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GENETIC DIVERSITY STUDY OF KABULI AND DESI TYPE CHICKPEA (*Cicer arietinum L.*) VARIETIES USING AGRO-MORPHOLOGICAL TRAITS, NUTRITIONAL COMPOSITION AND ISSR MARKER

A THESIS SUBMITTED TO THE DEPARTMENT OF MICROBIAL, CELLULAR AND MOLECULAR BIOLOGYIN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY (APPLIED GENETICS)

BY

ANSHA AHMED

MAY, 2020

ADDIS ABABA, ETHIOPIA

DECLARATION

First, I declare that this thesis is my work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for MSc degree in Applied Genetics at Addis Ababa University. I seriously declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate. Brief quotations from this thesis are acceptable without special permission provided that accurate acknowledgement of source is made. Requests for permission extended quotation from or reproduction of this document in whole or in part may be granted by the head of the major department or the Dean of the School of Graduate studies when in his or her judgment the future use of the material is in the interests of scholarship. In all other instances however. Permission must be obtained from the author.

Name: Ansha Ahmed Signature:___________________

Place: Addis Ababa University, Addis Ababa

Advisors: Dr. Kifle Dagne Signature: ___________________

Dr. Asnak Fiker Signature: __________________

Dr. Kassahun Tessfaye Signature: __________________

Abstract

Genetic diversity study of Kabuli and Desi type chickpea (Cicer arietinum L.) varieties using Agro-Morphological Traits, Nutritional Composition and ISSR Marker
Ansha Ahmed. MSc. Thesis
Addis Ababa University, January 2020

Chickpea (Cicer arietinum L.) is one of the oldest and most widely consumed legumes in the world due to its relatively high protein content and wide adaptability as a food grain. In Ethiopia an average chickpea yield on farmer's field is low. Even released varieties produce about 3.5t/ha in research area, although it's potential yield is more than 5t/ha. In Ethiopia, no studies were conducted on chickpea varieties genetic diversity assessment based on biochemical (nutritional and anti-nutritional) analysis and this study is designed to fill this gap. The study was designed to reveal the genetic diversity existing among Ethiopian chickpea varieties using phenotypic traits, biochemical analysis and ISSR markers. Field experiment was conducted at two environments' (irrigation, main seasons) for agro-morphological performance, while the molecular part was conducted at Addis Ababa University (ISSR) and biochemical experiment was conducted at Holeta Agricultural Research Center and Ethiopian public Health Institutions. A total of 28 chickpea varieties were used for biochemical and ISSR markers, whereas 7 varieties were used for phenotypic traits. A total of 10 phenotypic traits, 9 traits for biochemical 5 ISSR primers were used to assess genetic diversity and interrelationship among Ethiopian chickpea varieties. The genomic DNA extraction was done based on modified CTAB extraction methods. The combined analysis of variance over the two seasons 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 four major clusters and the first three principal components (PCs) accounted for 93.7% of the entire diversity among the varieties for all the 10 t0raits and the first two PCA revealed 73% of the total variation. UPGMA constructed based on nutritional and anti-nutritional traits revealed three major clusters and the five principal components (PCs) accounted for 90% of the entire diversity among the varieties for all the 9 traits and the first three PCA revealed 67% of the total variation. Five ISSR primers amplified 54 bands, of which 47 loci were polymorphic. The highest gene diversity (0.38) and Shannon index (0.55) were recorded by primer 889, while the least gene diversity (0.22) was revealed by primer 824. Therefore, the observed genetic variation in the study indicated the opportunity of using these materials in future Chickpea breeding program via introgression with other germplasm resources for improvement of better genotypes.

***Key Words**: Genetic diversity, ISSR, Phenotypic traits, nutritional traits, cicer arietinum*

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Acronyms of Abbreviations

AMOVA	Analysis of Molecular variance
ANOVA	Analysis of variance
ARARI	Amhara Agricultural Research Institute
CV	Coefficient of variations
Dz ARC	Debrezite Agricultural Research Center
GARC	Gondar Agricultural Research Center
I	Shannon information Index
LSD	Lest significant difference
NCS	Natural and Computational science
Nei’s(h)	Genetic diversity
NS	None Significant Difference
RPM	Revolution per Minute
SAS	Statistical Software
SD	Standard deviation

1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) belongs to the family *leguminosae*. It is one of the most important cool season food grain legumes in the world after common bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.) (Muehlbauer and Sarker, 2017). It is annual, self-pollinated and diploid species with 2n=2x=16 chromosomes (Van der Maesen, 1987). Chickpea is one of the first pulse crops domesticated in the Fertile Crescent about 7400 years ago and most probably originated in an area of South-eastern Turkey adjoining Syria (Van der Maesen, 1987). The crop later spread to other parts of the world. Ethiopia is designated as a secondary center of origin while South-west Asia and the Mediterranean are the two primary center of origin of chickpea according to Vavilov, (1926).

Chickpea is a source of carbohydrate ranged from 54 to 71% for kabuli and 51 to 65 % for desi type; protein from 12.6 to 29% for kabuli and from16.7 to 30.6 % for desi; lipid from 3.4 to 8.8% for kabuli and from 2.9 to 7.4% for desi; and energy from 357 to 447 kcal/100g and from 334 to 437 kcal/100g for kabuli and desi, respectively (Wood and Grusak, 2007). Chickpea also contain fiber, minerals (calcium, and phosphorus) vitamins and health-beneficial phyto chemicals (low in sodium and fat and cholesterol free) (Wood and Grusak, 2007). It also plays a significant role in maintaining soil fertility, can be grown as a second crop using residual moisture, used as animal feed, as fuel and source of cash (Legese*et al*., 2005). Recently since two decades the importance of kabuli chickpea is taking paramount importance. Asnake Fiker, (2014) reported that kabuli has accounted for at least one-third of total chickpea production in the country with increasing moment where as two-third of chickpea production were occupied by desi. Chickpea (desi and kabuli) are cultivated on 12.6million hectares with production of 12million tons and productivity of 0.95ton ha-1across the world (FAO, 2016).

Asia accounts for 80.3% of the global chickpea production and Africa accounts for 5.9%. The major producing countries in the world include India, Australia, Myanmar, Pakistan, Turkey, Ethiopia, Russian Federation, Iran, Mexico and USA. From this India is largest producing county contributing 64.6% of word production. In Africa area under chickpea cultivation is 606,363 hectare with production of 712,317 tons. Ethiopia is the sixth largest producing country contributing 3.67% of world total production and the first in Africa occupying about 62.3% of the

total production (FAO, 2016). In Ethiopia chickpea is the second in terms of production (499,425.5 tons) next to faba bean (921,761.53 tons) and the third in terms of productivity (2.05 ton ha-1), following soya bean (2.27 ton ha-1) and faba bean (2.10 ton ha-1) (CSA, 2017 /18). Although Ethiopia is major chickpea producer in Africa, the national average productivity of chickpea is low (about two tons per hectare) (CSA, 2017 /18). This is primarily due to cultivation of few improved varieties for varied eco-edaphic rain fed systems, poor adaptation, poor crop management, biotic (Ascochyta blight, Fusarium wilt, weed, cutworm and pod borer) and abiotic (drought, soil salinity and water logging) factors(Upadhyaya *et al*., 2002). To alleviate some of these problems, chickpea research was started forty years ago in Ethiopia (Tabikew*et al*., 2009) and as a result thirteen kabuli and fourteen desi type chickpea varieties were released (MoANR, 2016).

However, further development of desirable genotypes with high yield potential is essential for the improvement of production and productivity of the crop. These depend upon the extent of genetic variability in the base population (Singh, 2001). Large amounts of chickpea germplasms are introduced every year in order to taste their performance and adaptation in the country. In fact understanding the extent of genetic variability is important for the genetic improvement of the crop. In addition to genetic variation, identification of important characters and their interrelationship in population comprising diverse genotypes provides opportunity for selection and developing improved varieties (Falconer and Mackay, 1996).

Despite the large number of chickpea varieties released in Ethiopia, most of them have not been characterized either morphological or molecular levels (Tanto and Tefera, 2006). And not adequate studies based on nutritional and anti-nutritional composition study. From a few morphological, biochemical (isozyme) and RAPD marker –based studies in Ethiopia, it has been reported that there exists high morphological but low biochemical and molecular diversity (Workeye 2002; Dadi 2004). Another study was also conducted based on SSR markers but limited to a few varieties released in Ethiopia and additional varieties released in eight other countries (Sefera et al., 2011) this study was therefore, designed to assess morphological and molecular genetic diversity and nutritional composition of Ethiopian chickpea varieties.

1.1. Statement of the problem

In Ethiopia an average chickpea yield on farmer's field is usually low, even released varieties produce about 3.5t/ha under research area. Its potential is more than 5t/ha (Jagdish *et al*., 1995; Bejiga *et al*., 1998).

Modern plant breeding and agricultural systems have narrowed the base for the genetic diversity of cultivated chickpea (Robertson *et al.,* 1997*)*. Therefore, it is important to explore new sources of variation that might be used in plant breeding programmers. The value of germplasms relies upon the genetic variability of agronomic and yield components in the accessions of the crop. Genetic diversity among the parents is a pre-requisite for ensuring the chance of improved segregate selection for various characters (Dwevedi and Gaibriyal*,* 2009). Measures of genetic diversity using molecular markers are most rapid and versatile. Molecular markers have proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations (Bakht *et al.,* 2011).

Nutritional composition of legumes can be affected by many factors such as cultivars, cultural practices and locality or environmental conditions (Elshiekh *et al.,*1999*)*. Poor nutritive value of legume, due to the presence of certain anti‒nutritional factors such as tannins, Phytates and trypsin inhibitors has been also reported by some authors earlier (Siddhuraju *et al.,* 2000). Trypsin inhibitors and tannins inhibit, the digestibility of protein and starch, the Phytic acid reduces, the bioavailability of some essential minerals viz. iron and zinc etc (Rehman and Shah*,* 2001).The level of the anti-nutritional factors and the nutritional composition vary among different cultivars and varieties. There is limited information available in released varieties of Ethiopian chickpea. Assessing genetic diversity with morphological, nutritional composition and molecular bases are helps the breeder for releasing improved varieties. Therefore, the current study was done to address the following objectives.

Research Questions and Hypotheses

Research Questions

The research is focused towards answering the following main questions:

- ✓ Are there different types of chickpea)? What are they, and which ones are more frequently cultivated by farmers and which ones are most preferred for specific purpose?

- ✓ What are the difference interims of yield and yield components?
- ✓ Which variety contain high amount of protein content?
- ✓ Which varieties are contains low amount of anti-nutritional factors (tannin and phytic acid content).
- ✓ What is the genetic variation within and among the Ethiopian *chickpea varieties*?
- ✓ What is the genetic distance and similarity among different chickpea varieties?

Research Hypotheses

The major hypotheses to be tested include:

- ✓ There are different types of chickpea varieties are present interims their morphological, nutritional composition and molecular characteristics.
- ✓ There is high genetic diversity among different varieties of chickpea in Ethiopia.

1.2. Objectives

1.2.1. General objective

To evaluate the genetic diversity and nutritional composition of 14 kabuli and 14 desi varieties types Ethiopian chickpea.

1.2.2. Specific objectives include

- ✓ To study agro-morphological traits of 3 varieties of Ethiopian kabuli and 3 desi type chickpea varieties.
- ✓ To determine the nutritional composition and anti-nutritional composition
- ✓ To characterize the chickpea varieties using ISSR molecular markers

2. LITERATURE REVIEW

2.1. Origin and Distribution of Chickpea

Chickpea (*Cicer arietinum* L.) belongs to the family *Leguminoseae*; sub-family *Papilionaceae* and tribe *Cicereae.* It is cultivated as food and fodder in different parts of the world. Chickpea is the only cultivated species within genus *Cicer* and grown in relatively well-drained black soils, in the cool semi-arid areas of the tropics, sub-tropics as well as the temperate areas Chickpea is one of the first pulse crops domesticated in the Old World and most probably originated in an area of South-eastern, Turkey adjoining Syria . The crop later spread to other parts of the world, India, Europe and subsequently reached Africa. It has been apparently taken to the America soon after the discovery of the New World, and became an important food crop in the Pacific coastal regions of North, Central, and South America. Mediterranean area, the Indian subcontinent, north-east Africa and Europe were widely chickpea cultivating region (Atta and Shah, 2009).

2.2. Taxonomy and Botanical Description

Taxonomy, morphology and floral biology of *Cicer*, which was classified under the mono-generic tribe, *Cicereae.* The Genus includes 9 annuals and 34 perennial herbs (Vander Maesen, 1972); and (Muehlbauer, 1993). Cross ability and fertility of hybrids in inter-specific crosses have been used as a basis to classify the annuals into 4 cross ability groups.

Chickpea plants can be described as "stems are branched, erect or spreading, sometimes shrubby much branched, 0.2-1 m tall, glandular pubescent, olive, dark green or bluish green in color. Root system is robust, up to 2 m deep, but major portion up to 60 cm. Leaves imparipinnate, glandular-pubescent with 3-8 pairs of leaflets and a top leaflet (rachis ending in a leaflet); leaflets ovate toelliptic, 0.6-2.0 cm long, 0.3-1.4 cm wide; margin serrate, apex acuminate to aristate, base cuneate; stipules 2-5 toothed, stipules absent. Flowers solitary, sometimes 2 per inflorescence, axillary; peduncles 0.6-3 cm long, pedicels 0.5-1.3 cm long, bracts triangular or tripartite; calyx 7-10 mm long; corolla white, pink, purplish (fading to blue), or blue, 0.8-1.2 cm long. (van der Maesen, 1987). Pod rhomboid ellipsoid, 1-2 with three seeds as a maximum, and inflated, glandular-pubescent. Seed color cream, yellow, brown, black, or green, rounded to angular, seed coat smooth or wrinkled, or tuber culate, laterally compressed with a median groove around two-thirds of the seed, anterior beaked (Van der Maesen, 1987).

2.3. Types of cultivated chickpea

Two types of chickpea are known, namely kabuli and desi. The desi type chickpeas are characterized by small seed size of various colors, angular seed shape, pink flowers, anthocyan in pigmentation of stem, rough seed surface, either semi-erect or semi-spreading growth habit(Wood *et al*., 2012).It is the principal type grown in India, Pakistan, Iran, Afghanistan and Ethiopia, whereas the Kabuli types generally characterized by large seed size with whitish-cream or beige color, have large owl shaped seeds, white flowers, smooth seed surface, lack of anthocyan in pigmentation, semi-spreading growth habit and more suited to the temperate climates (Pundir*et al*., 1991). It is grown mainly in the Middle East, India, Mexico as well as in North America, Australia, Spain and recently in Ethiopia (Gaur *et al*., 2008).

2.4. Production Trend and Agro-Ecology of Chickpea in Ethiopia

Chickpea is mainly grown in the central, northern and eastern highland areas of the country at an altitude of 1400-2300 m.a.s.l., where annual rainfall ranges between 700 and 2000 mm (Yadeta and Geletu, 2002). The major chickpea producing areas are concentrated in Amhara and Oromia regional states. These two regions constitute about 93% of the total chickpea production. The top chickpea producing zones are North Gonder, South Gonder, South-West Shewa, East Shewa, North Shewa, West Shewa South Wollo, West Gojam and East Gojam; (CSA, 2016/17).In Ethiopia, chickpea is a low-input crop grown mainly on vertisoils that are prone to severe seasonal water logging. To overcome this problem farmers commonly plant chickpea late in the season on residual moisture (Legesse *et al*., 2005). However, the crop faces terminal drought, particularly during the critical period of pod filling stage. Planting in mid-August too early-September are recommended time for verti soil depending on intensity of rainfall. Chickpea harvesting is done manually, either for green pod consumption or for dry seed. Harvesting time extends from October to January for green pods and February to March for harvesting dried seed (Legesse *et al*., 2005).

Chickpea is one of the major pulses grown in Ethiopia, mainly by subsistence farmers usually under rain fed conditions. It is one of the main annual crops in Ethiopia both in terms of its share of the total pulse grown area and its role in direct human consumption. It is grown widely across the highlands and semi-arid regions of the country (Geletu *et al*., 1996). The total cultivated area

of chickpeas increased from 185 thousand hectare to 225 thousand hectare with productivity from 0.89 to 2.05 tons from 1999/00 to 2017/18 (CSA 2017).The consumption of chickpea is also increasing among the urban population mainly because of the growing recognition of its health benefits and affordable source of proteins. The crop provides an important source of food and nutritional security for the rural poor, especially those who cannot produce or cannot afford costly livestock products as source of essential proteins (Legesse *et al*., 2005).Chickpea contributes a significant portion of the total value of pulse exports and accounted about 25.02% of the pulse export volume in 2012. During this period of time, the exported volume accounts about 18.11% of the total quantity of chickpea production while the balance remains for domestic market.

2.5. Production Constraints

High and more stable yields are the major goals of plant breeding programs. However, the maximum yield of crop cultivars, determined by their genetic potential, is rarely achieved because of several limiting factors such as insufficient water and nutrients, damage by plant diseases and insect pests, poor traditional agronomic practices by subsistence farmers plus poor input availability, low prices, lack of sustainable marketing policy, lack of mechanization (Singh *et al*., 1994), and poor extension services. Low and unstable yields are observed from the chickpea crop. Biotic and abiotic stresses are responsible for the instability of chickpea yield (Halila and Beniwal, 1990).The general estimates of yield losses by biotic factors (pests, diseases or weeds) range from 5-10% in temperate regions and 50-100% in tropical regions (Van Emden, 1988).

Biotic stresses, especially *Helicoverpa* pod borer insect, Fusarium fungal wilt, Ascochyta blight, and Botrytis gray mold detract significantly from chickpea's potential productivity (ICRlSAT, 2000). Because chickpea leaves, stems and pods are heavily pubescent with glandular hairs that secrete malic and oxalic acid they suffer little direct damage from aphids and other insects (Oplinger *et al*., 1990). However, some of the important pests are: pod borer (*Helicove Jpaarmigera*) that feeds on leaves and developing seeds (Smithson *et al*., 1985). Occasionally it causes more than 80% pod damage on early sown chickpea in Ethiopia (Bejiga and Eshete, 1996). Cutworms (*Agrotissp.*) groundnut aphid (*Aphis craccivora*), pea aphid (*Acyrlhsosiphonpisum*), cowpea bean seed beetle (*Callosobruchusmaculalus*), and Adzuki bean seed beetle (*C, chinellsis*) are also some of the pests of this crop. Insects specifically *Bruchid sp*. that lowers seed viability is

a serious pest of stored chickpea. Wilt and root rots caused by *Fusarium oxysporiumf.sp.*ciceri, Rhizoclolliabalalicola and others are the most important chickpea diseases in the Nile valley and Red sea countries (Ethiopia, Egypt, Sudan and Yemen) and North Africa, particularly Tunisia (Bejiga*et al*., 1988). Generally Ascochyta blight, *Rhizoctonia* root rot, Pythium rot, Fusarium wilt, white mold, and bacterial blight are disease problems in production fields of chickpea. These are typical diseases that affect other legume crops and they are favoured by periods of high rainfall, humidity and high temperatures. There are also viruses isolated from chickpea that include alfalfa mosaic, pea leaf roll, pea streak, and bean yellow mosaic (Duke, 1981). These biotic constraints are best controlled by using good quality seed, proper crop rotations, proper tillage practices, burying diseased residue and using disease resistant varieties. For the storage pests some practices that have been recommended for control are, dusting with pesticides or fumigation with methyl bromide (Duke, 1981).

Abiotic Factors: Among the abiotic factors:-, drought stands to be the number one problem in major chickpea growing regions because the crop is grown on residual soil moisture and is eventually exposed to terminal drought (Johansen *et al*., 1994). The effects of drought are heightened, as the soil starts cracking and roots get pmned and the crop-growing periods shortened (Saxena, 1987, Bejiga and Eshete, 1996).

Breeding of chickpea for biotic stress: Annual Cicerspecies have been assessed for their reaction to ascochyta blight, fusarium wilt, cyst nematode, leaf miner and seed beetle at ICARDA (International Centre for Agricultural Research in the Dry Areas), and a high level of resistance to each stress has been identified (Kumar *et al*.,2011).

Breeding chickpea for resistance to diseases: Varieties with enhanced ascochyta blight resistance have been released in India, Pakistan, Syria, the USA, Canada and Australia (Ahmad *et al.,* 2005; Materne *et al*., 2011).Basandrai *et al.,* (2011) reported that sources resistant to ascochyta blight have been identified and used in breeding programmes (Malhotra *et al*., 2003; Pande *et al*., 2005, 2007; Basandrai*et al*., 2008). Lines with moderate resistance to this disease have continually been delivered to national programs from ICARDA and ICRISAT (Malhotra *et al*.,2003; Pande *et al*.,2005,2006; Basandrai *et al*.,2008; Kaur *et al*.,2008; Sarker *et al*.,2008).

Pande *et al.,* (2006) reported three accessions as being moderately resistant to ascochyta blight, but to date resistance sources have not been identified against pathotypes III and IV, as identified in Syria (Sarker *et al.,*2008). Gene pyramiding in lines has resulted in higher resistance (Kaur *et al*., 2008; Gaur *et al*., 2010). Materne *et al*., (2011) reported that varieties with stable resistance to fusarium wilt have been released in India. A number of varieties moderately resistant to dry root rot have been identified (Pande *et al.,* 2006; Kaur *et al*., 2008). Pande *et al*., (2006) reported six accessions with moderate resistance to dry root rot among 211 accessions in the desi chickpea mini-core collection under natural epiphy -totic conditions.

2.6. Nutritional value of chickpea and Chemical composition of chickpea

Chickpea is an excellent source of essential nutrient, proteins, fiber, unsaturated fatty acid and beta-carotene chickpea is considered as one of the most nutritious food grain legumes for human consumption with potential health benefits. High fiber content in chickpea has the ability to low cholesterols and prevents blood sugar levels from rising to rapidly after a meal and thus making it a healthy food for diabetic patient. Chickpea can also contain raffinose-type oligosaccharides which cause flatulence. However, those effects can be neutralized by boiling or soaking the seeds in water (Knights and Mailer, 1989).

The nutrient profile of chickpea varieties with smaller seeds is difficult, especially in fiber content, which is higher than the varieties with larger light colored seeds. In general, the cotyledons and embryo make up most of the nutritionally beneficial part of the seed, while the seed coat contains many of the anti-nutritional factors. The desi types have a thicker seed coat than the kabuli types, which reflected in greater fiber content of the desi types (Knights and Mailer, 1989).

Table 1 Chemical composition of chickpea

Item	Values are in 100 gram of edible chickpea
Carbohydrate	60.9
Protein	17.1
Calcium	2.02
Phosphors	3.12
Iron	4.6
Crud fiber	3.9
Moisture	9.8
Fat	5.3
Energy	360

Source national institute= of nutrition, 2007.

2.7. Anti nutritional factors tannins and trypsin Inhibitor in chickpea

Tannins are the polyphenolic compounds with various molecular weights and of varying complexity. They are present in a large number of products of vegetable origin used as human foods or animal feeds (Rana *et al.,* 2006). Their multiple phenolic hydroxyl groups lead to the formation of complexes with proteins, metal ions, and other Macromolecules like polysaccharides (Choct *et al.,* 1999; Ozkan and Sahin, 2006; Kondo *et al.,* 2007). Many previous studies have shown that tannins in the diet result in reduced weight gain and poor feed efficiencies in chicken (Ahmed *et al.*,1991) has indicated that digestive capacity and growth of chicken were depressed when diets containing sal seed meal were given to birds; the major contribution was due to the presence of tannins in the diets. Trypsin inhibitor (TI) is another anti-nutritional component in many legumes. It is a small protein (21 kDa) which inhibits the digestive enzyme trypsin with very high specificity and thereby impairs digestive functions in the lower gut several treatments have been employed to reduce or eliminate anti-nutritional effects associated with tannins and TI. The methods commonly used are physical removal of testa, soaking in water and aqueous alkali or acid solutions (Reichert *et al.,* 1980).

2.8. Origin of genotype × environment interaction

There are two different conceptions of the origin of gene×environment interaction (GEI). The two concepts are referred to as biometric and developmental interaction (Tabery, 2007) or statistical and common sense interaction (Sesardic, 2005). Fisher introduced the biometric concept of GEI, whereas Lance-lot Hogben introduced the developmental concept of GEI (Tabery, 2007).

The biometric (statistical) concept of GEI has its origins in research programs that seek to measure the relative proportions of genetic and environmental contributions to phenotypic variation within populations. Biometric gene×environment interaction has particular importance in population genetics and behavioral genetics (Tabery, 2007). Developmental GEI is a concept more commonly used by developmental geneticists and developmental psychobiologists. The developmental interaction is not seen merely as a statistical Phenomenon, but manifested in the causal interaction of genes and environments in producing an individual's phenotype (Tabery and Griffiths, 2010). Most of the subsequent history of research on GEI has largely been based on the Fisher and Lancelot Hogben's concepts (Tabery, 2007).

2.9. Uses of Chickpea

Chickpea seeds are eaten fresh as green vegetables, parched, fried, roasted, and boiled; as snack food, sweet and condiments; seeds are ground and the flour can be used as soup, dhal, and to make bread; prepared with pepper, salt and lemon it is served as a side dish. Dhal is the split chickpea without its seed coat, dried and cooked into a thick soup or ground into flour for snacks and sweetmeats (Hulse, 1991). "Sprouted seeds are eaten as a vegetable or added to salads. Young plants and green pods are eaten like spinach. A small proportion of canned chickpea is also used in Turkey and Latin America, and to produce fermented food.

Chickpea is also used as animal feed is another use of chickpea in many developing countries. An adhesive may also be prepared; although not water-resistant, it is suitable for plywood. Gram husks, and green or dried stems and leaves are used for stock feed; whole seeds may be milled directly for feed. Leaves are said to yield an indigo like dye. Acid exudates from the leaves can be applied medicinally or used as vinegar. In Chile, a cooked chickpea-milk (4:1) mixture was good for feeding infants, effectively controlling diarrhea.

Among the food legumes, chickpea is the most hypocholesteremic agent; germinated chickpea was reported to be effective in controlling cholesterol level in rats (Geervani, 1991). "Glandular secretion of the leaves, stems, and pods consists of malic and oxalic acids, giving a sour taste. In India these acids used to be harvested by spreading thin muslin over the crop during the night. In the morning the soaked cloth is wrung out, and the acids are collected in bottles. Medicinal applications include use for aphrodisiac, bronchitis, catarrh, cut amenia, cholera, constipation, diarrhea, dyspepsia, flatulence, snakebite, sunstroke, and warts. Acids are supposed to lower the blood cholesterol levels. Seeds are considered antibilious" (Duke, 1981).

2.10. Genetic Diversity and its Measurement

Genetic variation is essential for the development of improved cultivars, as well as survival of the species. Knowledge, access, and use of the available diversity in domesticated and wild relatives are essential for broadening the genetic base of cultivars to sustain improvement (Singh, 2005). Biological markers could have many different types but mainly classified into morphological, biochemical and molecular types. The morphological (classical or visible, agronomic traits) markers are phenotypic traits, while; biochemical markers are isozymes, and are based on gene product. The DNA (molecular) markers which reveal sites of variation in DNA are marker with better resolution of diversity. Each type of marker system has its own advantages and disadvantages. The type of marker is selected based on the species types, availability and cost benefit analysis (Semagn *et al.,* 2006). Morphological markers are usually visually described phenotypic characters such as flower color, seed shape, growth habits or pigmentation (Winter and Kahl, 1995).Morphological markers are phenotypic markers with distinguishable trait that have evident to human eyes (Bagali *et al.,* 2010).

Isozymes markers are differences in enzymes that are detected by electrophoresis and specific staining (Winter and Kahl, 1995).Biochemical analysis is based on the separation of proteins in to specific banding patterns. It is a fast method which requires only small amount of biological material; however only a limited number of enzymes are available and thus, the resolution of diversity is limited (Mondini *et al.,* 2009).

2.11. Marker Systems and their Applications in Genetic Diversity Study

Biological markers could have many different types but mainly classified into morphological and biochemical and molecular types. The morphological (classical or visible, agronomic traits) markers are phenotypic traits, while; biochemical markers are isozymes, and are based on gene product. The DNA (molecular) markers which reveal sites of variation in DNA is marker with better resolution of diversity. Each type of marker system has its own advantages and disadvantages. The type of marker is selected based on the species types, availability and cost benefit analysis (Winter and Kahl, 1995; Semagn *et al.,* 2006).

2.11.1. Morphological markers

Morphological markers are usually visually described phenotypic characters such as flower color, seed shape, growth habits or pigmentation (Winter and Kahl, 1995). Morphological markers are phenotypic markers with distinguishable trait that have evident to human eyes (Bagali *et al.,* 2010). 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. Its estimations are more dependent on environment and are more subjective than other measurements (Li *et al.,* 2009).

There are few studies on diversity analysis of Ethiopian chickpea landraces. Feven, (2002) Studied morphological and isoenzyme diversity of Ethiopian chickpea. Anbessa and Bejiga (2002) had also evaluated and screened 482 chickpea landraces collected from different regions for their tolerance to drought. Similarly, Bejiga and Anbessa (1994) evaluated chickpea genotypes for their drought tolerance. Characterizations of such genotypes via morphological, biochemical and DNA markers for their genetic variation will help plant breeder to utilize them in the chickpea improvement. However, there is no information on the genetic diversity of these drought tolerant and susceptible chickpea genotypes.

2.11.2. Biochemical (Isozyme) markers

Isozyme markers are differences in enzymes that are detected by electrophoresis and specific staining (Winter and Kahl, 1995). Biochemical analysis is based on the separation of proteins into

specific banding patterns. The technique is rapid, economical and co-dominant nature of allozyme data makes it useful for the characterization of genetic variation in plant species (Weising *et al.,* 2005).Although protein markers circumvent environmental effects, the numbers of detectable markers are limited and they are typically tissue and developmental stage-specific and have no power to discriminate genetic diversity between closely related varieties (Park *et al.,* 2009). For this reason, most researchers began to focus on the use of DNA marker systems for genetic and ecological analyses of plant populations.

2.11.3. Molecular markers

Molecular markers, also called DNA markers, are thought of as signs along the DNA trail that pinpoint the location of desirable genetic traits or indicate specific genetic differences. Characterizations of plant genetic resources have been greatly facilitated by using a number of molecular marker systems due to their abundance in the genome. Molecular markers are not subject to environmental influences; so assessment can be carried out at any time during plant development (DeVicente and Fulton, 2003). 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. It is a sequence of DNA or a gene, which is situated on a chromosome (Schulmann, 2007; Bagali *et al*. 2010). The common types of dominant DNA markers that are used for genetic diversity study are Random Amplified Length Polymorphism (RAPD), Inter Simple Sequence Repeat (ISSR), Amplified Fragment Length Polymorphism (AFLP), DNA amplification fingerprinting (DAF) and arbitrarily primed polymerase chain reaction (AP-PCR). Whereas the co-dominant markers includes RFLP, SSR, sequence characterized amplified region (SCAR), cleaved amplified polymorphic sequence (CAPS), expressed sequence tag (EST) and single nucleotide polymorphism (SNP) (De Vicente and Fulton, 2003).

2.11.3.1. Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) has much greater power and was originally developed for mapping human genes than anything previously available (Botstein *et al.,* 1980). RFLP analysis is based on the ability of restriction enzymes (also called restriction endo nucleases) to cleave DNA in to pieces (digested) at specific target nucleotide sequences consisting usually of a four or six nucleotide pairs. Variations in the characteristic pattern of a RFLP digest can be caused by base pair deletions, mutation, inversions, translocations, transpositions and unequal crossing over which result in the loss or gain of a recognition site resulting in a fragment of different length and polymorphism (Schlotterer and Tautz, 1992).

2.11.3.2. Random Amplified Polymorphic DNA (RAPD)

RAPD were the first PCR- based molecular markers to be employed in genetic variation analysis (Welsh and McClelland, 1990; Schierwater and Ender, 1993). It is a type of PCR reaction, but the segments of DNA that are amplified are random. This marker is first introduced by Williams *et al.* (1990). RAPD markers are useful DNA based method for initial assessment of genetic variation, especially the assessment of genetic diversity in plant species (Song, 2005).

2.11.3.3. Inter- Simple Sequence Repeat (ISSR)

ISSR are DNA fragments of about 100-3000 bp located between adjacent, oppositely oriented microsatellite regions (Joshi *et al.,* 2000; Bussell *et al.,* 2005). ISSR marker was first developed by Zietkiewicz *et al.* (1994). Primers based on microsatellites are utilized to amplify inter-SSR DNA sequences. ISSRs are amplified by PCR using microsatellite core sequences as primers with a few selective nucleotides as anchors into the non-repeat adjacent regions (16–18 bp). About 10–60 fragments from multiple loci are generated simultaneously, separated by gel electrophoresis and scored as the presence or absence of fragments of particular size.

ISSR markers are also more reliable and reproducible than RAPDs because of the higher annealing temperature contributed by longer primers (Zietkiewicz *et al.,* 1994; Domyati *et al.,* 1996; Powell *et al.,* 1996; Fang and Roose, 1997; Kojima *et al.,* 1998; Moreno *et al.,* 1998). ISSR markers target divergence in regions containing dispersed repetitive DNA and can rapidly differentiate closely related individuals, it is especially useful in detecting clonal variation and fingerprinting of related individuals.

It has great potential in the study of natural populations for addressing questions ranging from conservation biology to molecular ecology and systematic (Bornet and Branchard, 2001; Song, 2005; Mudibu *et al.,* 2011).

This technique is useful to endangered, rare and less commercially important species, which are generally little studied for their genetic structure (Song, 2005). So, ISSR markers have been widely used for detecting genetic diversity of rare and endangered plant species. Therefore, ISSR is mainly used in many respects such as the study of genetic diversity in many plant species (Brantestem *et al.,* 2004).

2.11.3.4. Amplified Fragment Length Polymorphism (AFLP)

Amplified Fragment Length Polymorphism is a DNA fingerprinting technique, which detects DNA restriction fragments by means of PCR amplification. AFLP involves the restriction of genomic DNA, followed by ligation of adaptors complementary to the restriction sites and selective PCR amplification of a subset of the adapted restriction fragments. These fragments are viewed on denaturing polyacrylamide gels either through auto radiographic or fluorescence methodologies (Jones *et al.,* 1997). AFLP gives high level of resolution to allow delineation of complex genetic structures, to differentiate individuals in a population gene flow experiments and also to register plant varieties. AFLP was first developed by (Vos *et al.* 1995).

2.11.3.5. Simple Sequence Repeats

Microsatellites are short nucleotide tandem repeats of a motif, usually one to six bases (Morgante and Olivieri, 1993; Bhat, 1999 and Kylin, 2010). Microsatellite repeats are sets repeated sequences found within eukaryotic genomes (Bell and Ecker, 1994; Gupta and Varshey, 2000) Microsatellite sequences are especially suited to distinguish closely related genotypes; because of their high degree of variability, they are favored in population studies and for the identification of closely related cultivars. If nucleotide sequences in the flanking regions of the microsatellite are known, specific primers (generally 20–25 bp) can be designed to amplify the microsatellite by PCR.

3. MATERIALS AND METHODS

3.1. Genetic Diversity Study of Ethiopian chickpea Varieties Using Phenotypic Markers

3.1.1. Description of the study

Area The field experiment was conducted at Debrezeit Agricultural Research Center (DZARC) during the 2018/19 growing season by one by irrigation and one by main season. DZARC is located 47 km south east of Addis Ababa 8° 44'N, 38° 58' E. Its altitude is 1860 m.a.s.l and it receives an annual rainfall ranging from 412.9 to 926.9 ml with annual mean of 682.08 ml. The temperature of this location ranges from 11.23^{o}C to 25.19^{o}C with mean annual temperature of 19°C. The dominant soil type of DZARC is verti soil.

3.1.2. Plant materials

A total of seven chickpea varieties were evaluated over two environmental conditions during 2018/2019(one by irrigation and one by main cropping season) at Debrzite agricultural research center. These varieties were collected from Debrzite Agricultural Research Center (DZARC).

Table 2 Detail information about the materials

No	Desi	Kabuli	Seed source
1	Dimtu	Dhara	D/z ARC
2	Dalota	Huora	D/z ARC
3	Teketaye	Ejere	D/z ARC
4	Local check		D/z ARC

3.1.3. Experimental design and field management for field study

The trial was laid out in Randomized Complete Block Design (RCBD) with three replications. The experimental plots were consisted of six rows each 4 m length with 0.30m row to row and 0.10 m plant-to-plant spacing. The total area of each plot was evaluated on 7.2 m^2 (0.30m x 6 rows x 4 m) and the harvestable area was 4.8m^2 which is the middle four in order to ignore the border effect. The total experimental area was 225m^2. Sowing was done by hand drilling. A total of 240 seeds were used for each plot. The varieties were assigned to plots randomly within each replication. All other crop management and protection practices were applied uniformly to all varieties 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. **Number of pods per plant (NP/PL):** The average numbers of pods counted from samples of five plants taken randomly from each plot.
b. **Number 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.
c. **Plant height (PH) (cm):**The average height of five plants taken randomly from each plot measured at physiological maturity
d. **Number of primary branches per plant (NBP/PL):** Numbers of branches per plant was counted from basal and mediated nodes.
e. **Number of secondary branches per plant (NSB/PL):** Numbers of branches raised directly from primary branches were counted at maturity.

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. **Hundred seed weight (HSW)(g):**The weight of thousand seeds taken randomly from the harvest seed lots of each plot

D. **Seed yield per plot (SY/P):** Seed yield (g) from the specified harvestable plot area. This value was converted to Kg/ha and used for analysis.

3.1.4. Data analysis

Analysis of variance (ANOVA):-The analysis was carried out in three replications for all parameters determinations. The data were analyzed by one way analysis of variance (ANOVA). A multiple comparison procedure of the treatment means was performed by Duncan's new multiple range test (Duncan, 1955). Significance of the differences was taken at $P <0.05$.using model GLM of SAS software.

Cluster Cluster analysis was made using SAS Software version 9.1.3 (SAS Institute, 2003). According to Sneath and Sokal (1973) records for all traits were pre-standardized to means of zero and variance of unit before clustering 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 in to a single new cluster) built to group the chickpea 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^2statistics based on the recommendation of (Singh and Chaudhary, 1996). The D^2statistics measures the force of differentiation at intra-cluster and inter-cluster levels (Singh, 2007). Hence, genetic distance between cluster as standardized Mahalanobis's D^2 statistics (Mahalanobis, 1936) and calculated as follow: -

$$D^2 ij = (X i - X j) \text{cov}-1(Xi-Xj)$$

Where, D^2ij is distance between class i and jXi and Xj are the vector means of the traits for the Ith and j^{th} group scov−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 ($\chi 2$) and was

tested for significance at 5% and 1% level of probability against the tabulated values of $\chi 2$ for p degrees of freedom, where p is the number of characters considered.

3.2. Genetic Diversity as Revealed biochemical

The laboratory experiment was conducted during the 2018/19, at Holeta Agricultural Research Center of Agricultural Laboratory (Dry matter content, Moisture content, Protein content Ash content, Fat content and Carbohydrate content) and the Ethiopian Public Health Institute (Tannin content Phytate content and Fiber content).

3.2.1. Plant Materials

Twenty eight cultivars of chickpea (*Cicer arietenum L.)* seeds in their dried state were acquired from DZARC. Those varieties include 14 desi and 14 kabuli varieties including a local check (Table 3). Seeds of each variety were used for the different analysis. The samples were cleaned by hand to remove dirty and broken grains and then packed in air tight plastics containers at room temperature. After that the seed samples were powdered using grinder and pass through -0.4 mm sieve for homogenization of the samples. They were kept in airtight containers. For different analysis all treatments were done in two replications.

Table 3 Randomization of the above varieties

Varieties	Randomization	Varieties	Randomization
Dimtu	1	Dz-10-4	15
Dalota	2	Habru	16
Teketaye	3	Akaki	17
Local	4	Chefe	18
Dhera	5	Mareye	19
Hora	6	Yelebie	20
Ejere	7	Teje	21
Shaho	8	Mastewal	22
Arerty	9	Dz-10-11	23
Kutaye	10	Worku	24
Dubie	11	Acosdubie	25
Menjare	12	Fetenech	26
Natoli	13	Kasech	27
Akuri	14	Kobo	28

Table 4 Detailed information of chickpea varieties

No	Desi	Color	Year of released	Kabuli	Color	Year of released	Source
1	Dimtu	Dark blue	2016	Dhera	Creamy	2016	DZARC
2	Dalota	Dark blue	2013	Hora	Creamy	2016	DZARC
3	Teketaye	Light blue	2013	Ejere	White	2005	DZARC
4	Local chick	Light yellowish		Shasho	White cream	1999	DZARC
5	Kutaye	Red	2005	Arerity	White cream	1999	DZARC
6	Dubie	Grey	1978	Akuri	Cream	2011	DZARC
7	Menjar	Golden	2010	Habru	White	2004	DZARC
8	Natoli	Light golden	2007	Dz-10-4	White cream	1974	DZARC
9	Akaki	Brown	1995	Chefe	White cream	2004	DZARC
10	Mareye	Brown	1985	Yelebie	Yellowish	2006	DZARC
11	Mastewal	Golden	2006	Teje	Yellowish	2006	DZARC
12	Dz -1011	Brown	1974	Acosdubie	White cream	2009	DZARC
13	Worku	Golden	1994	Kasech	White	2011	DZARC
14	Fetenech	Reddish	2006	Kobo	Yellowish	2012	DZARC

3.2.2. Methods of Analysis

Moisture content, total ash, crude protein, crude fiber, and crude fat of the seed flours were Determined according to Analysis of Official Analytical Chemistry.(AOAC). (2000).

Dry matter: Dry matter was determined by oven-drying at 105 °C to constant weight for 1 hour.

Moisture content (MC):2 g of the crush chickpea seeds were weighed into a crucible, after the crucible has been heated and weighed. Moisture content was determined by oven drying for 1 hour at 105°C. This was removed and cooled in desiccators and then weighed. The moisture content was calculated by the formula:

$$\%m.c = wsb - wsa * / wsb$$

$\%_{m.c}$=moisture content%

W_{sb}= Weight of sample before drying

W_{sa}=weight of sample after drying

Crude fiber content: Crude fiber was determined after digesting a known weight of chickpea flour by refluxing 1.25% boiling sulfuric acid and 28% boiling potassium hydroxide.

A sample of 1.5 g crashed chickpea flour was placed into a 600ml beaker, and 200ml of 1.25% HSO4 was added, and boiled gently exactly for 30 minutes, placing a watch glass over the mouth of the beaker. During boiling, the level of the sample solution was kept constant with hot distilled water. After 30 minute of boiling, 20ml of 28% KOH was added and boiled gently for a further 30 minute, with occasional stirring.

The bottom of a sintered glass crucible was covered with 10 mm sand layer and wetted with a little distilled water. The solution was poured from beaker into sintered glass crucible and then the vacuum pump was turned on. The wall of the beaker was rinsed with hot distilled water several times; washings were transferred to crucible, and filtered.

The residue in the crucible was washed with hot distilled water and filtered (repeated twice). The residue was washed with 1% H_2SO_4 and filtered, and then washed with hot distilled Water and filtered; and again washed with 1% NaOH and filtered. The residue was washed with hot distilled

water and filtered; and again washed with 1% H_2SO_4 and filtered. Finally the residue was washed with water- free acetone.

Drying combustion: The crucible with its content was dried for 2 hours in an electric drying oven at 130^0C and cooled for 30 min in the desiccator (with granular silica gel), and then Weighed. The crucible was transferred to a muffle furnace (Gallenkamp, size 3) and incinerated for 30 min at 550^0C. The crucible was cooled in the desiccators and weighed. Then the fiber was calculated as a residue after subtraction of the ash.

Crude fiber g /100 g= (W1-W2) x100]/W3

Where: W1 = weight of (crucible +sample) after drying;

W2 = weight of (crucible +sample) after ashing;

W3 = weight of fresh sample

Total Ash: 3 g of ground chickpea samples was weighed out into a crucible, after the crucible has been heated and weighed and was placed in a temperature controlled furnace at 500^oC for about 5hours for proper washing. The crucible was then cooled a desiccators and immediately weighed. Then % of ash was calculated as shown below

Calculation:

% Ash = wt of ash remaining x 100/Wt. of original sample

Crude fat:-3g of dry sample was weighed to within milligrams in an extraction thimble; it was placed in the extraction unit. The flask was connected to hexane containing at 2/3 of total volume to the extractor until 6 hours. When finished, the hexane was evaporated by distillation or in a rote evaporator. The flasks were cooled in a dryer and weigh them to within milligrams.

% oil = weight of sample – weight of residue after extraction x 100/ Weight of sample

Crude protein: 0.25g of sample was digested by adding 10ml of sulfuric acid with selenium mixture as catalyst for 2 hours. After light green color was observed the digest solution was cooled and transferred into 100mls volumetric flask which was made up to mark with distilled water. Micro Kjeldahl distillation apparatus was used to distill 25ml of the prepared digest by the addition of 70 ml 40% sodium hydroxide. The blue color changed to dark brown as distillation proceeded.

The released ammonia was condensed and collected into a receiver containing 30mls of boric acid with indicator solution. The condensed ammonia was then back titrated with 0.01M HCl to pink color end point.

% Nitrogen by weight N = (R-B) N*14*100/1000*W

% crude protein = N X 6.25

N=normality of the acid

R=volume of hydrochloric acid consumed to neutralized the sample.

B=the volume of the acid consumed to neutralized the blank

W= weight of nitrogen.

Carbohydrate:-Utilizable carbohydrate content was determined by difference. It was determined by subtracting the crude protein, crude fiber, moisture content, total ash and fat from the total dry weight of the sample.

3.2.3. Determination of Anti-nutritional Factor

3.2.3.1. Determination of phytate content

Phytate was determined by the method of Latta and Eskin (1980) and later modified by Vantraub and Lapteva, (1988). About 0.1g of fresh sample was extracted with 10ml 2.4% HCl in a mechanical shaker for 1hour at an ambient temperature and centrifuged at 3000rpm for 30 minute. The clear supernatant was used for phytate estimation. A 2ml of Wade reagent (containing 0.03% solution of $FeCl_3.6H_2O$ and 0.3% of sulfosalicilic acid in water) was added to 3ml of the sample solution (supernatant) and the mixture was mixed on a Vortex (Maxi Maxi II) for 5 seconds. The absorbance of the sample solutions were measured at 500 nm using UV-VIS spectrophotometer (Beckman DU-64- spectrophotometer, USA). A series of standard solution were prepared containing 0, 5, 10, 20 and 40 μg/ml of phytic acid (analytical grade sodium phytate) in 0.2N HCl. A 3ml of standard was added into 15ml of centrifuge tubes with 3ml of water which were used as a blank. A 1ml of the Wade reagent was added to each test tube and the solution was mixed on a Vortex mixer for 5 second. The mixtures were centrifuged for 10

minutes and the absorbances of the solutions (both the sample and standard) were measured at 500nm by using deionizer water as a blank. A standard curve was made from absorbance versus concentration and the slope and intercept were used for calculation. Phytate: mineral molar ratios were calculated using the molecular weight of PA=660.

Calculation:

Phytic acid in mg/100g = (absorbance-intercept)3/(slope x ρx wt. of Sample x10)

Where, ρ is density

3.2.3.2. Condensed tannin determination

Tannin content was determined by the method of Burns, (1971). About 1.0 gram of chickpea flour was weighed in a screw cap test tube. The chickpea flour was extracted with 10ml of 1% HCl in methanol for 24 hours at room temperature with mechanical shaking. After 24 hours shaking, the solution was centrifuged at 1000rpm for 5 minutes. A 1ml of supernatant was taken and mixed with 5 ml of vanillin-HCl reagent (prepared by combining equal volume of 8% concentrated HCl in methanol and 4% Vanillin in methanol).

D-catechin was used as standard for condensed tannin determination. A 40mg of D-catechin was weighed and dissolved in 1000 ml of 1% HCl in methanol, which was used as stock solution. A 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of stock solution was taken in test tube and the volume of each test tube was adjusted to 1ml with 1% HCl in methanol. A 5ml of vanillin-HCl reagent was added into each test tube. After 20 minutes, the absorbance of sample solutions and the standard solution were measured at 500nm by using water to zero the spectrophotometer, and the calibration curve was constructed from the series of standard solution using SPSS-15. A standard curve was made from absorbance versus concentration and the slope and intercept were used for calculation.

Calculation:

Concentration of tannin was read in mg of D-catechin per 100g of sample

Tannin in mg/100g = (absorbance-intercept)/ (slope x density x weight of samplex10)

3.2.4. Statistical analysis

3.2.4.1. Analysis of variance

The analysis was carried out in two replicates for all determinations. The means were calculated. The data were analyzed by one way analysis of variance (ANOVA) in SAS to analyze the data. A multiple comparison procedure of the treatment means was performed by Duncan's new multiple range test (Duncan, 1955). Significance of the differences was accepted at $P < 0.05$.

3.2.4.2. Cluster analysis principal component 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^2distances by average linkage method of agglomerative hierarchical clustering (the two closest clusters are merged into a single new cluster) built to group the chickpea 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^2statistics based on the recommend action of Singh and Chaudhary (1996). The D2statistics measures the force of differentiation at intra-cluster and inter-cluster levels (Singh, 2007). Hence, genetic distance between cluster as standardized Mahalanobis's D^2 statistics (Mahalanobis, 1936) were calculated as follow: -

$$D^2_{ij} = (X_i - X_j)\, cov^{-1} (X_i - X_j)$$

Where,D^2ij is distance between class i and jXi and Xj are the vector means of the traits for the Ith and jth groupscov−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 ($\chi 2$) and was tested for significance at 5% and 1% level of probability against the tabulated values of $\chi 2$ for p degrees of freedom, where p is the number of characters considered.

3.3. Genetic Diversity as Revealed by ISSR Marker

Molecular analysis experiment was done in Addis Ababa University AratKilo Campus in Plant Genetics Laboratory.

3.3.1. Working sample

Twenty eight cultivars of chickpea (*Cicerarietenum* L.) seeds in their dried state were obtained from DZARC. These varieties include fourteen desi and fourteen kabuli including the local check (18).The varieties were planted in pot at College of Natural Science, Addis Ababa University from which the young leaf samples were harvested and dried in silica gel.(refer table 3 of page 28).

3.3.2. DNA Extraction, quality and quantity determination

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. About 5mg of collected dried leaf samples from representative plants of twenty eight plants were separately placed 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 700µl 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 65°C for 30 minutes in water bath and centrifuged at 13000 rpm for 7 minutes. After taking the supernatant, 600µl chloroform was added and centrifuged at 13000rpm for 7 minutes and this step was repeated for better cleanup of the genomic DNA. Cooled (4°C) Iso-propanol (2/3 of volume) was added to solution and allowed to freeze for 2hrsat -20ºC and then centrifuged at 13000rpm 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°C.

Two micro-litre of the DNA was mixed with 2 µl distilled water and 1 µl of bromophenol blue dye on para-film sheet. The gel tray was filled with 1X TBE (running buffer) and the samples were loaded using 3µl micropipette. A known amount of condensed lambda phage DNA was loaded as control in the adjacent wells. The gel was run at5 v/cm for half an hour. The bromo-phenol blue dye was allowed to travel less than ⅔rd length of the gel, before removing the slab. The slab was viewed under UV light fluorescence using photo UV trans-illuminator and image was captured using gel documentation system. A single band near the wells indicated intact plant DNA.

Qualitative and quantitative Estimation of DNA was estimated by using UV spectroscopy and agarose gel electrophoresis.

Qualitative estimation of DNA: The quality of DNA samples was checked both by UV-spectrophotometer and agarose –gelelectrophoresis. Using spectrophotometer, the ratio of the absorbance at 260 and 280 nm was noted. Samples with a ratio of 1:8 -2 were considered of good quality.

Quantitative estimation of DNA Spectrophotometric method**:** The concentration of DNA was estimated by measuring the optical density at 260 nm using an UV visible spectrophotometer. The absorbance of various DNA samples was measured at awave-length of 260 nm against TE buffer blank in a quartz cuvette. 1µl an aliquot of DNA sample was taken and volume was made up to 1 ml with TE buffer. Optical density (O.D.) was measured at 260 and 280 nm. Using the relationship of O.D. unit of 1.0 at 260nmequivalent to 50 µg DNA per ml, the quantity of DNA was estimated by using the following formula*:*

Total quantity of DNA (µg/ml) = O.D. at 260 nm x 50 x dilution factor

3.3.3. Primer selection and optimization

A total of nine ISSR primers obtained from the Plant Genetics Research Laboratory Microbial, Cellular and Molecular Biology Department were used. ISSR primer selection was made following published methods on faba bean (Abdul-Razzak *et al*.,2012; Merji *et al*., 2012;Wang *et al*., 2012; Salazar-Laureles *et al*., 2014), and ,on chickpea (Bhagyawant and Srivastava, 2008), on Lentil (Edossa *et al*., 2007; Meenakshi *et al*., 2013) and on 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 50 ng/µl was made for

running of PCR to screen primers and optimize the reaction condition. Finally, five primers were selected based on their banding pattern, polymorphism and reproducibility for genotyping. ISSR are semi-arbitrary markers amplified by microsatellite primer that serve as both forward and back ward.

Table 5 List of primers, annealing temperature, Repeat motif and Nucleotide types used for optimization.

No	Primer	Repeat Motif	Annealing temp.(ºC)	Nucleotide types
1	UBC810	(GA)8TY	42 ºC	Di nucleotide
2	UBC812	(GA)8A	42 ºC	Di nucleotide
3	UBC824	(TC)8G	43ºC	Di nucleotide
4	UBC851	(GT)8YG	47ºC	Di nucleotide
5	UBC869	(GTT)6	50ºC	Tri-nucleotide

3.3.4. Polymerase Chain Reaction (PCR) Amplification of ISSSR Markers

The polymerase chain reaction was conducted in Biometra 2003 T3 Thermo cycler. PCR amplification was carried out in a 10 µl reaction mixture containing 1µl template DNA, 6µl H_20, 0.2µl dNTP (10x), 0.8µl PCR buffer (MgCl free), 0.3µl Taq buffer, 0.8µl $MgCl_2$ (50mM), 0.6µl primer and 0.3µl Taq Polymerase (5u/µl). The amplification program was 4 minutes preheating and initial denaturation at 94^0C, then Denaturation 94ºC for 30 second, Annealing 40-47ºC for 30 second based on primers used, 1 minutes extension at 72^0C and the final extension for 10 minutes at 72^0C. The amplification reaction was set to repeat the step (denaturations) to (extension) for 35 times and the amplified products were stored at-20°C till further use.

Agarose gel electrophoresis: Amplified DNA fragments were resolved by submerged horizontal electrophoresis in 1 %(w/v) agarose gel and visualized by staining with Ethidium bromide. Agarose solution was prepared in 1X TBE and ethidium bromide (3 mg/ml) was added in the gel at a concentration of 5 µl/100 ml of agarose solution and then mixed gently. It was poured in gel casting unit with appropriate comb with required well number and size. DNA samples were prepared by adding 0.20 volumes of 6X loading dye solution and loaded on wells using

micropipette. Electrophoresis was carried out at constant voltage (80 v/cm of gel) until dye migrated to other end of the gel. In order to determine the approximate size of a band, 100 bp DNA ladder was run along with the amplified products. PCR amplified products were viewed under UV light fluorescence using photo UV trans-illuminator and image was captured using gel documentation system.

3.3.5. Scoring of ISSR Amplicon and molecular data analysis

The banding pattern for each of the ISSR marker was recorded in each cultivar. The presence of band DNA markers run on agarose gel was taken as one and absence of band was read as zero. And the ambiguous or unclear bands were scored as (?) The size of the amplified bands (in nucleotides base pairs) was determined based on its migration relative to standard DNA marker (100 bp DNA ladder).

Polymorphic fragments (bands) were scored manually as binary data one (1) for present zero (0) for absent and for ambiguous data question mark (?).Various software's were used for analysis of the binary data matrix. Percentage of polymorphic bands (ppb),Nei's(1973) gene diversity(h), Shannon-weaver diversity index (I) were estimated using POPGENE version 1.32 *(*Yah *et al.,*1999)under the assumption of Hardy-Weinberg equilibrium. The two comparable estimators: Nei's gene diversity (h) and Shannon-Weaver diversity index (I) were used to calculate genetic diversity for each population. Jacard's similarity coefficient (Jaccard, 1908) was used, to estimate similarity between pairs of cultivars from free tree version 1.32. An analysis of molecular variation (AMOVA) was used to estimate genetic variance within and among each cultivar using Ariliquinvesion 3.01 *(*Excoffier *et al.,* 2006).

Cluster analysis was performed to construct dendrograms with Arithmetic averages (UPGMA) tree and neighbor joining (NJ) tree (Saitou and Nei1, 987*)* using free tree 0.9.1.50 *(*Pavlicek *et al.,* 1999) using Jaccard's coefficient of similarity.

4. RESULTS

4.1. Genetic Diversity Analysis based on Phenotypic Traits

4.1.1. Performance analysis of Ethiopian chickpea varieties

In this study, the morphological data of nine quantitative traits were measured to determine phenotypic diversity of Ethiopian chickpea varieties.

4.1.1.1. Mean performance and different Chickpea varieties across season

Estimated range for each location and combined over locations are presented in Table 5. At irrigation experiment, a wide range were recorded for days to 50% flowering, days to maturity, grain yield per plot, plant height , hundred seeds weight, numbers of pods per plant, numbers of seeds per plant, number of primary branch per plot, number of secondary branch per plant.

At main season experiment wide ranges were observed for days to 50% flowering, days to maturity, grain yield per plot, plant height , hundred seeds weight, numbers of pods per plant, numbers of seeds per plant, number of primary branch per plot, number of secondary branch per plant.

In combined seasons the range varies with the highest gaps for, days to maturity, grain yield per plot, plant height, hundred seeds weight, numbers of pods per plant, numbers of seeds per plant, number of primary branch per plot, number of secondary branch per plant. The minimum and maximum average seed yield combined over locations showed seed yield ranged from 12.2q$ha^{-1}$to 32.6qha^{-1}(Table 5).

Table 6 Minimum, Mean, and Maximum values of nine traits of seven chickpea varieties for irrigation, main season and combined over two seasons

Parameters	Irrigation Experiment			Main season experiment			Combined over two seasons		
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
DTF	50	52.4	58.6	49	51.8	63.5	49.5	52.1	60.6
DTM	86.3	105.5	120	83.3	105.6	130	84.8	105.5	121
NP/PL	18.3	29.7	41.8	29.7	49.3	87.6	21.2	39.2	56.4
NS/P	0.9	9.4	1.2	1.1	1.9	2.0	1.2	1.2	1.4
PH)(cm	33	40.4	48.3	45.3	50	67.8	40.2	45	56.1
NBP/PL	2.8	3.4	4.5	3.9	5.5	8.6	3.6	4.4	4.8
NSB/PL	3.9	5.1	6.8	15.8	22.7	42.2	9.6	14.5	22.5
HSW)(g	10.8	26.8	31	10.8	29.1	34.6	10.8	27.9	33.1
SY/P	10.4	13.6	28.9	14	28.6	38.6	12.2	22.8	32.06

4.1.1.2. Mean performance of varieties over locations

The mean performance of seven (7) Ethiopian chickpea varieties at irrigation, and main season experiment are presented in Table, 6, and 7, respectively. The combined mean performances over two seasons for all varieties are presented in a Table 8. The mean performance of seed yield of the seven chickpea varieties in this study were 13.6 qha^{-1} at irrigation experiment (Table 6), 28.6 qha^{-1}at main seasons experiment (Table 7), and 22.8 qha^{-1}over combined seasons (Table8). The lower mean value of seed yield was observed over all varieties at irrigation experiment as compared to the main season experiment.

At irrigation experiment the highest seed yield was obtained from Dimtu (28.9 qha^{-1}), and the lowest were obtained from Local check (10.4 qha^{-1}) (Table5.). Similarly, at main season experiment the highest and lowest seed yield was recorded from Teketaye (38.6qha^{-1}) and local check (14 qha^{-1}), respectively (Table 7).

The mean performance along withleast significant difference and coefficient of variation for combined data for all seasons are presented inTable8.The highest combined mean value was recorded for Dimtu(32.06 qha^{-1}), whereas the lowest mean value was recorded from local variety (12.2 qha^{-1}).The second higher yielding variety was Teketaye(27.7 qha^{-1}).

ON the other hand At irrigation experiment the highest seed yield was obtained from Dimtu (28.9 qha^{-1}), and the lowest were obtained from Local check (10.4 qha^{-1}) (Table6). Similarly, at main season experiment the highest and lowest seed yield was recorded from Teketaye (38.6qha^{-1}) and local check (14 qha^{-1}), respectively (Table 7).

The mean performance along with least significant difference and coefficient of variation for combined data for all seasons are presented in Table 8.The highest combined mean value was recorded for Dimtu(32.06 qha^{-1}), whereas the lowest mean value was recorded from local variety (12.2 qha^{-1}).The second higher yielding variety was Teketaye(27.7 qha^{-1}).

On the other hand, at irrigation experiment the days to 50% flowering ranged from 50.0 to 58.6 days with average of 52.4 days (Table 6). The minimum number of days taken by 50% of the variety to flower was by the Dimtu variety whereas; the maximum number of days was taken by

the variety Dhera (58.6). Similarly at main season experiment the days to 50% flowering ranged from 49 to63.5 days with average of 51.8 days (Table 7). The minimum number of days taken to 50% flowering was by the variety Dimtu (49.05) whereas, the maximum days was taken by the variety Dhera (63.5).

The highest combined mean for days to 50% flower ranged from 49.5 to60.6 days with average of 52.1days (Table 8). The minimum number of days taken to 50% flowering was by the variety Dimtu (49.5) whereas, the maximum days was taken by the variety Dhera (60.6).

On the other hand, at irrigation experiment days to maturity ranged from 86 to 120 days with an average of 105.5days (Table 8).The minimum number of days taken by the local check variety (86 days), whereas, the maximum number of days was taken by variety Dhera (120). Similarly at main season experiment the days to maturity ranged from 83.3to 130days with an average of 105.6days (Table 8). The minimum number of days taken to mature was by the local variety check (83.3 days), whereas, the maximum number of days taken was by the variety Dhera (130 days).

Combined mean performance for days to maturity ranged from 84.8to 119.5days with an average of 105.5days (Table 8). The minimum number of days taken to mature was by the local check (84.8 days), whereas, the maximum number of days taken was by the variety Dhera (119.5 days).
On the other hand based on plant height, at irrigation experiment Plant Height (cm) the range of plant height was from 33.66 to 48.3cm, with average plant height of 40.49cm (Table 8). The highest mean performance for plant height was observed in varieties Dhera and Teketaye (48.3cm), while, lowest mean performance for plant height was shown by Dalota (33.66). Similarly at main season experiment the range of plant height was from 44.5 to 67.8cm, with average plant height of 50cm (Table 8). The highest mean performance for plant height was shown by Dhera (67.8cm), while, lowest mean performance for plant height was shown by in local check (44.5). On the other hand the combined mean performance for plant height ranged from 40.2 to 56.1cm, with average plant height of 45cm (Table 8). The highest mean performance for plant height was shown by Dhera (56.1cm), while, lowest mean value for plant height was shown by in local check (40.2).

On the bases of number of pod per plant the range at irrigation experiment number of pod per plant, from 18.3 to 41.8 pods, with average 29.7 pods (Table 6). The highest mean performance for number of pods per plant was observed in varieties Dlota and Dimtu (41.8, 40.2), while, lowest mean performance for number of pods per plant was shown by Ejere (23.1). Similarly at main season experiment the range of number of pods per plant was from 29.7 to 87.6, with average pods of 49.3 (Table 7). The highest mean performance for number of pods per plant was shown by Teketaye (87.6), while, lowest mean performance for number of pods was shown by in local check (24.0).

In combined mean performance for number of pods per plant ranged from 56.4 to 12.6, with average number of 39.2 pods (Table 8). The highest mean performance for number of pods per plant was shown by Teketaye (56.4), while, lowest mean value for number of pods was shown by in local check (21.2).

Table 7. Men performance for irrigation experiment

No	Varieties	DTF	DTM	SC	PH(CM)	NPB\PL	NSB\PL	NPP/PL	NSP\PL	HSW	SY\P
1	Dimtu	50.0^{b}	98.3^{b}	76.0^{a}	38.0^{b}	3.0^{c}	5.4^{ab}	40.2^{a}	1.06^{ab}	30.6^{a}	28.9^{a}
2	Dalota	51.3^{b}	104.0^{b}	70.6^{b}	33.0^{b}	2.9^{c}	6.0^{a}	41.8^{a}	1.0^{b}	26.8^{b}	16.3^{b}
3	Teketaye	51.3^{b}	100.0^{b}	69.3^{b}	36.4^{a}	4.5^{a}	5.9^{a}	25.3^{c}	1.0^{b}	24.5^{c}	16.8^{b}
5	Dhera	58.6^{a}	115.0^{a}	77.0^{ab}	48.3^{b}	3.8^{ab}	3.9^{b}	27.0^{b}	1.0^{b}	32.8^{a}	17.0^{b}
6	Hora	53.0^{a}	115.0^{a}	98.0^{a}	46.2^{a}	3.4^{bc}	6.8^{a}	32.0^{b}	0.93^{b}	31.0^{a}	16.0^{b}
7	Ejere	50.3^{b}	120.0^{a}	76.6^{a}	44.2^{a}	2.8^{c}	4.06^{b}	23.1^{c}	0.9^{b}	31.0^{a}	15.3^{b}
Gm		52.4	105.5	75.4	40.4	3.4	5.1	29.7	1.0	26.8	17.3
CV		4.4	3.8	16.5	6.2	12.8	15.9	11.8	9.4	4.2	13.6
LSD		4.9**	7.25***	22.2ns	7***	4.3**	4.46**	7.7***	2.9ns	20.3***	7.3***

*: Means with the same letter in same column are not significantly different.

Table 8. Mean performances of 7 chickpea varieties for seed yield and its component for main season experiment

No	Varieties	DTF	DTM	SC	PH(CM)	NPB\PL	NSB\PL	NPP/PL	NSP\PL	HSW	SY\P
1	Dimtu	49.0^{b}	98.6^{cd}	80.0^{ab}	52.3^{b}	3.9^{d}	15.8^{c}	58.8^{b}	1.7^{ab}	34.6^{a}	35.1^{ab}
2	Dalota	51.3^{b}	104.0^{bc}	77.0^{ab}	47.7^{bc}	4.4^{d}	18.7^{bc}	50.7^{b}	2.0a	29.8^{b}	30.1^{c}
3	Teketaye	50.0^{b}	100.0^{c}	90.0^{ab}	51.4^{b}	4.5^{cd}	20.4^{bc}	87.6^{a}	1.5^{ab}	29.6^{b}	38.6^{a}
4	Local	53.0^{b}	83.3^{d}	71.6^{b}	44.5^{c}	4.6^{bc}	21.9^{b}	24.0^{c}	1.1^{c}	10.8^{c}	14.0^{e}
5	Dhera	63.5^{a}	130.0^{a}	84.5^{ab}	67.8^{a}	6.4^{b}	44.2^{a}	29.7^{c}	1.8A	33.7^{a}	24.5^{d}
6	Hora	49.3^{b}	112.6^{bc}	90.0^{ab}	46.9^{bc}	6.4^{bc}	21.7^{bc}	$56.^{b}$	1.4^{bc}	33.0^{a}	33.3^{bc}
7	Ejere	50.3^{b}	119.0^{ab}	91.6^{a}	45.3^{c}	8.6^{a}	23.5^{b}	31.0^{c}	1.6^{ab}	33.6^{a}	23.3^{D}
GM		51.8	105.6	83.5	50.0	5.5	22.7	49.3	1.9	29.1	28.6
CV		6.6	7.9	11.45	5.7	17.9	12.9	19.5	11.7	3.8	7.7
LSD		6.14^{**}	14.8^{***}	17^{ns}	5^{***}	1.76^{***}	5.2^{***}	17.1^{***}	0.3^{***}	1.95^{***}	3.9^{***}

Table 9. Mean performance of seven chickpea cultivars over two seasons

No	Varieties	DTF	DTM	SC	PH(CM)	NPB\PL	NSB\PL	NPP/PL	NSP\PL	HSW	SY\P
No	Dimtu	49.5^{b}	98.8^{b}	78^{bc}	45.1^{b}	3.9^{bc}	9.6^{c}	49.5^{ab}	1.4^{a}	32.6^{a}	32.06^{a}
1	Dalota	51.3^{b}	104^{b}	73^{bc}	40.7^{cd}	3.6^{c}	12.6^{b}	46.3^{ab}	1.4^{a}	28.3^{b}	23.2^{c}
2	Teketaye	50.6^{b}	100^{b}	79^{abc}	43.9^{bc}	4.3^{bc}	12.9^{b}	56.4^{a}	1.2^{ab}	27.0^{b}	27.7^{b}
3	Local	52.7^{b}	84.8^{c}	65.8^{c}	40.2^{d}	3.7^{c}	15.9^{b}	21.2^{c}	1.3^{bc}	10.8^{c}	12.2^{e}
4	Dhera	60.6^{a}	121^{a}	80^{abc}	56.1^{a}	4.5^{bc}	22.5^{a}	28.2^{C}	1.32^{ab}	33.1^{a}	20.0^{d}
5	Hora	51.1^{b}	113.8^{a}	94.1^{a}	46.5^{b}	4.8^{ab}	14.2^{b}	44.4^{b}	1.16^{ab}	32.0^{a}	24.7^{c}
6	Ejere	50.3^{b}	119.5^{a}	94.1^{a}	44.7^{b}	5.8^{a}	14.9^{b}	27.0^{c}	1.3^{b}	32.3a	19.3^{d}
7		52.1	105.5	79.3	45.0	4.4	14.5	39.2	1.2	27.9	22.8
Gm		5.4	5.9	14.3	6.2	20.4	17.1	20.6	13.7	4.7	9.6
CV		3.4**	7.5***	3.7**	3.3***	1**	2.9***	9.7***	0.2*	1.5**	2.6***

*: Means with the same letter in same column are not significantly different.

Kay: from no1to 4 are desi type and from 5-7 kabuli type chickpea.

Where * Significant at p= 0.05,***significant at p=0.001,**Significant at p= 0.01 ns= not significant deference Rep=Replication SV= Source of variation, DFM= Degree freedom LSD , Lest significant difference, DTF=50%DaysFloweringDTM=Daysto

4.1.1.3. Estimation of analysis of variance

Mean squares of the 9 characters from analysis of variance (ANOVA) at individual seasons (dz irrigation and dz main season) are presented in Table 9, and 10, respectively and mean square for combined over the two environments are presented in

Table 10 ANOVA tables of seven chickpea Cultivars for seed yield and its component for Co combined over two locations

Mean sum of square the characteristics											
Sv	Def	DF	DM	SC	PH(CM)	NPB\PL	NSB\PL	NP/PL	NS\P	HSW	SY\P
Rep	2	3.1	197.4*	1686.2***	95.3***	0.002 ns	13.8ns	100.9ns	0.02ns	3.9ns	9.8ns
Loc	1	0.01ns	10.0ns	524.1*	1051.5 ***	47.9 ***	2886.5***	3613.1 **	2.9***	63.4**	1239.4**
VARS	6	79.2	975.6***	459.8**	163.8***	3.7**	127.6***	1029.7 **	0.07*	372.7 **	241.9**
Trt*loc	6	9.2ns	36.4ns	135.9ns	69.5***	5.37 **	91.21***	623.7**	0.16**	4.4*	65.8**
Error	12	8.1	39.6	130.3	7.9	0.8	6.2	65.9	0.03	1.7	4.8

sv= Source of variation, def= Degree freedom, trt= treatment, loc=location, CV= Coefficient of Variations, LSD=Lest Significant Difference, DF=50% Days Flowering, DM= Days to Maturity, SC=Stand Count at, PH) (cm): =Plant Height, (NBP/PL)= Number of Primary Branches, (NSB/PL =Number of Secondary Branches, NP/PL = Number of Pod per Plant, NS/P= Number of Seed per Pod and SY/P=seed Yield Per plot.

At irrigation experiment significant differences among varieties (*p<0.01*) were observed for traits: days to 50% of flowering, days to maturity, Number of pods per plant, number of primary branch per plant, number of secondary branch per plant, hundred seed weight and seed yield per hectare. Contrary, traits such as stand cunt per plot and number of seed per pod showed non-significant difference (Table 11).

Table 11 ANOVA tables for Irrigation Experiment.

Mean sum of square											
SV	DFM	DF	DM	SC	PH(CM)	NPB\PL	NSB\PL	NPP	NSP	HSW	SY\P
REP	2	2.04ns	62.0ns	1472.7ns	78.2**	0.017ns	0.019ns	10.8ns	0.02ns	10. 1*	5.8ns
CUL	6	25.9**	420.9***	413.4ns	98.9***	1. 12**	4.2**	231.8***	0.02ns	172.9***	94.7***
ERROR	12	5.3	16.6	15.57	6.3	0.019	0.67	12.4	0.009	1.3	5.59

At main season experiment, highly significant differences among varieties (*p<0.01*) were observed for traits like days to 50% of flowering, days to maturity, plant height, number of primary branch per plant, number of secondary branch per plant, number of pod per plant, number of seed per plant, hundred seed weight and seed yield pre hectares, On the other hand, a traits such as stand cunt per plot was showed non-significant difference (Table 12).

Table 12 ANOVA Tables of seven chickpea Cultivars for seed yield and its component for main season

						Mean sum of square					
SV	DFM	DF	DM	SC	PH(CM)	NPB\PL	NSB\PL	NP/PL	NS/P	HSW	SY\P
REP	2	0.94ns	148.9ns	405.9*	33.3*	0.56 ns	25. 1ns	329.0ns	0.1*	0.68 ns	0.84ns
CUL	6	53.7**	555. 1***	175.5 ns	122.9***	8.3***	165.9***	1367.9***	0.2**	1.2***	211.3***
ERROR	12	11.9	69.5	91.4	8. 1	0.98	8.6	92.8	0.03	1.2	4.87

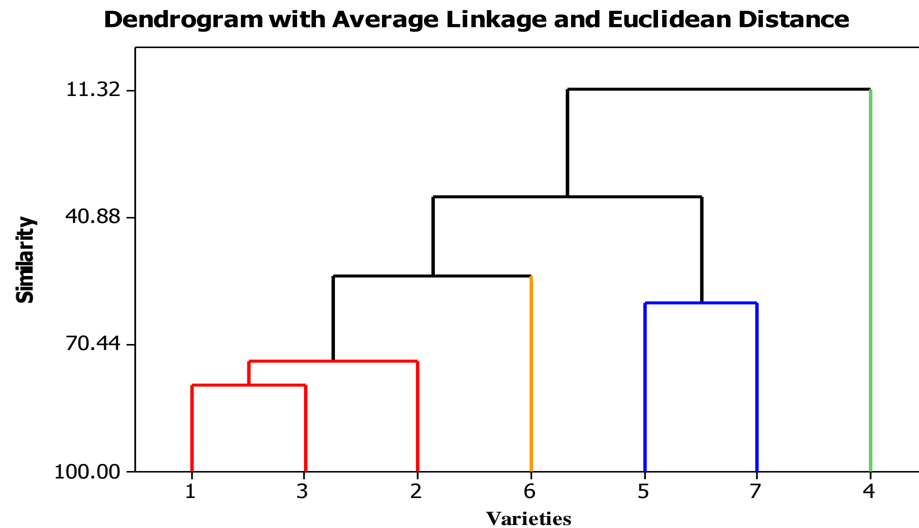
* Significant at p= 0.05,** Significant at p= 0.01, ***Significant at p= 0.001 and ns= not significant deference

cul=cultivars, dfm=degree freedom CV= Coefficient of Variations, LSD=Lest Significant Difference, DTF=50% Days Flowering, DTM= Days to Maturity, SC=Stand Count at, PH)(cm):Plant Height, (**NBP/PL**)= Number of Primary Branches, (**NSB/PL** =Number of Secondary Branches, NP/PL = Number of Pod per Plant, **NS/P**= Number of Seed per Pod and **SY/P**=seed Yield Per plot

The combined analysis over two seasons showed that there was highly significant difference between varieties, varieties by location interaction, interims of number of pod per plant, number of primary branch per plant, number of secondary branch per plant, number of pod per plant, plant height (cm)and seed yield per kg/ha. There was a non-significant difference between locations, varieties by location interim of 50%of days flowering, days to maturity. Height (cm)and seed yield per kg/ha. There was a non-significant difference between locations, varieties by location interim of 50%of days flowering, days to maturity.

On the other hand the analysis of variance for treatment by environment interaction showed that there was highly significant difference for traits such as, plant height, number of primary branches per plant, number of secondary branches per plant, number of pods per plant, number of seeds per pods, hundred seed weight and seed yield per hectares. Contrary a trait such as days to 50% of flowering, days to maturity and stand cunt per plot was shown non-significant difference.

Cluster:-Cluster analysis for the varieties revealed four distinct groups. The numbers of genotypes in each of the four clusters ranged from one to two in the smallest and largest cluster, respectively (Figure1). The first cluster contains desi type chickpea genotypes which were Dimtu and Teketaye. The second cluster was containing only one cultivar of desi type chickpea which was Dalota. The third cluster was also containing only one genotype of kabuli type chickpea which was Hora. The fourth cluster consists of two cultivars of kabuli type chickpea which were Dhera and Ejere. The fifth cluster contains only the local check.



*KEY:-1=Dimtu, 2=Dalota, 3=Teketaye, 4=Local check,5=Dhera,6=Hora and 7=Ejere.

Figure 1.Dendrogram with UPGMA and Euclidean distance showing similarity among seven Ethiopian chickpea genotypes using ten quantitative traits.

4.1.1.4. Principal component analysis (PCA) for phenotypic traits

The principal component analysis was applied to estimate the relative contribution of traits towards the variation in the 7 Ethiopian chickpea varieties. The first three principal components
(PCs) with Eigen value greater than 2.07 accounted for 93.7% of the entire diversity among tested varieties for all the 10traits (Ta

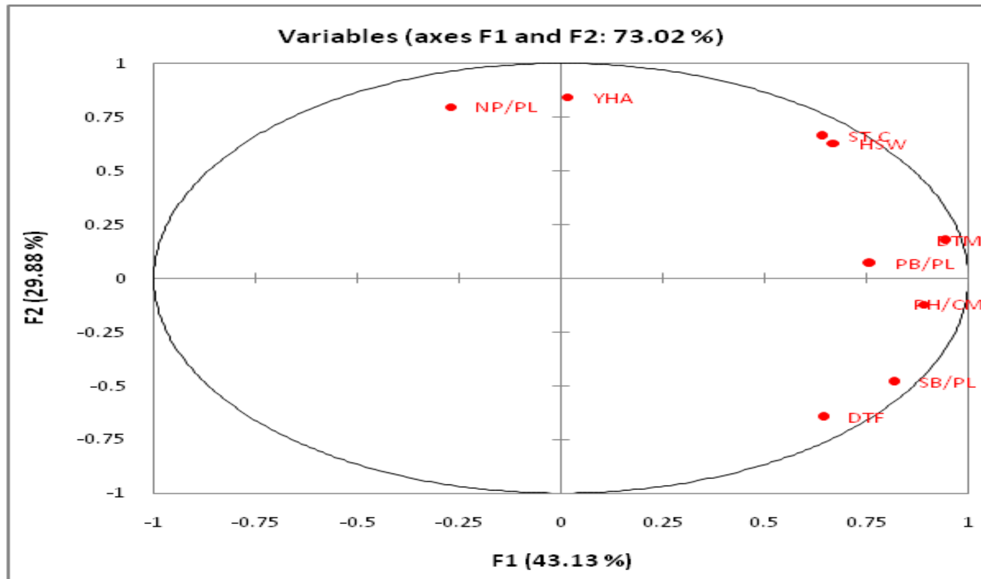
Table 13 Eigen values, variance percent, cumulative percent and eigenvectors of the first eight principal components of 9 qualitative traits of 7 Ethiopian chickpea varieties.

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	4.3132	2.9883	2.0719	0.3820	0.1391	0.1
Variability (%)	43.1316	29.8835	20.7186	3.8205	1.3911	1.1
Cumulative %	43.1316	73.0151	93.7337	97.5541	98.9452	100.0
Traits	Eigenvectors					
	PC1	PC2	PC3	PC4	PC5	PC6
DTF	0.31	-0.37	0.26	0.27	0.02	-0.20
DTM	0.45	0.10	-0.04	0.38	0.17	-0.38
ST.C	0.31	0.38	-0.17	0.17	0.71	-0.29
PH/CM	0.43	-0.07	0.25	0.22	0.21	0.58
PB/PL	0.37	0.04	-0.43	0.06	-0.49	0.19
SB/PL	0.39	-0.28	0.17	0.29	-0.13	-0.17
NP/PL	-0.13	0.46	0.27	0.53	-0.37	-0.38
NS/P	-0.09	-0.19	0.63	-0.33	0.05	-0.15
HSW	0.32	0.36	0.18	-0.47	-0.12	0.02
YHA	0.01	0.49	0.36	0.06	-0.09	0.41

The PC1contributed 43% of total variation among Ethiopian chickpea varieties and this variation were contributed by traits: days to maturity, plant height, days to flowering, secondary branch per plant and stand cunt per pot. Similarly, PC2 contributed 29.9 % of total variation and contributed by traits such as number of pods per plant, hundred seed weight and yield per hectors. On the other hand, PC3 was contributed about 20% of total variation and traits that caused this variation were number of seed per pod and yield. (Figure 2 and Table 13).

Table 14 Contribution of the variables (traits) (%)

	PC1	PC2	PC3	PC4	PC5	PC6
DTF	9.61	13.996	6.55	7.08	0.03	4.06
DTM	20.63	1.04	0.15	14.64	3.00	14.76
ST.C	9.55	14.63	2.89	3.01	50.95	8.35
PH/CM	18.41	0.49	6.35	4.82	4.39	33.29
PB/PL	13.36	0.16	18.34	0.39	23.98	3.93
SB/PL	15.56	7.82	2.80	8.26	1.78	2.71
NP/PL	1.69	21.32	7.14	28.34	13.43	14.04
NS/P	0.74	3.68	39.28	10.85	0.245	2.34
HSW	10.42	13.17	3.37	22.27	1.33	0.03
YHA	0.006	23.70	13.11	0.32	0.84	16.48



DTF=Days to 50% flowering, DTM=Days to maturity, ST.C=Stand cunt per plot, PH/CM=plant height, PB/PL=number of primary branch per plant, SB/PL=number of secondary branch per per plant, NS/P= number of seeds per pod, HSW=hundred seed weight and YHA=yield per hectors.

Figure 2.Relative position of the 10 traits of 7 Ethiopian chickpea varieties in the first and second principal axis

Based on the treatments, the PC1contributed 43% of total variation among Ethiopian chickpea varieties and this variation were contributed by varieties: Dhera, Localcheck, Dalota, and Dimtu. Similarly, PC2 contributed 29.9 % of total variation and contributed by varieties such as Localcheck, Dhera, Hora and Dimtu. On the other hand, PC3 was contributed about 20% of total variation and varieties that caused this variation were Ejere, Dimtu, Dhera, Hora and Dalota.(Figure 3 and Table 14)

Table 15 Contribution of Chickpea varieties (%):

Varieties	PC1	PC2	PC3	PC4	PC5	PC6
Dimtu	6.82	12.12	22.42	6.80	6.68	30.87
Dalota	7.43	0.0018	6.11	17.99	3.26	50.92
Teketaye	1.91	9.37	0.0222	38.45	35.78	0.18
Localchick	25.23	43.94	6.80	3.48	4.49	1.78
Dhera	46.43	20.20	17.94	0.72	0.22	0.20
Hora	5.37	13.67	7.35	8.22	41.30	9.81
Ejere	6.81	0.70	39.35	24.33	8.27	6.24

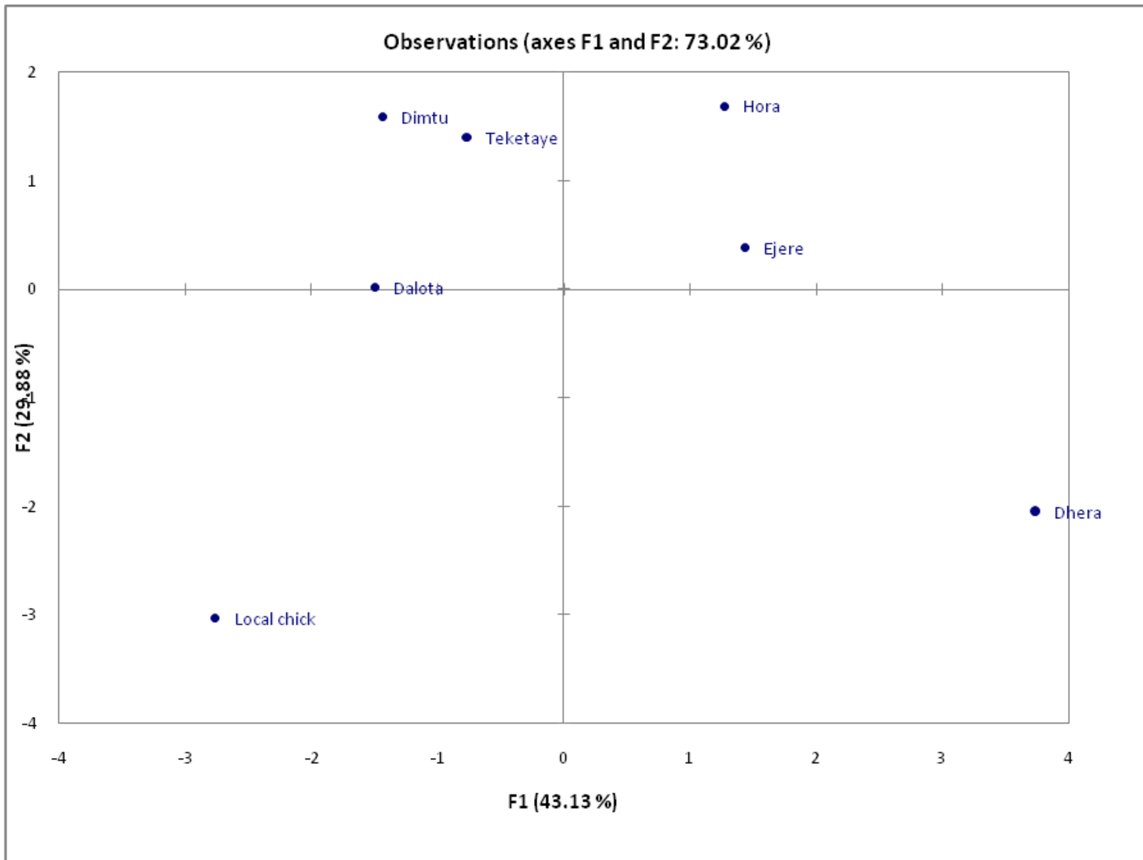


Figure 3 Relative positions of 7 Ethiopian chickpea varieties in the first and second principal axis

4.2. Genetic Diversity Analysis using Biochemical trait

4.2.1. Estimation of analysis of variance for biochemical experiment

The analysis of variance showed that there was highly significance difference was obtained between tasted varieties interims of dry mater, moisture, ash, fiber, protein, fat, carbohydrate, phytaic and tannin content.

Table 16 ANOVA for biochemical experiment

SV	Degree fridm	MC	DM	FAT	ASH	FR	PR	CAR	TAN	PHY
Cultivar	27	8.03 ***	8.6***	0.75***	0.41***	9.82**	27.7	34.7***	8688.9***	3584.9***
Error	756	1.33	1.18	0.9	0.05	0.014	7.1	7.3	26.04	45.5
F		6.04	7.33	8.39	8.26	710.8	3.89	4.7	333.9	78.8
P value		<.0001	<.0001	<.0001	<.0001	<.001	0.0003**	<.0001	<.0001	<.0001

*** Significant at p= 0.05,*** highly Significant at p= 0.001 ns= not significant deferenceF,and p values are for varieties**

Where MC=Moisture Content, DM=Dry mater, FAT=Fat content, ASH= Ash content, FR=fiber content, PR=protein content, CAR=Carbohydrate content, TAN=Tannin content and PHY=phyitic acid content.

4.2.2. Estimation of mean performance for biochemical experiment

Table 17 Top three mean performances for biochemical experiment

Cluster No	No of varieties	Names of varieties
1	7	Kob, Acosdubie, Kasech, Mareye, Yelebe, Chefe and Teji
2	4	kutaye,Dimtu,Dalota and Menjare
3	17	Teketaye,Local check, Dehera, Hora, Ejere, Shasho , Artery, Dubie, Natoli, Kauri, Habru, DZ -10- 4,Akaki,Mastewal, DZ-10-11,Worku and Fetenech

MC=Moisture Content, DM=Dry mater, FAT=Fat content, ASH= Ash content, FR=fiber content, PR=protein content, CAR=Carbohydrate content, TAN=Tannin content and PHY=phyitic acid content.

NB. See the appendix for the reset varieties performance.

4.2.3. Cluster and principal component analysis

4.2.3.1. Cluster analysis

Cluster analysis using the whole genotypes revealed three distinct groups. The number of genotypes in each of three clusters ranged from four to seventeen in the smallest and largest cluster, respectively (Table 15&Figure 2). The first cluster was the second largest cluster containing 7 genotypes (Table 15). The second cluster was consisting 4 genotypes. The third cluster was the largest cluster which contains Seventeen chickpea genotypes.

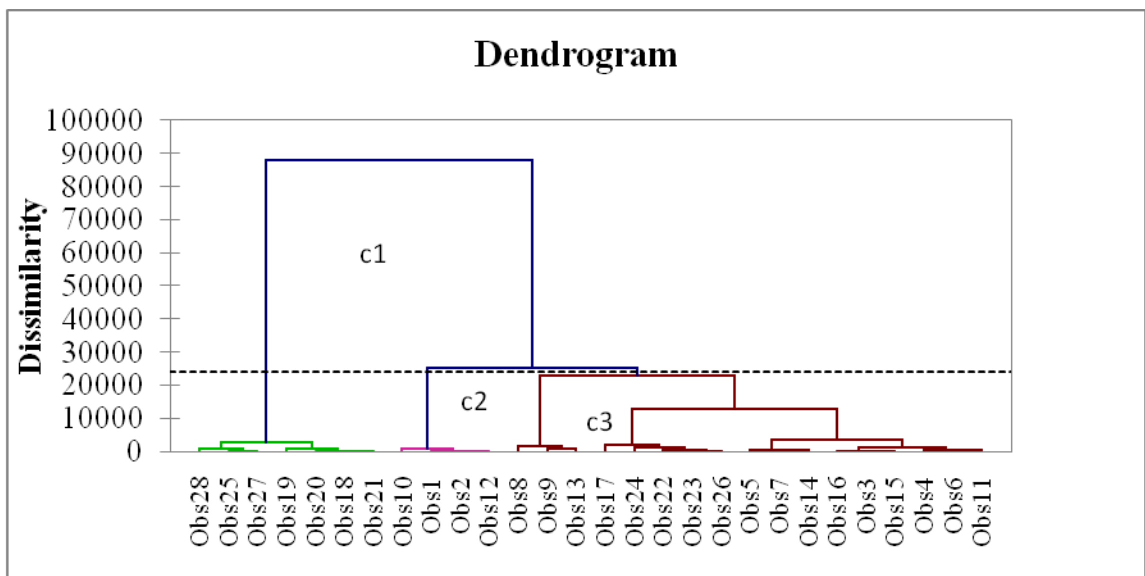


Figure 4.Dendrogram with UPGM and Euclidean distance showing similarity among twenty eight Ethiopian chickpea genotypes using nine qualitative traits.

Table 18 Lists of varieties grouped under different clusters

Cluster No	No of varieties	Names of varieties
1	7	Kob, Acosdubie, Kasech, Mareye, Yelebe, Chefe and Teji
2	4	kutaye,Dimtu,Dalota and Menjare
3	17	Teketaye,Local check, Dehera, Hora, Ejere, Shasho , Artery, Dubie, Natoli, Kauri, Habru, DZ -10- 4,Akaki,Mastewal, DZ-10-11,Worku and Fetenech

Table 19 Distances between three clusters of 28 chickpea varieties grown in Ethiopia

	C1	C2	C3
C1	0		
C2	96.8**	0	
C3	197.3**	101.7**	0

Genetic distance between clusters: Pair wise generalized distances (D^2) among the three clusters are presented in Table 14. 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= 197.3) was detected between C1&C3, while a minimum distance (D^2 = 96.80) was observed between C1 & C2.

4.2.3.2. Principal component analysis (PCA)

The principal component analysis was applied to estimate the relative contribution of traits towards the variation in the 28 Ethiopian chickpea varieties. The first five principal components (PCs) with Eigen value greater than 0.75 accounted for 90.3% of the entire diversity among tested varieties for all the 9 traits (Table 9).

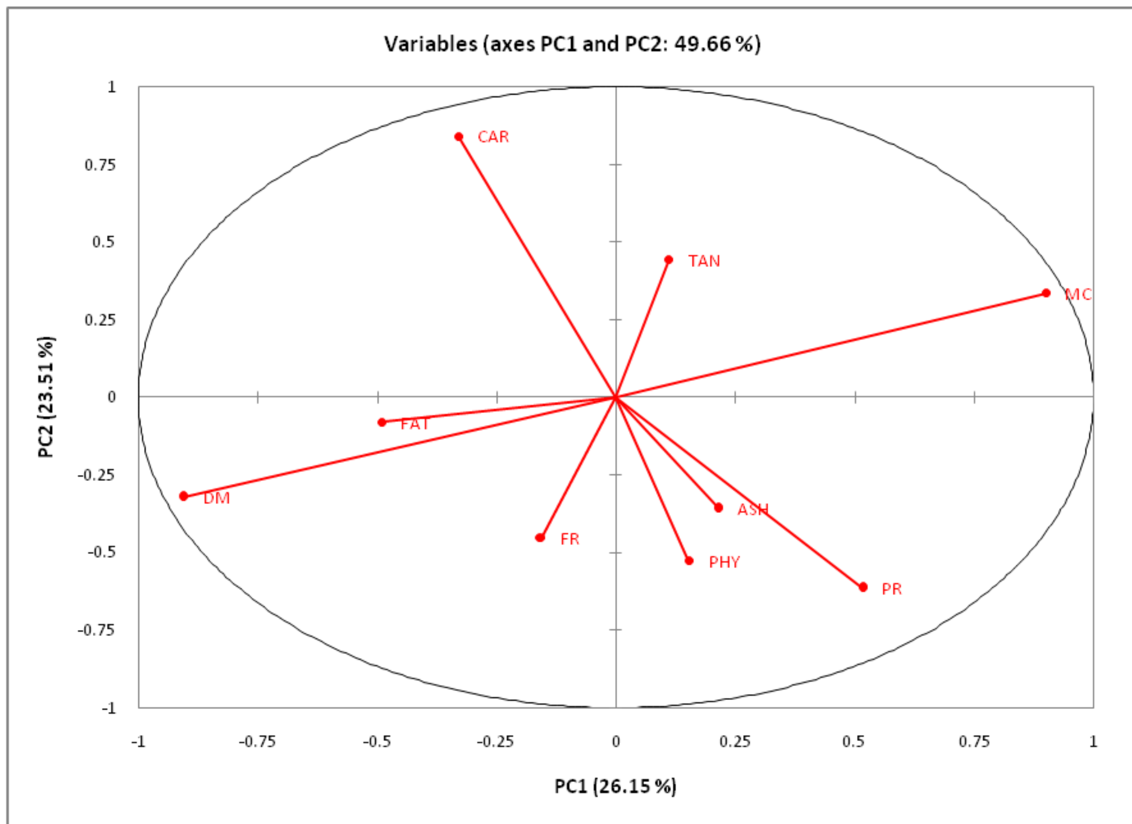
Table 20 Eigen values, variance percent, cumulative percent and eigenvectors of the first eight principal components of 9 qualitative traits of 28 Ethiopian chickpea varieties.

	Pc1	Pc2	Pc3	Pc4	Pc5	Pc6	Pc7	pc8
Eigenvalue	2.35	2.12	1.62	1.26	0.78	0.58	0.28	0.01
Variability	26.2	23.50	18.04	14.01	8.65	6.44	3.08	0.10
Cumulative	26.1	49.6	67.71	81.71	90.37	96.81	99.89	100.0

Traits	Eigenvectors:							
	Pc1	Pc2	Pc3	Pc4	Pc5	Pc6	Pc7	pc8
DM	-0.59	-0.22	0.02	0.05	-0.25	0.17	0.05	0.71
MC	0.59	0.23	-0.01	-0.01	0.25	-0.17	-0.12	0.70
FAT	-0.32	-0.05	-0.24	-0.36	0.77	-0.09	0.30	0.02
ASH	0.14	-0.25	0.44	0.28	0.41	0.68	-0.05	-0.01
FR	-0.10	-0.31	0.56	-0.20	0.06	-0.49	-0.38	-0.01
PR	0.34	-0.42	-0.32	-0.28	-0.26	0.20	0.20	0.02
CAR	-0.21	0.58	-0.03	0.38	0.04	0.02	-0.01	-0.01
TAN	0.07	0.30	0.55	-0.34	-0.19	0.03	0.67	0.04

FAT=Fat content, ASH= Ash content, FR=fiber content, PR=protein content, CAR=Carbohydrate content, TAN=Tannin content and PHY=phyitic acid content MC=Moisture Content, DM=Dry mat

The PC1contributed 26% of total variation among Ethiopian chickpea genotypes and this variation were contributed by traits: moisture, ash, protein, tannin and phytatic acid content Similarly, PC2 contributed 23 % of total variation and contributed by traits such as moisture, carbohydrate, and tannin content On the other hand, PC3,PC4 PC5 and PC6 were contributed about 41 % of total variation and traits that caused this variation were dry mater, ash, fiber, tannin, carbohydrate, moisture, and protein content. (Figure 4and Table 9)



Where DM =dry mater content, MC=moisture content, FR=fiber content, PHY=phytotic acid, PR=protein content, ASH= ash content, CAR=carbohydrate content, TAN =tannin content and FAT = fat content.

Figure 5.Relative position of the 9 traits of 28 Ethiopian chickpea varieties in the first and second principal axis

As it is visualized in Figure 6, the improved and local genotypes in this study were distributed in all four quadrants of the principal component axis. Marye (19) was plotted far apart from the group. Genotypes number 11(Dubie), 2(Dalota), 8(Shasho), 9 (arerity) and 10(Kutaye) highly contributed to PC1 in negative direction and genotype 4 (Local check), and 5 (Dhera) contributed highly in positive direction for PC1.On the other hand, the traits of genotypes number 19 (Mareye, 13 (Natoli), 22(Mastewal), 21(Teje), and 23(Dz-10-11) contributed highly and negatively to the PC2, whereas genotype 6 (Hora and 14 (Akuri) contributed highly and positively in this PC2.

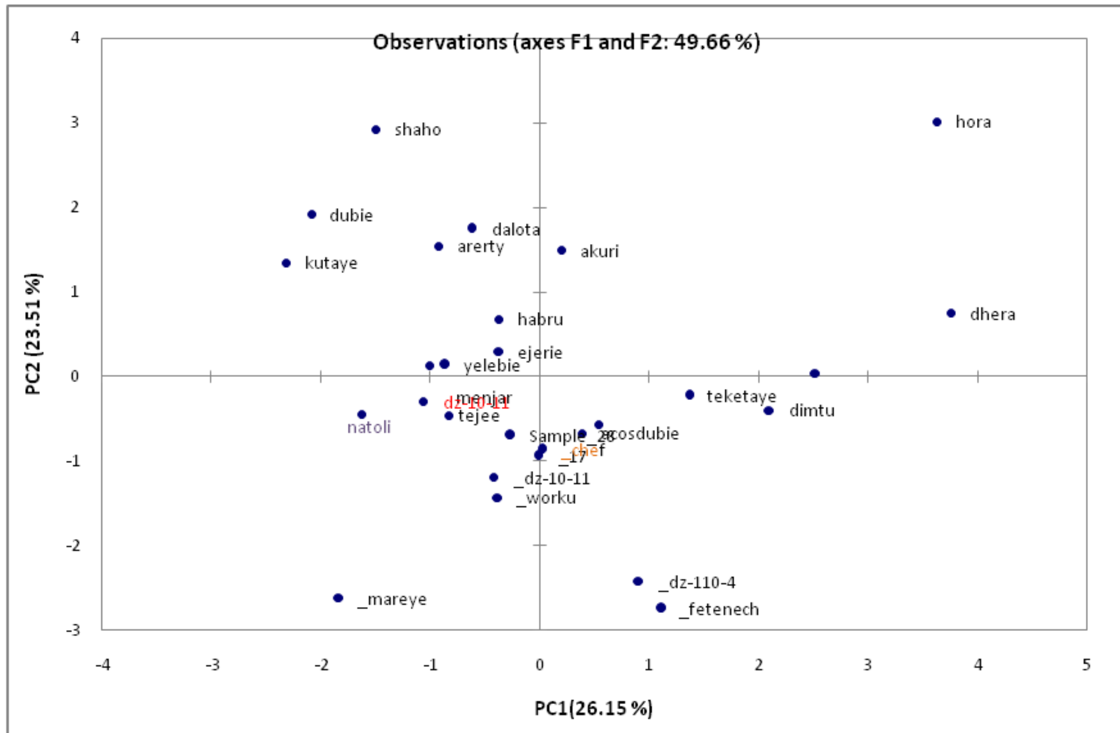


Figure 6.Relative position of the 28 Ethiopian chickpea varieties in the first and second principal axis

4.3. Genetic Diversity Analysis using ISSR molecular marker

4.3.1. ISSR primer percentage of polymorphism, band variation and level of polymorphism

Table 21 List of ISSR primers with their scored bands, polymorphism and gene diversity.

Sr.No.Primers	Base Sequences (5'--3')	Band size	Total band	NPB	PPB (%)	h+SD	I+SD
UBC-810	$(GA)_8TY$	150-1100	13	12	91.67	0.34+0.13	0.55+0.20
UBC-824	(TC)8G	550-1000	4	3	75	0.22+0.20	0.34+0.29
UBC-851	(GT)8YG	150-1100	15	15	100	0.28+0.14	0.43+0.18
UBC-889	DBD(AC)7	450-1200	12	11	91.67	0.38+0.15	0.55+0.2
UBC812	(GA)8A	650-1100	10	10	100	0.32+0.01	0.5+0.12
Total			54	51			
Mean			10.8	10.2	91.67	0.30+0.14	0.47+19

h=Nei's gene diversity, I=Shannon's information index, NPb =number of polymorphic bands ppb%= percentage of polymorphic bands.

The genetic fingerprinting of chickpea cultivars were performed using 5 ISSR primers. All the 5 primers tested were di-nucleotide repeat motif s(AG)8T4, DBD(AC)7, (Tc)8)Yc, (GA)8G and, (GT)8YG

A total of 54 bands were amplified across 28 cultivars of chickpea with 5 primers, revealing an average of 10.2 bands per primer (Table 20). The number of bands amplified by ISSR primers varied from 4to 15. The primer sequences UBC824, produced the least number of bands (4) , whereas, primers UBC-810, UBC-851, UBC889and UBC-812 amplified high number of bands (13, 15, 12,and 10) respectively. The ISSR fragment size ranged from 1200-1500 bp, the number of polymorphic bands ranged from 3 to15 contributing to 91.7 total polymorphic bands and 10.2 average polymorphic bands per primer. Percentage of polymorphic bands (PPB) ranged from 75-100%. All the five primers produced 91.7 % of polymorphic bands at specific level.(Table 19)

The highest Nei's gene diversity (0.37) and Shannon information index (0.55) were scored by primer 889. In contrast, primer 824 showed the least Nei's gene diversity and Shannon information index with values 0.2219, 0.3436 respectively. The mean Nei's gene diversity and Shannon information index for all primers were 0.305 and 0.474 respectively, (Table 19) the highest number of polymorphic bands (15) was obtained from primer 851. The largest (1200pb, 1100) band was produced by primers 889 and 810respectively.

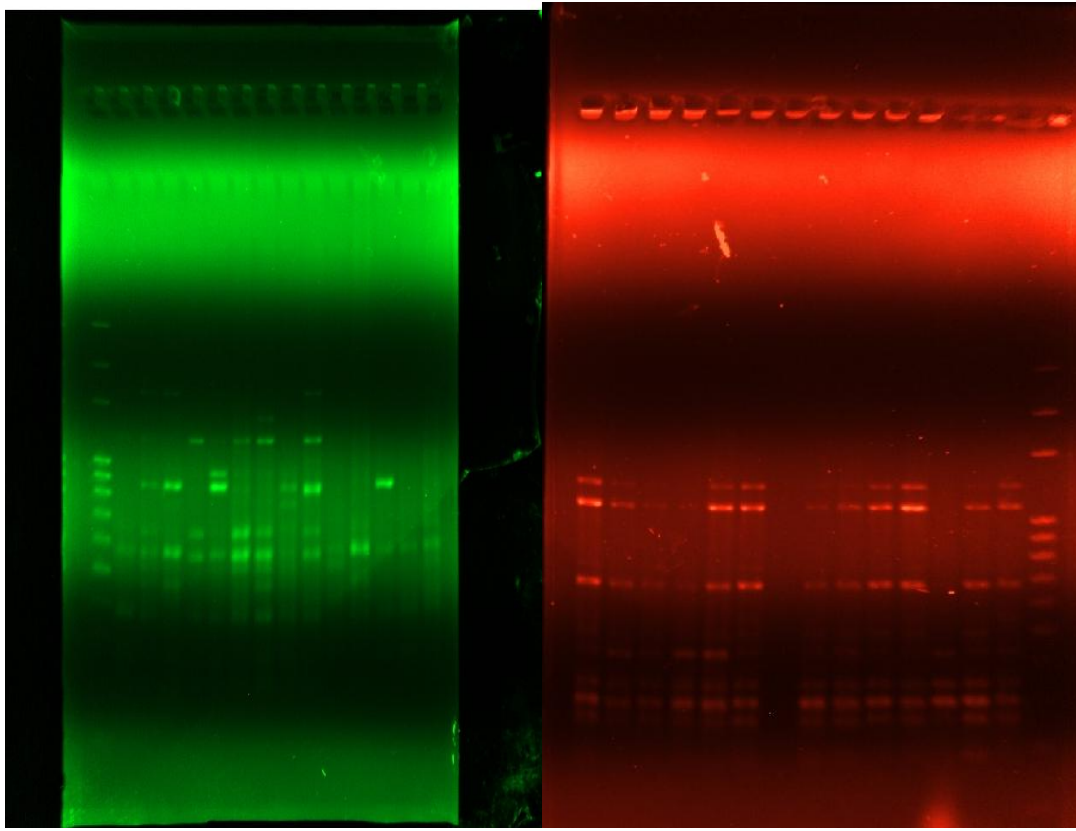


Figure7 ISSR finger print generating primer 851 and 812using agarose gel

Table 22 Measure of genetic diversity in two populations of chickpea, Desi vs Kabuli varieties

Pop	no of polym -orphic bands	%of polymorphic bands	h+SD	I+SD	GsT*	Nm*
Desi	47	87.04	0.28+0.17	0.42+0.23	-	-
Kabuli	47	87.04	0.31+0.16	0.46+0.22	-	-
Mean	94.4	87	0.29+0.75	0.29+45	0.05	8.71

Nm=estimation of gene flow from Gst.Nm=0.5(1-Gst)/Gst, h=Nei's gene diversity

4.3.2. Analysis of molecular variance (AMOVA)

Analysis of molecular vlariance was carried out on the overall ISSR data score of Ethiopian chickpea varieties. In the present study AMOVA revealed higher percentage of variation (56%) which is attributed to the within population variation, whereas the remaining variation is due to among group variation(44%) (Table 21)

Table 23 Result of Analysis of molecular variance (AMOVA) among two populations of chickpea, Desi and Kabuli

Source of variation	d.f	sum ofSquare	variance component	%of variation	fixation index
Among population	1	80.64	5.2 Va	44	0.44
Within population	26	173.86	6.6 Vb	56	
Totals	27	254.5	11.96		

Va variation among Vb= variation between.

4.3.3. Cluster analysis

Clustering among 28 Ethiopian chickpea varieties containing two groups were generated using UPGMA and NJ based on 54 amplified bands generated by 5 ISSR primers (Figure 13 and Figure 12). The varieties were categorized based on their types into two populations (desi and kabuli type varieties). All of the varieties were grouped into five clusters in a UPGMA-based dendrogram. The first major cluster mostly contained desi type's chickpea which includes: Teketaye, Local check, Akaki, Mareye and one variety derived from kabuli type chickpea (Kobo). The second major cluster also comprised three varieties from kabuli type chick pea (Habru, Chefe and Ejere). The third major cluster consisted of three varieties from desi type (Fetenech, Dimtu and Kutaye) and two varieties from kabuli types (Arerity and Yelebie). On the other hand cluster for consisted of three varieties from kabuli (Hora, Dhera and Dz-10-11) and one from desi type (Mastewal). Cluster five consisted of three varieties derived from kabuli type(Akuri, Acosdubie and Kasch) and two varieties from desi type (Dubie and Natoli).The Neighbor-joining (NJ) analysis of the same dataset showed five distinct clusters (cluster 1, cluster 2, cluster-3 cluster 4 and cluster 5) and sub-clusters within the third major cluster (cluster 3-a and cluster 3-b), cluster 4 (cluster 4a cluster 4b and cluster 4c, and cluster5 (cluster 5a, cluster 5b, cluster 5c and cluster 5d).Varieties such as

Dalota, Shaho, Dz-10- 4 and Teje was found as outlier in NJ the denderogram. Expect Dalota those outliers were kabuli type chickpea.

4.3.4. Population genetic diversity

Gene diversity ranged from 0.00 for Ejere, Shasho, Habru, DZ-10-4, and Teje (kabuli) to 0.15 for Mastewal (desi) with mean of 0.07 for DZ-10-11(desi). Varieties of Dalota (desi),Ejere Shasho, Habru Dz-10-4 and Teje (kabuli) show nearly equal gene diversity (0.02). Generally variety Mastewal and Kutaye (desi) showed the highest gene diversity (0.15). Over all Jaccared similarity coefficient that ranged from 0.00-0.15 was obtained. The pair wise comparison of Jaccar value showed that Dalota and Menjar (desi) are closest having similar coefficient of 0.02; Dalota and Mastewal (desi) are the most distantly related cultivars with 0.15 similarity coefficient (Table. 15).

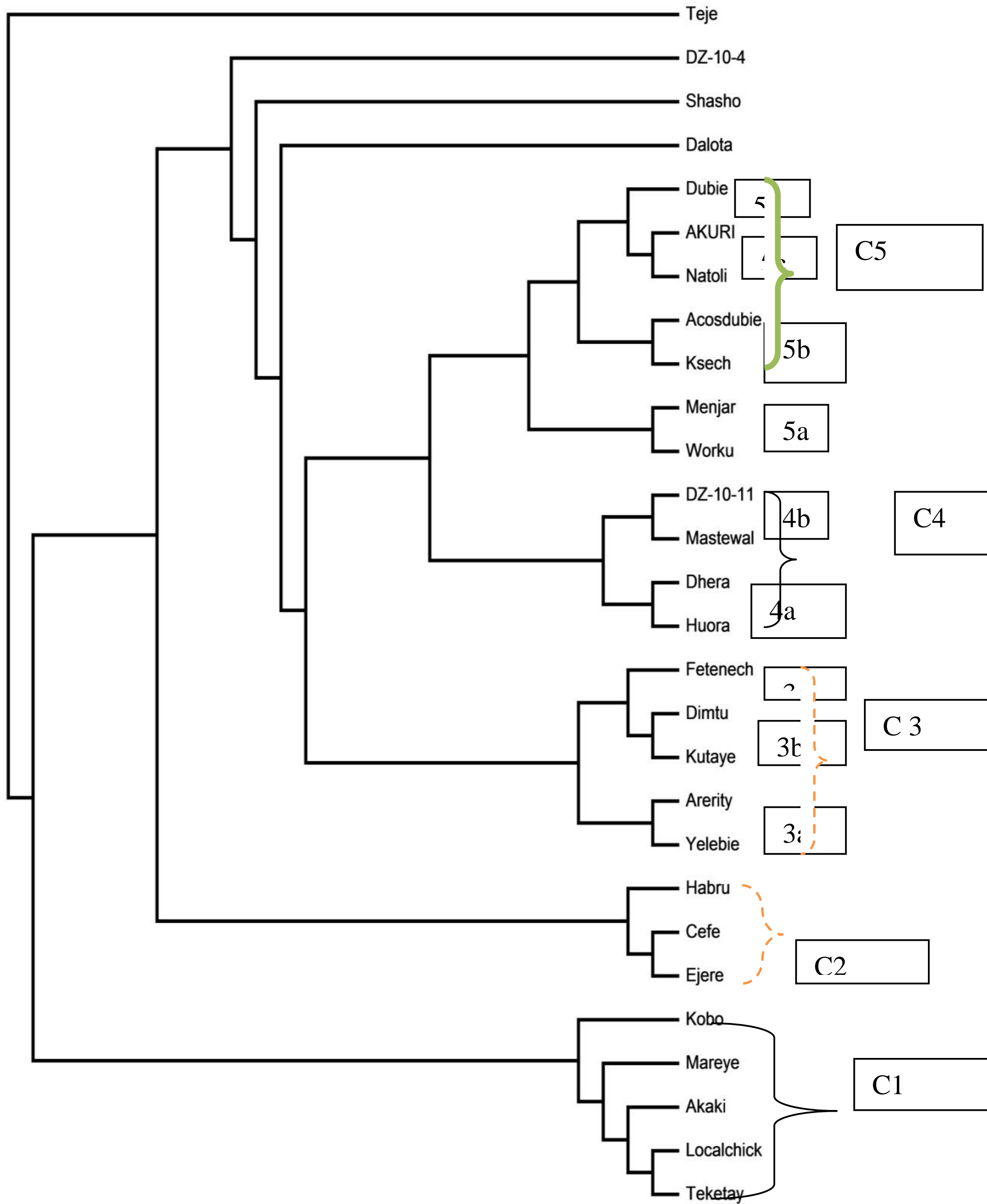


Figure 8 NJ based on 28 individuals of chickpea using Jaccared similarity

Table 24 Genetic similarity based on UPGMA and NJ of 28 varieties of chickpea using Jaccard similarity coefficient

1																											
2	0.02																										
3	0.05	0.03																									
4	0.06	0.05	0.05																								
5	0.05	0.03	0.06	0.08																							
6	0.05	0.03	0.06	0.08	0.03																						
7	0.02	0.0	0.03	0.05	0.03	0.03																					
8	0.02	0.00	0.03	0.05	0.03	0.03	0.00																				
9	0.06	0.05	0.08	0.09	0.08	0.08	0.05	0.05																			
10	0.05	0.06	0.09	0.11	0.09	0.09	0.06	0.06	0.08																		
11	0.05	0.03	0.06	0.08	0.06	0.06	0.03	0.03	0.08	0.09																	
12	0.03	0.02	0.05	0.06	0.05	0.05	0.02	0.02	0.06	0.08	0.02																
13	0.05	0.03	0.06	0.08	0.06	0.06	0.03	0.03	0.08	0.09	0.03	0.05															
14	0.05	0.03	0.06	0.08	0.06	0.06	0.03	0.03	0.08	0.09	0.03	0.05	0.03														
15	0.02	0	0.03	0.05	0.03	0.03	0	0	0.05	0.06	0.03	0.02	0.03	0.03													
16	0.02	0	0.03	0.05	0.03	0.03	0	0	0.05	0.06	0.03	0.02	0.03	0.03	0												
17	0.03	0.02	0.05	0.06	0.05	0.05	0.02	0.02	0.06	0.08	0.05	0.03	0.05	0.05	0.02	0.02											
18	0.05	0.03	0.06	0.08	0.06	0.06	0.03	0.03	0.08	0.09	0.06	0.05	0.06	0.06	0.03	0.03	0.05										
19	0.03	0.02	0.05	0.06	0.05	0.05	0.02	0.02	0.06	0.08	0.05	0.03	0.05	0.05	0.02	0.02	0.03	0.05									
20	0.05	0.03	0.06	0.08	0.06	0.03	0.03	0.03	0.05	0.06	0.06	0.05	0.06	0.06	0.03	0.03	0.05	0.06	0.05								
21	0.01515	0	0.03	0.05	0.03	0.03	0	0	0.05	0.06	0.03	0.02	0.03	0.03	0	0	0.02	0.03	0.02	0.03							
22	0.11	0.09	0.12	0.14	0.12	0.09	0.09	0.09	0.14	0.15	0.12	0.1	0.12	0.12	0.09	0.09	0.11	0.12	0.11	0.09	0.09						
23	0.05	0.03	0.06	0.08	0.06	0.06	0.03	0.03	0.08	0.09	0.06	0.05	0.06	0.06	0.03	0.03	0.05	0.06	0.05	0.06	0.03	0.09					
24	0.05	0.03	0.06	0.08	0.06	0.03	0.03	0.03	0.08	0.09	0.03	0.02	0.06	0.06	0.03	0.03	0.05	0.06	0.045	0.03	0.03	0.09	0.06				
25	0.03	0.02	0.05	0.06	0.05	0.05	0.02	0.02	0.06	0.08	0.05	0.03	0.05	0.05	0.02	0.02	0.03	0.05	0.03	0.05	0.02	0.11	0.05	0.05			
26	0.05	0.03	0.06	0.08	0.06	0.06	0.03	0.03	0.08	0.06	0.06	0.05	0.06	0.06	0.03	0.03	0.05	0.06	0.05	0.06	0.03	0.12	0.06	0.06	0.05		
27	0.06	0.05	0.08	0.09	0.08	0.08	0.05	0.05	0.09	0.11	0.05	0.06	0.05	0.05	0.05	0.05	0.06	0.08	0.06	0.08	0.05	0.14	0.08	0.08	0.03	0.08	
28	0.03	0.02	0.05	0.06	0.05	0.05	0.02	0.02	0.06	0.08	0.05	0.03	0.05	0.05	0.02	0.02	0.03	0.05	0.03	0.05	0.02	0.11	0.05	0.05	0.03	0.05	0.06

Sample 1=Dimtu,2=Dalota, 3=Teketay, 4=Local, 5=Dehera, 6=Huora, 7=Ejere, 8=Shasho, 9=Arerti, 10=Kutaye, 11=Dubie, 12=Mlenjar, 13=Natoli, 14=Akuri, 15=Habru, 16=DZ-10-4, 17=Akaki, 18=Chefe, 19=Mareye, 20=Yilebie, 21=Teje, 22=Mastewal, 23=DZ-10-11, 24=Worku, 25=Acous dubie,26=Fetenech, 27=Kasech and 28=Kobo

4.3.5. PCoA analysis for molecular experiment

PCOA analysis were undertaken for all data obtained from 5 ISSR primer using Jaccard's coefficients of similarity. PCoA analysis also categorized varieties into many different groups without following their genetic information, but the result was compatible with the UPGMA and NJ analysis. Individuals grouped in same cluster were grouped in similar within PCoA. Therefore, the grouping of individuals using two and three coordinates is indicated in Figure 13 and Figure 14, respectively.

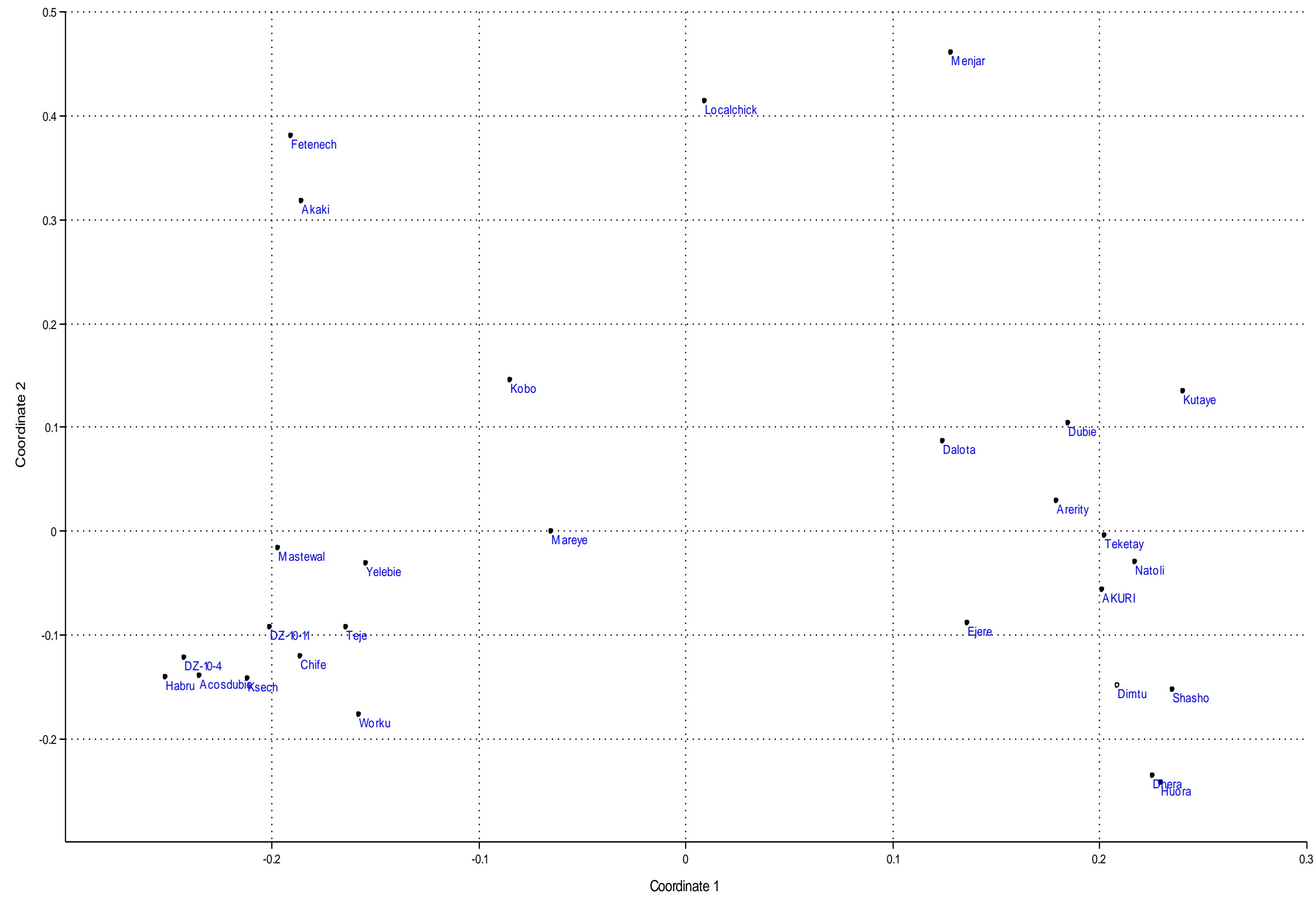
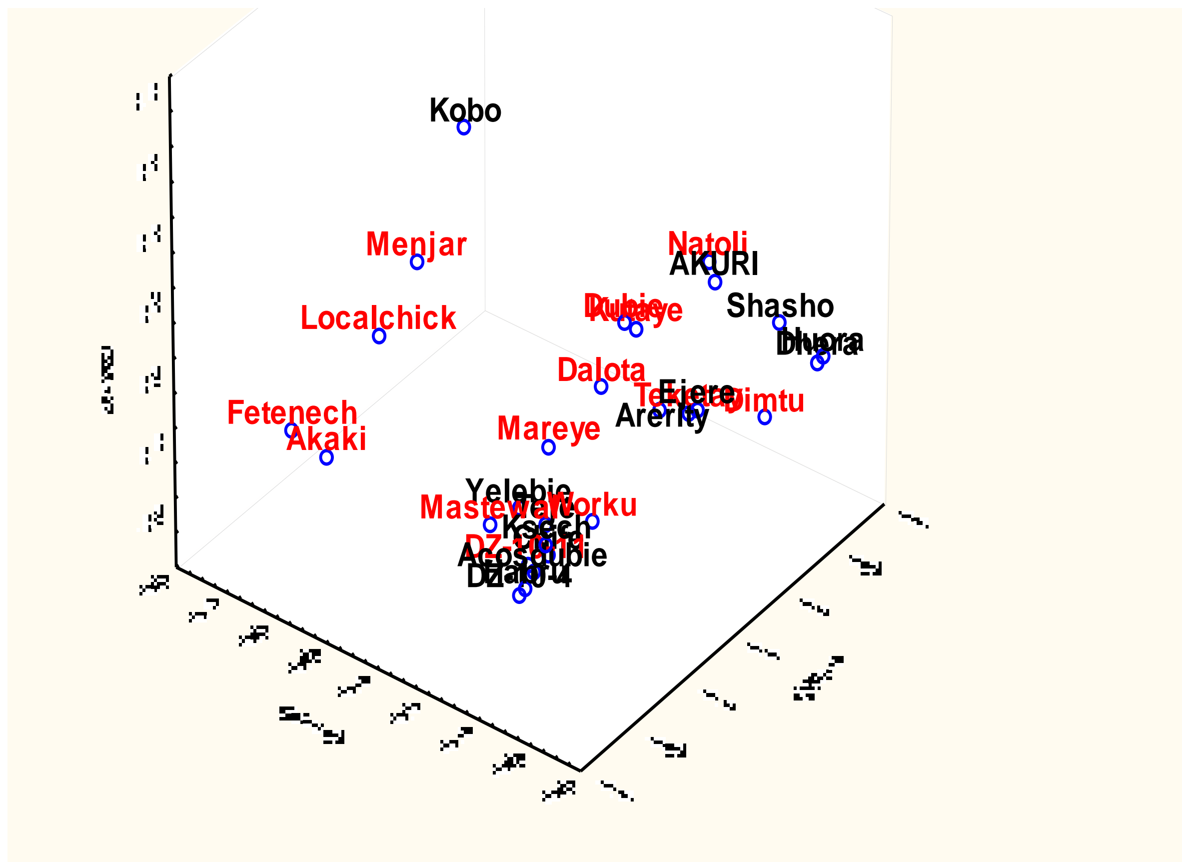


Figure 9.Three dimensional representations of 28 Individual chickpea varieties



Read colure was Desi type chickpea

Black colure was Kabuli type chickpea.

Figure 10. Three–dimensional representation of principal coordinate analysis of genetic relationships among 28 individual of released

5. DISCUSSIONS

5.1. Genetic Diversity based on Phenotypic Trait

5.1.1. Performance of Ethiopian desi and kabuli type 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 desi and kabuli types varieties regarding the measured traits and this implies that the availability of potential variation among the utilized chickpea materials.

The highest combined mean value for grain yield was recorded from variety Dimtu (desi) revealed that the variety has highly performed in all locations as compared to the others materials, whereas the lowest mean value was recorded from 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 variety (Teketaye) (desi) 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 Ejere (kabuli), and this variety had narrow adaptability in different locations and may grow best in their specific growing area. Based on yielding capacity desi type chickpea varieties are gives more yield than that of kabuli type chickpea.

Early flowering trait is essential in a chickpea crop improvement. Flowering stage is highly sensitive and may be influenced by extreme temperatures due to adverse effect on viability of pollen and pollination which could result in poor fertilization and low seed set the results from this study showed substantial differences among chickpea varieties for days to 50% flowering. Days to flowering ranged from 49.0 days to 63 days at individual seasons and the combined result across seasons revealed ranges from 49.5 to 60.6 days variation between varieties. Likewise, Atta *et al*., (2008), Khan *et al*., (2011) and Tesfamichael *et al*., (2014) reported significant and wide variation for days to flowering in chickpea germplasm. Based on this characteristic desi type are early flowering than that of kabuli type chickpea.

Days to maturity ranged from 83.3-130.4 days from individual seasons and from 84.8 to 119.5 for combined seasons. Earliness trait is one of the major breeding goals of chickpea as most farmers generally ask for early maturing varieties in order to enable the crop to mature within the growing season and give reasonable yield. Upadhyaya *et al*., (2007) reported that early-maturity helps chickpea crop to escape terminal heat and drought and increases its adaptation especially in the sub-tropics. The current results showed significant difference for days to maturity. Similar findings were reported by Atta *et al*., (2008), Khan *et al*., (2011) and Tesfamichael *et al*., (2014) in chickpea. The identified early maturing varieties (Local check, Dimtu and Teketaye) with reasonable yield traits could be utilized for development of early maturing kabuli and desi varieties and hence contribute to the promotion of chickpea in the region particularly during the short rain seasons. Based on this character desi type chickpea are earlier than that of kabuli type of chickpea.

The mean squares from the combined variance analysis over the two seasons 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 chickpea improvement programs. Sharifi (2015) reported highly significant difference with days to maturity, pod length, number of seeds per plant and thousand seeds weight.

Morphological, phonological and yield related attributes including plant height, pod number, days to 50% flowering and maturity, grain yield etc, are quantitative traits with high variance values and these could be efficiently employed for direct selection of agronomical traits or identification and inclusion of genotypes of desirable traits in breeding program (Malik *et al.,*2014). Diversity in these traits indicates great scope of these genotypes for use in different breeding approaches (Malik *et al,* 2010 and 2014).

Results of the present investigation are also in agreement with previous studies carried out on chickpea by several workers (Gemechu *et al.,*1997;Jeena and Arora, 2001; Kumar *et al.,* 2012).High genetic variations could provide effective selection of phenotypic trait for future chickpea improvement through hybridization (Mallu *et al.,* 2015).

5.2. Genetic Diversity based on Biochemical (nutritional and anti-nutritional) Traits

The moisture content showed significant difference among tested 28varieties of chickpea flours that ranged from4.45to13.700.The ash and crude fat contents of cultivars ranged from 2.45%-4.6% and 2.05%-4% respectively. The twenty eight varieties were showed highly significant which is in disagreement with findings of previous investigation which reported that there were no significant differences ($p \leq 0.05$) regarding ash and crude fat contents. The mean values reported for ash and crude fat contents were 2.7% and 5.9% respectively, for Canadian chickpea flours (Wang and Toews, 2011).

The protein content of chickpea cultivars differed significantly ($p \leq 0.0003$) between the two cultivars, among which Desi varieties showed the lowest amount (19.1%). This result is in agreement with that reported by Du *et al*., (2014) (22.37%). Differences in protein contents among chickpea cultivars can be related to the genotypic diversity, varietal characteristics and area of cultivation. The crude fibers and carbohydrates contents of chickpea varied from 8.7 to 10.5 and 59.2 to 73.3% for desi and 3.8 to7.0and 55.1 to 73.2 % respectively. Kabuli varieties were found to have the lowest fiber content and carbohydrates content. The concentration of crude fiber is related to seed coat content. The crude fibers and carbohydrates in the present study are in disagreement with those for Indian chickpea cultivars (Kaur and Singh 2005).

Tannin and phytic acid content shows highly significant difference, cultivar Kutaye (which is desi type) exhibited the highest tannin content (203.6%mg-1 100 g) whereas Teje (which is kabuli type) exhibited the lowest (-11.5 mg-1 100 g). Tannins are known as digestive enzyme inhibitor which, therefore, lowers the digestibility of proteins and starch. Tannins, being natural high molecular weight polyphenol compounds from plant sources are reported to play a defensive role in plants against both biotic and abiotic pathogenesis (Khattab and Arntfield, 2009). Phytic acid is a strong character of important minerals, thus, lowering mineral absorption and hence contribute towards mineral deficiency. It is known to bind zinc, calcium, magnesium, iron and other macro-elements (Doria *et al*., 2009). In this study genotype Akaki which was desi type exhibited the highest phytic acid content of 176 mg-1 100 g and Natoli (desi type) with -6.0 mg-1 100 g was the lowest. The total phytic acid content was greater in Kabuli type than Desi type. The wild bean has

the highest phytic acid level compared to edible bean (Awoyinka *et al.,* 2016). The Nigerian bean showed varying levels of anti-nutrients when unprocessed but decreased marginally after malting (Awoyinka *et al.,* 2016).

Same findings reported that there were same methods that are important for reduction of those anti-nutritional factors spatially for phytic acid. Elkhail *et al*., (2000) used both malting and natural fermentation process alternatively and achieved up to 83% reduction in phytate. Reduction in phytic acid contents of cereal and legume seeds with such processing treatments has been frequently reported (Ibrahim *et al.,* 2002). This has been attributed to an increase of phytase activities in fact; this enzyme makes the phytates soluble and released soluble protein and minerals. Phytates in chickpea seeds are more prone to hydrolysis during sprouting than other legumes (Chitra *et al.,* 1996). The present result will help the breeder too use those varieties with a small amount of phytic acid and tannin content (Teje, yelebie and Chefe for tannin (Natoli for phytic acid content) as parental material for future improvement of chickpea.

5.3. Genetic Diversity based on ISSR marker

Genetic diversity among crop plant species is important for efficient utilization of plant genetic resource since geographical isolation of population may cause its genome to drift away from other populations of the same species (Biron *et al.,* 2002*)*.The traditional method of identifying species by morphological character is now been replaced by DNA profiling which are more reliable due to several limitations of their morphological data *(*Nayak *et al.,* 2003).Genetic variation occurs within and among individual species as well as higher taxonomic groups (Hebert and Dewaard, 2003 and Amos, 2006). Factors that contribute to genetic variation include mutations, interactions with the environment, fitness selection and genetic drift (Bayratkar and Dolar, 2009).Five ISSR primers were used to estimate genetic diversity of chickpea. In the present investigation, size of amplified DNA fragments varied from approximately 150 bp to 1200 bp. Similarly, Irula *et al*.,(2002) obtained amplified DNA fragments that varied in size from 260 to 2500 bp; Aggarwal *et al* ., (2011)obtained from 150 to 3000 bp*;* Singh *et al.,* (2014*)*from 500 to 1500 bp and Aggarwal *et al.,(*2015) found from 120 to 2000 bp. In present study a total of 54bands were amplified across the 28 varieties of chickpea with the 5 primers, revealing an average of 10.2 bands per primer. And the number of bands amplified by five ISSR primers varied from 4 to 15. The present study is in

disagreement with that of ;Irula *et al., (*2002) who reported number of amplified bands from one to three with an average of one band per primer using seven primers in 75 chickpea genotypes. On the other hand Rao *et al.,(*2007)reported a total of 64 bands obtained using six primers in 19 chickpea genotypes. Similarly Fatemeh *et al.,*(2013) reported a total of 49 bands obtained using seven primers in 40 chickpea genotypes. The number of amplified bands varied from 5 to 11, with an average 7 bands per primer. On the bases of the number of polymorphic loci ranged from four (UBC-824) to 15 ((GT) 8YG primers). Overall 91.47% polymorphicloci were observed from the total loci scored. Among the di-nucleotide repeat motif primers, UBC-824 showed least polymorphism (75%),whereas, highest polymorphism showed by UBC-851and UBC 812 primer (100.00%).The present study is disagreement with that of Irula *et al., (*2002) who reported that only 31 fragments polymorphic out of 234 fragments and reported (13.2%) little polymorphism among 75 chickpea accessions. Similarly *Aggarwal et al., (*2015) obtained polymorphism% ranged from 63.60to 100.00% with an average 91.8%.

The cluster analysis led to the grouping of 28 chickpea varieties in five major clusters and nine sub-clusters. In the major cluster one and two, five and three varieties are exists respectively. The major cluster three, sub-divided into three sub-clusters. Clustering pattern revealed that cluster five was the largest consisting maximum number of seven varieties.

Similarly, Singh *et al*., 2014 reported that the genetic diversity in chickpea using 21 ISSR primers and UPGMA was used to calculate the generated bivariate data matrix and genetic distances. Similar results were reported by using microsatellites markers for assessment of genetic diversity among cultivars and their wild relatives of chickpea (Aggarwal *et al*., 2015).

Jaccard's similarity coefficient ranged from 0.00 to 0.15. Contrary, Irula *et al*., (2002) reported Jaccard's similarity coefficient from 0.30 to 0.98. (Rao *et al*., 2007) from 0.76 to 1.00 in cultivars, while, it varied from 0.58 to 0.76 in wild accessions. Aggarwal *et al*., (2011) reported from 0.01 to 0.90.

5.4. Comparisons of Diversity Analysis based on Morphological, Bio chemical and Molecular Markers

The results obtained in the present investigation showed that the ISSR primers are informative markers which can be examined to correlate biochemical characteristic and morphological characteristics. The relationship observed using molecular markers may provide information on the history and biology of varieties, but it does not necessarily reflect what may be observed with respect to morpho-agronomic traits (Métais *et al*., 2000). Previous studies have shown that the measurements of genetic divergence obtained from morpho-agronomical traits are not completely similar to molecular-based results (Talebi *et al*., 2008; Saeed *et al*., 2011).

The current study observed that no strong relationship between morphological, bio chemical and molecular characteristics because varieties from one cluster in biochemical character analysis entered into another cluster in molecular analysis and morphological characteristics. Molecular markers were not completely in accordance with the dendrogram based on biochemical traits reported for other crops (Fernandez *et al*., 2002). There are several possible explanations for such results: some of them connected with nature and structure of different molecular markers that designed from various regions of genome. Another problem was the possibility of over estimating genetic similarity because fragments with the same size could have different origins (Talebi *et al*., 2008). The magnitude and pattern of genetic variation detected in this study can be useful for more systematic germplasm management and utilization in breeding programs. The current study showed the importance of molecular studies (cheap, fast and informative markers) beside the morphological data and biochemical traits in detecting genetic variation among chickpea varieties in selecting diverse parents to carry out a new crossing program successfully.

6. CONCLUSION

Genetic diversity study plays a vital 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 chickpea varieties in Ethiopia is, therefore, the main concern of breeders as the effectiveness of selection depends on the proportion of the heritable variation. Regarding the agro-morphological experiment, it could be concluded that the 7 chickpea cultivars (Dimtu, Dalota, Teketaye, Local check, Dhera, Hora and Ejere) studied differed significantly for all the yield and yield component traits studied, indicating the high amount of genetic variability. The studied characteristics (number of pods per plant, number of primary branch per plant, number of seed per pod, plant height, number of secondary branch per plant, seed yield per plant and hundred seed weight.

The biochemical experiment with the 28 varieties of chickpea studied showed highly significant difference the varieties for the traits of moisture content, dry matter content ,ash content, fat content, protein content, carbohydrate content, fiber content, phytate content and tannin content and the result indicated that adequate amount of genetic variability exists among the studied varieties of chickpea. UPGMA clustering based on biochemical traits grouped 28chickpea varieties into three clusters. There was highly significant difference ($p<0.01$) among all inter cluster distances and maximum distance (197.3) was detected between clusters 1&3, while the minimum distance (96.8) was observed between cluster 1 (all are kabuli except Mareye) &2 (all are desi). Based on PCA analysis, the first five principal components accounted 90.3% of the total variation among chickpea varieties grown in Ethiopia.

On the basis molecular experiment, the genetic fingerprinting of chickpea genotypes was performed using ISSR marker. All the 5 primers tested were di-nucleotide repeat motifs. A total of 54 bands were amplified across 28 varieties of chickpea with 5 primers, revealing an average of 10.2 bands per primer. The total number of bands amplified by ISSR primers varied from 4 to 15. The primer sequences (TC)8 G, produces least number of bands (4) ,whereas, primers ($GA)_8TY$, (GT)8YG, DBD(AC)+ and (GA)8 A amplified maximum number of bands (13, 15, 12,and 10) respectively. The ISSR fragment size ranged from 150-1200 bp and the number of polymorphic bands ranged from 3 to15, contributing to 91.7 total polymorphic bands and 10.2 average

polymorphic bands per primer. Percentage of polymorphic bands (PPB) ranged from 75-100%. All the five primers produced 91.7 % of polymorphic bands at specific level.

Clusters formed in molecular analysis were more than that formed in morphological parameters analysis, and bio chemical analysis indicating the study at molecular level can detect narrow genetic base more efficiently. Based on morphological genetic diversity studies, the varieties Ejere and the local check showed sufficient amount of genetic diversity for yield and its component traits which may be used in chickpea breeding program for further improvement.

Overall, the three markers used in the present study regarding on all the diversity studied parameters signified the presence of high genetic diversity among Kabuli and Desi type chickpea varieties, especially in nutritional and morphological parameters which clearly indicated the variation existing among individual chickpea varieties grown in Ethiopia. Therefore, the presence of genetic diversity within a given population plays an essential role for designing better chickpea breeding strategies for genetic improvement aimed at solving the ultimate needs of the producers. Population plays an essential role for designing better chickpea breeding strategies for genetic improvement.

7. RECOMMENDATIONS

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

This study revealed highest amount of genetic variation among cultivated chickpea varieties. Therefore, breeders should consider these materials in future chickpea breeding program for Introgression with other germplasm resources.

- ✓ In the present study, sufficiently very high and moderately significance 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 chickpea varieties development program.
- ✓ The present study indicated large range of anti-nutritional factors tannin and phytate there for varieties with small amount of such anti-nutritional factors (Teje for tannin and Natoli for phytate) may be used as parent materials for future improvement.
- ✓ In this study, five ISSR primers successfully estimated the extent and pattern of genetic variability among released Ethiopian chickpea varieties. Therefore, these primers could be used in studies of genetic diversity, genomics and evolutionary studies, genome mapping and gene tagging of more chickpea landraces.
- ✓ Morphological and nutritional traits are affected by environmental condition there for breeders are considers future research with integrating those bio chemical, morphological and molecular research at a time.

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Appendix 1 Mean performance for biochemical analysis

Trt no	Varieties	MC	DrM	FAT	ASH	FR	PR	CAR	TAN	PHY
1	Dimtu	10.5^{CD}	91.0^{DE}	2.4^{IJ}	3.5^{BCDE}	8.0^{E}	24.1^{BC}	62.0^{FG}	197.5^{AB}	106.9^{HIJ}
2	Dalota	6.3^{EFG}	93.8^{ABC}	2.1J	2.9^{EFGHI}	6.0^{I}	16.9^{DEFG}	71.7^{BC}	187.9^{B}	100.5^{IJK}
3	Teketay	8.3^{DCE}	91.8^{CDE}	2.4^{J}	2.45^{J}	7.5^{E}	25.6^{B}	61.9F^{G}	124.1^{FGH}	77.7^{NM}
4	Localchick	10.5^{BC}	89.5^{DE}	2.6^{HIJ}	3.5^{BCD}	8.0^{E}	21.88^{BCDE}	64.0^{DEFG}	133.7^{DEF}	116.0$_{GH}$
5	Dhera	12.8^{AB}	86.8^{F}	3.5^{DEFG}	3.1^{CDEFG}	4.5^{L}	25.3^{BC}	63.6^{DEFG}	101.6^{I}	104.95^{HIJ}
6	Huora	13.7^{A}	86.0^{F}	3.1^{FGH}	2.9^{EFGHI}	3.5^{O}	20.1^{BCDE}	70.18^{ABCD}	159.1^{C}	95.6^{JKL}
7	Ejere	6.7^{DEFG}	93.4 ^{ABCD}	3.45^{DEFG}	3.0^{DEFGH}	3.8^{N}	22.6^{BCDE}	66.9^{ABCDEF}	77.3^{L}	86.9^{KLM}
8	Shasho	6.5 ^{DEFG}	93.0^{ABCD}	3.5^{DEFG}	2.6^{GHI}	4.1^{M}	16.5^{EFG}	73.2^{AB}	138.9^{ED}	24.7^{O}
9	Arerity	7.4^{DEF}	92.6^{BCD}	3.8^{CDEF}	2.5^{HI}	6.3I	19.1^{BCDEF}	68.1^{ABCDEF}	99.4^{IJ}	26.3^{O}
10	Kutaye	6.0^{EFG}	94.0^{ABC}	4.3^{AB}	2.9^{EFGH}	9.8^{CI}	13.6^{FG}	69.2^{ABCDE}	203.6^{A}	70.6^{NM}
11	Dubie	6.0^{EFG}	94.0^{ABC}	3.6^{CDEFG}	3.2^{BCDEF}	7.1^{G}	12.66^{G}	73.3^{A}	140.4^{D}	91.468^{JKLM}
12	Menjar	5.3^{FG}	94.7^{AB}	3.3^{DEFG}	3.6^{B}	5.7^{J}	20.^{5BCDE}	66.7^{BCDEF}	196.8^{AB}	92.4^{JKLM}
13	Natoli	5.8^{EFG}	94.2^{ABC}	4.5^{ABC}	2.9^{FGHI}	8.38^{D}	22.4^{BCDE}	61.7^{FG}	83.0^{KL}	-6.0^{P}
14	AKURI	8.1^{CDE}	91.^{6CDE}	3.35^{DEFG}	2.9^{EFGHI}	4.1^{M}	19.8^{BCDEF}	69.7^{ABCDE}	96.7^{IJ}	83.3^{LMN}
15	Habru	6.500^{DEFG}	93.500^{ABCD}	3.2500^{EFGH}	3.5^{BC}	3.5^{O}	20.9^{BCDE}	68.68^{ABCDE}	117.9^{GH}	83.0^{LMN}
16	DZ-10-4	6.9^{DEFG}	92.6^{BCD}	4.0^{BCD}	2.9^{FGHI}	6.88^{H}	31.1^{A}	55.1^{H}	128.3^{EFG}	88.1^{KLM}
17	Akaki	7.4^{DEF}	92.6^{BCD}	3.4^{DEFG}	3.6^{BC}	8.0^{E}	18.8^{CDEF}	66.19^{CDEF}	66.2^{M}	176.9^{A}
18	Chefe	7.1^{DEFG}	92.9^{ABCD}	3.5^{DEFG}	3.1^{CDEFG}	4.55^{L}	23.1^{BCD}	65.8^{CDEFG}	-7.3^{OP}	144.6^{CD}
19	Mareye	4.5^{G}	95.550^{A}	4.0^{BCDE}	2.9^{FGHI}	7.2G	24.2^{BC}	61.68^{FG}	19.8^{N}	161.0^{B}
20	Yelebie	6.7^{DEFG}	93.4^{ABCD}	3.6^{CDEFG}	2.7^{FGHI}	4.19^{M}	19.8^{BCDEF}	69.7^{ABCDE}	-3.13O^{P}	156.2^{BC}
21	Teje	6.8^{DEFG}	93.3^{ABCD}	3.7^{CDEF}	2.8^{FGHT}	4.07^{MN}	22.1^{BCDE}	67.3^{ABCDEF}	-11.5^{OP}	138.2^{DE}
22	Mastewal	7.1^{DEFG}	92.9^{ABCD}	4.7^{A}	2.9^{FGHI}	7.16^{G}	19.8^{BCDEF}	65.3^{CDEFG}	88.9^{JK}	121.9^{FG}
23	DZ-10-11	6.4 ^{DEFG}	93.6^{ABC}	2.9^{GHI}	2.6^{GHI}	10.3^{AB}	21.0^{BCDE}	63.1^{EFG}	90.6^{IJK}	143.09^{CD}
24	Worku	6.6^{DEFG}	93.400 ^{ABCD}	3.2500^{EFGH}	3.5^{BCDE}	10.5^{A}	19.27^{BCDEF}	63.5^{EFG}	114.6^{H}	156.8^{BC}
25	Acosdubie	7.0^{DEFG}	93.000^{ABCD}	2.9^{GHI}	2.9^{FGHI}	4.0^{MN}	24.9^{BC}	65.2C^{DEFG}	-6.2O^{P}	112.0^{GHI}
26	Fetenech	7.6^{DEF}	92.400^{BCD}	3.1000^{FGH}	4.6^{A}	10.1^{B}	22.8^{BCDE}	59.2^{G}	82.4^{KL}	134.1^{DEF}
27	Ksech	7.8^{DEF}	92.3^{BCD}	3.2^{FGH}	3.2^{BCDE}	4.88^{K}	22.9^{BCDE}	65.8^{CDEFG}	2.0^{O}	124.08^{EFG}
28	Kobo	7.4^{DEFG}	92.9^{BCD}	3.5^{DEFG}	3.6^{N}	7.^{G}	20.2^{BCDE}	65.4^{CDEFG}	17.8^{N}	94.5^{JLK}
Mean		92.5	7.3	3.3	3.0	6.45	21	65.9	94.3	103.8
CV		1.2	14.8	8.9	7.18	1.8	12.6	4.1	5.41	6.4
LSD		2.36	2.2	1.9	0.4	0.24	5.4	5.5	10.5	13.8