1220	546	Ser to Ile	non-synonmous
18	Medicago truncatula plant/F20M13-60 protein mRNA	4.00E-54	91%	XM_013591181.1	Exon	1	1899	755	Gly to Gly	synonmous

NI = Not indicated in data base

Appendix 7. Cont….,

SN	Gene name	E-value	Similarity	Accession nuumber	Gene region	Region number	Total gene sequence	Position of SNP on sequence	Mutation Type	
									Amino acid	effects
20	Mt- RAB GTPase-like protein A1D mRNA	3.00E-75	92%	XM_003601883.2	Exon	1	1187	636	Gly to Val	non-synonmous
21	Mt- mammalian STARD2 lipid-binding START domain protein mRNA	9.00E-89	97%	XM_013597882.1	Exon	1	2333	1242	Val to val	synonmous
22	Medicago truncatula hypothetical protein mRNA	2.00E-45	86%	XM_003611597.2	Exon	1	1016	148	Ser to Ser	synonmous
23	Medicago truncatula transmembrane protein, putative mRNA	3.00E-64	88%	XM_013606986.1	Exon	1	1167	617	Asp to Asp	synonmous
24	Medicago truncatula elongation factor Tu protein mRNA	2.00E-66	89%	XM_003601112.2	Exon	1	2065	618	Thr to Thr	synonmous
25	Mt-RNA-binding (RRM/RBD/RNP motif) family protein mRNA	1.00E-48	90%	XM_003601609.2	Exon	1	1008	135	Thr to Thr	synonmous
26	Medicago truncatula aspartic proteinase nepenthesin-like protein mRNA	2.00E-38	86%	XM_003602883.2	Exon	1	1812	431	Val to val	synonmous
27	Medicago truncatula PPP4R2 protein mRNA	1.00E-41	80%	XM_003607352.2	Exon	1	1278	687	Glu to Asp	non-synonmous
28	Medicago truncatula RAN GTPase activating protein partial mRNA	2.00E-53	85%	XM_003592400.1	Exon	1	1602	340	Ala to Ser	non-synonmous
29	Medicago truncatula enhancer of rudimentary-like protein mRNA	1.00E-48	86%	XM_003588828.2	CDS	NI	978	499	NI	NI
30	Medicago truncatula sigma factor sigb regulation rsbq-like protein mRNA	8.00E-58	87%	XM_003589038.2	Exon	1	1521	580	Lys to Ser	non-synonmous
31	Medicago truncatula Ras-GTPase-activating protein-binding protein mRNA	1.00E-81	94%	XM_003590147.2	Exon	1	1829	525	Ile to Leu	non-synonmous
32	Medicago truncatula salt stress response/antifungal domain protein mRNA	2.00E-66	89%	XM_003616220.2	Exon	1	1460	587	Val to val	synonmous
33	Medicago truncatula Pre-mRNA splicing factor partial mRNA	3.00E-89	97%	XM_013602205.1	Exon	1	1803	1239	Thr to Thr	synonmous
34	Medicago truncatula 28S ribosomal protein S29 mRNA	3.00E-69	91%	XM_003599244.2	Exon	1	1695	941	Val to Leu	non-synonmous
35	M.truncatula DNA sequence from clone MTH2-116D19 on chromosome 3, complete sequence	3.00E-69	91%	XM_003599244.2	NI	NI	120115	118174	NI	NI
36	Medicago truncatula superkiller viralicidic activity-like protein mRNA	1.00E-60	94%	XM_003624183.2	Exon	1	3604	1281	Tyr to Ser	non-synonmous
37	Medicago truncatula interactor of constitutive active ROPs-like protein mRNA	3.00E-70	90%	XM_003625535.2	Exon	1	2293	573	Ser to Ser	synonmous

NI = Not indicated in data base

Declaration

I certify that the thesis hereby submitted by me for the Degree Master of Science in Biology (Applied Genetics) to School of Graduate Studies of Addis Ababa University is my own work and all helpful materials used in this thesis have been duly acknowledged. I solemnly declare that the material has not been submitted, either in whole or in part, for the award of any academic degree, diploma or certificate to any other institution.

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This thesis has been submitted for examination with my approval as a university advisor.

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