



GENETIC VARIABILITY AND GENOTYPE BY ENVIRONMENT INTERACTIONS
AMONG RELEASED VARIETIES AND ADVANCED LINES OF DESI TYPE
CHICKPEA GENOTYPES UNDER ACIDIC SOILS OF WESTERN ETHIOPIA

BIRU ALEMU CHALI

A THESIS SUBMITTED TO THE DEPARTMENT OF MICROBIAL, CELLULAR
AND MOLECULAR BIOLOGY

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE
DEGREE OF MASTERS OF SCIENCE IN BIOLOGY (APPLIED GENETICS)

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DECLARATION

I declare that this thesis is submitted to the School of Graduate Studies of Addis Ababa University for the Master's Degree in Biology (Applied Genetics). I would like to prove through my signature affixed underneath that it is my own independent work and has not beforehand been submitted elsewhere by me or anybody else. All authors of the references cited in the current study were duly acknowledged.

Signed on 20th of November 2017, The School of Graduate Studies,
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ABSTRACT

GENETIC VARIABILITY AND GENOTYPE BY ENVIRONMENT
INTERACTIONS AMONG RELEASED VARIETIES AND ADVANCED LINES
OF DESI TYPE CHICKPEA GENOTYPES UNDER ACIDIC SOILS OF
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Addis Ababa University, 2017

The importance of pulses such as chickpea (Cicer arietinum L.) cannot be overstated because of their significant role in sustaining food security, balancing the ecosystem and generating revenue. A field experiment was conducted on sixteen desi type chickpea genotypes under field condition at five locations viz., Shambu, Hawa Galan, Mata, Alaku Belle and Badesso in western Ethiopia during the main cropping season of 2016/2017 to examine the magnitude and pattern of environmental effect on Desi-type chickpea genotypes and genetic variability at molecular and morpho-agronomic levels. Pooled analysis of variance indicated highly significant differences for genotypes, environments, and genotype by environment interaction. The combined mean of genotypes indicated that variety Natoli and advanced line DZ-2012-CK-20113-2-0042 were top yielders among the sixteen genotypes tested at five environments of western Ethiopia. Significant statistical differences among the genotypes were observed for a number of characters. Genetic similarity matrix based on Jaccard's similarity coefficient using inter simple sequence repeat (ISSR) markers displayed an average range from 0.21 to 0.98. Based on yield performance, Additive Main Effect and Multiplicative Interaction (AMMI), AMMI stability value (ASV), Genotype selection index (GSI), Genotype main effect and Genotype by Environment Interaction (GGE) biplot analysis Natoli and DZ-2012-CK-20113-2-0042 were stable and high yielding genotypes and thus they are recommended for wider production in test locations and similar agro-ecologies.

Key words: - Chickpea (Cicer arietinum L.), Genetic variability, Adaptability and stability, Inter simple sequence repeat (ISSR)

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ABBREVIATIONS AND ACRONYMS

AMMI	Additive Main Effects and Multiplicative Interaction
ANOVA	Analysis of Variance
ASV	AMMI stability value
CIAT	International Center for Tropical Agriculture
CSA	Central Statistical Authority
CTAB	Cetyltrimethylammonium Ammonium Bromide
DAP	Diammonium Phosphate
BARC	Bako Agricultural Research Center
DZARC	Debre Zeit Agricultural Research Center
FAOSTAT	Food and Agriculture Organization Statistics
HSARC	Haro Sabu Agricultural Research Center
GGE	Genotype Main Effect and Genotype by Environment Interaction
G×E	Genotype by Environment Interactions
GSI	Genotype Selection Index
IBPGR	International Board for Plant Genetic Resources
ICARDA	International Center for Agricultural Research in the Dry Areas
ICRISAT	International Crop Research Institute for the Semi-Arid Tropics
ISSR	Inter Simple Sequence Repeat
IRRI	International Rice Research Institute
M.a.s.l.	Meter above sea level
MS	Mean square
MSE	Mean square of error
NJ	Neighbor joining
NTSYS	Numerical Taxonomy System
OARI	Oromia Agricultural Research Institute
PCA	Principal Component Analysis
PCoA	Principal Coordinated Analysis

PCR	Polymerase Chain Reaction
SAS	Statistical Analysis System
SSR	Simple Sequence Repeat
SS	Sum Square
TBE	Tris-borate-EDTA
TE	Tris-EDTA
Tris	Tris-hydroxymethyl amino methane
UPGMA	Unweighted Pair Group Method with Arithmetic mean

1. INTRODUCTION

1.1. Background and justification

Population growth, dwindling agricultural land, and climate change present increasing risks to crop production. The impact of these factors can easily be felt in a country like Ethiopia where the overall economic growth hinge on the success of the agriculture sector. Particularly, the importance of pulses such as chickpea cannot be overemphasized because of their significance in sustaining food security, balancing ecosystem, and generating revenue. Pulses are important local food crops in Ethiopia. They account for about 11.89% of the annual crop production and chickpea is the third in total production (4,097,331.63 ton), yield per unit area (1.71 t ha⁻¹) and in area coverage (239,512.43 ha) among all the pulses preceded only by Faba bean and Haricot bean (CSA, 2013). In farming systems, pulses contribute for input saving and resource conserving due to their biological nitrogen fixations that reduce the necessity of chemical fertilizer (Keneni *et al.*, 2012).

Chickpea (*Cicer arietinum* L.; $2n = 2x = 16$) is self-pollinating grain legume of Near-East origin with unique natural history. Currently, the crop is grown in over 50 countries of the world and it is one of the ancient crops in Ethiopia. Archaeological evidence from Lalibela caves discovered seed samples dated to Iron Age (Dombrowski, 1970). The ecological and economic contribution of chickpea is high in Ethiopia; however, its productivity is far below the potential because of several biophysical and socioeconomic constraints. It is believed that genetic potential yields of chickpea under the ideal condition on experimental plots may reach 5 t ha⁻¹ (Muehlbauer and Tullu, 1997). Improved varieties released in Ethiopia are reported to yield 2.8 t ha⁻¹ on research stations (Kassie *et al.*, 2009) and 1.8 t ha⁻¹ on farmers' fields (Jarso *et al.*, 2011; Keneni *et al.*, 2012). Nevertheless, the national average yield is low (1.71t ha⁻¹) (CSA, 2013).

Biotic and abiotic stresses cause significant economic losses to crop plants. Stress due to acidity was one of the most important abiotic stresses with

worldwide distribution. Agricultural practices and climate changes increase the amount of land affected by acidity and thus limit legume crop productivity including chickpea. Acidic soils limit crop production on 30-40% of the world's arable land and up to 70% of the world's potentially arable land. Soil acidity is one visible obstruction to agricultural production across most parts of Ethiopia where an estimated 40% of arable land is currently affected (Batjes, 1995; Abebe, 2007). Although, acid soils occur widely in nature, human activities such as acid rain resulting from atmospheric pollution by industry, the use of nitrogenous fertilizers applied in excess of immediate crop requirements, the removal of basic nutrients (calcium, magnesium and potassium) in harvested crops and animal products and accelerated decomposition of soil organic matter as a result of tillage markedly accelerate acidification.

Soil acidity problem that occurs particularly in central and western zones of Oromia is very critical and deserves immediate intervention to amend the soils for crop production (Abdenna *et al.*, 2007). Soil pH is probably the most important principal chemical soil parameter and it mirrors the overall chemical status of the soil and influences a whole range of chemical and biological processes occurring in the soils. Most plants and soil organisms prefer pH range between 6.0 and 7.5 (Hazelton and Murphy, 2007; Hall, 2008). Different scholars reported the pH of the soils in western Ethiopia is out of this normal pH range (Chimdi *et al.*, 2012; Deressa *et al.*, 2013). Under such low pH, the availability of essential nutrients is critically affected. As pH falls, Aluminum is increasingly released from natural soil minerals into the plant-available soil solution. Aluminum in acidic soil solutions is bound to cell walls in emerging root tips resulting in root stunting. Toxicity of aluminum to plants greatly affects root and shoot growth as well as nutrients and water absorption (Abebe, 2007). Moreover, the activities of microorganisms which play pivotal roles in nutrient cycling in agroecosystems are affected (Addisu, 2007).

Adding lime to the soil can ameliorate soil acidity effects on plant growth. However, they are both costly and ecologically unsound. The most efficient

way to cope with soil acidity is breeding tolerant cultivars. Genetic variation for tolerance to soil acidity has been reported in several studies using different germplasm, different traits, and different genetic analyses. Plants generally vary significantly in acid soil tolerance and this variation makes it possible to breed tolerant cultivars. All the variability present in biological systems can be attributed to genotype and/or environment (Allard, 1960; Welsh, 1981) where variability may be phenotypic and genotypic. Genotypic variability is the component of variation, which is due to the genotypic differences among individuals within a population and is the main concern of plant breeders all over the world (Johnson *et al.*, 1955). Consequently, our understanding of organism's genetics now extends from phenotypes to molecular levels, which can lead to new or improved screening methods for selecting superior genotypes more efficiently and improve the decision-making process in breeding strategies. To this effect, understanding of the genetics and its information warrants the success of breeding programs on acidic soil.

The phenotype of an organism is not only determined by the genetic makeup of organism but also with the effect of the environment and the interaction of genotype with an environment with one another. One way to estimate the genetic variability is through morphological traits study. It is the earliest genetic marker used for assessment of variation and still has great importance. Nevertheless, low heritability and high genotype by environment interactions make it difficult to determine genetic variation precisely for the discrete morphological traits (Gilbert *et al.*, 1999).

Genotype by environment interaction ($G \times E$) is one of the main difficulties in the selection of broad adaptation in most breeding schemes. In most cases $G \times E$ is regarded as a complication for crop improvement program. However, it offers an opportunity of selecting and using genotypes that show positive interactions with locations and the prevailing environmental conditions thereby exploiting specific adaptability or yield stability (Ceccareli, 1996; Annicchiarico, 2002). When environmental differences are large like as in Ethiopia, it may be expected that the interaction of genotype by environment will also be higher. As a result, one cultivar may have the highest yield in

some environments while another cultivar may still surpass in others. These fluctuations are often referred as $G \times E$.

Various methods have been proposed by different authors to measure the stability of genotypes over environments. However, two multivariate models viz., additive main effects and multiplicative interaction models (AMMI) and the genotype main effects and genotype by environment interaction effect (GGE) model were used prevalently (Rad *et al.*, 2013). The AMMI Model appeared to be able to extract a large part of the $G \times E$ and was efficient in analyzing interaction patterns. The GGE- methodology was used to visually analyze the multi-environment yield trial data (Rad *et al.*, 2013).

Determining genetic variation accurately through morphological traits was complicated by environmental effect, and, therefore, DNA markers have proved valuable in crop breeding, especially in studies on genetic diversity and gene mapping. The applications of Inter simple sequence repeated (ISSR) markers mainly in plant genetics was for genetic variation studies. ISSRs have been successfully used to estimate the extent of genetic diversity at inter- and intra-specific level in a wide range of crop species (Zietkiewicz *et al.*, 1994).

Despite the paramount importance of $G \times E$, in a country like Ethiopia, the information on the genotype by environment interaction effects in acid soils is quite limited. In a similar manner, the study that combines DNA marker and morphological tool in determining genetic variability that exists in chickpea genotypes in Ethiopia are quite limited. Therefore, the current study was undertaken to fill the above-mentioned gaps.

2. OBJECTIVES OF THE STUDY

2.1. General objective

To examine the magnitude of environmental effect and genetic variability in some released and advanced Desi-type chickpea genotypes at molecular and morpho-agronomic levels.

2.2. Specific objectives

The specific objectives of the study were: -

- ❖ To study the nature and extent of genotype by environment interaction on Desi-type chickpea genotypes,
- ❖ To investigate adaptation and stability of some released, and advanced chickpea genotypes under acidic soils of western Ethiopia, and
- ❖ To investigate prevailing genetic variability in Desi chickpea genotypes using morphological and ISSR markers.

3. LITERATURE REVIEW

3.1. Distribution of chickpea

Chickpea (*Cicer arietinum* L.; $2n = 2x = 16$), is an ancient crop that belongs to the legume family. It has been grown in Africa, the Middle East, and India for centuries. Chickpea is now widely cultivated in South America, Australia, both the European and African Mediterranean regions, the Balkans, East Africa, southern Asia from Myanmar to Iran, and the Middle East encompassing Iraq, Israel, and Turkey (Van der Maesen, 1972). Of the 17,250 accessions in the largest gene bank of chickpea landraces at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India, about 930 are from Ethiopia. At the International Centre for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria, there is also a report that ample number of accession exist collected from Ethiopia (Redden and Berger, 2007). Both gene banks broadly reflect the available sources of genetic diversity of chickpea landraces in Ethiopia.

3.2. Chickpea utilizations

Chickpea (*Cicer arietinum* L.) plays an important role in the diets of vegetarians around the world and provides affordable protein especially to rural populations. Energy values for chickpea have been reported to be 14–18 MJ/kg (334–437 Kcal/100 g) for desi types. The protein concentration of chickpea seed ranges from 16.7% to 30.6% for Desi and is commonly 2–3 times higher than cereal grains. The total lipid concentration of Desi ranges from 2.9% to 7.4% and it is most of the beneficial kind (monounsaturated and polyunsaturated) rather than saturated fats that have been linked to heart and circulatory diseases (Yadav and Chen, 2007). Starch is the principal carbohydrate constituent in chickpea seeds (30–57%) and is the main dietary energy source derived from chickpea. Although the concentrations of any given mineral vary depending on genotype and environmental constraints, chickpea contributes significantly to several minerals in the human diet. Chickpea also provides a substantial amount of vitamin and fibers that are essential for normal bodily growth and many metabolic processes. But it is

not to mean that chickpea consumption has no side effect. For example, the alpha-galactosidase that are found in chickpea is well known as anti-nutritional factors for causing flatulence (Yadav and Chen, 2007).

In Ethiopia, the green chickpea seed is utilized as vegetable or cooked and salted to be served as snacks. It is consumed mainly in the form of sauces made of kik (dehulled split and spiced) and shiro (slightly roasted, powdered and spiced). It is also consumed as kollo (soaked and roasted), nifro (boiled), shimbira (unleavened bread of chickpea with spice) and kitta (leavened bread). It is used to simulate fish where it is not available as a source of protein during the fasting period when animal products are not used up (Kassie *et al.*, 2009; Keneni *et al.*, 2012). Chickpea also generates significant amount of cash to the rural population in Ethiopia.

3.3. Chickpea production in Ethiopia and in developing countries

The Sub-Saharan Africa region accounts for about 3.5% of world's total area of chickpea production. Ethiopia, followed by Malawi and Tanzania, is accountable for 52% of total area and 76% of total production in Sub Saharan Africa (FAOSTAT, 2010). It is also the sixth largest producer worldwide and contributes about 3% to the total world chickpea production (Akibode and Maredia, 2012). In Ethiopia, chickpea research collaboration began in the early 1980's (ICARDA, 2010). To date, joint evaluation of ICARDA breeding lines led to the release of more than four improved wilt resistant Kabuli and Desi varieties, targeted primarily at mid-altitude areas in central Ethiopia. Improved production packages were also developed, combining appropriate planting date, weed control, pest management and other practices to maximize yields from the new varieties (ICARDA, 2010).

About 95% of the total chickpea cultivation area is in developing countries. India, Pakistan, Turkey, Iran, and Syria account for about 90% of the world chickpea production. In Africa, the major producers of chickpea are Ethiopia, Malawi, Morocco, and Tanzania, accounting for about 80% of total production. Latin America accounts for about 3% of total chickpea production with Mexico accounting for 99% of the area under the crop (Joshi *et al.*, 2001).

Worldwide there was a minimum yield increment of chickpea from 0.74-ton ha⁻¹ in the 1990's to 0.81-ton ha⁻¹ in 2000's (Akibode and Maredia, 2012). In the same manner from 1994 to 2008 chickpea production in developing countries increased in a small amount from 7.82 to 8.28 million tons. Yield increment per hectare is in the range of 0.74 to 0.79 tons per hectare, and it is extremely very low (FAOSTAT, 2010).

3.4. Genotype by Environment Interaction

Due to the inter-play between genes and the environment, cultivars grown in different environments will frequently show significant flux in yield performance (Kandus *et al.*, 2010). Crop researchers and breeders are conscious of differences in performance among crop cultivars, both geographically and yearly, indicating the presence of genotype by environment interactions. In the absence of G×E, the best cultivar in any one trial would yield more than all cultivars at all locations every year. But this might not be realized and hence breeders usually come across situations where the relative rankings of varieties change from location to location and/or from year to year thereby imposing problems in identifying superior cultivars (Kandus *et al.*, 2010). Therefore, understanding genotype by environment interaction (G×E) can be used to establish breeding objectives, to identify ideal test conditions, and to formulate recommendations for areas of optimal cultivar adaptation (testing general and specific cultivar adaptation). It can also help to reduce the cost of extensive genotype evaluation by eliminating unnecessary testing sites and by fine tuning the breeding schemes (Alberts, 2004). The presence of a large G×E may dictate establishment of additional testing sites, thus increasing the cost of developing commercially important varieties (Magari and Kang, 1993).

In fact, the effect of G×E cannot be eliminated where the genotypes are grown not even on diverse ecology but also in a vicinity that looks similar. The best thing is, therefore, to reduce its influence in a very fashionable way. There are two major strategies for developing genotypes with low G×E. The first is sub-division or stratification of a heterogeneous area into smaller more identical sub-regions, with breeding programs aimed at developing genotypes for

specific sub-regions. However, even with this amendment, the impact of interaction cannot be avoided, because the breeding area does not reduce the interaction of genotypes with locations and years. The year effect on genotypes cannot be controlled, and thus the genotype \times year (G \times Y) interaction could be ignored for practicality and/or making the situation simpler so that only the G \times E is evaluated. The second strategy for reducing G \times E involves selecting genotypes with better stability across a wide range of environments (Eberhart and Russell, 1966).

Therefore, better understanding of the level of G \times E and performance stability in crops serves as a decision tool, particularly at the final stage of variety development process to generate essential information on pattern of adaptation in breeding lines, new varieties for release, and to determine the recommendation domains for released varieties (Alberts, 2004).

Successful cultivars might have a good yield and other essential agronomic characters and in the meantime, their performance should be reliable over a wide range of environmental conditions. The study that involves G \times E were reported on maize (Alberts, 2004; Issa, 2009; Kandus *et al.*, 2010), cotton (Blanche, 2005), wheat (Purchase, 1997; Akcura and Kaya, 2008) and chickpea (Getachew *et al.*, 2015). However, their studies did not involve acidic soils as a concern. Furthermore, characterizations and evaluations of germplasm lonely based on quantitative morpho-agronomical characters are not stable because of polygenic nature of the quantitative characters and the genotype by environment interaction effect. Therefore, considering G \times E is important in studies that aim to improve chickpea for tolerance to acidic soils in the country.

3.4.1. Statistical methods to measure G \times E Interaction

There are many statistical methods available to analyses the G \times E interaction: for example, combined ANOVA, stability analysis and multivariate methods. These statistical methods can be classified into two groups: univariate and multivariate.

Multivariate analysis has three main purposes: 1) to eliminate noise from the data pattern (for example, to distinguish systematic and non-systematic variation); 2) to summarize the data, 3) to reveal the structure in the data (Wolfinger *et al.*, 2000). Due to this peculiar character, Multivariate models have been widely used to analyze genotype by environment interaction (G×E). Multivariate models include a wide range of methods such as principal component analysis (PCA), cluster analysis (Mungomery *et al.*, 1974), genotype main effect plus genotype by environment interaction (GGE) biplot analysis (Ding *et al.*, 2008), and additive main effects and multiplicative interaction (AMMI) models (Gauch and Zobel, 1988).

The additive main effect and multiplicative interaction (AMMI) method integrate analysis of variance (ANOVA) and principal component analysis (PCA) into a unified approach that can be used to analyze multi-location trials (Zobel *et al.*, 1988; Gauch and Zobel, 1988; Crossa *et al.*, 2002). AMMI uses analysis of variance to study the main effects of genotypes and environments and principal component analysis for the residual multiplicative interaction among genotypes and environments. In addition, AMMI simultaneously quantifies the contribution of each genotype and environment to the sum square of G×E and provides an easy graphical interpretation of the results by the AMMI biplot technique (Kempton, 1984; Zobel *et al.*, 1988).

On the other hand, GGE which refers to genotype main effect (G) and G×E biplot could display mean performance and stability of cultivars. Therefore, with this technique, one can readily identify productive cultivars with wide adaptability and mega-environments, as well as define the agronomic zoning of cultivars with specific adaptability and identify environments in which to conduct tests (Lule *et al.*, 2012). A number of studies were undertaken using these models to exploit G×E using different crops (Alberts, 2004; Kandus *et al.*, 2010; Hongyu *et al.*, 2014; Tadesse and Abay, 2011; Tolessa, 2015; Getachew *et al.*, 2015; Dabessa *et al.*, 2016).

3.5. Soil acidity

3.5.1. Global distribution of acidic soils

More than 1.5 billion hectares – about 12 percent of the world's land area are used for crop production. However, a significant proportion of this land is lost due to soil acidity. In the 1970's, there were about 3 billion hectares of acid soils in the world. A survey conducted twenty years later indicated the projection of acidic soil to 4 billion hectares. Furthermore, it is now estimated that approximately about 50% of the world's arable land is acidic (Kochian *et al.*, 2015). Acid soils occur widely in nature, human activities such as acid rain resulting from atmospheric pollution by industry, the use of nitrogenous fertilizers applied in excess of immediate crop requirements, the removal of basic nutrients (calcium, magnesium and potassium) in harvested crops and animal products and accelerated decomposition of soil organic matter as a result of tillage markedly accelerate acidification. Consequently, soil acidity results in declining crop production all over the world (Samac and Tesfaye, 2003).

In Africa, 22% or 659 million ha of the total 3.01-billion-hectare land area has soil acidity problem (Von Uexk"ull and Mutert, 1995). In Ethiopia, it was estimated that more than 40% of Ethiopian land was affected by acidic soil out of which 33% of this area was due to Al-toxicity (Tolessa and Beshir, 2009). Abebe (2007) noted that the soil acidity problem of Ethiopia is mainly related to some of the Alfisols, and most Oxisols and Ultisols soil classes that occur in the west, north-western, south-western and southern parts of the country.

3.5.2. Principal causes of soil acidity, crop responses and remedies

A number of factors contribute to acid soil toxicity depending on soil composition (Samac and Tesfaye, 2003). One of the major acidity inducing factor is leaching of nitrate nitrogen sourced from legume fixation or from ammonium fertilizers (Hede *et al.*, 2001). This acidity is neutralized by plants discharging an alkaline substance as they take up nitrate and to a smaller extent by conversion of nitrate to nitrogen gas (Hede *et al.*, 2001). While the

plants continue to take up all the nitrate nitrogen, the acid/alkali balance of the soil surrounding the roots remains in balance (Clough *et al.*, 2013). However, nitrate is very soluble and easily leached and, therefore, leaching breaks the balance of the acid/alkali processes and results in increased soil acidity. Rapid acidification problem in China was associated with the overuse of nitrogen fertilizer because of depletion of soil (Guo *et al.*, 2010) and Ethiopia is not an exception to such trend (Deressa *et al.*, 2013).

Soil acidification, or a decrease in soil pH, is also caused by a natural process that is accelerated by crop production practices, primarily the use of nitrogen (N) fertilizers such as urea, ammonium sulfate, or other fertilizers containing Ammonium-N and high rainfall (Brett *et al.*, 2013).

Crop tolerance of low soil pH has become extremely important in the agricultural development of the humid tropics because so many of those soils have low pH (Kamprath and Foy, 1985). Some crops are more tolerant than others, and even between varieties within each crop species, there can be wide ranges in tolerance of soil acidity. Generally, legumes are the most sensitive to soil acidity while wheat and barley are less sensitive, followed by triticale and grass hay/seed crops, which can tolerate more acidic soil. For legumes, acid soils also pose an additional challenge because their symbiotic rhizobia are acid sensitive (Hartel and Bouton, 1991).

In acid soils with a high mineral content, the primary factor limiting plant growth is Aluminum toxicity. For most agriculturally important plants, Aluminum ions rapidly inhibit root growth at micromolar concentrations. In response to aluminum, developing root cells accumulate callose, suggesting that the inhibition of root elongation is in part due to use of sugar substrates for callose formation instead of cellulose formation (Huang *et al.*, 2013). In organic acid soils, H ions dominate in the soil solution. Although organic acid soils make up a large proportion of acid soils worldwide, the effect of H⁺ ions on plant growth has not been well studied.

The genetic and physiological basis of Acid tolerance has been investigated in several crop and model plant species. For instance, two main types of Al

resistance mechanisms have been documented: (a) Al exclusion mechanisms, which aim at preventing Al from entering the root apex (both apoplastic and symplast), and (b) Al tolerance mechanisms, in which Al enters the plant and is detoxified and sequestered (Delhaize and Ryan, 1995; Kochian *et al.*, 2015). Al resistance via Al exclusion from the root apex, the primary site of Al toxicity, involves the regulated release of organic compounds from the root tip (Delhaize and Ryan, 1993; Kochian *et al.*, 2015). By far the well-characterized Al exclusion mechanism is Al-mediated root exudation of organic acid (OA) anions into the rhizosphere, where they chelate Al³⁺ ions, forming non-toxic compounds that do not enter the root (Kochian *et al.*, 2015).

Numerous studies in a range of monocot and dicot species demonstrated similar correlations between variation in Al resistance and the Al-activated exudation of malate, citrate, and (in some species) oxalate from roots (Mayer *et al.*, 2012). The primary differences between plant species that employ this Al resistance mechanism are (a) the identity of the organic acids released (malate, citrate, or oxalate), (b) the magnitude of the organic acids exudation, and (c) the time course of Al induction and activation of the organic acid exudation. Although many plant species release one organic acid, it is not unusual to find plant species that release more than one organic acid in response to Al stress [e.g., malate and citrate in wheat and *Arabidopsis* (Liu *et al.*, 2009), indicating that multiple release mechanisms or transporters are operating in tandem in these species (Osawa and Matsumoto, 2002; Liu *et al.*, 2009).

In recent time, with an advancement of technology, the search for novel genes that are responsible for resistance of crops under the acidic soil, bring a new area of concern and tremendous studies are conducted in biotechnology and genetics with fruitful results. In this regard, the first Al resistance gene, TaALMT1, which encodes an Al-activated malate efflux transporter in wheat root apices was identified (Sasaki *et al.*, 2004). In the same manner, Al-activated anion channels that underlie Al-activated malate efflux and Al resistance in wheat (Ryan *et al.*, 2009) and the first rice Al resistance genes (STAR1 and STAR2) involved in transporting cell wall-modifying substrates

from the root cytoplasm was reported (Huang *et al.*, 2009). Two international agricultural research centers, CIAT (Colombia) and IRRI (Philippines), have collaborated in the development of modern rice varieties that tolerate Al saturation of 50–75 percent with scarcely any yield reduction and achieve a tremendous and promising result (Marschner, 1991).

Several strategies have been pursued to manage acid soils including an application of lime (calcium carbonate) to raise soil pH and cause conversion of Al to less toxic forms. However, these soil amendments are not practical in many locations, such as highly erodible slopes, nor are they economical where large areas require amendment or where transportation costs are prohibitive. In addition, soil pH below the plow layer is raised very slowly by liming (Dall'Agnol *et al.*, 1996). Therefore, morphological, physiological and molecular characterization of plants that better tolerate acidity under the usual condition and give rise better yield and economic turn for poor farmers is a promising alternative or supplement to liming and related agronomic practices.

3.6. Inter simple sequence repeat (ISSR) marker

ISSR involves amplification of DNA regions present at an amplifiable distance in between two identical microsatellites repeat regions oriented in opposite direction (Reddy *et al.*, 2002). ISSR is simply inherited as dominant and reliable marker system for many organisms. ISSRs use longer primers (15–30 mers) as compared to RAPD primers (10 mers), which permit the subsequent use of high annealing temperature leading to higher stringency.

ISSR is a fast and simple system with a cost-efficient as well as it does not require any prior knowledge about the sequences to be amplified making it an ideal genetic marker for beginners and for organisms whose genetic information is lacking and thus it is immensely useful molecular marker in genetic diversity, phylogeny, genomics and evolutionary studies (Bhagyawant and Srivastava, 2008).

ISSR markers usually show high polymorphism (Kojima *et al.*, 1998) although the level of polymorphism has been shown to vary with the detection method

used. Fang and Roose (1997) reported a reproducibility level of more than 99% after performing repeatability tests for ISSR markers by using DNA samples of the same cultivar grown in different locations, DNA extracted from different aged leaves of the same individual, and by performing separate PCR runs. In other cases, the reproducibility of ISSRs amplification products ranged from 86 to 94%, with the maximum amplification when polyacrylamide gel electrophoresis and AgNO₃ staining were used and weak bands excluded from scoring (Moreno *et al.*, 1998). These features make ISSR an ideal genetic marker for various studies, such as genetic diversity, DNA fingerprinting and phylogenetic studies. Consequently, ISSR fingerprinting has commonly been used in different crops such as lentil (Toklu *et al.*, 2009), soybean (Brick and Sivolap, 2001), peanut and chickpea (Bayraktar and Dolar, 2009).

4. MATERIALS AND METHODS

4.1. Description of the study areas and planting materials

A field experiment was conducted under field condition at five locations viz., Shambu, Hawa Galan, Mata, Alaku Belle and Badesso in western Ethiopia for one year during the main cropping season of 2016/2017 (Figure 1). A total of sixteen (Table 1) Desi chickpea varieties viz., eight cultivars released over three decades, one local variety and seven advanced lines acquired from Debre Zeit Agricultural Research Center (DZARC) were grown at the above-mentioned locations. At each site the experiment was laid out in a randomized complete block design (RCBD) with three replicates. The plot size was six rows of three-meter length (5.4 m²). The central four rows were harvested to determine seed yield. Diammonium phosphate fertilizer (DAP) with a rate of 100 Kg per hectare was used and all other crop management and protection practices were applied uniformly to all varieties as necessary.

Table 1. Passport description of the test genotypes.

Codes	Genotype names	Status	Year of release
G1	Akaki	Released	1995
G2	Dalota	Released	2013
G3	Dimtu	Released	2012
G4	Dubie	Released	1978
G5	Local	Local variety	-
G6	Mariye	Released	1985
G7	Minjar	Released	2010
G8	Natoli	Released	2007
G9	Teketay	Released	2013
G10	DZ-2012-CK-0032	Advanced line	-
G11	DZ-2012-CK-0034	Advanced line	-
G12	DZ-2012-CK-0233	Advanced line	-
G13	DZ-2012-CK-0237	Advanced line	-
G14	DZ-2012-CK-0312	Advanced line	-
G15	DZ-2012-CK-0313	Advanced line	-
G16	DZ-2012-CK-20113-2-0042	Advanced line	-

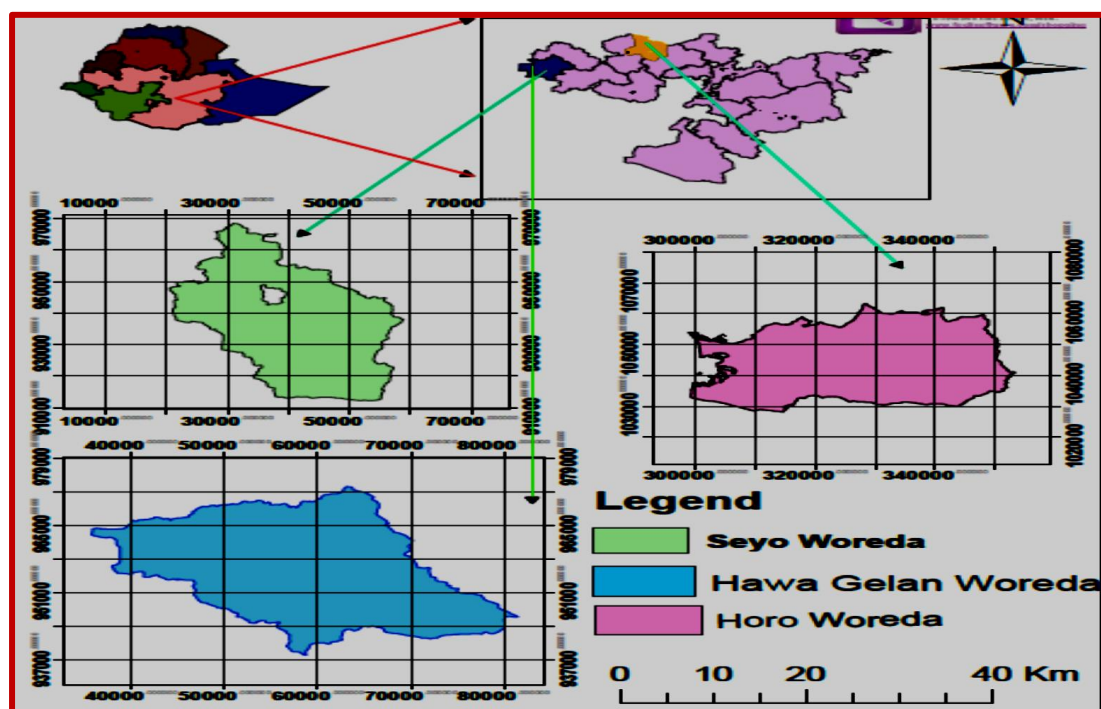


Figure 1. Map showing the experimental sites.

Table 2. Description of experimental sites for geographical locations.

Parameters	Sites				
	Shambu	H. Galan	Mata	A. Belle	Badesso
Latitude	09° 32'N	08° 38' N	08° 34' N	08° 37'N	08° 40' N
Longitude	037° 04'E	034° 50'E	034° 44'E	034° 42'E	034°47'E
Altitude (m.a.s.l.)	2776	1905	2016	2050	2054

4.2. Soil sampling and analysis

Soil sample was taken at a depth of 0-20 cm in a zigzag pattern randomly from each experimental field. Soil pH was measured potentiometrically with a digital pH meter in the supernatant suspension of 1:2.5 soils to water ratio and KCl extract. The electrical conductivity was also measured for the same supernatant suspension using conductivity meter. Cation Exchange Capacity (CEC) and exchangeable bases (Ca, Mg, K and Na) were determined by extracting with 1.0 M ammonium acetate (NH₄OAc) solution at pH 7 (Chapman, 1965). The extracts of Ca and Mg ions were determined using atomic absorption spectrophotometer (AAS) while K and Na were determined

by flame photometer. Organic carbon was determined following the wet digestion method as described by Walkley and Black (1934). Total nitrogen (TN) was determined by the Micro-kjeldahl procedure as described by Jackson (1958). Exchangeable acidity was determined by extracting the soil samples with 1M KCl solutions and titrating with standardized NaOH as described by McLean (1965). Exchangeable aluminum was determined by Volumetric-KCl extraction method.

4.3. Agronomic data collections

The following morphological data were collected conferring chickpea (*Cicer arietinum* L.) descriptor (IBPGR, ICRISAT and ICARDA, 1993) as follows: -

Data collected on plant basis as follows: -

Number of pods per plant (NPPP): The average numbers of pods counted from samples of five plants taken randomly from each plot.

Numbers of seeds per pod (NSPP): 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.

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

Data collected on plot basis as follows: -

Days to 50% flowering (DF): Number of days from sowing to the stage when 50% of plants have begun to flower.

Days to maturity (DM): The number of days from sowing to the stage when over 90% pods have matured and turned yellow.

Grain filling period (GFP): The number of days from flowering to maturity (i.e. the number of days to maturity minus the number of days to flowering).

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

Grain yield (GYLD): Grain yield (g) from the specified harvestable plot area and adjusted to its recommended (10%) moisture content. This value was converted to ton ha⁻¹ and used for analysis.

Harvest index (HI): harvest index as the ratio of grain yield to above ground biological yield.

4.4. DNA extraction

Equal amount of seeds from each genotype were planted under greenhouse conditions at Addis Ababa University. Two weeks after planting approximately equal amount of bulk leave samples was collected mainly from actively growing leaves and preserved by silica gel. The samples were ground using a mixer mill (Restch MM 400) and genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method (Borsch *et al.*, 2003) with some minor modifications. About 700 μ l of a warm CTAB solution was added to each sample to break open plant cells and solubilize the contents. The sample was shortly mixed using a vortex, kept in gently shaking water bath (CU 420) for 30 minutes at 65°C and centrifuged (Micro 20 centrifuge) for 7 minutes at 1300 rpm to separate an organic phase. The supernatant was transferred in a new Eppendorf-cap. Chlorophyll and some denatured proteins were removed from the samples by dissolving in 600 μ l chloroform. About 2/3 of total solution volume isopropanol was added, frozen for more than 2 hours at -20°C and the DNA was precipitated by centrifugation for 15 minutes at 13000rpm. The isopropanol was removed and the DNA was cleaned by repeated washing with 70% ethanol and centrifugation. Then the pellet was air dried and dissolved in 100 μ l of 1X TE.

4.5. Polymerase chain reaction (PCR)

The quality and quantity of DNA were tested spectrophotometrically using a Nanodrop® 1000 (Thermos Scientific, USA) and the amount in each sample was quantified on an agarose gel (0.8%). PCR reaction was performed in a thermal cycler (Biometrical T Personal Thermocycler) in a total volume of 25 μ l containing 2 μ l DNA, 0.8 μ l dNTPs, 2.5 μ l buffer 10x, 0.4 μ l primer and 0.3 μ l Taq DNA polymerase in 16 μ l dH₂O.

The PCR was programmed at an initial denaturation step of 4 minutes preheating and initial denaturation at 94°C followed by 40 cycles of 15 seconds denaturation at 94°C, 60 seconds primer annealing at 45 - 53°C

(depending on the primer), 90 seconds extension at 72°C, 7 minutes' final elongation at 72°C and holding temperature at 4°C until loading on gel for electrophoresis. Polymerase chain reaction (PCR) optimization for primers amplification was carried out.

Samples were loaded on 1.67% agarose gel with a 6x DNA loading dye. Electrophoresis was carried out on an electrophoresis (Biometra® standard power pack p25) using a standard DNA ladder (*Hi MEDIA* 100bp) with known reference bands.

The gel was stained with Ethidium bromide for 20 minutes and visualized under UV light (BIO-RAD GEL DOC™ EZ Imager) on a gel documentation system and saved in an attached computer for further analysis. Primers with unclear and missing bands were sorted and rejected. Clearly distinguishable and reproducible bands were selected for scoring.

Table 3. List of ISSR primers used in this study with repeat motifs and amplification.

Primers	Repeat motif	Amplification
UBC-810	(GA)8T	Amplified
UBC-811	(GA)8C	Not amplified
UBC-815	(CT)8G	Not amplified
UBC-834	(AG)8YT	Amplified
UBC-835	(AG)8YC	Not amplified
UBC-855	(AC)8TT	Not amplified
UBC-873	(GACA)4	Not amplified
UBC-880	(GGAGA)3	Not amplified
UBC881	(GGGGT)3	Amplified

4.6. Data analysis

4.6.1. Morphological data analysis

Analyses of variances were performed for each environment and combined across environments for each trait using SAS 9.0 Version (SAS Inc., 2003). The combined analysis was used to create an analysis of variance (ANOVA) table to determine the presence or absence of genotype by environment interactions (G×E). Bartlett's test of homogeneity was used to check the

homogeneity of variances between environments before performing combined analyses of variance.

The percentage of total variation attributed to environment, genotype, or genotype-environment interaction (G×E) was calculated using the sums of squares from the ANOVA table. Additive Main Effects and Multiplicative Interaction Model (AMMI) which help to visualize relationships among genotypes and environments indicating both main and interaction effects were analyzed using GenStat software.

AMMI method as described in Zobel et al. (1988) was used to analyze adaptability and phenotypic stability using the following statistical model:

$$y_{ij} = \mu + g_i + e_j + \sum_{k=1}^n \lambda_k \alpha_{ik} \gamma_{kj} + r_{ij} + \varepsilon_{ij}$$

Where, Y_{ij} is the yield of the i^{th} genotype in the j^{th} environment; μ is the grand mean; g_i and e_j are the genotype and environment deviations from the grand mean, respectively; λ_k is the eigen value of the PCA analysis axis k ; α_{ik} and γ_{kj} are the genotype and environment principal component scores for axis k ; n is the number of principal components retained in the model and ε_{ij} is the error term.

AMMI Stability value was used for determining stability value of each genotype and ranking as suggested by Purchase et al. (2000) given below.

$$ASV = \sqrt{[(SS_{IPCA1} \div SS_{IPCA2})(IPCA1score)]^2 + (IPCA2score)^2}$$

Genotype Selection Index (GSI) that incorporate both mean yield and stability in a single index (Kang, 1993; Farshadfar, 2008) was calculated as: -

$$GSI = RASV + RY$$

Where, RASV is the rank of AMMI stability value and RY is the rank of mean grain yield of genotypes across environments.

Site regression analysis, SREG (Cornelius *et al.*, 1996; Crossa *et al.*, 2002), also called GGE (Genotype Main Effect and Genotype by Environment Interaction), which is suggested when the environments are the main source of variation in relation to the contributions of the genotypes and the genotype by environment interaction (G×E) with respect to the total variability was used to allow the determination of mega-environments, and detection of genotype by environment interaction (G×E) in terms of cross over and non-cross over effect (Casanoves *et al.*, 2005). The GenStat software (GenStat® 15th edition) was also used to generate graphs showing "which-won-where" pattern, the ranking of cultivars on the basis of yield and stability, location vectors, and comparison of locations to ideal location (Yan and Kang, 2003).

GGE biplot which is mostly useful for cultivar evaluation of the multi environmental trial was computed as suggested by Yan and Kang (2003) as follows,

$$\hat{y}_{ij} = \mu + \alpha_i + \beta_j + \phi_{ij}$$

Whereas,

\hat{y}_{ij} = the expected yield of genotype i in environment j ,

μ = the grand mean of all observations,

α_i = the main effect of genotype i ,

β_j = the main effect of environment j , and

ϕ_{ij} = the interaction between genotype i and environment j .

Hierarchical clustering of average linkage method with squared Euclidian distance was performed using MINITAB14 software (Minitab Inc., 2003). Data of all quantitative traits were standardized to a mean of zero and a variance of one before clustering to avoid bias that arises due to differences in measurement scales. The distances between clusters were assessed using Mahalanobis distance (D^2) for the standardized data.

Correlation coefficients between characters were estimated based on the standard procedure. Principal component analysis for ten standardized

quantitative traits was computed by using MINITAB14 software (MINITAB, 2003) to identify the most important contributing traits to the total variations observed among the genotypes. As suggested by Johnson and Wichern (1988), principal components with eigenvalues greater than one were considered.

4.6.2. Molecular data analysis

Molecular data scoring was performed as discrete statistics using a binary matrix as "0" for the absence of a band, "1" for the presence of a band, and "?" for the ambiguous band. POPGENE version 1.32 (Yeh *et al.*, 1999) was used to investigate and characterize genetic diversity in terms of a number of polymorphic loci, percent polymorphism, means of Nei's genetic diversity and Shannon index. NTSYS- pc version 2.02 (Rohlf, 2000) and Free Tree 0.9.1.50 (Pavlicek *et al.*, 1999) software were used to compute Jaccard's similarity coefficient by using the following formula: -

$$S_{ij} = \frac{a}{a + b + c}$$

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

NTSYS- pc (Numerical Taxonomy System, version 2.02 (Rohlf, 2000) was used to generate cluster analysis based on the Unweighted Pair Group Method with Arithmetic mean (UPGMA) for analyzing and comparing of individual genotypes and standardized genetic distance analysis (Nei, 1972). Neighbor joining (NJ) clustering method was computed by using Free Tree 0.9.1.50 Software (Pavlicek *et al.*, 1999) to compare individual genotypes and evaluate patterns of genetic diversity. Principal coordinate analysis (PCoA) was figured using PAST version 1.18 (Hammer *et al.*, 2001) software based on Jaccard's coefficient similarity for further examining the patterns of variation among individual genotypes.

5. RESULTS AND DISCUSSIONS

5.1 Chemical properties of the soils

The analyzed soil samples indicated very strongly acidic across all location with varying degree (Chapman, 1965). The soil had total nitrogen content that range from 0.22 to 0.40% which was high according to Bruce and Rayment (1982) where they classified soil total nitrogen availability of <0.05% as very low, 0.05-0.15% as low, 0.15-0.25% as medium, 0.25%-0.5% as high and >0.5% as very high. Organic carbon content of the soil ranges from 3.27 to 4.01 which is very high according to the classification of Bruce and Rayment (1982) where soils with organic carbon content of >3, 3-1.8, 1.8-1, 1- 0.6, 0.6-0.4, and <0.4% were classified as very high, high, moderate, low, very low and extremely low, respectively. Exchangeable Ca and Mg in the soil was high, whereas exchangeable Na was low in these soils. According to Melson (1961), top soils having CEC greater than >40, 40-25, 25-12, 12-6, and < 6 Cmol (+)/kg of soil are rated as very high, high, medium, low and very low, respectively. Depending on this delineation, the CEC that ranges 28.11- 41.53 Cmol (+)/kg is rated as high and very high.

Table 4. Analyzed chemical properties of soils.

Parameters	Sites				
	Shambu	H. Galan	Mata	A. Belle	Badesso
Organic C (%)	4.01	3.27	3.64	3.95	3.61
TN (%)	0.40	0.22	0.33	0.33	0.37
pH (1:2:5 H ₂ O)	4.59	4.96	5.3	5.19	5.26
pH (1:2:5 KCl)	4.09	4.3	4.59	4.44	4.65
Exch. Acidity	1.35	0.3	0.07	0.24	0.14
Exch. Al ³⁺	0.66	ND	ND	ND	ND
EC(dS/m)	0.27	0.06	0.06	0.17	0.09
CEC	41.53	28.52	36.16	35.74	36.07
Na	0.16	0.1	0.13	0.24	0.09
K	0.34	0.81	0.06	1.13	1.26
Ca	8.1	11.55	16.69	19.11	11.77
Mg	4.6	3.85	7.7	5.46	9.42

Key: - ND = results with less than the method's detection limit.

5.2. Analysis of variance

Pooled analysis of variance (Table 5) indicated that highly significant differences exist for genotypes, environments and most importantly genotype by environment interaction. Variance component of sum squares for treatments were 11% for genotypes, 55% for environments and 12% for genotype by environment interaction (Table 5). This indicates that environments were a much larger source of variability than genotypes in western Ethiopia. In addition to the environmental factors, the contribution of genotype by environment interaction was also appreciable.

The significant genotype by environment interaction (G×E) suggests that grain yield of genotypes varied across environmental conditions. Significant differences for genotypes, environments, and G×E indicated the effect of environments in G×E, genetic variability among genotypes, and the possibility of selecting stable genotypes. G×E with the location is more important than G×E with the year (Yan & Kang, 2003). Since G×E was significant, we, therefore, move further to estimate phenotypic stability (Farshadfar and Sutka, 2006).

Table 5. Combined analysis of variance of grain yield ha⁻¹ of chickpea genotypes tested across five environments.

Source of Variation	DF	SS	MS
Environments(E)	4	79.62	19.9***
Block(Env)	10	10.62	1.06***
Genotypes(G)	15	15.89	1.05***
G×E	60	17.56	0.29***
Error	150	19.76	0.13
Total	239	143.45	
CV	21.7		
LSD	0.262		
R²	87%		
Grand Mean	1.67		

The mean grain yield (ton ha⁻¹) of the sixteen genotypes tested at five environments of Western Ethiopia indicated statistically significant difference among genotypes. At Alaku Belle, genotype DZ-2012-CK-20113-2-0042 and Natoli were better performers while Dubie was the worst performer where the best performer genotypes exceeded it by more than twofold. At Badesso, genotype DZ-2012-CK-0237 performed better than any other genotype and Akaki was found to be the poorest of all at this location. At Hawa Galan and Shambu, genotype DZ-2012-CK-0032 out-performed the rest of genotypes while local variety was the poorest performer at both locations. At Mata, genotype, DZ-2012-CK-20113-2-0042 was the best performer as in Alaku Belle and the performance of the local variety was the poorest in a similar fashion it displayed in Shambu and Hawa Galan (Refer Table 6).

Table 6. Pooled over location mean grain yields (ton ha⁻¹) of chickpea genotypes tested in five environments.

Genotypes Code	Environments/sites					Mean
	AB	BD	HG	MT	SH	
G1	1.44	0.49	1.34	2.26	0.59	1.23 ^e
G2	1.76	1.31	1.48	2.51	0.73	1.56 ^{dc}
G3	1.94	1.88	1.49	2.62	0.75	1.74 ^{bc}
G4	1.22	1.24	1.46	2.25	0.68	1.37 ^{de}
G5	1.25	1.80	1.17	2.15	0.40	1.35 ^{de}
G6	1.55	2.13	1.25	2.33	0.49	1.55 ^{dc}
G7	2.06	2.11	1.60	2.75	0.86	1.88 ^{ab}
G8	2.46	2.20	1.65	2.95	0.94	2.04^a
G9	1.81	2.45	1.80	2.74	1.03	1.97 ^{ab}
G10	1.40	2.39	1.85	2.58	1.04	1.85 ^{ab}
G11	1.47	1.25	1.39	2.33	0.64	1.42 ^{de}
G12	1.66	2.43	1.42	2.48	0.66	1.73 ^{bc}
G13	1.87	3.07	1.38	2.59	0.63	1.91 ^{ab}
G14	1.28	1.53	1.30	2.21	0.53	1.37 ^{de}
G15	1.50	1.98	1.66	2.51	0.88	1.71 ^{bc}
G16	2.48	1.97	1.71	2.98	0.99	2.02 ^a
Mean	1.69	1.89	1.49	2.52	0.74	1.67

N.B. Different letters indicate significant differences among genotypes at $p < 0.05$, AB=Alaku Belle, BD= Badesso, HG= Hawa Galan, MT= Mata, and SH= Shambu.

This result is in agreement with the report of Getachew et al. (2015) who showed inconsistent performances of chickpea genotypes in central and eastern Ethiopia. Tamene (2015) conducted multi-locational studies of seventeen faba bean varieties and reported that the varieties responded differentially in southeastern and central Oromia. Similar result was noted on sesame in northern Ethiopia (Tadesse and Abay, 2011).

5.3. Agronomic performance

Differences among the genotypes were significant for a number of characters (Table 7). A local variety included in this investigation was the earliest to flower (60.07 days). However, this local landrace was intermediate for maturity relative to other genotypes. This indicates that local landraces had relatively a longer grain filling period. This result was in agreement with Summerfield and Roberts (1988) whose report indicated that early flowering genotypes do not certainly mature early and some late flowering genotypes have a short reproductive period and mature concurrently with earlier flowering ones. Wakeyo (2012) also tested one hundred fifty-five chickpea germplasms including landraces, improved varieties and some introduced pipelines indicating that landraces had a relatively shorter period of vegetative growth and longer grain filling periods. Except DZ-2012-CK-0032 (G10) and DZ-2012-CK-0233 (G12), all other improved varieties and advanced lines included in this study showed delayed flowering. This might be due to higher asset they employ at vegetative growth. In contrast to this, though Natoli was late to flower (71.60); it pays its late flower by filling the grain as short as possible (64.3). Mariye (72.33), Minjar (72.47), DZ-2012-CK-20113-2-0042 /G16 (73.80) and Teketay (74.07) acquired a short grain filling time whereas DZ-2012-CK-0312 (G14) was the late maturing genotype accompanied with a long grain filling period (78.27).

Minjar, Mariye and local landrace developed relatively higher number of pods than other genotypes. Even though improved genotypes display small difference among themselves for seed per pod; the difference with landraces was very high. On the other hand, in terms of plant height Mariye, Akaki,

Natoli and local landraces were the shorter whereas DZ-2012-CK-0313 (G15), Teketay (G9), DZ-2012-CK-0312 (G14), Dubie (G4) and Dimtu (G3) were comparatively the taller genotypes. Nevertheless, the high number pods bearing character of local landrace (G5), Natoli (G8), Mariye (G6) and Minjar (G7) were not only accounted from their respective height. Thus, the pod bearing character of these genotypes may emanate from branches they possess. The local landraces included in this study were by far inferior by their seed weight. Wakeyo (2012) also reported that the seed size of landraces was not comparable to improved genotypes and released varieties. However, among released and advanced genotypes there were differential seed weights. Some of the released cultivars, namely, Akaki (G1), Dubie (G4) and Minjar (G7) do also possess small seed weights. In contrary, DZ-2012-CK-0312 (G14) and Dimtu (G3) showed higher seed weight than all tested materials.

Table 7. Pooled over location mean of phenological traits, yield and yield components of chickpea genotypes grown in western Ethiopia at five locations.

Geno	DF	DM	GFP	NPPP	SPP	PH	BRN	HSW
1	63.47 ^{bcd}	138.33 ^{abcde}	74.87 ^{cde}	29.67 ^{cd}	1.34 ^{ab}	46.41 ^{ef}	3.78 ^c	23.64 ^g
2	62.87 ^{cde}	139.13 ^{abc}	76.27 ^{abcd}	28.07 ^{cd}	1.24 ^b	53.39 ^{abc}	4.04 ^{bc}	33.64 ^b
3	60.73 ^{fg}	138.73 ^{abcd}	78.00 ^{ab}	26.59 ^{cd}	1.16 ^b	55.47 ^{ab}	4.16 ^{bc}	36.56 ^a
4	61.53 ^{efg}	138.93 ^{abcd}	77.40 ^{abc}	32.15 ^{bcd}	1.15 ^b	54.04 ^{abc}	4.19 ^{bc}	23.46 ^g
5	60.07 ^g	138.07 ^{abcde}	78.00 ^{ab}	41.74 ^a	1.47 ^a	46.81 ^{def}	4.25 ^{bc}	14.01 ^h
6	64.87 ^b	137.20 ^{cde}	72.33 ^e	39.17 ^{ab}	1.23 ^b	44.35 ^f	4.52 ^{ab}	26.20 ^f
7	64.20 ^{bc}	136.67 ^{de}	72.47 ^e	42.60 ^a	1.26 ^b	52.11 ^{abc}	4.95 ^a	23.10 ^g
8	71.60 ^a	135.93 ^e	64.33 ^f	33.41 ^{bc}	1.28 ^{ab}	46.68 ^{def}	3.89 ^{bc}	32.40 ^{bcd}
9	64.87 ^b	138.93 ^{abcd}	74.07 ^{de}	29.36 ^{cd}	1.20 ^b	56.14 ^a	4.09 ^{bc}	31.70 ^{cd}
10	60.67 ^{fg}	138.60 ^{abcd}	77.93 ^{abc}	31.97 ^{bcd}	1.18 ^b	52.80 ^{abc}	4.13 ^{bc}	29.05 ^e
11	61.73 ^{defg}	139.80 ^{ab}	78.07 ^{ab}	26.05 ^{cd}	1.22 ^b	50.17 ^{cde}	3.88 ^{bc}	33.77 ^b
12	60.93 ^{fg}	138.47 ^{abcd}	77.53 ^{abc}	29.09 ^{cd}	1.22 ^b	53.94 ^{abc}	4.07 ^{bc}	31.10 ^d
13	64.80 ^b	139.93 ^{ab}	75.13 ^{bcd}	30.08 ^{cd}	1.16 ^b	51.15 ^{bcd}	4.36 ^{abc}	33.21 ^{bc}
14	62.20 ^{def}	140.47 ^a	78.27 ^a	26.37 ^{cd}	1.18 ^b	56.08 ^a	4.29 ^{bc}	38.35 ^a
15	61.8 ^{defg}	139.07 ^{abcd}	77.27 ^{abc}	29.46 ^{cd}	1.24 ^b	56.51 ^a	4.39 ^{abc}	32.47 ^{bcd}
16	64.07 ^{bc}	137.87 ^{bcd}	73.80 ^{de}	25.12 ^d	1.18 ^b	53.02 ^{abc}	3.75 ^c	31.47 ^{cd}
CV	4.02	2.43	5.75	32.78	21.82	11.96	21.71	8.55
R	90%	74%	80%	53%	33%	83%	45%	92%
LSD	1.83	2.43	3.12	7.4	0.19	4.47	0.65	1.83

Key: -DF=Days to flower, DM = Days to mature, GFP=Grain filling period, NPPP=Number of pod per plant, SPP=Seed per pod, PH =Plant Height, BRN=Branch Number, HSW=Hundred seed weight, GYLD=Grain yield ton per hectare. Within column different letters indicate significance difference (p<0.05).

Overall, Natoli (G8), G7 (Minjar), G9 (Teketay), G10 (DZ-2012-CK-0032), G13 (DZ-2012-CK-0237) and G16 (DZ-2012-CK-20113-2-0042) were the best performing genotypes. Akaki (G1), a variety released two decades ago, was the poorest variety across all tested environments followed by Dubie (G4), Local variety (G5), DZ-2012-CK-0034 (G11) and DZ-2012-CK-0312 (G14).

5.4. Additive Main Effects and Multiple Interaction (AMMI) Model

Only the first IPCA-1 explaining 63% of the total genotype by environment interaction ($G \times E$) was significant in the AMMI analysis of variance and thus indicate that the AMMI-1 model was the best fit for the data set. In AMMI-1 Biplot the x-coordinate indicates the main effects (means) and the y-coordinate indicates the effects of the interaction (IPCA-1). In AMMI-1 biplot the differences among genotypes in terms of direction and magnitude along the X-axis (yield) and Y axis (IPCA-1 scores) are important. The AMMI analysis of variance (ANOVA'S) with the fittest AMMI model was indicated in Table 8.

The first principal component attained over 63% of the genotype by environment interaction ($G \times E$) sum squares with 30% of the corresponding degrees of freedom while the second principal component declare 19% of the interaction, and the remaining 17% is due to residual (noise) and it is difficult to interpret and thus need to be rejected.

Table 8. Partitioning of the explained sum of square (SS) and mean of square (MS) from AMMI analysis of variance for grain yield of 16 chickpea varieties evaluated at five environments.

Source of Variation	DF	SS	Explained % SS	MS
Total	239	143.45	100	0.6
Treatments	79	113.08	78.83	1.43***
Genotypes	15	15.89	11.08	1.06***
Environments	4	79.62	55.50	19.9***
Block	10	10.62	7.40	1.06***
Interactions	60	17.56	12.25	0.29***
IPCA1	18	11.15	63.49	0.62***
IPCA2	16	3.41	19.40	0.21 ^{ns}
Residuals	26	3.01	17.11	0.12
Pooled Error	150	19.76		0.13

Different authors suggest that to obtain the accurate information it is desirable that most of the structural pattern of sum square of genotype by environment interaction ($G \times E$) be captured in the first axis (e.g., Gauch and Zobel, 1988). According to Kempton (1984) in AMMI model the first two interactions principal component axis was a best predictive model that explains the interaction sum of squares. Furthermore, Purchase (1997) illustrated that not only large IPCA but also significant parameters in the model are interpretable in terms of biological cause.

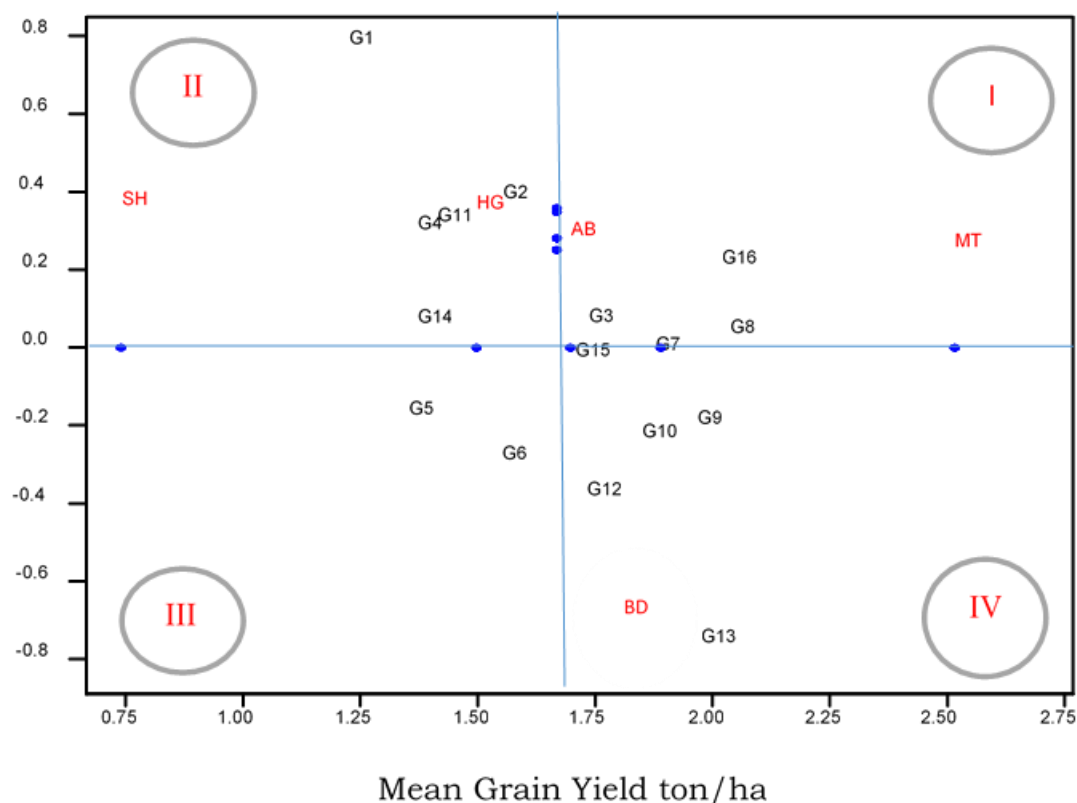
The IPCA scores of a genotype in the AMMI analysis is an indication of the stability of genotypes over environments. The greater the IPCA scores, either negative or positive, the more specifically adapted a genotype is to certain environments. Values closer to the origin of the axis (IPCA-1) provide a smaller contribution to the interaction than those that are further away. That is the more IPCA scores approximate to zero, the more stable the genotype is all over the environments sampled (Purchase, 1997). In other words, the ideal genotype is one with high productivity and IPCA-1 values close to zero and undesirable genotype has low stability associated with low productivity (Kempton, 1984; Gauch and Zobel, 1988).

In the AMMI biplot display, genotypes or environments that appear almost on a perpendicular line of the graph had similar mean yields and those that fall almost on a horizontal line had similar interaction (Alberts, 2004). Genotypes or environments on the right side of the midpoint of the perpendicular line have higher yields than those on the left side. The score and sign of IPCA-1 reflect the magnitude of the contribution of both genotypes and environments to genotype by environment interaction ($G \times E$), where scores near zero are characteristic of stability, and the higher score (absolute value) considered as unstable and specific adapted to a certain environment (Gollob, 1968).

The classification of the tested genotypes based on mean grain yield and their contribution to $G \times E$ by mean of IPCA-1 indicated that Natoli (G8), and DZ-2012-CK-20113-2-0042 (G16) were specifically adapted to high yielding environments Mata (MT) and Alaku Belle (AB) having a grain yield more than

grand mean yield; but with respect to their contribution to G×E (the IPCA-1 score i.e. stability), Natoli (G8) and DZ-2012-CK-20113-2-0042 (G16) were intermediately stable genotypes (Figure 2). However, Dimtu (G3), Minjar (G7) and DZ-2012-CK-0313 (G15) were shown to have a higher stability for yield than any other genotypes because these genotypes were positioned near the origin of the biplot.

However, any genotypes to be considered as best genotypes must combine a good grain yield and stable performance across a range of production environments. In this regard, Natoli (G8) and DZ-2012-CK-20113-2-0042 (G16) were higher yielder genotypes, and relatively stable. On the other hand, Dalota (G2), Dubie (G4), DZ-2012-CK-0034 (G11) and DZ-2012-CK-0312 (G14) were adapted to the low yielding environment and unstable. Local variety (G5) and Mariye (G6) were poor yielder genotypes and also phenotypically unstable. Akaki (G1) and DZ-2012-CK-0237 (G13) were the most unstable; that is, these genotypes had specific adaptations, because they were more distant from the biplot origin. Differential responses of chickpea genotypes in terms of yield and yield related traits were perceived both within and across locations. This signposted that the productivity of a breeding program intended at yield improvement is diminished due to genotype by environment interaction, which obscures the course of variety development specially when varieties are selected in one environment and recommended for wider cultivation. Similar result was reported in chickpea (Getachew *et al.*, 2015), maize (Issa, 2009), Sesame (Tadesse and Abay, 2011), and Faba bean (Mulusew *et al.*, 2008; Tolessa, 2015) in Ethiopia.



Key: - SH= Shambu, HG= Hawa Galan, MT= Mata, AB= Alaku Belle, BD= Badesso

Figure 2. Biplot of interaction principal component axis (IPCA-1) against mean yield of chickpea varieties evaluated across five environments.

Genotypes and environments positioned close to each other in the biplot have positive associations, and thus these enable us the creation of agronomic zones with relative ease. DZ-2012-CK-0237 (G13) had a specific adaptation to Badesso (BD), whereas Akaki (G1) was adapted to Shambu (SH) and Hawa Galan (HG) environments. The current result indicated that even under very heterogeneous environments (be it due to soil character and other agro-ecological condition) cultivars with wide geographic adaptation and high productivity ($> 2\text{-ton ha}^{-1}$) were identified. In addition, the suitable growing environments with better productivity were also recognized for each variety and genotype tested in the present study.

The environments showed much variability in both additive main effects and interactions (Table 5 and 8). In AMMI biplot environments are more scattered than the genotypes indicating that variability due to environments is higher

than that due to genotypes difference which is in complete agreement of ANOVA (Table 5).

The contribution of the environments to the interaction is high for Badesso (BD) and intermediate for others. The average yield in environments Mata (MT), Alaku Belle (AB), and Badesso (BD) exceed the grand mean (1.67-ton ha⁻¹). This might be directly related to the intensity of soil acidity of these locations.

The most potential environment, Mata (MT), having positive IPCA-1 score showed a differential performance of genotypes for grain yield. The lowest yielding environment, Shambu (SH), had recorded but positive IPCA-1 score suggesting that all the genotypes poorly performed under this environment. Alaku Belle (AB), Hawa Galan (HG), Mata (MT), and Shambu (SH) are less responsive whereas Badesso (BD) was the most differentiating environment since it is positioned distant from the origin. Similar result was reported in chickpea (Getachew *et al.*, 2015), maize (Issa, 2009), sesame (Tadesse and Abay, 2011), and faba bean (Mulusew *et al.*, 2008; Tolessa, 2015) in Ethiopia.

5.5. AMMI stability value (ASV) and Genotype Selection Index (GSI)

In AMMI model, the genotype with least AMMI stability value (ASV) score was considered as the most stable. Accordingly, Minjar (G7), DZ-2012-CK-0312 (G14), Dimtu (G3), Local variety (G5), Natoli (G8), Teketay (G9) and DZ-2012-CK-20113-2-0042 (G16) had general adaptation, while Akaki (G1), Dalota (G2), DZ-2012-CK-0233 (G12) and DZ-2012-CK-0237 (G13) were the most unstable and/or they are specifically adapted to certain environments. This result was in agreement with that of AMMI Biplot. Nevertheless, stable genotypes would not inevitably give the best yield performance and henceforth there is a need to find for approaches that integrate together both mean and stability in a single criterion.

In this regard, genotype selection index (GSI) was used to further identify stable genotypes with better yield performance. Consequently, Minjar (G7), Natoli (G8), DZ-2012-CK-20113-2-0042 (G16), Teketay (G9) and Dimtu (G3)

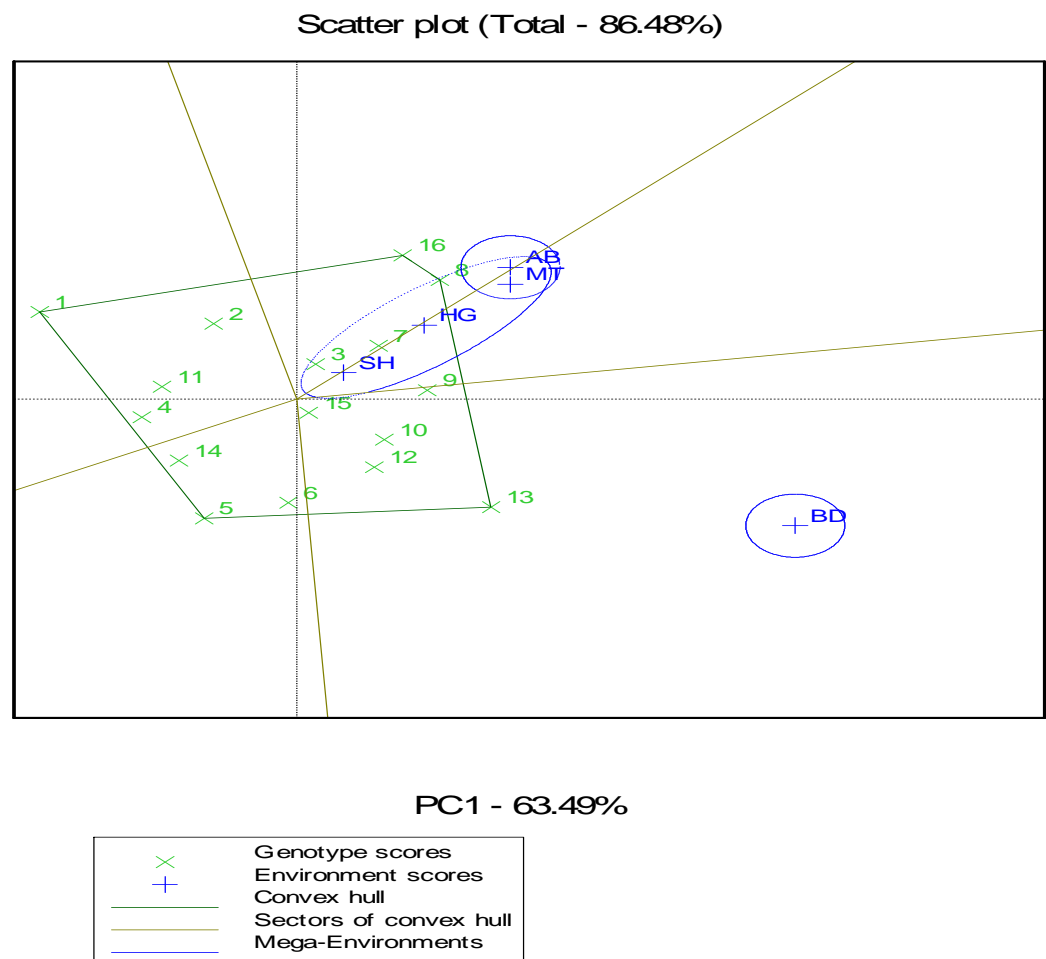
were considered as most stable genotypes, whereas, Akaki (G1), Dalota (G2), Dubie (G4), Local variety (G5), Mariye (G6), DZ-2012-CK-0034 (G11), DZ-2012-CK-0233 (G12) and DZ-2012-CK-0237 (G13) are the least stable genotypes.

Table 9. AMMI stability value, genotype selection index, yield rank and principal component axis.

Name of genotypes	Mean	Yield R.	ASV	ASV R.	GSI	IPCA-1	IPCA-2
G1	1.23	15	2.52	16	31	0.77	0.04
G2	1.56	10	1.23	13	23	0.38	-0.08
G3	1.74	7	0.28	3	10	0.06	-0.20
G4	1.38	12	1.02	11	23	0.30	0.33
G5	1.36	14	0.60	6	20	-0.18	0.10
G6	1.55	11	0.97	10	21	-0.30	-0.06
G7	1.88	5	0.21	1	6	-0.01	-0.20
G8	2.04	1	0.48	5	6	0.03	-0.47
G9	1.97	3	0.69	7	10	-0.20	0.16
G10	1.85	6	0.93	9	15	-0.24	0.51
G11	1.42	13	1.04	12	25	0.32	0.08
G12	1.73	8	1.26	14	22	-0.39	-0.02
G13	1.91	4	2.51	15	19	-0.76	-0.19
G14	1.37	13	0.25	2	15	0.06	0.17
G15	1.71	9	0.30	4	13	-0.03	0.28
G16	2.02	2	0.82	8	10	0.21	-0.45

5.6. Genotype and Genotype by Environment Interaction (GGE) Biplot Analysis

The sum of squares for the effects of the environment was by far greater than the sum of squares for the genotypes plus the genotype by environment interaction, being 55.5% and 23.33% of the total sum of squares, respectively (Table 8). This allowed the evaluation of the data using Site Regression analysis also called GGE (Genotype Main Effect and Genotype by Environment Interaction). The first two principal components for this model explained a proportion as high as 86.48% of the data variability. The right side of the biplot shows the higher yielding genotypes and vice-versa.



Key: - SH= Shambu, HG= Hawa Galan, MT= Mata, AB= Alaku Belle, BD= Badesso

Figure 3. Scatter plot showing the which-won-where pattern of the GGE biplot.

The polygon is drawn by joining the cultivars (G1, G5, G8, G16, and G13) that are located farthest from the biplot origin so that all other cultivars are contained in the polygon. The polygon can be called a convex hull, and the cultivars at the corner of the polygon can be called the vertex cultivars. Therefore, the vertex cultivars are those located farthest from the origin. They have the elongated vectors in their particular directions, which is a measure of responsiveness to environments. The vertex cultivars are, therefore, among the most responsive cultivars; while cultivars that positioned close to the origin are less responsive in their respective directions. Each sector has a vertex cultivar; a sector can, therefore, be named after the vertex cultivar. In this regard, the sector with vertex cultivar G13 may be referred to as the G13 sector; and environment BD fell in this sector. As a rule, the vertex cultivar is the highest-yielding cultivar in all environments that share the sector with it. In the same manner in G8 and G16 sector, four environments fell. No environments fell in the sectors with G1 and G5 as vertex cultivars and this indicates that these vertex cultivars were not the best in any of the test environments. Moreover, this indicates that these cultivars were the poorest in some or all of the environments. A cultivar located at the origin would rank the same in all environments and is not at all responsive to the environments.

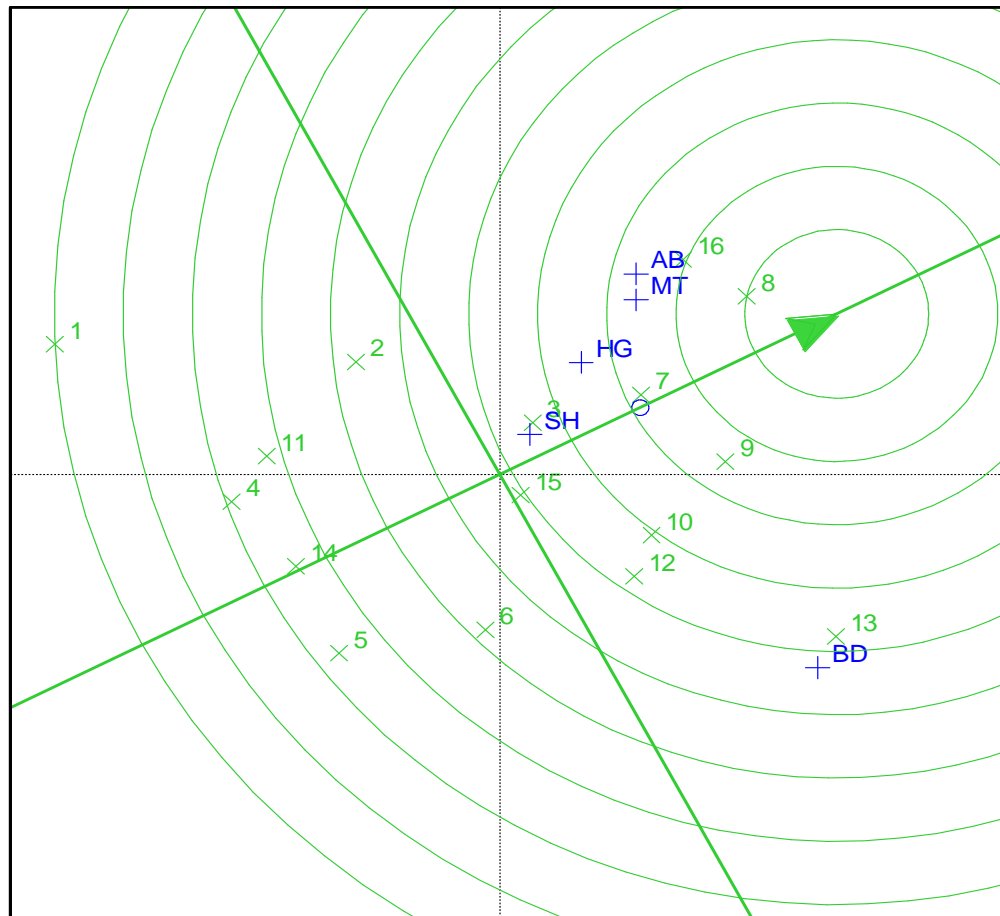
The polygon view of a GGE biplot not only shows the best cultivar for each test environment but also divides the test environments into groups. Though the existence of different mega-environments could be validated by multiple year data and specifically the "Which-Won-Where" pattern observed in one year should be repeatable in another year to conclude the existence of mega environment, the current study reveals two different mega-environments. The first Mega-environment consists SH, HG, AB and MT environments and while the second mega-environment consists BD environment. Genotypes 8, 16 and 13 were highest yielders. Genotypes 1 and 5 were lowest yielders and were the most extreme genotypes, and hence produced a greater contribution to $G \times E$. Genotypes located near the plot origin were less responsive than the vertex genotypes. Depending on this delineations genotype 15 and 3 were less responsive genotypes.

5.7. Evaluation of test environments and genotypes using GGE Biplot

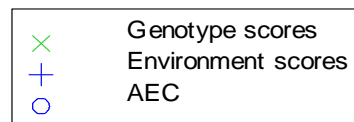
Visualization of the mean and stability of genotypes is achieved by drawing an average environment coordinate (AEC) on the genotype-focused biplot. First, an average environment, represented by the small circle, is defined by the mean PC1 and PC2 scores of the environments. Analogous to visualizing cultivar performances in a given environment, the line that passes through the biplot origin and the average environment may be called the average environment axis — it is the abscissa of the AEC. The AEC ordinate is the double-arrowed line that passes through the biplot origin and is perpendicular to the AEC abscissa. Therefore, if the AEC abscissa represents the genotype (G), the AEC ordinate must approximate the genotype by environment interaction (G×E) associated with each genotype, which is a measure of variability or instability of the genotypes. The double arrow indicates that a greater projection onto the AEC ordinate, regardless of the direction, means greater instability.

Therefore, environments and genotypes that fall in the central (concentric) circle are considered as ideal environments and stable genotypes, respectively. Genotype is more desirable if it is located closer to the ideal genotype. Though such an ideal genotype hardly exists in reality, the distance between any cultivar and ideal cultivar can be used as a measure of its desirability. Here, G8 (Natoli) fall in the central (concentric) circle and it is therefore considered as ideal genotype. On the other hand, G16 (DZ-2012-CK-20113-2-0042), G9 (Teketay) and G7 (Minjar) fall near to the ideal cultivar and are, therefore, most desirable of all the other tested cultivars. Most importantly, the genotype-focused scaling pattern of GGE biplot indicates that advanced genotype G16 (DZ-2012-CK-20113-2-0042) was desirable genotype in that it has broad adaptability. This result agrees with that of AMMI biplot.

Comparison biplot (Total - 86.48%)



PC1 - 63.49%



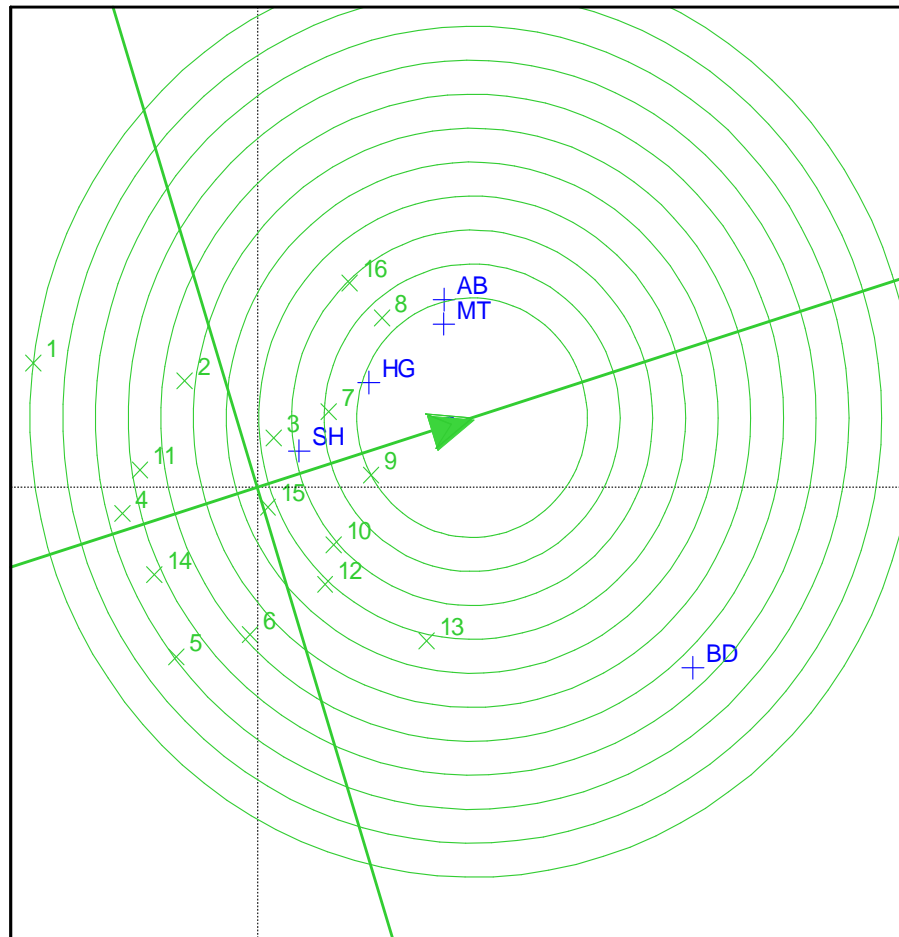
Key: - SH= Shambu, HG= Hawa Galan, MT= Mata, AB= Alaku Belle, BD= Badesso

Figure 4. GGE biplot based on genotype-focused scaling for comparison of genotypes for their yield potential and stability.

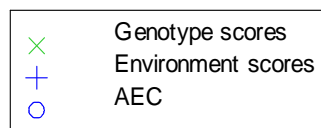
The scenario is parallel for the environments too. An environment is more desirable and discriminating when positioned nearer to the center circle or to an ideal environment (Dabessa *et al.*, 2016). This study clearly discloses that Mata (MT), as the ideal environment and Alaku Belle (AB) and Hawa Galan (HG) as desirable environments and therefore they are both discriminating

and representative environments. On the other hand, Badesso (BD) was positioned distant from centric circle and therefore, it is not an ideal environment.

Comparison biplot (Total - 86.41%)



PC1 - 63.46%



Key: - SH= Shambu, HG= Hawa Galan, MT= Mata, AB= Alaku Belle, BD= Badesso

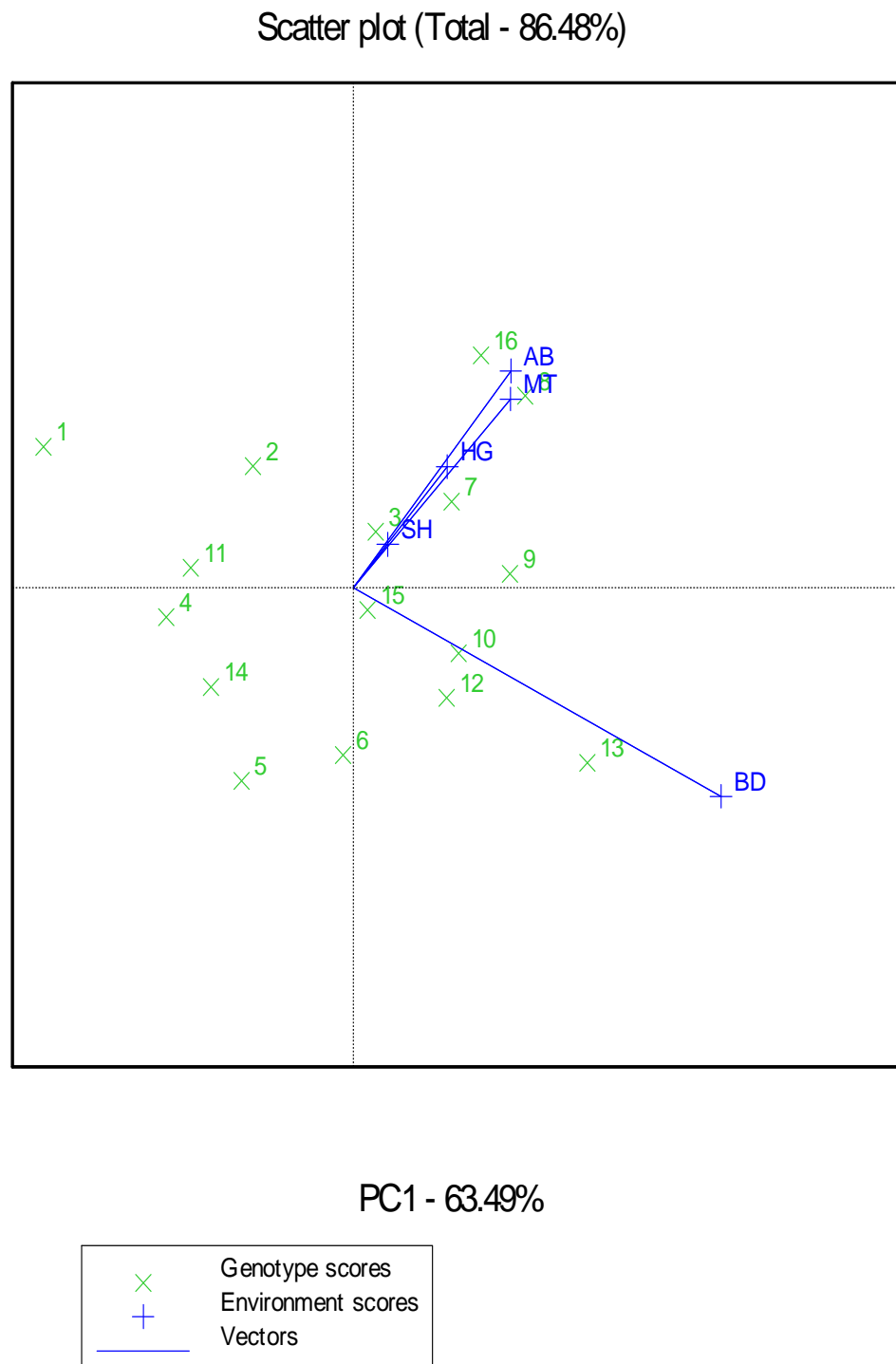
Figure 5. GGE biplot based on environment-focused scaling.

As usual one of the primary objective to conduct multi environmental trials are to evaluate cultivars and equally evaluating test environments. Plant

breeders are usually restrained by resource limitations, and conducting selections in a few locations without losing information will therefore be considered as best alternative. The test environment that lacks discriminating ability offer no information about genotypes tested on it. The length of the environment vectors of the biplot approximates the standard deviation within each environment which is a measure of their discriminating ability.

Thus, this study reveals Badesso (BD) as most discriminating test environment whereas Mata (MT) and Alaku Belle (AB) were also importantly discriminating testing sites. On the other hand, Shambu (SH) was the least discriminating testing site.

GGE biplot also helps us to understand the interrelationships among the environments. Notably, the cosine of the angle between the vectors of two environments is used to approximate the correlation coefficient between them. The cosine of the angles does not necessarily translate to correlation coefficients; However, they are informative to know about the interrelationships amid test environment. That is if some environments have small angles (acute angle) and are, therefore, highly positively correlated; information on genotypes attained from these environments must be comparable. If this resemblance is repeatable across years, these environments are redundant implying that a single environment should suffice. Obtaining the equivalent or better information by using fewer test environments will reduce the cost of production and surge breeding efficiency (Dabessa *et al.*, 2016).



Key: - SH= Shambu, HG= Hawa Galan, MT= Mata, AB= Alaku Belle, BD= Badesso

Figure 6. GGE Biplot indicating relationship among test environments.

The vector view of the GGE-biplot delivers a concise summary of the interrelationships among the environments. All environments except Badesso (BD) were positively correlated because angles among them were smaller than

90°. Therefore, Badesso (BD) was negatively correlated with all the remaining sites indicating that it is a representative environment. However, Shambu (SH), Hawa Galan (HG), Alaku Belle (AB) and Mata (MT) were positively correlated environments suggesting that some of these environments were not representative test environments.

Combining both discriminating and representativeness of test environment suggests that Badesso (BD) is both representative and discriminant test environment. On the other hand, though the similarity between test environments necessitate repeatability across years to conclude redundant test environment, the current study discloses that Shambu (SH) was the least discriminant and less representative test environment and thus needs to be rejected. Likewise, Dabessa et al. (2016) reported that two environments were stable, representative and discriminating among six environments for the performance of groundnut genotypes evaluated in western Ethiopia.

Ideal environments Mata (MT) are most effective in identifying the superior cultivars, whereas discriminating but not representative sites such as Hawa Galan (HG) and Alaku Belle (AB) are convenient for testing stable cultivars and environments. Environments with such characters are sometimes called killer or culling environment (Yan & Kang, 2003). In the same manner, environments which are not representative Shambu can be used for testing inferior genotypes (Yan & Kang, 2003).

5.8. Phenotypic variability

5.8.1. Cluster and distance analyses

The D^2 values based on the pooled mean of genotypes resulted in classifying the sixteen genotypes in to five distinct clusters (Table 10). Two clusters and three solitary groups were formed at 80% similarity level (Figure 6). It is usually expected that characters with larger absolute values closer to unity within the first principal component influence the clustering more than those with lower absolute values closer to zero (Chahal and Gosal, 2002). The mean value of the nine quantitative characters in each cluster is presented (Table 11).

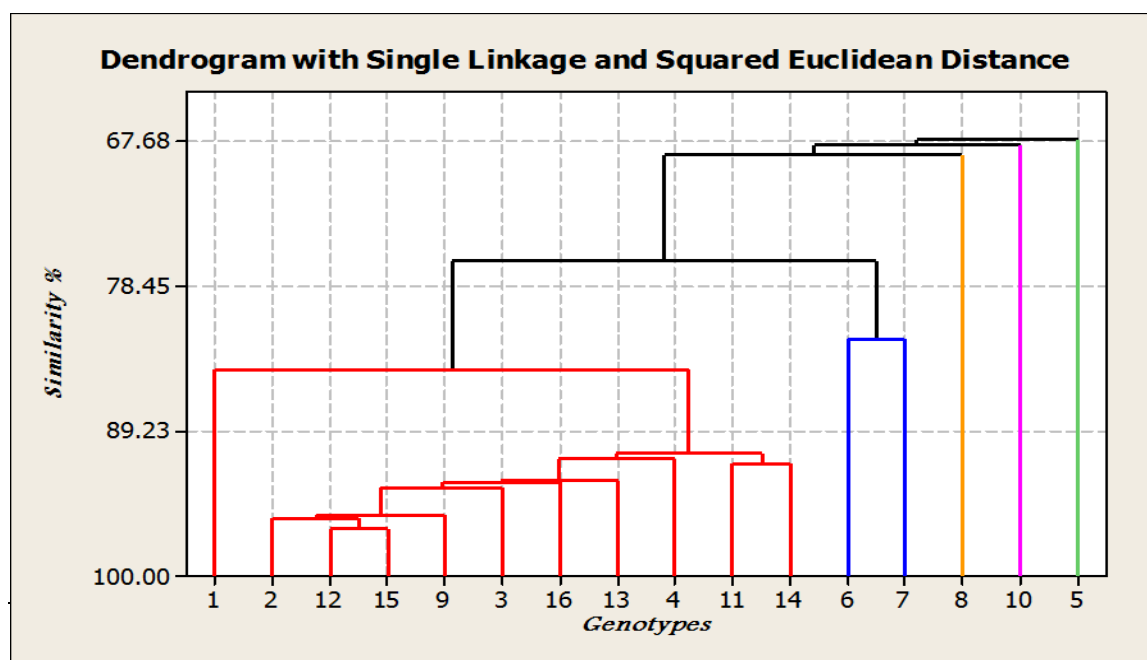


Figure 7. Dendrogram showing the similarities among sixteen chickpea genotypes for eleven quantitative traits.

Inter-cluster Euclidian distance measured based on the standardized means for the eleven quantitative traits revealed that relatively wider genetic variation (D^2) between cluster two and cluster four followed by cluster four and cluster five (Table 10). This indicates that the hybridization between the genotypes of these clusters would yield desirable segregates with accumulation of favorable genes in the segregating generations. The greater the distance between two clusters shows wider genetic distance between their genotypes.

Table 10. Distances between Cluster Centroids

	Cluster1	Cluster2	Cluster3	Cluster4	Cluster5
Cluster1	0.00	6.37	4.11	5.53	4.47
Cluster2		0.00	5.42	7.93	5.53
Cluster3			0.00	5.39	5.34
Cluster4				0.00	7.13
Cluster5					0.00

The number of member genotypes varied from cluster to cluster. Cluster (C1), comprised a total of 11 members or 69% of the total population. The first cluster accommodated the largest number of genotypes than any other cluster. This variety was distinguished by late in days to mature, high plant height and hundred seed weight. Cluster (C2) with 2 members or 12% of the total population constituted two chickpea varieties that are distinguished by their number of pod per plant and seed per pod. Cluster (C3), with only one member or 6% of the total population, was improved genotypes that give better yield than any other varieties and advanced pipe lines. In addition to yield, this variety was distinguished by late flower and early maturity and short grain filling period. Cluster (C4), with only one member or 6% of the total population, was advanced pipe lines characterized with intermediate hundred seed weight and early flower genotype among test genotypes. Cluster (C5), was a local landrace distinguished by early flowering and late maturity and long grain filling period tied with inferior yield (Table 11).

Table 11. Differences among the five clusters of sixteen Desi chickpea genotypes for mean agronomic characters' performance.

Character	Clusters					Mean
	C1	C2	C3	C4	C5	
Days to flowering	62.63	64.54	71.6	60.67	60.07	63.90
Days to maturity	139.06	136.94	135.93	138.60	138.07	137.72
Grain filling period	76.43	72.4	64.33	77.93	78	73.82
N ^o of pod per plant	28.36	40.89	33.41	31.97	41.74	35.27
N ^o of seed per pod	1.21	1.25	1.28	1.18	1.47	1.28
Plant height	53.30	48.23	46.68	52.8	46.81	49.56
N ^o of branches	4.09	4.74	3.89	4.13	4.25	4.22
Hundred seed weight	31.76	24.65	32.4	29.05	14.01	26.37
Grain yield	1.64	1.715	2.04	1.85	1.35	1.72

5.8.2. Principal component analysis

Principal component analysis indicated that the first four components with an eigenvalue greater than one explained a substantial portion of the total variations and accounted for 88.4 % of the total variation among sixteen chickpea genotypes for ten agronomic and yield related traits.

As suggested by Johnson and Wichern (1988), considering the first four principal components with eigenvalues greater than one constituted a variation of 36%, 25.8%, 14.8% and 11.8%, respectively (Table 12). A summary of the characters in the first five components, eigenvalues, the percentage of variances, cumulative percentage variances are presented (Table 12).

Some agronomic and yield related traits had contributed more in variability that prevails in these genotypes. Grain yield, hundred seed weight, number of pod per plant, plant height, and grain filling period showed greater absolute values of eigenvectors either in the first, second and/or third principal components. This indicated that these traits had higher contributions in clustering of these genotypes according to their class. In other word, selection efforts based on these traits are effective and productive.

Mekbib and Buta (2011) reported contribution of number of seed per plant and number of pods per plants in differentiating chickpea varieties. Similar report was made by Keneni et al. (2012) indicating that traits such as pod and seed setting, dry matter accumulation, nitrogen and grain yields, growth rate and grain production efficiency and nitrogen fixation had higher contribution in clustering of chickpea genotypes. According to Nawab et al. (2013) number of pods per plant, seeds per plant, biological yield, and grain yield had great contribution in clustering chickpea genotypes.

Table 12. Eigenvalue, proportion and cumulative variances and eigenvectors on the first five principal components for 10 agronomic traits in sixteen chickpea genotypes.

Parameter	-----Principal components (PCs)-----				
	PC1	PC2	PC3	PC4	PC5
Eigenvalue	3.6030	2.5787	1.4818	1.1754	0.4981
Proportion (%)	36	25.8	14.8	11.8	5
Cumulative (%)	36	61.8	76.6	88.4	93.4
Variables	----- Eigenvectors -----				
Days to flowering	-0.094	-0.497	0.141	0.484	-0.009
Days to maturity	-0.389	-0.065	0.302	0.453	0.310
Grain filling period	-0.294	0.465	0.156	-0.058	0.323
N ^o of pod per plant	0.424	-0.126	0.440	-0.113	0.000
N ^o of seed per pod	0.369	0.041	0.012	0.518	-0.110
Plant height	0.442	0.073	0.189	-0.217	-0.317
N ^o of branches	0.045	-0.058	0.783	-0.208	-0.068
Harvest index	0.005	-0.470	-0.102	-0.323	0.712
Hundred seed weight	-0.468	-0.141	-0.044	0.128	-0.121
Grain yield	0.451	-0.514	-0.096	-0.255	-0.402

In disparity to this, other traits such as days to flowering, days to maturity, seed per pod and harvest index contribute more in fourth and five principal components. Nevertheless, the gross contribution of variation accounted on these coordinates are as low as 16.8% and therefore, had a very restricted role in clustering these genotypes.

5.8.3. Correlations between characters

In fact, grain yield and its components are polygenic traits that are strongly influenced by the environment. Thus, the direct evaluation and improvement of grain yield by itself may be difficult due to the influence of the environmental component. Therefore, it is important to analyze the data for the relative contribution (correlation/association) of various components to yield performance.

Grain yield exhibited significant ($P \leq 0.01$) negative correlation with days to flowering ($r = -0.44$) and maturity ($r = -0.48$). Similar results were reported

from moisture stressed areas in Australia where higher yields were manifested by genotypes characterized by early flowering and rapidly setting pods (Berger *et al.*, 2003). Nevertheless, this scenario may not be the case in areas where moisture is available thereby supporting late flowering and maturing genotypes to exploit the available moisture and perform better than early genotypes (Wallace and Yan, 1998).

Grain yield showed significant positive association with plant height ($r = 0.40$), branch number ($r=0.33$) and hundred seed weight ($r=0.32$). Phadnis *et al.* (1970) tested 45 chickpea lines and reported that seed weight had a direct effect on seed yield. Numerous findings indicated that any positive increase of plant height, branch number and hundred seed weight improve grain yield of chickpea (Saleem *et al.*, 2002; Raval and Dobariya, 2003; Toker, 2004; Farshadfar and Farshadfar, 2008; Ali *et al.*, 2009; Zali *et al.*, 2010; Malik *et al.*, 2010; Keneni *et al.*, 2012).

Among these traits, plant height had the strongest association with the grain yield, suggesting that this trait may be important yield predictors and perhaps it is the most important for yield improvement in chickpea. Even if it was not significant, grain yield was positively associated with a number of pod per plant and seed per pod. A weak association between grain yield and these characters may have been emanated from the impact environmental factor that was expected when genotypes are grown in a diverse environment.

Table 13. Pearson correlation coefficient for major quantitative traits of sixteen chickpea genotypes.

Traits	DF	DM	NPPP	SPP	PH	BRN	HSW	GYLD
DF	1	0.12	0.12	-0.07	-0.46***	-0.23***	-0.23***	-0.44***
DM		1	0.02	-0.08	-0.15**	-0.37***	0.01	-0.48***
NPPP			1	0.04	-0.02	0.12	-0.38***	0.06
SPP				1	0.137*	0.088	-0.14*	0.07
PH					1	0.297***	0.34***	0.41***
BRN						1	0.04	0.32***
HSW							1	0.32***
GLYD								1

Key: -DF=Days to flower, DM = Days to mature, NPPP=Number of pod per plant, SPP=Seed per pod, PH =Plant Height, BRN=Branch Number, HSW=Hundred seed weight, GYLD=Grain yield.

5.9. Genetic diversity analysis based on ISSR markers

5.9.1. ISSR-PCR amplification and banding pattern

A total of nine ISSR primers were initially screened for molecular analysis to depict genetic diversity, among which only three primers that produced relatively clear amplification, explicit bands were selected and considered for statistical analysis (Table 3). The scope of all amplified bands ranged from about 300 to 2000 base pairs (Figure 8). A total of 25 bands were scored from three primers *viz.* nine for 834, eight for 810 and 880 primers. Mekbib and Buta (2011) also reported 38 bands using four primers. Amplification of one hundred fifty-five bands from fifteen individuals of three genuine species of rhubarb using the 15 ISSR primers was reported (Wang, 2011).

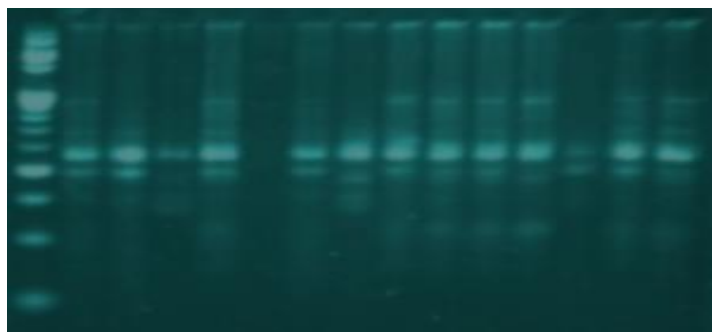


Figure 8. ISSR fingerprint created from 16 desi type chickpea genotypes using primer 880.

Twenty-one polymorphic loci were found in three assessed primers with a total of 84% polymorphisms. One hundred seven polymorphic loci were stated in 11 assayed primers with the total of 90% polymorphisms in faba bean (Behailu, 2016).

In the present study, Nei's gene diversity and Shannon's Information index with values of 0.29 ± 0.19 and 0.44 ± 0.26 , were noticed among the chickpea genotypes tested. Parallel with this result, Nei's gene diversity and Shannon index with values of 0.27 and 0.41 was also reported among Ethiopian Faba bean varieties (Behailu, 2016). The mean number of alleles and effective number of alleles was documented to be 1.84 ± 0.37 and 1.51 ± 0.39 , respectively.

5.9.2. Genetic similarity

Genetic similarities were calculated according to the method developed by Jaccard (1908). UPGMA and neighbor joining analysis were used to construct dendrogram for desi type chickpea genotypes by using Jaccard's similarity coefficient. The average of Jaccard genetic similarity among chickpea cultivars and advanced lines varied from 0.21 to 0.98 (Table 14). Lower and upper values of genetic similarity coefficient indicate the minimum and maximum similarity, respectively. Higher genetic similarity (98%) were detected between G1 and G2, G6 and G10, and G13 and G14 whereas lowest similarity (0.21) was between G3 and G15.

Table 14. Genetic similarity matrix between 16 desi type chickpea genotypes based on Jaccard's similarity coefficient from ISSR primer data fingerprinting.

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16
G1	1.00															
G2	0.98	1.00														
G3	0.42	0.58	1.00													
G4	0.71	0.79	0.73	1.00												
G5	0.62	0.62	0.60	0.62	1.00											
G6	0.71	0.71	0.50	0.75	0.64	1.00										
G7	0.86	0.79	0.60	0.83	0.67	0.92	1.00									
G8	0.86	0.79	0.43	0.73	0.60	0.93	0.79	1.00								
G9	0.86	0.79	0.43	0.73	0.60	0.93	0.86	0.88	1.00							
G10	0.89	0.73	0.40	0.69	0.56	0.98	0.86	0.94	0.94	1.00						
G11	0.89	0.89	0.60	0.90	0.50	0.82	0.80	0.91	0.91	0.83	1.00					
G12	0.70	0.70	0.67	0.64	0.56	0.64	0.78	0.50	0.64	0.58	0.55	1.00				
G13	0.77	0.77	0.54	0.79	0.54	0.92	0.91	0.85	0.85	0.92	0.75	0.75	1.00			
G14	0.83	0.83	0.58	0.85	0.58	0.92	0.91	0.83	0.92	0.92	0.82	0.82	0.98	1.00		
G15	0.44	0.42	0.21	0.42	0.26	0.53	0.47	0.47	0.56	0.53	0.40	0.25	0.39	0.41	1.00	
G16	0.33	0.32	0.27	0.33	0.31	0.42	0.35	0.37	0.44	0.42	0.29	0.31	0.29	0.31	0.69	1.00

Above all, a genetic similarity coefficient indicates that varieties or groups that are genetically similar could not be used in the hybridization as they are not

fine in attaining the required traits. A similarity coefficient varied from 0.41 to 0.97 was also attested among 19 released chickpea varieties (Mekbib and Buta, 2011). Jaccard's similarity coefficients which ranged from 0.01 to 0.90 were also reported among 125 cultivars of chickpea (*Cicer arietinum* L.) of Indian origin using ISSR markers (Aggarwal *et al.*, 2015).

5.9.3. Cluster analysis

The chickpea genotypes were grouped into five clusters in a UPGMA-based dendrogram. In the first cluster, G15 and G16 were encompassed whereas a local landrace (G5) was clustered in the second cluster. G3 and G12 were grouped in the third cluster whereas G13, G14, G7, G9, G8, G10, and G6 were grouped in the fourth cluster. G1, G2, G4, and G9 were placed in the fifth cluster. The clustering pattern observed indicated the differentiations of released varieties, advanced lines and local landraces except in few cases where released varieties and advanced lines were amalgamated. The current clustering pattern observed in this study concedes with Aggarwal *et al.* (2011) in their studies of genetic diversity in 115 chickpea genotypes from India using ISSR and assembled the individuals into five clusters depending on their genetic similarity. Similarly, Gautam *et al.* (2016) reported three broad clusters of chickpea genotypes obtained from dendrogram of 13 chickpea accession including accessions from Ethiopia.

Neighbor-joining based clustering grouped the 16 Desi type chickpea genotypes into three major clusters from the main node (Figure 10) comprising, seven, three, and six chickpea genotypes in the first, second, and third cluster, respectively. In the first cluster, one advanced pipeline, and six released varieties were included. In the second cluster, a local variety and two released varieties were grouped and in the third cluster, two released varieties and four advanced pipeline genotypes were grouped together.

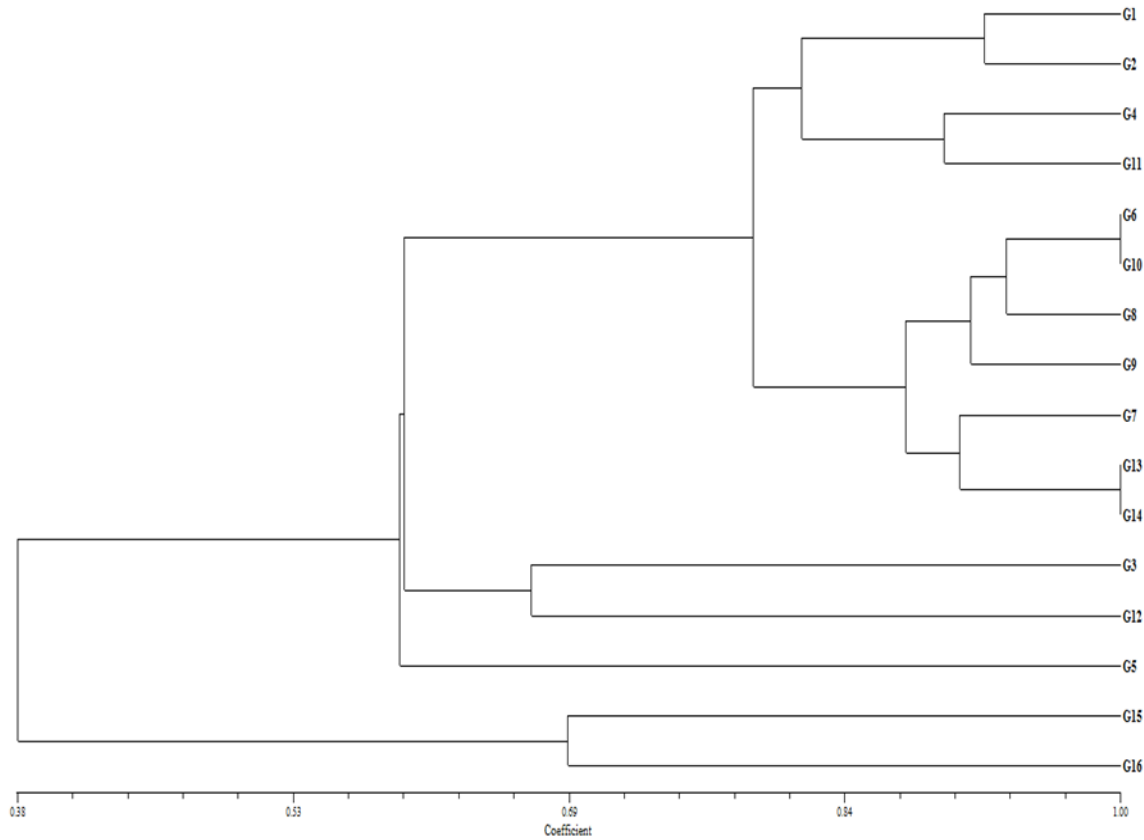


Figure 9. Dendrogram for 16 Desi type chickpea genotypes based on UPGMA analysis of amplified bands by ISSR primers.

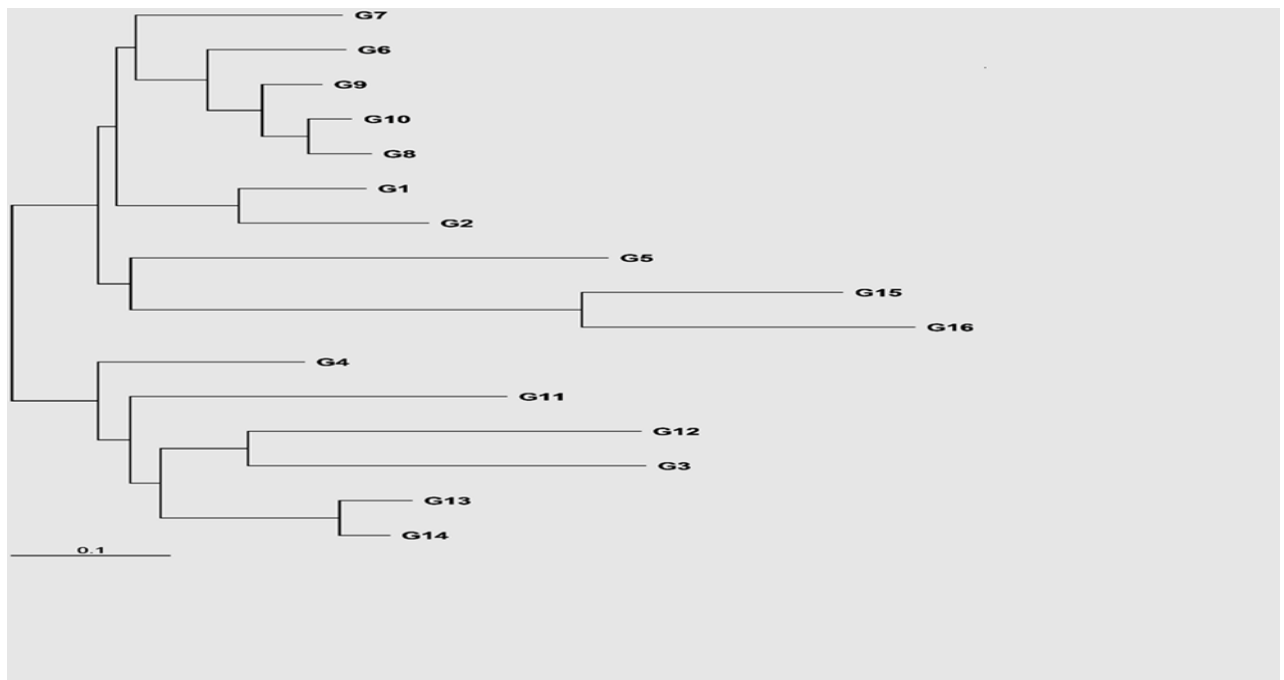


Figure 10. Neighbor joining clustering of 16 Desi type chickpea genotypes in Ethiopia.

5.9.4. Principal coordinate analysis

In the first three coordinates of the PCO eigenvalues of 1.95, 1.61 and 1.16 with variance of 18.24%, 15.11% and 10.88%, respectively, were recorded. Except in a few cases of where there were intermixed clusters, the clustering pattern observed in two-dimensional Principal Coordinate Analysis fully agrees with that of the clustering pattern observed in UPGMA and Neighbor joining clustering. Behailu (2016) also reported the resemblances of clustering pattern among UPGMA, NJ and principal coordinate analysis in Faba bean varieties.

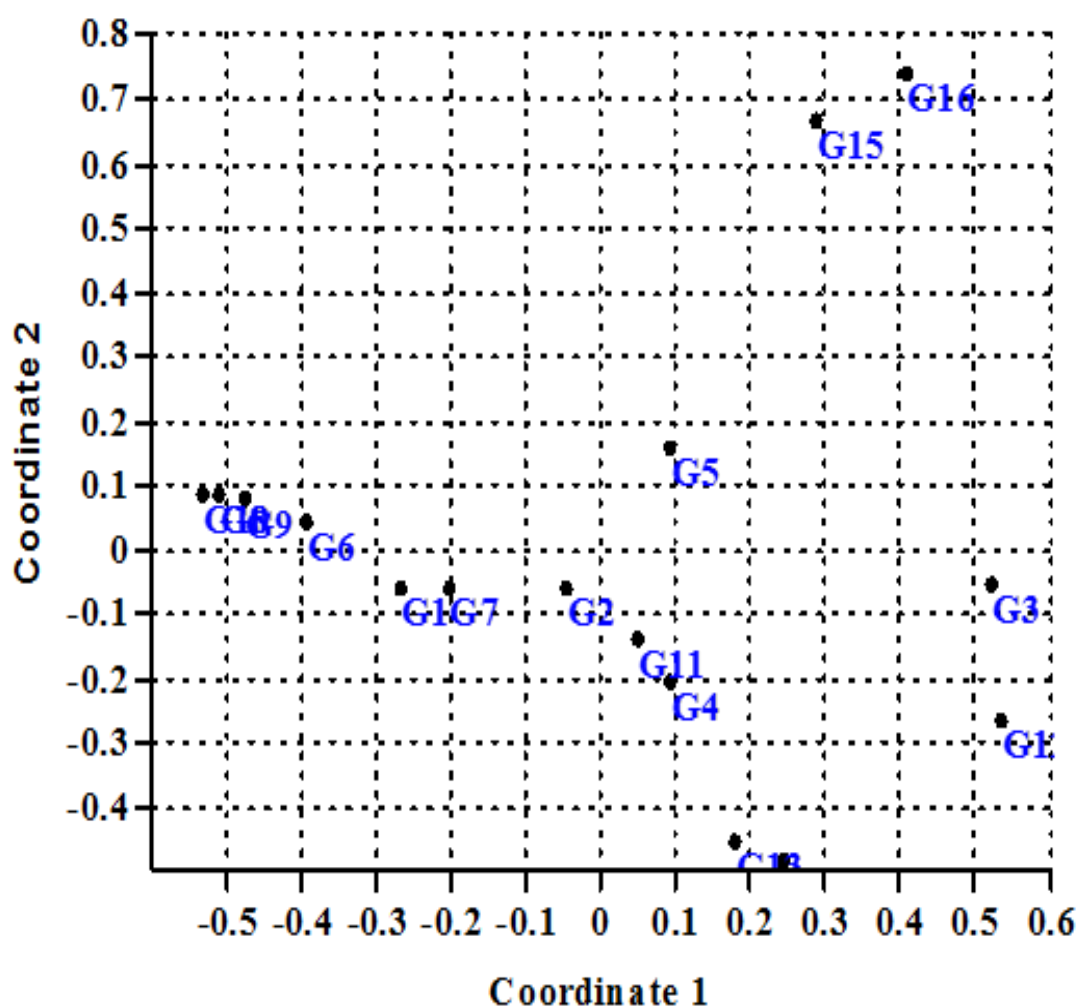


Figure 11. Two-dimensional representation of principal coordinate analysis of genetic relationships among sixteen chickpea genotypes inferred from similarity matrix using the Jaccard's index.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

Analyzing the pattern and causes of phenotypic and genetic variation might help to understand variability in plants, to predict their responses to changing environmental conditions and thereby planning proper breeding schemes for crop improvement. Inter simple sequence repeat marker (ISSR) discriminate the pattern of genetic variability among chickpea genotypes than morphological based clustering in the present study.

Morphological and molecular evaluation of the chickpea genotypes in this study showed the existence of genetic variability. Moreover, the genetic variability of sixteen chickpea genotypes exhibited a good potential to screen large germplasm of chickpea for soil acidity tolerance and develop a cultivar that is tolerant to soil acidity with better grain on such acidic soils in the future.

This study also revealed that chickpea genotypes differ in tolerance to soil acidity. Although some genotypes exhibited an outstanding performance in terms of grain yield and yield related traits, soil fertility improvement through lime application would still be useful in places with strong acid soils as the ones used in this study and other similar environments, if it is found economical.

Chickpea genotypes responded differentially across a diverse growing environment. Some genotypes showed wide adaptation and had high stability coupled with good grain yield across diverse growing environmental conditions.

Generally, differential response of chickpea genotypes indicates the possibilities of designing better chickpea breeding strategies that aim at screening large germplasm of chickpea for soil acidity tolerance and thereby developing a cultivar(s) with wider adaptations.

This study indicates that both yield and stability should be considered instantaneously to reduce the effect of genotype by environment interaction

(G×E) and to make selection of the genotypes more precise and refined. The analysis of variance based on the AMMI model for grain yield showed that genotypes, environments, genotype by environment interaction (G×E) and Interaction Principal Component Axis (IPCA-1) were significant. Thus, the first IPCA was used to conduct a biplot analysis with grain yield because of its significance in contributing to the greater percentage of genotype by environment interaction (G×E).

A graphical interpretation of the AMMI biplot, GGE biplot and GSI index incorporating ASV and yield capacity of the different genotypes in a single non-parametric index were useful for discriminating genotypes with superior and stable grain yield. Overall, the current result indicated that, based on yield performance, AMMI and GGE biplot, ASV and GSI indices G16 (DZ-2012-CK-20113-2-0042) and Natoli (G8) variety showed better grain yield with better stability across environments and thus they are recommended for wider production in test locations and similar agro-ecologies in Ethiopia.

6.2. Recommendations

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

- ✓ The morphological and molecular tools have been playing decisive roles in discriminating genetic variability in chickpea. This study revealed the presence of genetic variability among released chickpea varieties and advanced lines. Therefore, it is recommended that future study should consider these materials in their breeding programs.
- ✓ Further studies of chickpea genotypes with larger sample size in across growing environments and seasons can give additional information on chickpea genotypes tolerance to acidic soils and thereby selecting stable genotypes.
- ✓ Further study of ISSR marker by using large number of primers and additional studies using other molecular markers are recommended in order to give confirmative results of genetic variability in chickpea genotypes.

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8. APPENDICES

Table 1. Combined analysis of variance for grain yield and other agronomic traits of sixteen chickpea genotypes grown at Shambu, Hawa Galan, Badesso, Mata and Alaku Belle in 2016.

Source of Variation	Mean squares								
	D2F	D2M	GF	PPP	SPP	PH	BR	HSW	GYL
Rep (2)	10.4 ^{ns}	43.83 ^{**}	96.53 ^{**}	84.84 ^{ns}	0.22 [*]	4.74 ^{ns}	3.32 ^{**}	22.8 [*]	2685.7 ^{***}
Rep (Loc) (8)	1.29 ^{ns}	97.22 ^{***}	107.8 ^{***}	552.12 ^{***}	0.18 ^{**}	187.2 ^{***}	2.55 ^{**}	10.7 ^{ns}	655.5 ^{***}
Loc (4)	1806.8 ^{***}	1032.2 ^{***}	2017.1 ^{***}	1546.8 ^{***}	0.35 ^{**}	5303.8 ^{***}	12.45 ^{***}	671.2 ^{***}	19905.6 ^{***}
Geno (15)	115.9 ^{***}	21.03 ^{***}	193.2 ^{***}	443.41 ^{***}	0.96 ^{ns}	226.3 ^{***}	1.36 [*]	568.4 ^{***}	1059.2 ^{***}
G × Loc (60)	11.26 [*]	13.25 ^{**}	27.89 ^{**}	95.88 ^{ns}	0.05 ^{ns}	79.4 ^{***}	0.61 ^{ns}	12.8 ^{**}	292.7 ^{***}
Error (150)	6.67	6.39	13.02	81.76	0.06	30.94	0.69	5.9	131.7
Cv	4.09	1.82	4.78	28.88	20.6	10.73	19.98	8.2	21.76
R	0.90	0.86	0.87	0.6	0.43	0.869	0.56	0.9	0.86

Table 2. First four AMMI selections per environment.

Environment	Mean	Score	1	2	3	4
SH	740	11.32	G10	G9	G16	G8
HG	1497	11.03	G10	G9	G16	G15
AB	1697	8.89	G16	G8	G7	G3
MT	2516	7.94	G16	G8	G7	G9
BD	1889	-39.19	G13	G9	G12	G10