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Genetic Diversity and Population Differentiation Analysis of Ethiopian Barley (*Hordeum vulgare* L.) Landraces using Morphological Traits and SSR Markers

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I dedicated this thesis to my late Mother, Abebech Bekele, who always wished to see this day!

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Abbreviations

Abbreviation	Explanation
AMOVA	Analysis of Molecular Variance
BLUPs	Best Linear Unbiased Predicators
CI	Confidence Interval
F_{ST}	Genetic differentiation
Nm	Gene flow
PCA	Principal Component Analysis
PCoA	Principal Coordinate analysis
PCR	Polymerase Chain Reaction
PCs	Principal Components
PIC	Polymorphic Information Content
Q_{ST}	Quantitative differentiation
REML	Restricted Maximum Likelihood
SSR	Simple Sequence Repeats
UPGMA	Unweighted Pair Group Method with Arithmetic Average
UTM	Universal Transverse Mercator

Zusammenfassung

In dieser Arbeit wurden 199 Sommergerstenlandsorten und vier Kultursommergersten aus zehn unterschiedlichen Regionen Äthiopiens mit morphologischen und molekularen Markern (SSR) untersucht. Die Feldversuche zur Erhebung der morphologischen Marker fand im „Holetta und Bekoji Agriculture research center“ in Äthiopien statt, die genetischen Untersuchungen wurden im Labor des INRES-Pflanzenzüchtung der Universität Bonn durchgeführt. Neben der Abschätzung der morphologischen und genetischen Varianz der Gerstenakzessionen hinsichtlich ihrer regionalen Herkunft und Höhenlage, die Einteilung der Gerstenakzessionen in homologe Cluster, Bestimmung von genetisch hoch variablen Merkmalen, Abschätzung der Variation innerhalb der Populationen war auch die Identifizierung von Merkmalen mit hohem Selektionsdruck Ziel der vorliegenden Arbeit.Die Abschätzung der genetischen Varianz der Regionen und Höhenlagen deutet auf eine hohe genetische Varianz zwischen den Akzessionen bezüglich der untersuchten Merkmale hin, die hohe morphologische Varianz weist auf das Potential der Regionen und Höhenlagen über 2000m über NN zur Verbesserung und Erhaltung der Gerstenakzessionen hin. Basierend auf morphologischen Merkmalen ist die Clustereinteilung der Akzessionen unabhängig ihrer geographischen Herkunftsregion. Durch die Hauptkoordinatenanalyse können die Akzessionen anhand der Merkmale Ährenschieben, Tage bis zur Abreife, Wuchshöhe und Ährenlänge gruppiert werden. Die molekulare Varianzanalyse der SSR-Marker verdeutlicht eine höhere Variation innerhalb der Regionen, Höhenlagen und Akzessionen als zwischen den Regionen, Höhenlagen und Akzessionen.Die durchgeführte Analyse zur Bestimmung der Variation zwischen den Regionen und Höhenlagen resultiert in mittlere Variation zwischen den Regionen und nicht signifikanten Unterschieden zwischen den Höhenlagen, welches auf eine hohe Migrationsrate der selbstbefruchtenden Arten hindeutet. Die molekulare genetische Varianz wurde mit der Varianz qualitativer Merkmale zur Bestimmung der Selektionsrichtung, gerichtet oder ungerichtet, verglichen. Daraus resultiert die Erkenntnis, dass die Glucangehalt nicht dem Selektionsdruck durch Landwirte unterliegen.Die Ergebnisse aus der vorliegenden Arbeit machen deutlich, dass die genetischen Ressourcen der Gerste in Äthiopien hoch divers sind. Allerdings wirkt eine starke Migrationsrate zwischen den Regionen und Höhenlagen der lokalen Merkmalsanpassung von Akzessionen durch Selektion entgege.

Abstract

Morphological and simple sequence repeats (SSR) were used to analyze a total of 199 landraces collected from 10 administrative regions of Ethiopia and four released cultivars of barley. For the morphological data; experiments were conducted in Holetta and Bekoji agricultural research centers of Ethiopia, and the genetic analysis using molecular marker (SSR) were conducted in laboratory of INRES, University of Bonn. The objectives of this study were (1) to assess the extent of morphological and molecular variation in barley accessions in respect to regions and altitude of collection, (2) to cluster the accessions into relatively homogenous groups and to identify the major traits contributing to the overall diversity of the germplasm, (3) to estimate the population differentiation and (4) finally to identify traits which undergo selection pressure. Genetic variance estimates of regions and altitudes indicated a wide variation among accessions depending on the traits involved. Presence of high morphological variation within regions and altitudes above 2000 meter above sea level (m.a.s.l) indicated the potential of each region and higher altitudes in contributing towards barley improvement and conservation activities. Clustering of accessions was not dependant on geographic regions whereas the well adapted traits like days to heading, days to maturity, plant height and awn length, played a role in differentiating accessions collected from different regions and altitude classes into principal components. Molecular variance analysis from SSR data resulted in a high variation within region, altitude and accessions as compared to the variation among region, altitude and accessions variation. The Analysis conducted to determine the differentiation among regions and altitude classes resulted in a medium differentiation among regions accompanied by a high gene flow. While there was no significant genetic differentiation among altitude classes and high gene flow was estimated. To test if the quantitative traits were under directional or homogenizing selection molecular marker genetic differentiation was compared with qualitative trait differentiation which resulted in glucan content as traits which was not undergoing selection pressure of farmers. From this study it can be concluded that high genetic variability exists in the barley germplasm of Ethiopia. However, a high gene flow among altitudes and regions counteracts the gene frequency for local adaption of traits created by selection.

1. Introduction

Barley (*Hordeum vulgare* L. subsp. *vulgare*) is one of the earliest domesticated crop plants (Zohary and Hopf 1993). The genus *Hordeum* comprises over 32 species, including diploid and polyploidy, perennial and annual types, which are spread throughout the world (Von Bothmer et al. 1995). In terms of area and production worldwide, barley is the fourth most important cereal after wheat, rice and maize. Barley is adapted to a broad range of agro-ecological environments and it is tolerant to soil salinity, drought and frost to a considerable level. The crop successfully grows in the arid climates of the Sahara, the Tibetan plateaus, the highlands of the Himalayas, and the Andean countries, the tropical plains of India and the mountains of Ethiopia.

Ethiopia is a center of origin and diversity for many cultivated crops and their wild relatives. The country is located near the Equator, but due to high altitudes it experiences a temperate climate, specially at altitudes of more than 2000 meters above sea level. In addition, soil variation, ecological diversity, substantial temperature and rainfall variations, and diverse social and cultural conditions are some of the possible explanations for the existence of large genetic variation of crop varieties in the country. Plant genetic diversity is a useful character in plants that can be transmitted genetically from parents to offspring. The sources of tremendous variation in plants support all other forms of life on land. Plant genetic diversity covers a wide range, at both the evolutionary and ecological level. Ecologically the variation ranges from the natural ecosystems and traditional low-input agriculture to modern, intensive production systems. At the crop evolutionary level it covers a wide range of diversity from wild ancestors to modern cultivars. The resulting diversity in plants has been the basis for providing food and satisfying other human needs for millennia and it continues to do so for the development of plant characters required to adapt barley to the increasingly and rapidly changing environmental situations and socio-economic conditions. Since Ethiopia is characterized by a wide range of agro-climatic conditions and barley is one the major cereals grown in wide agro-ecology of the country with its economic and social importance so identification of regions with high allelic variation and important traits from farmers' point of view is vital aspect for *in situ* conservation as well for improvement of barley.

Objectives of the study

In general currently dynamic *in-situ* conservation of cultivated plants is being actively discussed as an essential conservation strategy. A dynamic on farm conservation is being implemented in Ethiopia and other countries and barley is one of the crops in focus. Identification of better agronomic characteristics, disease resistance, earliness, good quality and higher biological yield are necessary for barley improvement program. The availability of such important traits depends on the identification of areas with high genetic diversity concentration of different valuable morphological traits. The identification of these sites has paramount importance for the collection mission and appropriate *in-situ* conservation. So this study aimed to choose sites for *in-situ* conservation which depends on high diversity estimates based on SSRs and knowledge of adaptive traits linked to certain ecological conditions and agricultural practices of farmers. In addition proper knowledge of the nature and extent of genetic variation was crucial since successful conservation and utilization of germplasm depends on the prior assessment of variation within and among populations. Comparing of quantitative traits statistics (Q_{ST}) and neutral traits statistics (F_{ST}) is crucial to identify traits which were important from farmers point of view and undergoes selection pressure. Therefore, the specific objectives of this study were:

- to assess the extent of genetic diversity in barley accessions in respect to regions and altitude of collection
- to classify the accessions into relatively homogeneous groups
- to identify the major traits contributing to the overall diversity of the germplasm
- to estimate population differentiation
- to identify traits important from farmers point of view and detect sites of high allelic diversity for germplasm collection and *in situ* conservation

2. Literature Review

2.1 Origin and Domestication of Barley (*Hordeum vulgare* L.)

Domestication of wheat and barley probably took place prior to 7000 B.C. in the region of the Near East known as 'Fertile Crescent'. The Fertile Crescent includes parts of Jordan, Lebanon, Palestine, Syria, Southeastern Turkey, Iraq and Western Iran. The wild progenitor of cultivated barley (*Hordeum vulgare* subsp. *spontaneum*) is still widely distributed along the Fertile Crescent, particularly in the driest areas (Harlan and Zohary 1966). Archeological evidence indicated that emmer and einkorn wheat led to the foundation of the old world agriculture (Zohary and Hopf 1993). The domestication of barley is assumed to have taken place from two-rowed wild barley *Hordeum vulgare* L. subsp. *spontaneum* in the Near East (Harlan and Zohary 1966). However, evidences suggesting alternative ways of barley domestication have been reported (Tanno et al. 2002; Molina-Cano et al. 2005). The controversies surrounding the origin of cultivated barley in the last centuries can be summarized: (1) the six-rowed barley originated repeatedly at different times and in different regions, through independent mutations of *Vrs1* allele (two-rowed spike) (Komatsuda et al. 2007); (2) Tibet is unlikely to be a center of origin for cultivated barley (Yang et al. 2008); (3) the numerous other forms are either direct descendents of one or other ancestral forms *H. agriocrithon or H. spontaneum*, or derived from hybridization between the two ancestral forms (Li et al. 2004).

With the development and advancement of molecular markers in recent years, more precise information on origin and domestication history of barley is emerging. Badr et al. (2000) demonstrated a monophyletic nature of barley origin based on allele frequency at 400 polymorphic AFLP loci studied in a world collection of wild and cultivated barley and showed that the Israel-Jordan area in the southern part of the Fertile Crescent has the highest probability of being the geographical area within which wild barley was domesticated. The hypothesis of monophyletic origin of barley is further supported by Li et al. (2004), who analyzed the

rDNA polymorphism in wild barley accessions derived from Tibet and other parts of the world. However, Molina-Cano et al. (1999) suggested barley domestication could have taken place outside the Fertile Crescent, particularly in Morocco. This proposition however, was not substantiated by the RAPD analyses of wild and cultivated barley samples derived from the western Mediterranean basin including Morocco (Blattner and Mendez 2001) and the authors concluded in favor of a monophyletic origin of barley. In contrast to this, (Tanno et al. 2002) based on DNA sequence analysis at a marker closely linked to the *vrs1* locus (row-type gene), and more recently, (Molina-Cano et al. 2005) with chloroplast SSRs analysis, have shown strong evidences that cultivated barley may have multiple origins. The later authors proposed Ethiopia and the western Mediterranean as possible centers of barley origin. It is now generally accepted that *H. spontaneum* is the progenitor of cultivated barley, however, it is not clear whether cultivated barley is of monophyletic origin or the domestication events happened in other parts of the word besides the Fertile Crescent.

2.2 Barley genome

The DNA content of *Hordeum* species ranges from 6.85 to 10.67 pg in diploids (2n=14) and up to 29.85 pg in hexaploid species (2n=42) (Jakob et al. 2004). The cultivated barley is a self-pollinating diploid species (2n=2x=14) with a genome size of approximately 5.3 x 10^9 bp equivalent to 5.5 pg DNA of a haploid nucleus (Bennett and Smith 1976). The barley genome consists of a complex mixture of unique and repeated nucleotide sequences, and approximately 10 to 20 % are tandem arranged repeated sequences while 50 to 60 % are repeated sequences interspersed among one another or among unique nucleotide sequences (Rimpau et al. 1980). The interspersed copia-like retrotransposon BARE-1 comprises almost 7 % of the barley genome (Manninen and Schulman 1993). The genome of barley has seven pairs of distinct chromosomes and chromosome 2H is considered the longest, followed in length by 5H, 3H, 7H, 4H, 6H and 1H (Pedersen et al. 1995) which differ in their sizes measured at mitotic metaphase. Comparative mapping has revealed that barley chromosomes 1, 2, 3, 4, 5, 6, and 7 are homeologous to wheat chromosomes 7H, 2H, 3H, 4H, 1H, 6H, and 5H, respectively. It has been recommended that barley chromosomes be designated according to their homeologous relationships with chromosomes of other *Triticeae* species (Linde-Laursen 1997).

2.3 Barley cultivation and use

2.3.1 Global Distribution

The largest area under barley cultivation is in Europe (ca. 27.3 million hectares) and Asia (ca. 12.16 million hectares). The barley area in other parts of the world is significantly lower than in these two continents, e.g., North and South America account for about 6.64 million hectares, Africa 4.87 million hectares and Oceania about 4.46 million hectares. About 41.5 % of the world barley production is contributed by the top five barley producing countries that are Russia, Ukraine, Canada, Turkey and Spain respectively (FAOSTAT 2007).

Barley grains are used as human food, to feed farm animals and for malt production, which in turn is used to make beer, whisky or other processed food products. In Japan, barley grains are used for special preparations, e.g., barley tea, shochu, miso and as a rice extender (Kays et al. 2005). In the Western world barley is becoming less important as a human food, and it is mainly used to feed farm animals or for malt production. On the other hand, in the highlands of Tibet, Nepal and Ethiopia, in the Andean countries, and also in some areas of North Africa, China and Russia, barley is still an important human food. Because of its low demand as a human food and its lower yield potential compared to other cereals like wheat and maize, the barley area in the major barley producing countries is decreasing. However, barley is a high value crop in large parts of arid and drought inflicted regions (Fertile Crescent region), the Tibetan plateau and the Himalayas, the marginal areas of many developing countries, and Ireland, Scotland and the Nordic region of Europe (Denmark, Finland, Norway and Finland), where the agricultural activities are restricted by a very short vegetation period (Ortiz et al. 2002). Diets containing barley are effective in lowering blood cholesterol in hypercholesterolemic people with a higher risk of cardiovascular diseases (Behall et al. 2004; Ripple et al. 2009). More recently, whole grain barley and barley containing products have been allowed to claim that they reduce the risk of coronary heart diseases by the US Food and Drug Administration (FDA, http://www.fda.gov). The nutritional and clinical importance of barley foods and public consciousness regarding quality of daily diet, i.e., cereal diversification, may have a positive impact on the demand of barley as a human food in the future.

β-Glucans occur in the bran of grasses (*Gramineae*) such as barley, oats, rye and wheat, generally in amounts of about 7%, 5%, 2% and less than 1%, respectively. The main use of β-glucans is in texturizing as fat substitutes. They are recognized as having important positive health benefits centered around their benefits in coronary heart disease, cholesterol lowering and reducing the glycemic response. Such health benefits are linked to its high viscosity although it may be that some of these effects are due to appetite suppression. High molecular weight β-glucans are viscous due to labile cooperative associations whereas lower molecular weight β-glucans can form soft gels as the chains are easier to rearrange to maximize linkages. Barley β-glucan is highly viscous and pseudoplastic, both properties decreasing with increasing temperature (Burkus and Temelli 2005). These properties cause difficulty in the brewing industry, negatively affecting fermentation and filtration.

2.3.2 Distribution and Importance of Barley in Ethiopia

Ethiopia, with its diverse agro-ecological and climatic features, is well known for being one of the 12 Vavilovian Centers of Diversity (Harlan 1969; Vavilov 1951). The altitudinal variation, which ranges from 110m below sea level in areas of Kobar Sink to 4,620 m.a.s.l. at Ras Dashen (IPGRI 1996), temperature and rainfall differences coupled with edaphic factors creates a wide range of ecological conditions in the country. This complex topography and environmental heterogeneity provide sustainable environment for a wide range of life forms. As a result, Ethiopia is considered as one of the richest genetic resources centers in the world. Crop plants such as Coffee and 'Tef' are known to originate in Ethiopia (Harlan 1971). Landraces of several major crops such as barley, wheat, sorghum, field pea, faba bean and wild relatives of some of the world's important food crops are abundant in Ethiopia.

Barley (*Hordeum vulgare* L.) is one of the oldest cultivated crops and has been grown in Ethiopia for at least 5000 years (Harlan 1969) and it is cultivated in all regions of the country. The most important barley producing regions are Shewa, Arsi, Gojam, Gonder, Welo, Bale and Tigray where more than 85% of the country's total production comes from (CSA 2005). Barley is produced twice a year i.e., during the main season, *Meher* (August-December), and the short rainy season, *Belg* (March-July). *Belg* barley is important in Welo, Bale and North

Shewa (Yirga et al. 1998). Barley can be cultivated at altitudes between 1500 and 3500m, but is predominantly grown between altitudes of 2000m and 3000m (Lakew et al. 1996). This wide distribution demonstrates the wide ecological amplitude throughout the country (Asfaw 1988; Asfaw 1989; Lakew et al. 1996).

In Ethiopia barley growing areas gradually diminish due to the expansion of wheat and rye cultivation in some regions. Currently the crop is pushed to marginal areas (to very high altitudes where frost prevails) and threatened by genetic erosion (IBC 2007). Therefore, rare morphotypes are declining in frequency of occurrence through time. Some morphotypes, which were reported by Orlov 1929s to occur in abundance in a given region or locality were either never encountered or found only in a rare admixture (Asfaw 1988).

Early efforts in studying the agro-morphological variability of Ethiopian barley indicated wide morphological variation. Ward (1962) showed that the Ethiopian barley posses a large diversity of forms, and he regarded Ethiopia as center of concentration for deficiens, irregular and short rachilla types. Asfaw (1988) also recognized a total of 64 botanical forms. Variation of disease resistance in Ethiopian barley was reported by different authors (Fukuyama and Takeda 1992; Jorgensen 1992; Qualset 1975). Genes related with high lysine (Munck et al. 1970), malting and brewing quality (Lance and Nilan 1980) were reported for Ethiopian barley. Demissie and Bjørnstad (1996) found morphological diversity for different traits in relation with regions, altitudes and agro-ecological zones for a total of 51 barley populations.

Barley is grown primarily for local food and beverage consumption. For small scale highland farmers, barley is the predominant subsistence crop (Asfaw 2000). The mode of consumption and overall barley utilization was studied by Asfaw and Bothmer (1990). According to this report in Ethiopia the highest level of barley consumption is in highland areas where it is widely cultivated. It is further shown that consumption begins at milky stage of grain maturation when youngsters remove the awns from the green unripe spikes, crush them between the palms and blow away the fragments of the rachis and glumes, and consumes the tasty raw green grains in the field in limited quantities. Such unripe spikes many also be green roasted over fire.

Similarly a sheaf of ripe barley can be roasted in the fire, crushed between the palms and the grains eaten as a supplementary or snacks. As for the type of total food preparations, it is shown that different kinds of bread, dough balls, porridge, soup and gruel are made in every household from any barley type, but there are preferred types for different food categories. Concerning drinks the study showed that many alcoholic and non-alcoholic local beverages are brewed in the household from barley grains for daily consumption or for holidays and celebrations. The barley straw is used in the construction of traditional huts and grain stores either as thatching or as a mud plaster. A barley residue is used as fodder mainly for cattle and equines. The small grain that fail to fill up and those crushed in the process of threshing mix with the chaff are kept for chicken feed. Some barley types are purposely cultivated for their special use (e.g. naked barley for roasted grain) while many other are more of multipurpose (Asfaw 2000)

2.4 Genetic Diversity

Genetic diversity is one of the three pillars of biodiversity, diversity within species, between species and of ecosystems (CBD, Article 2), which was defined at the Rio de Janeiro Earth Summit as the variability among living organisms from all sources including, interalia, marine and other aquatic ecosystems and the ecological complexes of which they are part. Crop genetic diversity can be viewed at different geographical scales or levels of analysis. Variation manifests itself both among the crops and varieties grown by individual farmers and at a community level (Almekinders and Struik 2000). Today it is a common phenomenon to encounter both farmers' varieties and improved varieties in rural parts of developing countries. The relationship between variety names and genetic variation is not well defined (Benin et al. 2003). Within crops, "variety" is simply understood as crop population recognized by farmers. Farmers' varieties are defined as varieties that have been grown and selected by farmers for many years and modern varieties are varieties that meet International Union for the Protection of New Varieties of Plants (UPOV) definition. UPOV's definition of improved varieties states that the varieties should be of distinct, uniform and stable as well as "rusticated" or "creolized" types that are the product of deliberate or natural mixing of the two (Bellon and Risopoulos 2001). Usually "name" given by farmers have agro-morphological characters that farmers use to distinguish among them and that are an expression of their genetic diversity.

Centre of origin is defined as the primary centre of *in situ* diversity for a given crop and continued gene flow between crops and their wild relatives can occur. Centre of diversity is defined as geographical area where a wide genetic diversity is found for particular crops and related species (FAO 1996). This definition is, however, difficult to rely on. In some cases, different species of the same crop might have been domesticated in different places, for example yams were domesticated in West Africa, Southeast Asia and in tropical America (Harlan 1976). Furthermore, since evolution outside the centers of origin has resulted in different genetic constitution of the materials, it can be argued that these materials originate from the farms where they were further shaped and maintained. With present sophisticated methods of looking at genetic diversity, such as isozyme and molecular analysis, it has become clear that most genetic diversity in a crop is not necessarily found in its centre of origin.

It is generally accepted that Ethiopia is an important domestication and genetic diversification centers of crop species and their wild relatives (Hancock 2004; Purseglove 2004; Vavilov 1951). Local cultivars/farmers' varieties of several major crops, e.g. durum wheat, bread wheat, barley, sorghum, field pea, faba bean, chick pea, cowpea, linseed, castor bean and wild relatives of some of the world's important crops are abundant in Ethiopia. In Ethiopia the main cereal staples include durum wheat, bread wheat, barley, tef, finger millet, maize and sorghum grown in varying proportions according to soils, altitude and the prevailing climatic and market conditions during planting seasons. The continued interaction of cultivated crop plants with their wild relatives under diverse ecological, social, and economic conditions has made the country one of the most heterogeneous areas of the world in terms of genetic diversity of farmers' varieties. For instance, crops that were originally domesticated outside of the East African highlands exhibit extreme secondary diversification in Ethiopia (Harlan 1969; Vavilov 1951). Vavilov (1951) and Harlan (1969) regarded Ethiopia as a centre of origin of many cultivated species such as *Eragrostis tef*, *Guizotia abyssinica*, *Rhamnus prinoides*, Hygenia *abyssinica*, *Ensete ventricosum*, *Catha edulis* and *Coffea arabica*. According to Harlan sorghum, finger millet, okra, castor bean, and sesame could be of Ethiopian origin. Numerous useful genetic variations of global significance have evolved at the local farm and farming community in the country. These diverse genetic resources are used and managed in various ways by communities.

2.5 Population structure

Population structure occurs when dispersal rates between local populations are sufficiently low to allow genetic differentiation (Slatkin 1987). Thus population structure is intimately linked with gene flow. Population structuring can occur due to the effects of distance, geographic barriers to dispersal or due to mate preferences or behavioral specialization. A central theme of molecular ecology is the detection of population structure for the purposes of defining conservation units and for assessment of evolutionary potential. Statistics employed to detect gene flow or structure typically assume neutrality and are based upon a theoretical model of population dispersal. Population structure is typically measured using (Wright 1951) fixation index, F_{ST} and its analogues, to determine if there is a significant difference in the variance of haplotype frequencies between two populations. The F_{ST} statistic measures the difference in similarity (either as heterozygosity or probability of identity-by descent) of two alleles (haplotypes) drawn from the same population compared to the two alleles drawn at random from the total population and is standardized to a range of 0-1. The greater the diversity - the lower the F_{ST}. This problem is discussed (Hedrick 1999) but in general this leads to F_{ST} statistics being considered to indicate only if gene flow is “high”, “moderate” or “low” and not as an absolute solution. In similar assumption, Spitze (1993) and Prout and Barker (1993) introduced Q_{ST}, a quantitative genetic analog of WRIGHT's F_{ST}. In principle, the average Q_{ST} of a neutral additive quantitative trait is expected to be equal to the mean value of F_{ST} for neutral genetic loci (Whitlock 2008). F_{ST} can be readily measured on commonly available genetic markers, and Q_{ST} can be measured as well with an appropriate breeding design in a common-garden setting.

As a result, Q_{ST} promises to be an index of the effect of selection on the quantitative trait. If Q_{ST} is higher than F_{ST}, then this is taken as evidence of spatially divergent selection on the trait. If Q_{ST} is much smaller than F_{ST}, then this has been taken as evidence of spatially uniform stabilizing selection, which makes the trait diverge less than expected by chance.

2.6 Genetic resources conservation strategies

2.6.1 *Ex situ* conservation

Ex situ conservation of plant genetic resources is mainly based in gene banks. It aims to conserve as much as possible of existing genetic diversity of cultivated species with their infra-specific taxa and wild species of potential use outside agro-ecosystems (Alvarez et al. 2005). *Ex situ* conservation, strategy is limited in that there could be loss of genes or materials in case of failing infrastructure and low level of knowledge regarding optimum storage conditions and seed biology. Furthermore, an important characteristic of gene banks is that they 'freeze' the evolution of the stored genetic materials. It arrests the most complex interaction of genetically diverse farmers' varieties with the associated pests, diseases, climatic factors and wild and weedy relatives. It also fails to retain traditional knowledge associated with landraces, which can be instrumental in the management of genetic resources. The Institute of Biodiversity Conservation (IBC) in Ethiopia is actively implementing conservation of crop species, largely in *ex situ* gene banks. So far the Institute holds about 61,000 accessions of plant genetic resources of which 90 % consist of germplasm of field crops (IBC 2001). However, not all farmers' varieties from all regions and wild relatives of these crop species, which are also of socio-economic value as gene donors to crop species (Hoyt 1988; Cooper et al. 2001), are sufficiently collected and conserved.

2.6.2 *In situ* conservation

In situ conservation is an ecosystem and habitat-based conservation strategy, which allows the maintenance of organisms in their natural habitat. Maintaining genetic variation *in situ* as a complementary strategy to conservation in gene banks has re-emerged as a scientific question in recent years (Brush 2000). For cultivated crops, conservation of genetic resources *in situ* refers to the continued cultivation and management by farmers of crop populations in the open, genetically dynamic systems where the crop has evolved (see on-farm conservation below). Under this system, crops co-evolve with diseases, pests, and weeds by developing mechanisms of co-existence through time. The diversity of crops maintained on farms has both inter-specific and infra-specific components. Inter-specific diversity is the diversity among crop species, while

infra-specific diversity is the repertoire of varieties of a crop that farmers grow simultaneously (Bellon 1996). For conservation of wild relatives of crop species, in *situ* method is given priority and community seed conservation practice on-farm has been taken as a tool for agro biodiversity rural development projects in many countries around the world. However, *in situ* conservation is facing challenge from the expanding human population with irreversible influence on native environment. In Ethiopia the Institute of Biodiversity Conservation is mandated to conserve biodiversity in general and has established various *in situ* sites (field gene banks) for conservation of coffee and other horticultural crop genetic resources in particular (IBC 2001). Understanding the population management processes involved to ensure inter-specific and infra-specific components of genetic conservation *in situ* and the attempt to develop practical techniques to achieve this goal is useful for plant conservationists. For cultivated plant species, this concept is being used in on-farm seed conservation practices.

2.6.3 On-farm conservation

On-farm conservation involves farmers' continued cultivation and management of a diverse set of crop populations and accompanied taxa in the agro-ecosystem where the crop evolved, or in secondary centers of diversity. *In situ* conservation of genetic resources can also specifically target the conservation of local varieties or landraces (Alvarez et al. 2005). In this case, the farm or agro-ecosystem is considered the habitat where the genetic diversity developed or originated. Conservation at farm level allows continuing farmer selection, interaction with environment and gene exchange with wild species so that evolution of the landraces may continue. On-farm conservation practices by farmers, therefore, influence evolutionary forces acting on crop plant populations. Farmers' criteria in seed selection and the goals of selection, choices among varieties, and spatial arrangement of planting in ways that encourage hybridization between varieties that are associated with on-farm activities maintain continuous evolutionary genetics of crop plants in traditional agro-ecosystems (Alvarez et al. 2005). By default, this conservation practice still exists in marginal areas of most developing countries.

Ethiopia is one of the world's richest regions of crop diversity and its genetic resources are of considerable value both within and outside Africa. Among numerous examples are the yellow dwarf virus (BYDV) resistance gene found in Ethiopian barley, on which California's US $160 million annual barley crop depends (Tolba and Rummel-Bulska 1998), as well as the high lysine gene in sorghum. Much of this diversity is still in the hands of the farmers, despite the depletion of some. The recognition of the situation has served as a basis for the inception of on-farm farmers' varieties maintenance and enhancement strategy, in the year 1989 through the project entitled "A dynamic Farmer Based Approach to the Conservation of Ethiopia's Plant Genetic Resources" funded by the Global Environmental Facility (GEF). With this project it was possible to establish 12 on-farm conservation sites and community gene banks to link farming communities and their varieties with the existing formal genetic resource conservation undertaken by the IBC in six agro-ecological regions (IBC 2001). A consortium of Canadian NGO's headed by the Unitarian Service Committee of Canada (USC/C) also supported on-farm conservation of farmers' varieties in drought-prone areas of Ethiopia. However, such projects seem to lose their long-term sustainability because of lack of connection to the socio-cultural basis of on-farm conservation in project implementation sites. Generally, on-farm conservation program encourages smallholder farmers in selected areas to continue growing landraces of several staple crops, which are in danger of being displaced by high yielding varieties. Nevertheless, intensification and mechanization of agriculture accompanied with the fast changing land use system have affected the success of *in situ* conservation. As the result in today's conservation and practical development of crop genetic resource *ex situ* and *in situ* conservation strategies are used complementarily.

2.6.4 *Ex situ* and *in situ*/on-farm as complementary conservation strategy

Dynamic (*in situ*) and static (*ex situ*) conservation strategies have a complementary function in genetic resource management and utilization (Worede 1997). Many gene banks were created to provide the material to breeders and other potential users. Nowadays, the need to ensure the availability of genetic material with information for future generation is gaining importance. Therefore, *ex situ* conservation could play a buffering role and could be a back up against some unpredictable evolution (Berthaud 1997) and could also be involved in the availability and use of

germplasm for the improvement of a variety of crops. For sustainable genetic resource development, part of *ex situ* conservation should be envisaged as connected with dynamic, *in situ*/on-farm conservation. In some cases, short or medium term established *ex situ* conservation might serve restoration of crop varieties on-farm beyond its role to maintain valuable genes for future use.

In situ conservation is particularly useful for conserving semi-wild species or wild relatives of crop species. It is particularly relevant for habitats where crops and their wild relatives occur together, and which are under such pressure that the wild relatives might disappear. *In situ* conservation could help preserve and maintain knowledge, innovation and practices of indigenous and local communities embodying traditional lifestyles relevant for conservation and sustainable use of crop genetic resources (Kebebew 1997). The combination of these dynamic and static conservation strategies, therefore, maintains evolution of materials conserved and associated traditional knowledge. Local plant development with integration of plant breeding and seed system is the basis for such conservation strategies maintaining, stimulating, and enhancing the dynamics of the community management of plant genetic resources.

2.7 DNA Markers in Plant Breeding

Plant Breeding is a dynamic area of applied science. It relies on genetic variation and uses selection to gradually improve plants for traits and characteristics that are of interest for the grower and the consumer. Practical breeding of many economic important crops is performed by commercial companies that strive in a fierce competition for the favor of agricultural producers and consumers (Zuurbier 1994). These improvements were partly realized through an efficient use of existing variability, present within the available material. Another important way of improvement is the introduction of new genetic material (e.g. genes for disease resistance) from other sources, such as gene bank accessions and related plant species. Although current breeding practices have been very successful in producing a continuous range of improved varieties, recent developments in the field of biotechnology and molecular biology can be employed to enhance plant breeding efforts and to speed up the creation of cultivars. Also, new ways and

methods that allow an easier introduction of genetic material from related and unrelated plant species, without the drawbacks that are normally associated with the introduction of "wild genes" through conventional methods, become feasible. Biochemical and Molecular techniques have shortened the duration of breeding programs from years to months, weeks, or eliminated the need for them all together. The use of molecular markers in conventional breeding techniques has also improved the accuracy of crosses and allowed breeders to produce strains with combined traits that were impossible before the advent of DNA technology (Stuber et al. 1999).

Breeding is simply defined as the selective mating of individuals of a population to isolate or combine desired morphological, physiological or genetic traits such as appearance, yield, and disease resistance. This is performed with the assistance of identifiable traits. When a detectable mutant is identified within a population, the gene causing the mutation was placed on a genetic map through a series of crosses that would establish its recombination frequency relative to other genes that had previously discovered and mapped. If the mutant gene was in close proximity to the gene for a desired trait, the mutant gene or "marker" was said to be linked to it because the marker and the gene tend to co-segregate. In a breeding cross, this mutant gene could be used to detect whether or not a breeding cross had been successful in transferring the desired trait. If the mutant gene is observed being expressed in the progeny, it is most likely that the progeny also has the desired trait due to its link to the mutant gene. This is the phenomenon of co-inheritance and the selection of these mutant genes for the tracking of desired traits is called indirect selection.

2.7.1 Morphological Markers

Morphological markers generally correspond to the qualitative traits that can be scored visually. They have been found in nature or as the result of mutagenesis experiments. There are several undesirable factors that are associated with morphological markers. The first is the high dependency on environmental factors. Often the conditions that a plant is grown in can influence the expression of these markers and lead to false determination (Chawla 2004). Second, these mutant traits often have undesirable features such as dwarfism or albinism. And lastly, performing breeding experiments with these markers is time consuming, labour intensive and the

large populations of plants required need large plots of land and/or greenhouse space in which to be grown (Stuber et al. 1999).

2.7.2 Biochemical Markers

Biochemical markers are proteins produced by gene expression. Isozymes, the different molecular forms of the same enzyme that catalyze the same reaction, are proteins. They are the products of the various alleles of one or several genes (Chawla 2004). Isozymes are used as biochemical markers in plant breeding and are common enzymes expressed in the cells of plants. The enzymes are extracted, and run on denaturing electrophoresis gels. The denaturing component in the gels (usually SDS) unravels the secondary and tertiary structure of the enzymes and they are then separated on the basis of net charge and mass. The sequence of nucleotides of the DNA is converted to a sequence of amino acids of polypeptides by the process of transcription and translation (Reiner and Hans 2007). Polymorphic differences occur on the amino acid level allowing singular peptide polymorphism to be detected and utilized as a polymorphic biochemical marker. Biochemical markers are superior to morphological markers in that they are generally independent of environmental growth conditions. The only problem with isozymes in marker assisted selection is that most cultivars (commercial breeds of plants) are genetically very similar and isozymes do not produce a great amount of polymorphism and polymorphism in the protein primary structure may still cause an alteration in protein function or expression.

2.7.3 Molecular Markers

The discovery of restriction enzymes (Smith and Wilcox 1970) and the polymerase chain reaction (PCR) (Mullis and Faloona 1987) have created the opportunity to visualize the composition of organisms at the DNA level, and obtain a so-called genetic fingerprint (Kearsey and Pooni 1996). The visualization is routinely performed by the separation, on a gel, of DNA-fragments that result from a selective digestion with enzymes or fragments that result from a selective amplification using PCR. DNA-fragments that result in different gel patterns between samples or individuals are called polymorphic markers. The visible differences on the gel result from differences at the DNA level. Not all types of markers are the same, the information content

depends on the method that was used to obtain the marker data and the population in which the markers were 'scored'. For instance, it is not always possible to distinguish genome fragments that are present in homozygous condition from heterozygous fragments. In a heterogeneous population like an F_2, co-dominant markers like RFLPs (Botstein et al. 1980) and co-dominantly scored AFLPs (Vos et al. 1995) yield more information than dominant markers like RAPDs (Welsh and McClelland 1990) and dominantly scored AFLPs. Advanced tools for the retrieval of marker data and the subsequent analysis have been developed and allow a quick and reliable analysis in most plant species. These developments have opened up a new era for genetics and selection (Moreau 1998).

Molecular markers are based on naturally occurring polymorphisms in DNA sequences (i.e.: base pair deletions, substitutions, additions or patterns). There are various methods to detect and amplify these polymorphisms so that they can be used for breeding analysis. Molecular markers are superior to other forms of marker assisted selection because they are relatively simple to detect, abundant throughout the genome even in highly bred cultivars, completely independent of environmental conditions and can be detected at virtually any stage of plant development. Molecular markers can be used for several different applications including: germplasm characterization, genetic diagnostics, characterization of transformants, study of genome organization and phylogenic analysis.

Different kinds of molecular markers exist, such as RFLPs, RAPDs, AFLPs, SSRs and SNPs. They may differ in a variety of ways - such as their technical requirements; the amount of time, money and labour needed; the number of genetic markers that can be detected throughout the genome; and the amount of genetic variation found at each marker in a given population (Table 1). The information provided by the markers for the breeder will vary depending on the type of marker system used. Each one has its advantages and disadvantages and, in the future, other systems are also likely to be developed (Korzun 2003).

A brief overview of the major marker systems as follows:

Restriction Fragment Length Polymorphisms

Restriction Fragment Length Polymorphisms (RFLPs) are markers detected by treating DNA with restriction enzymes (enzymes that cut DNA at a specific sequence) (Botstein et al. 1980). For example, the EcoR1 restriction enzyme cuts DNA whenever the base sequence GAATTC is found. Differences in the lengths of DNA fragments will then be seen if, for example, the DNA of one individual contains that sequence at a specific part of the genome (e.g. tip of chromosome 3) whereas another individual has the sequence GAATTT (Which is not cut by EcoR1). RFLPs were the first molecular markers to be widely used. Their use is, however, time-consuming and expensive and simpler marker systems have subsequently been developed.

Table 1: Comparison of marker systems

Feature	RFLPs	RAPDs	AFLPs	SSRs	SNPs
Amount of DNA required (μg)	10	0.02	0.5-1.0	0.05	0.05
Quality of DNA required	high	high	moderate	moderate	high
PCR-based	no	yes	yes	yes	yes
Number of polymorphic loci analyzed per analysis	1.0-3.0	1.5-50	20-100	1.0-3.0	1
Ease of use	not easy	easy	easy	easy	easy
Amenable to automation	low	moderate	moderate	high	high
Reproducibility	high	unreliable	high	high	high
Development cost	low	low	moderate	high	high
Cost per analysis	high	low	moderate	low	low

Random Amplified Polymorphic DNA

Random Amplified Polymorphic DNA (RAPD) markers were first described in 1990 (Williams et al. 1990). They are detected using the polymerase chain reaction (PCR), a widespread molecular biology procedure allowing the production of multiple copies (amplification) of specific DNA sequences. The analysis for RAPD markers is quick and simple, although results are sensitive to laboratory conditions.

Amplified Fragment Length Polymorphism

PCR-based method of generating molecular markers was described by (Vos et al. 1995), giving rise to Amplified Fragment Length Polymorphism (AFLP) markers. With this technique, DNA treated with restriction enzymes is amplified with PCR. It allows selective amplification of restriction fragments giving rise to large numbers of useful markers which can be located on the genome relatively quickly and reliably.

Simple Sequence Repeats

Simple Sequence Repeats (SSRs) also known as microsatellites are simple DNA sequences (e.g. AC), usually 2 or 3 bases long, repeated a variable number of times in tandem. They are easy to detect with PCR and a typical microsatellite marker has more variants than those from other marker systems. Initial identification of SSR is time-consuming. Simple sequence repeat markers have emerged as very attractive tools for genetic studies (Saghai-Maroof et al. 1994; Liu et al. 1996), because they are PCR-based, relatively inexpensive, primarily co-dominant, reproducible across mapping populations, and multi-allelic. They are generally recognized as neutral, so that they do not influence the expression of a linked gene. However, SSR markers in barley are also derived from EST sequences and are thus embedded in coding sequences where mutations are likely to have an effect on gene function, e.g. by shifting the open reading frame. It has been found that mutations in SSR repeat number cause quantitative variation in transcriptional activity and biological function of human and mammalian genes (Kashi et al. 1997). Data from SSR markers are primarily used as single loci (if they are unlinked), but may also be employed for haplotyping (if they are physically linked). Moreover, it must be highlighted that SSR convey an extra amount of information, compared to other classes of markers, thanks to the underlying stepwise mutational model (Slatkin 1995). SSR markers were first used in genetic analysis of humans (Tauz 1989) and later in plant studies (Morgante and Olivieri 1993). The variability at SSR marker loci is due to the differences in the number of repeat units, e.g. di-, tri- or tetranucleotide repeats. In barley there are currently over 1045 SSRs have been mapped (Saghai-Maroof et al. 1994; Liu et al. 1996; Pillen et al. 2000; Ramsay et al. 2000; Li et al. 2003; Thiel et al. 2003; Marcel et al. 2007; Rostoks et al. 2007; Varshney et al. 2006; Varshney et al. 2007). In the majority of the studies mentioned above, the SSR markers were developed after screening

small insert or microsatellite enriched genomic libraries for SSR motifs. In recent years, however, because of the availability of large expressed sequence tag (EST) datasets for a number of plant species and the development of several bioinformatics tools, it has been possible to identify and develop SSR markers from ESTs (Pillen et al. 2000; Thiel et al. 2003; Ramsay et al. 2004; Varshney et al. 2006). The SSR markers derived from ESTs are commonly known as ‘‘EST-SSRs’’. The development of such markers, in contrast to the earlier genomic SSRs, is easier, faster and cheaper (Varshney et al. 2005).

Single Nucleotide Polymorphisms

Single Nucleotide Polymorphisms (SNPs) (Gupta et al. 2001; Marcel et al. 2007) i.e. single base changes in DNA sequence, have become an increasingly important class of molecular marker. The potential number of SNP markers is very high, meaning that it should be possible to find them in all parts of the genome, and micro-array procedures have been developed for automatically scoring hundreds of SNP loci simultaneously at a low cost per sample.

3. Materials and Methods

The present study was aimed to analyze the presence of diversity among Ethiopian barley landraces using morphological and molecular marker SSRs. Area of origin and altitude of collection were the basis for studying. The detail information on the structure of data and the methods used will be presented below.

3.1 Morphological Study

3.1.1 Experimental Materials

Barely germplasm for this study were provided by Institute of Biodiversity Conservation (Ethiopia) with their passport data (Appendix 1). The accessions were originally collected from 10 regions of Ethiopia, which covers an altitudinal range from 1740 to 3430 m.a.s.l. and collected from year 1983 to 2005. A total of 199 barley (*Hordeum vulgare* L.) accessions and four released cultivars (Shege, Ardu 12-60B, HB-42 and HB-1307), which were commonly used and adapted to the experimental sites, were used for the study (Table 2). Accessions (130 accessions) which have longitudes and latitudes were plotted on Ethiopian map (Fig.1).

Table 2: Regions in Ethiopia, number of accessions and altitude ranges used for collecting barley germplasm in present study

	Altitude classes				
Regions	**Class I <2000**	**Class II 2001-2500**	**Class III 2501-3000**	**Class IV >3000**	**Total**
Arsi	-	5	8	2	15
Bale	3	20	3	-	26
Gamo Gofa	2	6	16	1	25
Gojam	1	5	4	1	11
Gonder	-	9	8	1	18
Harerge	3	16	3	-	22
Shewa	1	5	7	6	19
Sidamo	7	18	2	-	27
Tigray	4	8	4	-	16
Welo	-	3	9	8	20
Total	21	95	64	19	199

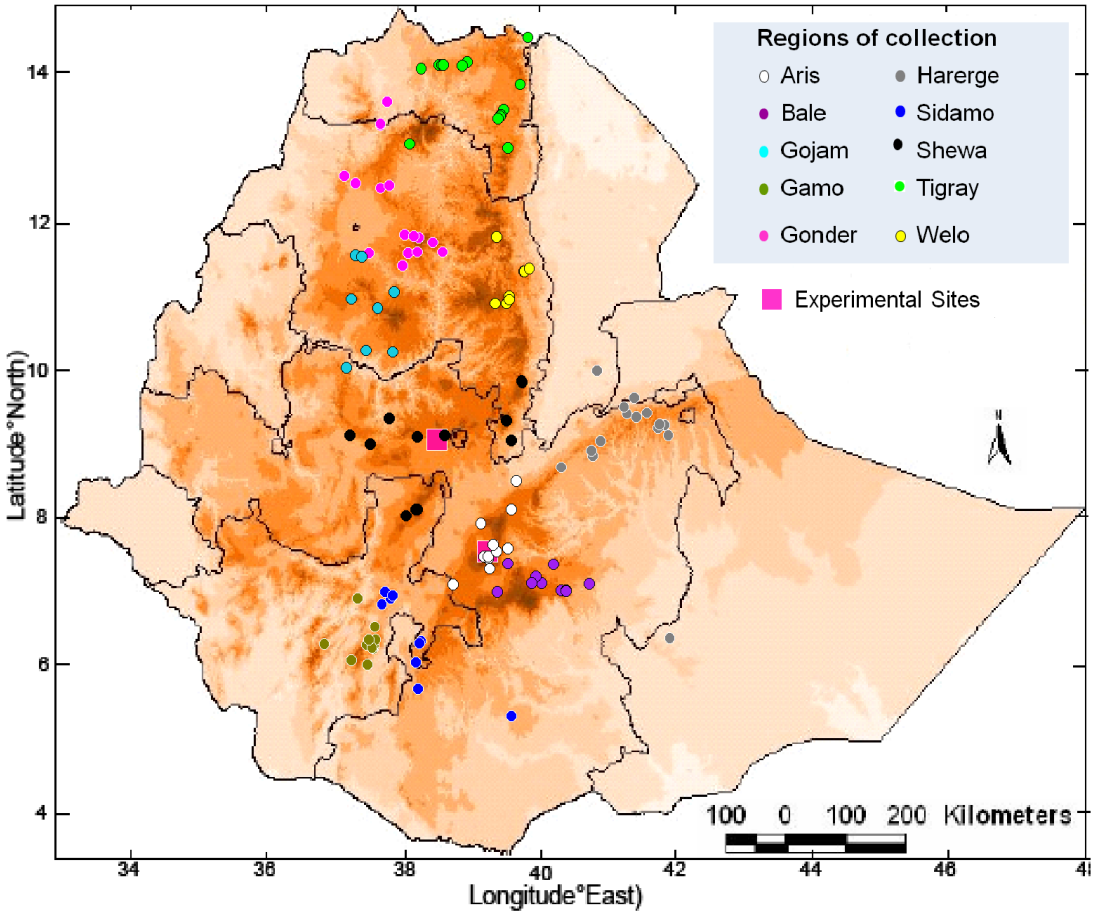

Fig. 1: Geographical distribution of 130 barley accessions (○) and location of experimental sites (♦) (Map adapted from (Tamene 2005))

Accessions were originally collected from regions Arsi, Bale, Gamo Gofa, Gojam, Gonder, Harerge, Shewa, Sidamo, Tigray and Welo. The original samples were collected from farmers' fields, by use of random sampling technique (Hawkes 1976).

3.1.2 Experimental Sites

The accessions were tested at Holetta and Bekoji Agricultural Research Centers, Ethiopia, in main cropping season of 2006. Holetta Agricultural Research Center (9 degree 3'N and 38 degree 30'E) is located 39 km west of Addis Ababa. It is one of the research centers known for highland crops and located at an altitude of 2400 m.a.s.l. with annual average rainfall of 1055 m^2 most falling between March and October with peaks in July and August. The temperature ranges from 22^0C to 6^0C with the soil type classified as Eutric Nitisol with a pH of 4.92.

Bekoji (7 degrees 32'N and 39 degrees 15'E) research site located in Arsi region of Ethiopia. The station soil is classified as Eutric Nitosol, exhibiting a clay content of approximately 48.5% (Tanner et al. 1993) and is relatively deficient in Phosphorus with pH of 5.3. The site receives an annual average rainfall of 1020 m^2 which occurs from June-October growing season. The station is situated at an altitude of 2780 m.a.s.l. with an annual average temperature ranges from 8^0C to 20^0C.

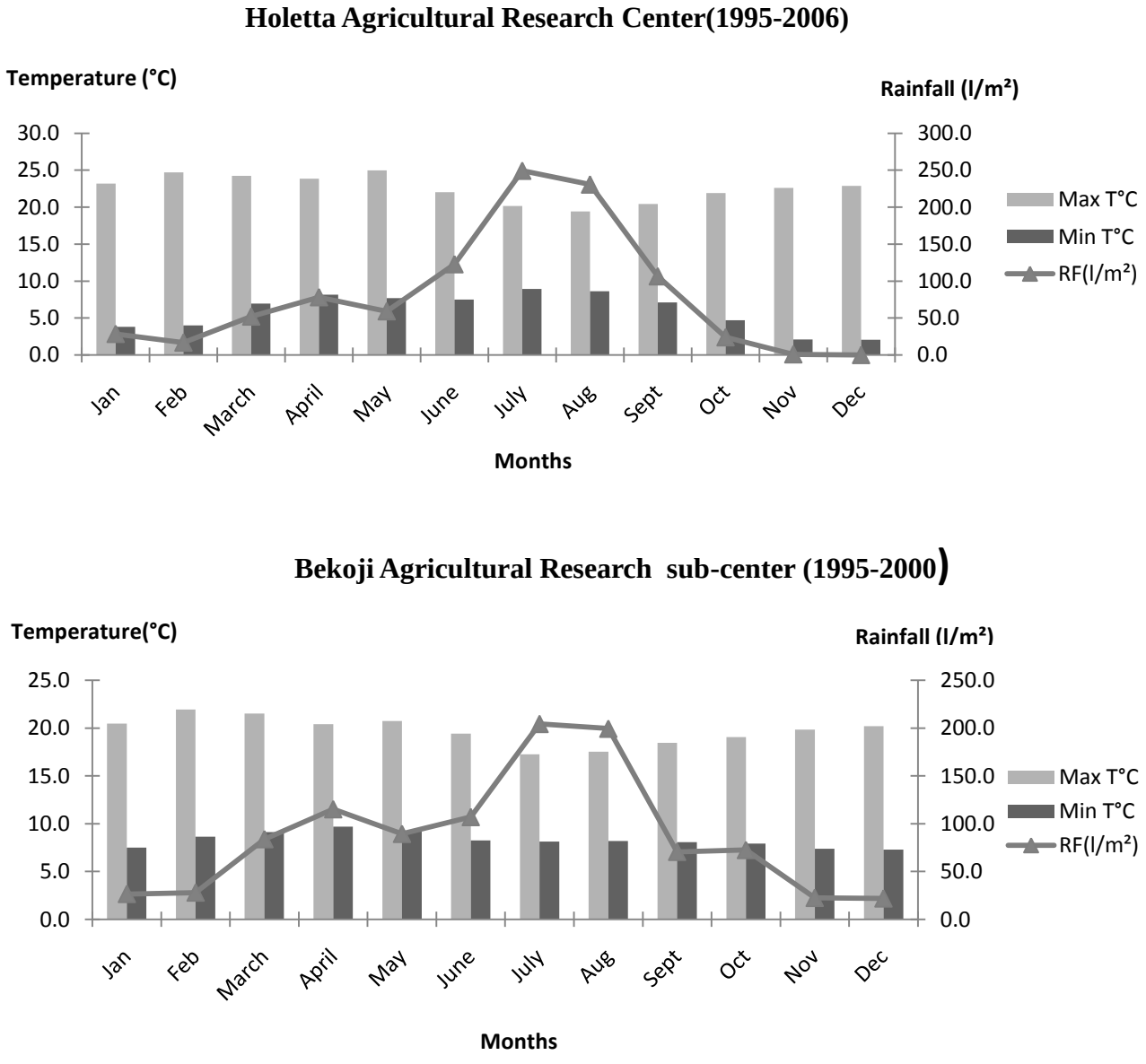

Fig. 2: Climate data of the experimental sites at Holetta and Bekoji (minimum and maximum temperature as well as monthly rainfall)

The trial was conducted in augmented design with four blocks and four cultivars, which were placed after each 7 accessions. Each accession was grown in a single row with 2.5 m length and 0.4m width between rows.

3.1.3 Agronomic data collected

Data were recorded for nine quantitative characters using barley Descriptors (IPGRI 1994).

1. **Days to Heading (DH):** the number of days from planting up to heading.
2. **Days to Maturity (DM):** the number of days from planting up to physiological maturity.
3. **Thousand Seed weight (TSW):** weight of 1000 seeds randomly taken from each plot in gram.
4. **Flag Leaf Length (FLL):** the length of the flag leaf from its bottom to tip in cm.
5. **Awn Length (AL):** distance from the tip of the spike to the end of the awn
6. **Spike Length (SL):** distance from the base of the spike to the tip of the highest spikelet (excluding own) in cm.
7. **Number of Seeds per Spike (NSS):** the actual count of the number of spikelet of the mother spike
8. **Plant Height (PLH):** the distance between the ground level to the tip of the terminal spikelet in cm of the mother plant
9. **Number of Fertile Tiller per Plant (NFTP):** the actual count of the fertile numbers of tillers (spike bearing) per plant

For each accession, 10 individual plants were used to record data except for days to heading, days to maturity, thousand seed weight and biomass, in which data were recorded on plot basis.

10. **Beta Glucan (BG):** 199 barley samples were milled and incubated at 33^0C for 3days. 10 ml enzyme solution (9.9 ml of bidest water and 0.1 ml α-amylase) were added on 50mg of milled barley samples. Vortexed samples incubated in 100^0c in water bath for 1hour. The samples were cooled in cold water and 0.75 M of H_2SO_4 were added and vortexed. Again the samples incubated in 100^0C in water bath for 16 minutes sharply. The samples again cooled in cold water (10°C) and centrifuged 3000 rpm for 10 minutes. Finally the supernatant pored to new labeled tube. Beta glucan measured using the tecator β-glucan 5700 analyzer.

3.2 Molecular study

3.2.1 DNA extraction

DNA from 199 barley accessions representing plot and 197 barley lines representing accessions were isolated according to Cetyl trimethylammonium bromide (CTAB) protocol (Saghai-Maroof et al. 1984). For plot data one single spike representing one accession was used for DNA extraction and analysis were conducted based on this data. While two accessions from each regions represented by 10 spikes were used to determine the variation exists within accessions.

Fresh leaves were harvested from two weeks old barley seedlings grown on peteridish. From each sample four plants were used to handle the possible existed variation. The leaves were stored in -80^{0}c freezer until DNA extraction. Approximately 100 mg leaves were transferred in to 96 deep well plates. One tungsten bead was added in each well and freezed in liquid nitrogen. Freezed samples homogenized in tissuelyser at 20 Hz for 1 minute followed by centrifugation at 3000 rpm for 2 minutes. In each well 200 µl extraction buffer added and shaked gently without flipping. The samples incubated at 60^{0}C for 60 minutes followed by cooling at 4^{0}C for 15 minutes. Subsequently 200 µl chloroform/isoamyl alcohol (24:1) was added under the hood and centrifuged with 3000 rpm for 30 minutes. Again 150 µl of extraction buffer added and centrifuged 30 minutes with 3000 rpm. Then 100 µl of the supernatant transferred into sterile 96 deep well plates, which contained 200 µl cold isopropanol. The precipitated DNA then collected by centrifugation for 30 minutes with 3000 rpm. The supernatant drained off immediately and then pellets washed with 100 µl 70% cold ethanol. Finally the DNA pellet was incubated at 60^{0}C for 45 minutes and resolved in 100 µl of bidest water.

Buffers and solutions

Buffers and solutions used for extraction of DNA will be presented in detail.

CTAB buffer for 96 samples

Soribitol buffer 15.75 ml

Lysis buffer 15.75 ml

5% laurylsarcosine 6 ml

Na bisulphate 300 mg

Sorbitol buffer, pH 7.5

Sorbitol	350 mM
Tris	100 mM
EDTA	5 mM
H_2O (high purity)	ad 2 L

Adjust to pH 7.5 with HCl and store at 4°C

Lauryl sarcosine 5%

Lauryl sarcosine	170.42 mM
H_2O (high purity)	ad 500 ml

Keep at room temperature

Nucleic lysis buffer

Tris	200 mM
EDTA	50 mM
$NaCl_2$	2 M
CTAB	2 %
H_2O (high purity)	ad 5 L

Keep at room temperature

Tris-borate-EDTA-buffer (5x TBE), pH 8.3

Tris	450 mM
Boric acid	450 mM
EDTA	10 mM
H_2O (high purity)	ad 5 L

Adjust to pH 8.3 with NaOH at room temperature

Chloroform/isoamyl alcohol 24:1

24 volumes chloroform and 1 volume isoamyl alcohol were mixed.

The DNA was run on a 2.5 % agarose gel to check its quality and to quantify the amount of DNA by ethidium bromide after electrophoresis. Nanodrop spectrophotometer was also run to check the concentration of DNA.

3.2.2 SSR analysis

A total of 15 SSR markers selected with an even coverage of the barley chromosomes (Table 3).

Table 3: List of SSR markers used for genotyping barley samples

Locus	Chrom.	Position (cM)	Repeat	Theor. Size	References
GBM1042	1HS	46.0	$(AAC)_5$	296	Thiel et al. 2003
Bmag0579	1HL	132.8	(AC)6(AG)15	126	Ramsay et al. 2000
Bmag0211	1H	59.8	(CT)16	174	Pillen et al. 2000
HVM36	2HS	31.0	(GA)13	114	Liu et al. 1996
GMS003	2H	66.1	(GT)15	144	Struss and Plieske 1996
HVM54	2HL	122.4	(GA)14	159	Liu et al. 1996
EBmac0541	3HL	137.2	(AC)9	106	Ramsay et al. 2000
HVLTPPB	3H	20.5	(AC)10	216	Pillen et al. 2000
HVM67	4HL	120.5	(GA)11	116	Liu et al. 1996
Bmag0337	5H	45.0	(AG)22	145	Ramsay et al. 2000
Bmag0222	5H	144.9	(AC)9(AG)17	179	Ramsay et al. 2000
Bmac0316	6HS	7.2	(AC)19	135	Ramsay et al. 2000
Bmac0040	6HL	113.2	(AC)20	236	Ramsay et al. 2000
Bmag0007	7H	22.2	(AG)16(AC)16	185	Ramsay et al. 2000
Bmag0135	7HL	147.5	(AG)10GG(AG)12	161	Ramsay et al. 2000

3.2.3 Polymerase Chain Reaction (PCR)

PCR amplification was performed on 15 µl final volume reactions containing 5µl template DNA (10ng/µl), including 0.5 µl Taq polymerase (5units/µl, Promega), 1.5 µl of 10 × PCR buffer, 0.75 µl of 25 mM $MgCl_2$, 0.75 µl of dNTP, 0.075 µl of the forward and reverse oligonucleotide primers (10 µM) and 0.5 µl of the M13 universal forward primer (1pmol/µl). Each forward oligonucleotide primer was tailed by adding the M13 universal forward primer sequence at the 5' end. The M13 primer was labeled with either IRD700 or IRD800 at the 5'end for visualization. The samples of PCR amplification were stored at -20°C before loading.

The amplification profile started with 10 cycles of denaturing at 94 °C for 1 min, annealing at 64- 55 °C (touch-down PCR) for 1 min and extension for 1 min at 72 °C followed by 30 cycles of 1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C with a final extension step at 72 °C for 5 min.

3.2.4 Licor analysis

The LI-COR DNA Sequencer 4200 was used as an automated DNA detection device. The Li-COR system employs infrared fluorescence to detect DNA. During the PCR reaction, the DNA polymerase incorporates an infrared dye (IRD)-labelled primer into the PCR fragments. The IRD labelled fragments separate according to size on an acrylamide gel. A solid-state diode excites the infrared dye on DNA fragments as they pass the detector window. A focusing fluorescence microscope containing a solid-state silicon avalanche photodiode scans back and forth across the width of the gel collecting data in real time. The raw image data are series of bands displayed autoradiogram-like on the computer screen.

IRD800 is a heptamethine cyanine dye absorbing and fluorescing in the near infrared region of the spectrum. The absorption maximum at 795 nm is well-matched to the 785 nm laser of the DNA sequencer. The extremely high absorptivity and good quantum efficiency of the dye provide excellent sensitivity (LI-COR 1999).

IRD700 is a pentamethine carbocyanine dye fluorescing in the near infrared region of the spectrum. The absorption maximum (685 nm) is just outside the visible region and matches the 685 nm laser of DNA sequencer. While the absorptivity of IRD700 is slightly less than that of IRD800, the higher fluorescence efficiency compensates for the absorption difference (LI-COR 1999).

3.2.5 Genotyping score

For each SSR marker and each sample, fragment sizes were visualized by the e-seq software and genotype scoring was carried out manually 1 as presence and 0 as absence of bands. Genotype data was archived in Excel tables for further analysis.

3.3 Statistical analyses

Since the barley accessions used for the study was several in number (199 accessions) and less amount of seeds for running complete block design, field experiment was conducted with an augmented design consisting of four blocks without replications. In addition to the accessions, four improved cultivars (checks) were also included which were replicated in each block for estimation of error variance. So all Statistical analyses for morphological data were conducted using SAS software (SAS 2004). Data were analyzed by restricted maximum likelihood (REML) to fit a mixed model with checks and experimental sites as a fixed effect and unreplicated accessions as random effect (Little et al. 1996; Comadran 2008). The REML model produced best linear unbiased predictors (BLUPs), which can handle unbalanced data while accounting for differences in the amount of data available for each accessions (Etten et al. 2008; Bernardo 2002), for the data of each accessions in both sites to be used in subsequent analyses. PROC MIXED was conducted to estimate genotypic as well as residual variance component.

MIXED used to fit model of the form

$$Y_{ijk} = \mu + L_i + S_j + A_k + L_i*A_k + e_{ijk}$$

Where Y_{ijk} is response variable; μ is general mean; L_i is the fixed effect of i^{th} location; S_j is the fixed effect of j^{th} standard checks; A_k is the random effect of k^{th} accession; L_i*A_k is the random interaction effect of i^{th} location with j^{th} accession and e_{ijk} is random errors.

Heritability (h^2) for accessions was calculated following (Comstock & Moll, 1963) from components of variance.

$$h^2 = \delta_g^2 / (\delta_g^2 + (\delta_{g*l}^2/2) + \delta_e^2)$$

Where δ_g^2 is genetic variance of accessions; δ_{g*l}^2 genetic variance of accessions by location interactions and δ_e^2 error variance.

Before undertaking multivariate analysis, in which two or more variables analyzed at a time, the data were standardized to mean of zero and a variance of one to avoid differences in scales used for recording data (Sneath and Sokal 1973). PROC Princomp was employed to group the variables into subsets that are relatively independent from each other as well as for reducing the dimensionality of the structure. To examine the validity of the origin based groupings of the accessions PROC Discrim were utilized. Using PROC Cluster 199 accessions and 10 regions of origin were clustered into respective classes. Values of the Cubic Clustering Criterion (CCC), pseudo F statistic (PSF) and Hotelling's pseudo T^2 statistic were considered for defining optimum cluster numbers (SAS 2004). The measure of dissimilarity was Euclidean distance and the accessions were clustered using WARD method. Principal Components for regions was plotted along the first three axes to graphically display relationships among regions.

Genotypic data were exposed for different analysis using different software. To refer to the informativeness of microsatellites, Polymorphic Information Content (PIC) was employed based on the following formula:

$$PIC = 1 - \left(\sum_{i=1}^{n} Pi^2 \right)$$

Where *Pi* is the frequency of the i^{th} alleles and *n* is the number of alleles and this value referred as heterozygosity and gene diversity (Weir 1990; Anderson et al. 1993). The binary data generated by SSR analysis was used to calculate polymorphism percentage by dividing amplified polymorphic band by total number of bands observed. To examine the relative proportion of variation among and within regions, altitude and accessions Analysis of Molecular Variance (AMOVA) were computed using ARLEQUIN ver. 3.11 (Excoffier et al. 2005). Population differentiation was used the F statistics which measures how much of the total heterozygosity (H_T) is explained by within population heterozygosity (H_S):

$F_{ST} = (H_T - H_S)/H_T$

Gene flow among populations was estimated with Nm, the number of migrants per generation between pairs of populations. Nm was estimated from the formula $Nm = 1/(4F_{ST}) - 1/4$ (Slatkin

1993). The Nm values were grouped into three categories: high (Nm≥1), intermediate (0.250-0.99) and low (0.00-0.249) (Slatkin 1981, 1985; Caccone 1985; Waples 1987). The genotypic data were arranged in appropriate format using convert (Glaubitz 2005) before analyzed in AMOVA. Among 199 accessions used for the study only 130 accessions which have full data of longitudes and latitudes used to find Universal Transverse Mercator (UTM) which further involved to calculate geographic distance using Euclidean distance. To see the correlation between geographic distance and dice similarity matrix was conducted using XLSTAT (Agresti 1990; Saporta 1991) and the significance of the P value was calculated based on 10000 permutation. PAST (Hammer at al. 2001) version 1.95 software were employed to construct neighbor joining tree for population pair wise F_{ST}.

Just as F_{ST} gives a standardized measure of the genetic differentiation among populations for a genetic locus, Q_{ST} measures the amount of genetic variance among populations relative to the total genetic variance (Spitze 1993):

$$Q_{ST}=V_b/(V_b+V_w)$$

Where V_b and V_w are the variance between population and within population respectively.

Comparison of Q_{ST} and F_{ST} estimates of population differentiation was made using the overall loci estimates of SSR markers as a null hypothesis to test quantitative differentiation divergences (Yang et al. 1996). The 95% confidence interval (CI) for F_{ST} and QST estimated by bootstrapping accessions and loci 1000 times using the program R package (http://www.r-project.org). Q_{ST} was considered to be statistically different from F_{ST} when 95% confidence intervals of Q_{ST} did not overlap 95% confidence intervals of F_{ST} (Sahli et al. 2008).

The software STRUCTURE ver 2.3.1 (Pritchard et al. 2000) was used to analyze the genetic structure of the population and to perform an assignment test on the studied individuals. This program implements a model-based Bayesian clustering method for inferring population structure using genotype data of markers. Here it was also applied to assign individuals to each subpopulation or cluster. Analyses were performed using the admixture model with correlated marker allele frequencies (Falush et al. 2003) in cases of subtle population structure. When alpha is close to zero, most individuals are essentially from one population or another, while alpha > 1

means that most individuals are admixed (Falush et al. 2003). To choose the appropriate number of inferred clusters 20 independent job was performed with K=2 to K=20. All computations used a burn-in period of 50 000 and 10000 Markov Chain Monte Carlo (MCMC) iterations for data collection.

Similarity matrix used for clustering was estimated using Dice coefficient equation:

$G_{ij}=2a/ (2a+b+c)$

Where 'a' refers to alleles shared between two accessions and 'b' and 'c' to alleles present either in accession *i* or accession *j* respectively (Dice 1945).

The degree of genetic relationship among the studied barley accessions as revealed by Dice's similarity coefficient was represented through cluster analysis using the algorism of Unweighted Pair Group Method with Arithmetic Average (UPGMA). The degree of association between the similarity estimates based on SSR and Euclidean distance; and SSR and geographic distance was done by Mantel test (Mantel 1967) using MXCOMP in NTSYS-pc (Rohlf 1998) thereby to determine the significance level of correlation coefficient between the matrices. The analysis was conducted with the null hypothesis which stated that there is no correlation between both variables. The analysis was performed with 5000 permutations to estimate a significance level.

The goodness of fit of the clustering compared to the basic data matrix was also tested by computing co-phenetic correlation using software NTSYS-pc program (Rohlf 1998). Additionally principal coordinate analysis (PCoA) was carried out based on the pairwise genetic similarity matrix using software XLSTAT (Agresti 1990; Saporta 1991).

4. Results

4.1 Phenotypic variation

To determine the variation existing among and within accessions collected from different regions of origin and different altitudes, different statistical analyses was conducted and the results are presented thereafter.

4.1.1 Analysis of Variance

Results obtained from analysis of variance indicate significant differences between accessions for all traits (Table 4). The same result was obtained for all traits in testing location, except for flag leaf length, while no genetic-location interaction was observed for plant height. All traits showed medium (0.1-0.3) to high heritability (>0.3) with low border values for flag leaf length (0.12) and number of fertile tiller per plant (0.12). Days to heading (0.83), days to maturity (0.73), number of seeds per spike (0.77) and thousand seed weight (0.67) were traits which showed highest heritability.

Table 4: Results of variance components and heritability with standard error for 199 barley accessions

Source of variation	δ_g^2	δ_{g*l}^2	δ_e^2	Heritability
DH	33.46**	0.62*	6.59	0.83±3.76
DM	21.97*	1.53*	7.54	0.73±2.71
TSW	25.37**	4.11**	10.6	0.67±3.38
FLL	0.19*	0.14*	1.29	0.12±0.11
AL	0.16**	0.0035	0.29	0.36±0.03
SL	0.53**	0.01	0.31	0.62±0.07
NSS	59.22*	0.21	17.42	0.77±6.93
PH	14.53**	0	19.89	0.42±2.63
NFTP	0.06*	0.01	0.42	0.12±0.03

Where: *, ** significant at P=0.05 level and P=0.01 respectively, δ_g^2, δ_{g*l}^2, δ_e^2, genetic, genotype by location interaction and error variance respectively; DH=Days to Head, DM=Days to Maturity, TSW=Thousand Seed Weight, FLL=Flag Leaf Length, AL=Awn Length, SL=Spike Length, NSS=Number of Seeds per Spike, PH=Plant Height, NFTP=Number of Fertile Tiller per Plant

4.1.1.1 Regional Variation

Though analysis of variance indicated statistically no significant variation between regions (Table 5) for all traits, high variation for each region was observed, which indicated wide differences for each character within region. Except Harerge all regions showed high variation for days to heading and maturity where as none of the regions showed significant variation for number of fertile tiller per plant except Shewa. Accessions collected from all regions revealed statistically significant variation for number of seeds per spike. Sidamo showed significant variation for all traits except flag leaf length and number of fertile tiller followed by Shewa, which showed significant variation for all traits except plant height indicating wide variation within these regions. Gojam showed significant variation for days to heading, days to maturity and number of seeds per spike while Harerge showed no variation for all traits except for flag leaf length and number of seeds per spike (Table 6).

Table 5: Mean, minimum and maximum values of selected traits of accession with the significance test for regions and altitude classes

Traits	Minimum	Maximum	Mean	Regions	Altitude classes
DH	63.06	100.13	81.5	NS	**
DM	118.34	144.53	181.4	NS	**
TSW	28.39	58.21	41.66	NS	*
FLL	15.16	21.38	18.48	NS	NS
AL	9.46	12.89	11.33	NS	*
SL	4.63	10.49	8.07	NS	NS
NSS	21.32	64.29	40.48	NS	*
PH	99.43	135.18	120.51	NS	**
NFTP	2.63	5.75	3.99	NS	NS

Where: *, **, NS: significant at P=0.05 P=0.01 level and Non significant at P>0.05respectively; DH=Days to Head, DM=Days to Maturity, TSW=Thousand Seed Weight, FLL=Flag Leaf Length, AL=Awn Length, SL=Spike Length, NSS=Number of Seeds per Spike, PH=Plant Height, NFTP=Number of Fertile Tiller per Plant

Region based heritability analysis showed wide variation for each region depending on the traits. Shewa, Sidamo, Gonder and Bale showed medium to high heritability for all traits and flag leaf with lowest heritability as compared to other traits (Table 7). Medium to high heritability was

estimated for days to heading, days to maturity, thousand seed weight, and number of seeds per spike and plant height while other traits have no heritability estimates in one region or more.

4.1.1.2 Altitudinal variation

Analysis based on different altitude classes (Table 5) showed significant variation among altitudes classes for all traits except flag leaf length, spike length and number of fertile tiller plant. High genetic variation observed within each altitude classes based on the traits involved (Table 6). Statistically high significant variation was observed between all altitude classes for days to heading, thousand seed weight, awn length, spike length and number of seeds per spike. Altitude class II and III showed statistically significant variation for all traits. While altitude class I and IV showed no significant variation for flag leaf length, number of fertile tiller per plant and in addition statistically no significant variation observed for accessions collected from altitude class IV for days to maturity.

In contrast to regions all altitude classes have heritability estimates ranged from low to high. While altitude class II and III showed from medium to high heritability for all traits but lowest heritability observed for number of fertile tiller per plant in altitude class II. Altitude classes I and IV showed relatively low heritability as compared to altitude class II and III depending on the traits. As compared to altitude class IV, altitude class I showed less heritability estimates which is associated with less genetic variation for these altitude classes (Table 7).

To see if longitudes and latitudes have any association with heading and maturity date, correlation analysis was conducted but the results showed there was no correlation between longitudes, latitudes and other morphological traits (Table 8).

Table 6: Genetic variance estimates for 10 regions and altitude classes based on nine quantitative traits

Region	DH	DM	TSW	FLL	AL	SL	NSS	PH	NFTP
Arsi	47.51*	58.92*	26.40*	1.02*	0.59*	0.73*	76.98*	1.22	0.24
Bale	8.32**	7.71**	4.17	0.11	0.05	0.31**	28.16**	3.82	0.15
Gamo	19.79**	9.01*	52.69**	0.00	0.14*	0.18**	84.74**	1.32	0.09
Gojam	63.84*	24.45*	18.91	0.00	0.04	0.26	55.62*	23.87	0.00
Gonder	120.04**	57.44*	13.04	1.73*	0.11	0.16	50.67*	37.84*	0.26
Harerge	2.07	0.33	1.96	0.68*	0.00	0.05	11.94*	1.12	0.00
Shewa	13.27*	8.80**	43.66*	0.61*	0.25*	0.94**	91.44**	14.45	0.48*
Sidamo	18.08**	18.47**	51.02**	0.14	0.60**	2.04**	76.96**	47.49**	0.14
Tigray	11.88*	4.75*	9.92*	0.00	0.09*	0.06	54.75**	3.93	0.00
Welo	20.58**	12.98*	22.28**	0.18	0.21*	0.29*	26.77*	3.53	0.00
Altitude Class I	14.33**	9.34**	25.29**	0.17	0.18*	0.59*	49.47**	10.73*	0.04
Altitude Class II	31.21**	22.27**	16.23**	0.46**	0.24**	0.63**	50.85**	17.38**	0.10*
Altitude Class III	47.22**	29.89**	38.01**	0.48*	0.17**	0.43**	77.54**	15.09**	0.20**
Altitude Class IV	29.17**	11.99	33.13**	0.11	0.17*	0.55**	51.77**	15.09*	0.08

Where: *, ** significant at P=0.05 level and P= 0. 01 respectively; DH=Days to Head, DM=Days to Maturity, TSW=Thousand Seed Weight, FLL=Flag Leaf Length, AL=Awn Length, SL=Spike Length, NSS=Number of Seeds per Spike, PH=Plant Height, NFTP=Number of Fertile Tiller per Plant; Altitude Class I= < 2000m, Altitude Class II=2001-2500m, Altitude Class III=2501-3000m, Altitude Class IV= >3000m

Table 7: Heritability estimates with standard error for 10 regions and 4 altitude classes

Regions	DH	DM	TSW	FLL	AL	SL	NSS	PH	NFTP
Arsi	0.92±20.82	0.87±27.56	0.87±12.14	0.52±0.63	0.73±0.29	0	0.88±35.68	0.12±4.98	0.49±0.16
Bale	0.81±2.87	0.77±2.79	0.35±3.89	0.15±0.19	0.16±0.07	0.56±0.13	0.78±10.7	0.42±2.87	0.29±0.11
Gamo Gofa	0.92±6.48	0.69±4.03	0.90±17.95	0	0.41±0.08	0	0.86±30.35	0.10±5.35	0.30±0.07
Gojam	0.96±35.26	0.92±13.94	0.64±16.21	0	0.20±0.07	0.54±0.21	0.95±30.96	0.62±21.46	0
Gonder	0.96±45.37	0.85±24.76	0.43±12.36	0.56±0.91	0.41±0.07	0.27±0.16	0.83±22.45	0.78±17.78	0.38±0.18
Harerge	0.45±1.48	0.22±0.48	0.17±4.51	0.41±0.41	0	0.13±0.09	0.57±7.13	0.13±3.48	0
Shewa	0.69±7.07	0.44±7.98	0.85±18.81	0.44±0.39	0.67±0.11	0.81±0.38	0.92±6.07	0.46±12.48	0.57±0.24
Sidamo	0.84±6.15	0.85±6.0	0.86±17.16	0.15±0.25	0.57±0.25	0.85±0.65	0.87±25.69	0.90±15.18	0.37±0.08
Tigray	0.79±5.72	0.61±3.03	0.69±5.68	0	0.51±0.05	0.23±0.07	0.97±22.04	0.37±4.59	0
Welo	0.85±8.24	0.75±5.90	0.88±8.49	0.18±0.3	0.43±0.13	0.58±0.14	0.68±13.82	0.24±5.92	0
Altitude Class I	0.81±5.49	0.76±3.78	0.88±8.96	0.17±0.28	0.43±0.09	0.58±0.26	0.85±18.41	0.66±5.27	0.13±0.09
Altitude Class II	0.93±4.83	0.89±3.60	0.62±4.02	0.40±0.15	0.49±0.06	0.73±0.11	0.86±8.58	0.70±3.71	0.25±0.04
Altitude Class III	0.93±8.98	0.85±6.19	0.87±7.74	0.34±0.21	0.46±0.05	0.61±0.10	0.88±15.73	0.54±2.28	0.41±0.06
Altitude Class IV	0.86±11.30	0.47±9.14	0.88±12.48	0.14±0.23	0.51±0.08	0.78±0.21	0.84±20.51	0.60±6.89	0.25±0.07

Where: DH=Days to Head, DM=Days to Maturity, TSW=Thousand Seed Weight, FLL=Flag Leaf Length, AL=Awn Length, SL=Spike Length, NSS=Number of Seeds per Spike, PH=Plant Height, NFTP=Number of Fertile Tiller per Plant; Altitude Class I= < 2000m, Altitude Class II=2001-2500m, Altitude Class III=2501-3000m, Altitude Class IV= >3000m

Table 8: Correlation between longitude, latitude and morphological traits

	DH	DM	TSW	FLL	ANL	SL	SPS	PLH	FTP	Longitude	Latitude
DH	1										
DM	0.919	1									
	<.0001										
TSW	0.400	0.428	1								
	<.0001	<.0001									
FLL	0.247	0.192	-0.056	1							
	0.005	0.029	0.532								
ANL	0.243	0.285	0.481	-0.110	1						
	0.006	0.001	<.0001	0.214							
SL	-0.037	-0.057	0.414	0.147	0.246	1					
	0.674	0.522	<.0001	0.096	0.005						
SPS	0.441	0.407	-0.337	0.282	0.047	-0.365	1				
	<.0001	<.0001	<.0001	0.001	0.596	<.0001					
PLH	0.405	0.352	0.544	0.161	0.336	0.367	0.023	1			
	<.0001	<.0001	<.0001	0.068	<.0001	<.0001	0.793				
FTP	-0.212	-0.209	0.313	-0.205	0.032	0.366	-0.606	0.140	1		
	0.016	0.018	0.000	0.020	0.723	<.0001	<.0001	0.113			
Longitude	-0.002	-0.001	-0.038	0.081	-0.088	-0.137	0.056	-0.103	-0.002	1	
	0.980	0.993	0.668	0.363	0.324	0.122	0.530	0.245	0.986		
Latitude	0.042	0.054	0.086	-0.042	0.097	0.103	-0.162	-0.002	0.109	-0.037	1
	0.640	0.541	0.333	0.637	0.277	0.247	0.066	0.986	0.217	0.677	

Where: DH=Days to Head, DM=Days to Maturity, TSW=Thousand Seed Weight, FLL=Flag Leaf Length, AL=Awn Length, SL=Spike Length, NSS=Number of Seeds per Spike, PH=Plant Height, NFTP=Number of Fertile Tiller per Plant

4.1.2 Principal Component analysis

The first three principal components, with eigenvalues greater than unity, explained 72.85% of the total variation among accessions for the 9 quantitative traits (Table 9). The relative magnitude of eigenvectors from the first principal component (31.47 %) indicated that thousand seed weight and plant height followed by awn length and spike length was the most important contributing traits. From the second principal component, which contributed 28.23% of the total variation, the most predominant characters were seeds per spike, days to heading and days to maturity, which were opposite and almost equal with fertile tiller per plant, indicated negative correlation. Third principal component explained 13.15% of total variation with high loadings from flag length and spike length.

Table 9: Eigenvectors and eigenvalues of the first three principal components of nine quantitative traits of 199 barley accessions from Ethiopia

	Eigen vectors		
Characters	**PC1**	**PC2**	**PC3**
DH	0.31	0.47	-0.18
DM	0.31	0.46	-0.22
TSW	0.51	-0.10	-0.21
FLL	0.10	0.22	0.76
AL	0.38	-0.02	-0.16
SL	0.36	-0.27	0.43
NSS	-0.13	0.52	0.14
PH	0.46	-0.04	0.23
NFTP	0.18	-0.40	-0.12
Eigen value	2.83	2.54	1.18
% of total variance	31.47	28.23	13.15
% cumulative variance	31.47	59.70	72.85

Where: DH=Days to Head, DM=Days to Maturity, TSW=Thousand Seed Weight, FLL=Flag Leaf Length, AL=Awn Length, SL=Spike Length, NSS=Number of Seeds per Spike, PH=Plant Height, NFTP=Number of Fertile Tiller per Plant

4.1.2.1 Principal Component analysis for region

Principal component analysis was employed to assess the regional diversity pattern of barley accessions using the respective regional means for the 9 quantitative traits assessed.

Considering an eigenvalue greater than one, the total variance among regions was explained on the basis of three eigenvalues (Table 10). It extracted 86.26% of the total regional

Table 10: Eigenvectors and eigenvalues of the first three and two principal components of regions of origin of accessions and altitude classes, respectively

	Regions			Altitude classes	
Traits	**PC1**	**PC2**	**PC3**	**PC1**	**PC2**
DH	0.08	0.54	-0.21	0.35	0.23
DM	0.10	0.56	-0.12	0.35	0.29
TSW	0.51	0.03	-0.02	0.36	-0.17
FLL	-0.22	0.31	-0.51	-0.02	0.80
ANL	0.10	0.19	0.69	0.37	0.04
SL	0.48	0.14	0.11	0.35	-0.25
SPS	-0.43	0.28	0.22	0.36	0.23
PLH	0.11	0.38	0.27	0.34	-0.28
FTP	0.48	-0.11	-0.26	-0.35	0.04
Eigenvalue	3.71	2.71	1.34	7.19	1.52
% of total variance	41.26	30.07	14.93	79.99	16.93
%cumulative variance	41.26	71.33	86.26	79.99	96.92

Where: DH=Days to Heading, DM=Days to Maturity, TSW=Thousand Seed Weight, FLL=Flag Leaf Length, AL=Awn Length, SL=Spike Length, NSS=Number of Seeds per Spike, PH=Plant Height, NFTP=Number of Fertile Tiller per Plant

variation. Thousand seed weight, spike length and fertile tiller per plant were the highest contributors for the first principal component which explain 41.26% of total variation while seeds per spike and flag leaf length had negative correlation with other traits. Similarly, 30.07% of the variation, which was accounted by the second principal component, explained by days to maturity and days to heading followed by flag leaf length. The third principal component explained 14.93% of the variation with high loadings through awn length, followed by plant height and seeds per spike while highest negative loadings from flag leaf length observed. The three dimensional plot of the first three principal components of regions was shown in Fig. 2.

Further using the mean of 9 quantitative traits evaluated in the study, principal component analyses were performed to examine the variation among the accessions within each region of origin. In the analysis most of the variation was explained with the first three eigenvalues, which are greater than one, for all the regions (Table 11). Based on PCA analysis for

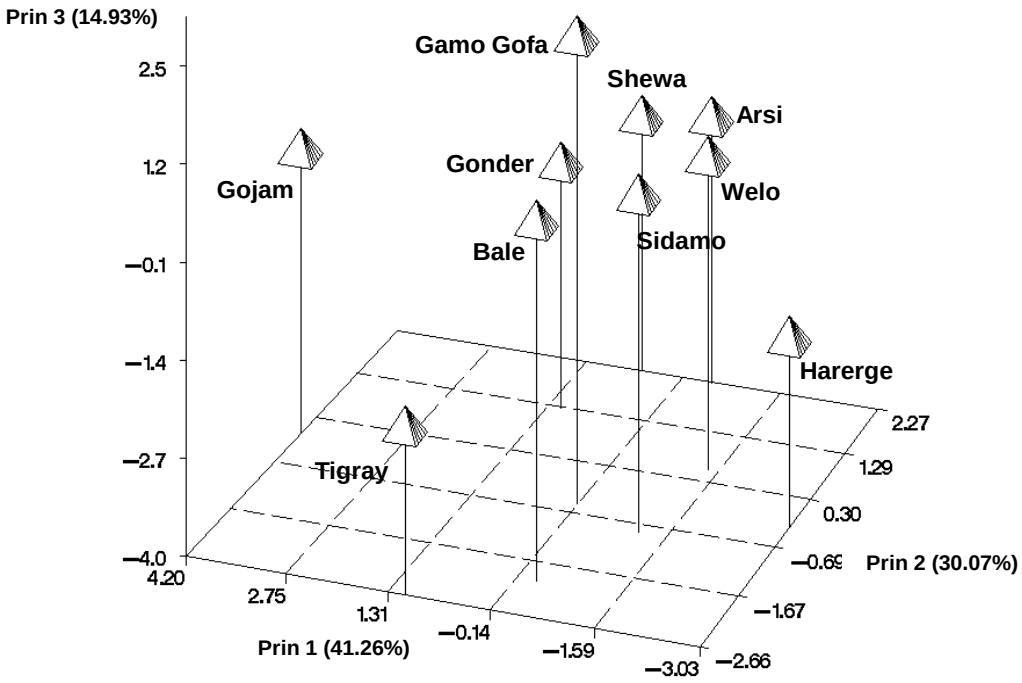

Fig. 3: 3D diagram showing the relationships among 10 regions based on the first three principal components

regions Gojam (85.15%), Gonder (82.17), Sidamo (81.27) and Shewa (83.64%) were the regions which showed high variation with the first three eigenvalues. Whereas Harerge (66.11%) was the region with the smallest cumulative variance as compared with other regions.

Days to heading and days to maturity were the two traits which have high loadings on the first principal component of all regions except in Gamo, Shewa and Sidamo (Table 12).

Whereas thousand seed weight, spike length and plant height were the important traits in Gamo, Shewa and Sidamo. For Harerge, the regions with the lowest cumulative variance, days to heading and days to maturity were the traits with the highest loadings as compared with the other traits for the first principal component.

Table 11: The first three eigenvalues and cumulative variance of barley accessions for 10 Regions and four altitude classes

Regions	Eigenvalues			% Cumulative variance
Arsi	3.83	2.04	1.33	80.12
Bale	3.52	2.30	1.08	76.66
Gamo Gofa	3.61	2.07	1.11	75.47
Gojam	4.44	1.70	1.38	83.64
Gonder	3.55	2.64	1.17	81.79
Harerge	2.94	1.85	1.15	66.11
Shewa	3.38	2.31	1.72	82.39
Sidamo	4.05	2.24	1.12	82.39
Tigray	4.15	1.86	1.20	80.28
Welo	3.42	2.09	1.33	76.19
Altitude class I	4.05	2.28	1.08	82.28
Altitude class II	2.79	2.77	1.08	73.84
Altitude class III	2.93	2.25	1.29	71.96
Altitude class IV	3.14	2.45	1.17	75.16

Where: DH=Days to Heading, DM=Days to Maturity, TSW=Thousand Seed Weight, FLL=Flag Leaf Length, AL=Awn Length, SL=Spike Length, NSS=Number of Seeds per Spike, PH=Plant Height, NFTP=Number of Fertile Tiller per Plant; Altitude Class I= < 2000m, Altitude Class II=2001-2500m, Altitude Class III=2501-3000m, Altitude Class IV= >3000m

4.1.2.2 Principal component analysis for altitude classes

Considering altitude classes for analysis of principal component resulted in two eigenvalues which were greater than one and it explained 96.92% of the total variation (Table 10). All traits except flag leaf length and fertile tiller per plant had high loadings for the first principal component which contributed 79.99% of variation. Flag leaf length was the one with the highest lodgings for the second principal component (15.24%) followed by days to

heading and days to maturity and seeds per spike. The negative value of spike length, plant height and thousand seed weight showed their negative association with other traits. Principal component analysis based on altitude classes showed three eigenvalues could explain most of the variation depending on the altitudes classes. The highest cumulative variation (82.28%) was observed for altitude class I (Table 11).

Whereas the other three altitude classes showed nearly equal variation with the lowest for altitude class III (71.96%).The loadings of each traits on the first principal component for altitude classes showed on Table 10. Thousand seed weight, awn length, fertile tiller per plant and spike length were the traits with high loadings on the first principal component of altitude class I and II except awn length in altitude class II. Days to heading and days to maturity with negative loadings observed in altitude class I and II while the highest negative value was for number of seeds per spike which indicated their negative association with the rest of the traits except flag leaf length in altitude class II. Thousand seed weight, plant height, awn length and fertile tiller per plant were traits with high loadings on first principal component of altitude class III where as for altitude class IV days to heading, days to maturity, thousand seed weight and plant height were the traits with high loadings. For altitude classes all traits except flag leaf length and fertile tiller per plant could differentiate among the classes.

To observe the general pattern for variation of traits, both regions and altitude classes combined together and analysed for principal components. In general the three principal components contributed 80.35% of the total variation with 51.08%, 18.08% and 11.19% respectively. The relationship between traits and regions and altitude classes were presented on the two dimensional graph (Fig 4).

Table 12: Eigenvectors and eigenvalues of the first principal component of region of origin and altitude classes

Traits	Arsi	Bale	Gamo Gofa	Gojam	Gonder	Harerge	Shewa	Sidamo	Tigray	Welo	Altitude class I	Altitude class II	Altitude class III	Altitude class IV
DH	0.48	0.44	0.14	0.42	0.49	0.51	-0.09	-0.08	0.46	0.49	-0.20	-0.22	0.28	0.46
DM	0.47	0.43	0.14	0.38	0.48	0.53	-0.02	-0.05	0.43	0.50	-0.15	-0.20	0.25	0.50
TSW	0.30	0.41	0.47	0.26	0.18	0.30	0.46	0.44	0.08	0.47	0.41	0.37	0.53	0.45
FLL	-0.09	0.21	-0.09	0.34	0.41	0.05	-0.05	0.10	-0.27	0.08	0.19	-0.06	-0.13	0.03
ANL	0.35	0.33	0.33	0.10	0.14	0.24	0.25	0.34	0.14	0.27	0.35	0.19	0.37	0.19
SL	-0.28	0.26	0.34	0.18	0.23	0.00	0.48	0.46	-0.30	0.01	0.40	0.50	0.23	0.11
SPS	0.43	0.26	-0.44	0.38	0.42	0.32	-0.43	-0.40	0.37	-0.19	-0.45	-0.49	-0.27	0.06
PLH	0.03	0.41	0.46	0.42	0.25	-0.07	0.45	0.42	0.34	0.39	0.29	0.31	0.43	0.47
FTP	-0.24	0.07	0.33	-0.36	-0.16	-0.45	0.33	0.35	-0.41	0.13	0.40	0.39	0.34	-0.23
Eigen value	3.83	3.52	3.61	4.44	3.55	2.94	3.38	4.05	4.15	3.42	4.05	2.79	2.93	3.14
% of total variance	42.64	39.08	40.13	49.39	39.48	32.63	37.6	45.03	46.17	38.08	44.97	31.02	32.61	34.92

Where: DH=Days to Heading, DM=Days to Maturity, TSW=Thousand Seed Weight, FLL=Flag Leaf Length, AL=Awn Length, SL=Spike Length, NSS=Number of Seeds per Spike, PH=Plant Height, NFTP=Number of Fertile Tiller per Plant; Altitude class I= < 2000m, Altitude class II=2001-2500m, Altitude class III=2501-3000m, Altitude class IV= >3000m

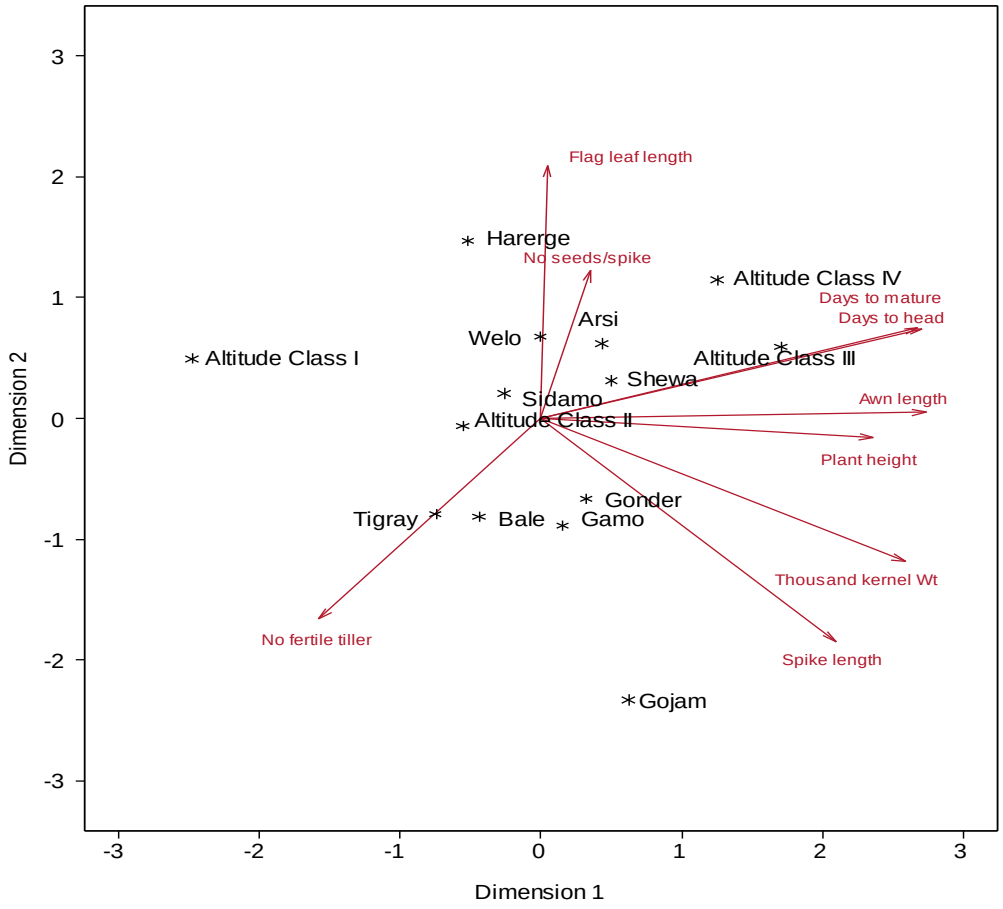

Fig. 4: Diagram showing the pattern among 10 regions and four altitude classes combined together based on the first two principal components

4.1.3 Cluster analysis

Hierarchical cluster analysis was used to examine the aggregation patterns of 199 barley accessions. All the accessions in the study grouped into 7 clusters (Table 13). The number of accessions per cluster varied from 55 accessions in cluster II to 11 accessions in cluster V. In Cluster I 55% and 44% of accessions from Gojam and Tigray clustered and all of them matured relatively earlier with short plant height and small seed size since the majority of the accessions were from altitude class II. While cluster II included accessions from all regions with the highest percentage from Harerge (55%) and Bale (42%), the regions which contributed highest percentage of accessions collected from altitude class II and 85% of the accessions matured less

than the average maturity day with tall plant height, flag leaf length and spike length . Early matured accessions with small seeds and short plant height clustered under cluster III in which Bale contributed the highest percentage (23%) followed by Gamo Gofa (20%). Cluster IV were the only cluster in which less number of regions contributed and among these Sidamo shared the highest percentage (26%). Most of the accessions included in Cluster IV were matured less than the average day and had the biggest seed size associated with small number of seeds per spike and long spike length and its is the only cluster without out accessions from altitude class IV. Cluster V consisted of smallest number of accessions as compared to other clusters and these accessions were small seeded with shortest plant height, short spike and awn length with most accessions with high number of seeds per spike. Each of cluster VI and VII were consisted of 13% of the total accessions in the study where cluster VI contained accessions which matured relatively late with small seeds, longer flag leaf length and high number of seeds per spike with medium sized plant height. While all accessions grouped in the last cluster matured late in which 65% of accessions originated from altitude class III with bigger seed size and longer plant height accompanied by longest awn length.

Table 13: Distribution of 199 barley accessions over 7 clusters by 10 regions and 4 altitude classes

Regions	Clusters							Accessions
	I	II	III	IV	V	VI	VII	
Arsi	4	1	1	1	3	3	5	15
Bale	5	11	6	1	3	1	2	26
Gamo Gofa	2	7	5	4	1	3	3	25
Gojam	6	2	-	-	-	2	1	11
Gonder	5	4	-	-	-	5	4	18
Harerge	2	12	4	-	1	3	-	22
Shewa	3	5	-	-	4	3	4	19
Sidamo	2	4	5	7	5	2	2	27
Tigray	7	4	2	-	-	1	2	16
Welo	4	5	5	-	-	3	3	20
total	40	55	28	13	11	26	26	199
Altitude class								
I	6	4	6	1	3	-	1	21
II	24	29	13	7	4	13	5	95
III	7	20	5	5	1	9	17	64
IV	3	2	4	-	3	4	3	19

Where: Altitude Class I= < 2000m, Altitude Class II=2001-2500m, Altitude Class III=2501-3000m, Altitude Class IV= >3000m

Further clustering analysis for regions resulted in 5 clusters based on the variation of the 10 quantitative traits (Fig 5). Cluster I included Arsi, Shewa and Gonder, regions in which late matured with taller plant height accessions were observed. While cluster II contained regions with accessions of medium sized seeds with taller plant height, which were Bale, Gamo Gofa, Sidamo and Welo. Harerge, region from which medium plant height with medium sized spike length accessions with high number of seeds per spike of smallest seed size observed, grouped under cluster III. Gojam which exhibited the tallest accessions with bigger seed size and small number of seeds per spike was grouped into Cluster IV. Cluster V, encompassed earlier matured accessions from Tigray with shortest plant height and smallest number of seeds per spike.

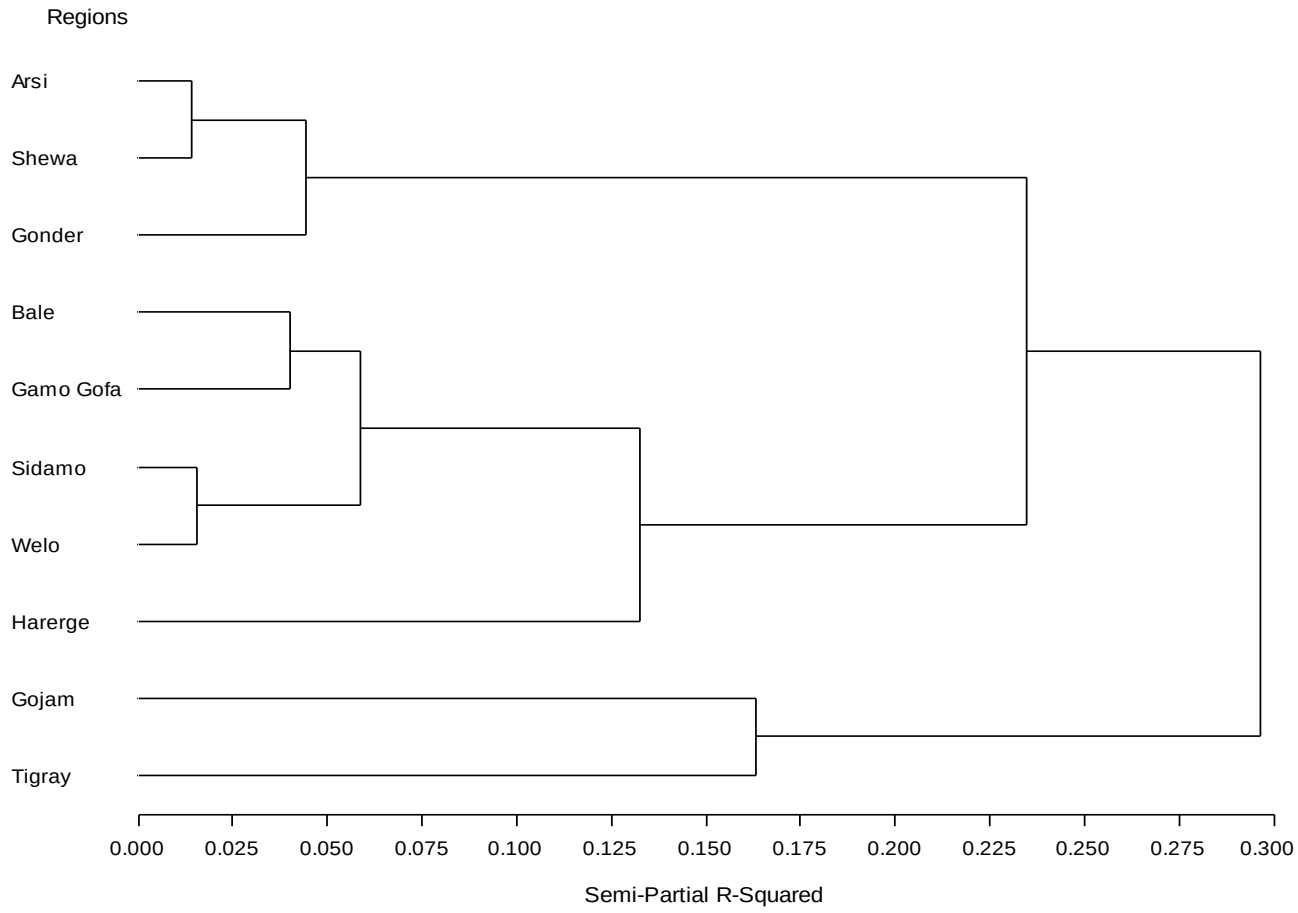

Fig. 5: Dendrogram showing the relationships among 10 regions

4.1.4 Discriminant analysis

Discriminant analysis using the regions of origin of the accessions as a grouping variable revealed that only 32 accessions out of 199 (16.02%) were classified in their respective regions. The percentage of accessions correctly classified varies with regions (Table 14). Tigray (56.25%) and Gojam (36.6%) were the only two regions with relatively high number of accessions in their respective regions, while most of the accessions from the rest of regions were scattered all over

the regions. Among these Sidamo (3.7%), Welo (5.0%) and Bale (7.7%) were the regions with smallest percentage of accessions in their respective region of origin whereas none of the accessions from Shewa grouped in same region of origin.29.63% of accessions from Sidamo and 23.08% of accessions from Bale grouped under Gojam. With the same pattern 24.0% of accessions from Gamo grouped under Welo. Except Sidamo accessions collected from Shewa grouped under all regions with the highest percentage (26.3%) under Harerge which is the proximity region geographically. Despite the highest percentage of accessions grouped in same region of origin, accessions from Tigray grouped under all regions except Sidamo and Welo.

Table 14: Summary of discriminant analysis for barley accessions

Regions	**Original number of accessions**	**Regions**										
		1	2	3	4	5	6	7	8	9	10	%
Arsi	15	2	0	5	3	0	3	0	0	2	0	13.30
Bale	26	2	2	3	6	3	2	2	1	4	1	7.70
Gamo Gofa	25	1	4	3	1	2	1	1	0	6	6	12.00
Gojam	11	0	0	0	4	1	1	0	1	4	0	36.60
Gonder	18	1	3	2	3	3	0	2	0	3	1	16.70
Harerge	22	3	4	2	1	2	7	0	0	2	1	31.82
Shewa	19	2	1	1	3	1	5	0	0	3	3	0.00
Sidamo	27	0	2	2	8	1	7	2	1	3	1	3.70
Tigray	16	1	1	1	1	1	1	1	0	9	0	56.25
Welo	20	2	1	3	1	1	3	1	0	7	1	5.00

4.2 Genotypic diversity

Genetic diversity analysis based on 15 SSR markers detected a total of 58 alleles for 199 accessions collected from 10 regions of Ethiopia ranging from 2 (*GBM1042* and *HVM36*) to 7 (B*mac0040*) and 6 (*Bmag0007*) (Table 15) with an average value of 3.86 alleles per locus. The average polymorphic information content (PIC) was ranging from 0.03 (*EBmac0541*) to 0.79 (*Bmag0007*). As compared to *EBmac0541* (0.03), *Bmag0222* (0.62) showed high PIC, also suggested as gene diversity (Anderson et al. 1993), while both markers amplified equal number of alleles. This low PIC of *EBmac0541* (0.03) was related with the presence of rare alleles which reduce the frequency. The highest numbers of alleles were detected for chromosome 1H, 6H and 7H which were equal in number, 11.

Table 15: Molecular diversity of the 15 SSR loci

		Allelic richness		
Locus	**Chromosome**	**Per locus**	**Per chromosome**	**PIC**
GBM1042	1H	2	11	0.15
Bmag0579	1H	4		0.17
Bmag0211	1H	5		0.70
HVM36	2H	2	9	0.27
GMS003	2H	4		0.49
HVM54	2H	3		0.42
EBmac0541	3H	3	8	0.03
HVLTPPB	3H	5		0.52
HVM67	4H	3	3	0.29
Bmag0337	5H	4	7	0.28
Bmag0222	5H	3		0.62
Bmac0316	6H	4	11	0.37
Bmac0040	6H	7		0.66
Bmag0135	7H	5	11	0.57
Bmag0007	7H	6		0.79
Total		58		
Average		3.86		0.43

Where: PIC=Polymorphic Information Content

Table 16: Diversity of 15 SSR markers across 10 regions

Locus	Arsi	Bale	Gamo	Gojam	Gonder	Harerge	Shewa	Sidamo	Tigray	Welo	Total Band/ locus	Polymorphic band	Polymorphism %
GBM1042	1	1	2	2	2	2	2	2	2	1	17	14	82
Bmag0579	3	2	2	1	2	2	1	2	1	3	19	16	84
Bmag0211	4	4	4	4	4	4	4	4	2	4	38	38	100
GMS003	4	4	3	3	4	4	2	3	2	3	32	32	100
HVM36	1	2	2	2	2	2	2	2	2	2	19	18	95
HVM54	3	2	3	2	2	3	3	2	3	3	26	26	100
EBmac0541	2	1	1	1	1	1	2	1	1	2	13	6	46
HVLTPPB	3	3	2	2	3	2	3	3	3	2	26	26	100
HVM67	3	2	2	2	3	3	3	2	2	2	24	24	100
Bmag0337	3	3	2	2	2	2	2	2	2	1	21	20	95
Bmag0222	3	3	3	3	3	2	3	3	3	3	29	29	100
Bmac0040	6	4	5	4	4	4	4	6	3	5	45	45	100
Bmac0316	4	4	3	2	2	3	4	4	2	3	31	31	100
Bmag0135	2	4	3	3	4	4	3	4	3	4	34	34	100
Bmag0007	5	5	5	5	4	5	5	5	4	5	48	48	100
Total alleles	47	44	42	38	42	43	43	45	35	43	422	407	100
Mean	3.10	2.80	2.73	2.40	2.73	2.80	2.80	2.93	2.20	2.73			
SD	1.60	1.47	1.28	1.35	1.16	1.27	1.21	1.49	1.08	1.49			

Where: SD=Standard Deviation

Genetic diversity between barley landraces for each geographic region was assed using 15 SSR markers. As the data of SSR markers summarized in Table 16 showed a total of 422 bands were observed among which 407 were polymorphic and 10 of the analyzed markers showed 100% polymorphic percentage while *Ebmac0541* showed the least polymorphic percentage (46%). Among the 10 regions analyzed only Arsi, Shewa and Welo were polymorphic for loci *Ebmac0541*. As compared to other regions Tigray showed lowest band (35) over all loci while Arsi with the highest number of bands (47).

4.2 Genetic diversity and population differentiation

4.2.1 Regional Diversity and genetic differentiation

A hierarchical AMOVA was performed to examine hierarchical population structure based on regions and altitude of collection setting regions and altitude classes as population. The AMOVA result obtained setting regions as population showed that among regions variation accounted for 7.82% while the most variation ascribed to within regions (91.95%) (Table 17).

Mean while the degree of population differentiation was measured with fixation index F_{ST} and can be presumed that a value lying in the range 0 to 0.05 indicates little genetic differentiation, 0.05 to 0.15 indicates moderate differentiation, 0.15 to 0.25 a large degree of differentiation and values above 0.25 very great differentiation (Wright 1978; Hartl and Clark 1997). The overall population differentiation among regions were significantly different from zero ($P<0.0001$) with F_{ST} value of 0.08 which indicated medium differentiation. Contribution of seed dispersal to overall gene flow can be estimated by comparing levels of interpopulational differentiation (i.e., F_{ST} or G_{ST}). So gene flow ($Nm=2.95$) was estimated for regions from population differentiation (Table 18).

Table 17: Analysis of molecular variance for regions and altitude of collection

Source of Variation	DF	Sum of squares	Variance component	Percent of variation (%)
Among regions	9	111.68	0.24	7.82
Within regions	390	1107.72	2.84	92.18
Total	399	1219.40	3.09	100
F_{ST}=0.07819 (P<0.0001) Nm=2.947				
Among altitude classes	3	18.08	0.03	1.10
Within altitude classes	396	1215.55	3.07	98.90
Total	399	1233.63	3.10	100
F_{ST}=0.011(P<0.5384) Nm=22.477				

Where: DF=Degree of Freedom, Nm=Gene Flow

Level of geographic differentiations was determined by estimating pairwise F_{ST} among regions (Table 18). The lowest and non significant population differentiation was observed among Arsi and Bale (F_{ST}=0.028), Arsi and Harerge (F_{ST}=0.040), Gamo Gofa and Gonder (F_{ST}=0.037), Gamo Gofa and Shewa (0.016) and Gojam and Harerge (0.023) which indicated these regions were more similar. While Tigray were strongly differentiated from Arsi (F_{ST}=0.188), Bale (F_{ST}=0.132), Gamo (F_{ST}=0.143), Harerge (F_{ST}=0.136), Shewa (0.128), Gonder (F_{ST}=0.097) and Sidamo (F_{ST}=0.078) which showed from medium to high fixation index and region specific fixation index also showed relatively high F_{ST} estimates for Tigray (Table 18). Tigray and Welo were the two regions which were significantly differentiated from the rest of the regions while Sidamo were significantly differentiated from all regions except Gojam. From region specific F_{ST} estimates it was observed that Welo and Arsi was the region with relatively low F_{ST}. Bale was significantly differentiated from all regions except Arsi and Gojam.

Table 18: Pairwise F_{ST} among regions (below diagonal) and significance test level (above diagonal)

	Arsi	Bale	Gamo	Gonder	Gojam	Harerge	Shewa	Sidamo	Tigray	Welo	F_{ST}^
Arsi		-	*	***	-	-	-	***	***	*	0.076
Bale	0.028		***	***	-	***	**	***	***	***	0.077
Gamo	0.070	0.074		-	*	-	-	***	***	**	0.079
Gonder	0.095	0.073	0.037		-	***	-	***	***	***	0.078
Gojam	0.075	0.059	0.079	0.052		-	-	-	*	*	0.079
Harerge	0.040	0.058	0.050	0.069	0.023		-	***	***	***	0.077
Shewa	0.055	0.071	0.016	0.045	0.050	0.042		**	***	*	0.080
Sidamo	0.105	0.087	0.093	0.069	0.055	0.099	0.063		***	***	0.078
Tigray	0.188	0.132	0.143	0.097	0.092	0.136	0.128	0.078		***	0.082
Welo	0.059	0.078	0.078	0.078	0.086	0.071	0.058	0.109	0.162		0.076

Significance levels: - P> 0.05,*0.01<P<0.05, **0.001<P<0.01, ***P<0.001, ^ population specific fixation indices

Finally pairwise population F_{ST} estimate (Table 18) was used to construct Neighbor Joining tree (Fig. 6) to see the relationship between regions. The tree showed that Tigray was highly differentiated from all other regions where as Arsi and Bale was less differentiated.

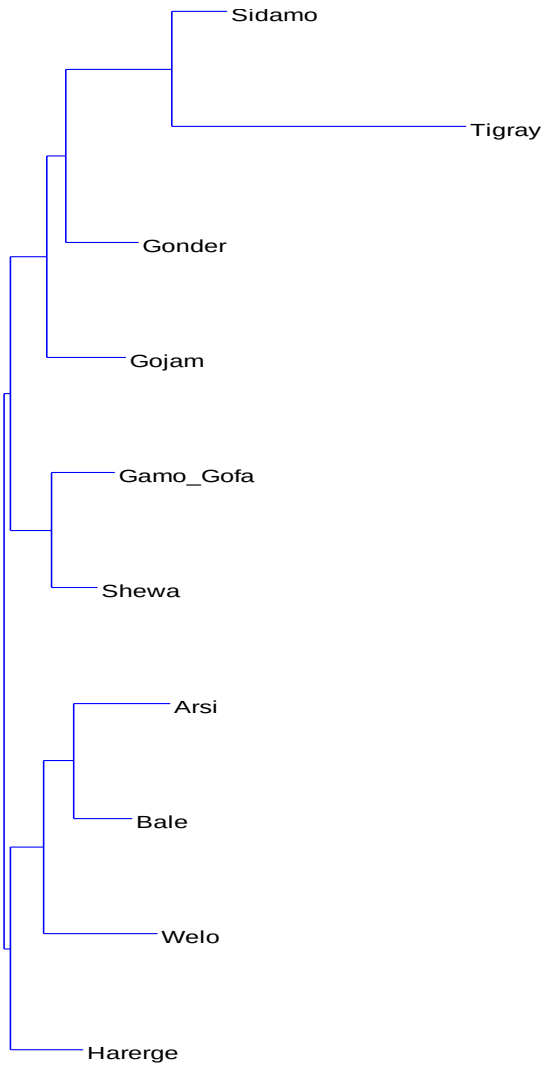

Fig. 6: Neighbor-Joining Tree based on pairwise F_{ST} values, showing phylogenetic relationship among regions

Locus by locus analysis of variance for regions showed loci which were effective in differentiating population. Loci *HVLTPPB* (0.145), *HVM36* (0.138), *Bmag0337* (0.13) *Bmag0135* (108) and *Bmag0211* (0.105) have showed high fixation index and could differentiate among population (P<0.0001) (Table 18). While *Bmac0316* (0.024) and *EBmac0541* (0.026),

GBM1042 (0.032), *Bmag0222* (0.040) *HVM54* (0.048), *Bmag0579* (0.053) were not able to differentiate among population and not significantly different from zero (P<0.05).

Table 19: Locus by locus analysis of variance for regions of origin using 15 loci

	Among regions				Within regions				Fixation indices	
Locus	**SSD**	**DF**	**Va**	**% variation**	**SSD**	**DF**	**Vb**	**% variation**	**F_{ST}**	**P-value**
GBM1042	1.232	9	0.002	3.200	23.078	390	0.059	96.800	0.032	0.3297
Bmag0579	2.286	9	0.004	5.252	30.944	390	0.079	94.748	0.053	0.1021
Bmag0211	16.115	9	0.037	10.492	123.475	390	0.317	89.509	0.105	0.0001
GMS003	10.625	9	0.024	9.709	87.264	388	0.225	90.291	0.097	0.0008
HVM36	7.984	9	0.019	13.768	47.126	390	0.121	86.232	0.138	0.0006
HVM54	5.377	9	0.010	4.807	77.513	390	0.199	95.193	0.048	0.1378
EBmac0541	0.269	9	0.000	2.589	5.661	390	0.015	97.411	0.026	0.3661
HVLTPPB	15.710	9	0.038	14.500	88.000	390	0.226	85.500	0.145	0.0001
HVM67	4.679	9	0.010	6.362	54.811	390	0.141	93.638	0.064	0.0504
Bmag0337	7.705	9	0.018	12.951	48.325	390	0.124	87.049	0.130	0.0009
Bmag0222	7.018	9	0.012	3.961	114.318	382	0.299	96.039	0.040	0.2132
Bmac0040	9.029	9	0.018	5.440	117.697	376	0.313	94.560	0.054	0.0323
Bmac0316	3.185	9	0.004	2.376	69.815	386	0.181	97.624	0.024	0.5122
Bmag0135	13.050	9	0.031	10.822	96.473	376	0.257	89.178	0.108	0.0001
Bmag0007	10.414	9	0.021	5.191	139.638	370	0.377	94.809	0.052	0.0344

Where: SSD: Standard deviation, DF: degree of Freedom, Va: variance among regions, Vb: variance within regions

4.2.2 Altitudinal diversity and genetic differentiation

Allelic polymorphism based on 15 SSRs markers for altitude classes were summarized in Table 19. Polymorphism percentage showed 100% for all loci except for locus *EBmac0541* which was monomorphic for altitude class I unlike in regions where 40% of the locus was monomorphic. Total number of bands over all loci was higher and equal for altitude class II (55) and III (55) as compared to the extreme altitude class I and altitude class IV showed less number of bands 45 and 49 respectively.

Table 20: Diversity of 15 SSR markers along altitude classes

Locus	Class I	Class II	Class III	Class IV	Total bands/ locus	Polymorphic band	Polymorphism (%)
GBM1042	2	2	2	2	8	8	100
Bmag0579	2	2	4	2	10	10	100
Bmag0211	4	5	5	5	19	19	100
GMS003	3	4	4	4	15	15	100
HVM36	2	2	2	2	8	8	100
HVM54	3	3	3	3	12	12	100
EBmac0541	1	3	2	2	8	7	87.5
HVLTPPB	3	4	4	3	14	14	100
HVM67	2	3	3	3	11	11	100
Bmag0337	2	3	3	3	11	11	100
Bmag0222	3	4	3	3	13	13	100
Bmac0040	6	7	7	5	25	25	100
Bmac0316	3	4	4	3	14	14	100
Bmag0135	4	4	4	4	16	16	100
Bmag0007	5	5	5	5	20	20	100
Total bands	45	55	55	49	204	203	99.5
Mean	2.933	3.667	3.667	3.267			
SD	1.438	1.345	1.345	1.1			

Where: SD=Standard Deviation; Altitude Class I= < 2000m, Altitude Class II=2001-2500m, Altitude Class III=2501-3000m, Altitude Class IV= >3000m

For estimation of population differentiation along altitude classes hierarchical AMOVA was conducted. According to the result obtained for altitude classes 98.90% of the total variation attributed to within altitude class while only 1.10% among altitude class. The population differentiation considering altitude class as population resulted in low and non significant ($P>0.05$) fixation index ($F_{ST}=0.011$) (Table 17). As compared with regions for altitude classes high gene flow ($Nm=22.48$) was estimated.

Locus by locus analysis of variance also showed none of the loci were significantly different from zero and failed to differentiate among population (Table 21).

Table 21: Locus by locus analysis of variance for altitude classes

	Among altitude classes				Within altitude classes				Fixation indices	
Locus	**SSD**	**DF**	**Va**	**variation**	**SSD**	**DF**	**Vb**	**Variation**	**F_{ST}**	**P-value**
GBM1042	0.418	3	0.001	1.496	23.892	396	0.060	98.504	0.015	0.33267
Bmag0579	0.256	3	0.000	-0.080	36.274	396	0.092	100.080	-0.001	0.76149
Bmag0211	3.678	3	0.010	2.892	135.982	396	0.343	97.108	0.029	0.07535
GMS003	1.444	3	0.003	1.112	96.696	394	0.245	98.888	0.011	0.42663
HVM36	0.339	3	0.000	-0.213	54.771	396	0.138	100.213	-0.002	0.75743
HVM54	1.220	3	0.002	1.087	82.670	396	0.209	98.913	0.011	0.41782
EBmac0541	0.069	3	0.000	0.194	7.801	396	0.020	99.806	0.002	0.70426
HVLTPPB	0.637	3	-0.001	-0.218	103.443	396	0.261	100.218	-0.002	0.80218
HVM67	1.508	3	0.004	2.562	60.902	396	0.154	97.438	0.026	0.16139
Bmag0337	0.657	3	0.001	0.608	56.733	396	0.143	99.392	0.006	0.53624
Bmag0222	2.504	3	0.006	2.000	119.149	388	0.307	98.000	0.020	0.2302
Bmac0040	1.343	3	0.001	0.442	125.709	384	0.327	99.558	0.004	0.75604
Bmac0316	0.873	3	0.001	0.638	73.713	392	0.188	99.362	0.006	0.59881
Bmag0135	1.838	3	0.004	1.393	107.686	382	0.282	98.607	0.014	0.36931
Bmag0007	2.102	3	0.004	0.940	148.150	376	0.394	99.060	0.009	0.57584

Where: SSD: Standard Deviation, DF: Degree of Freedom, Va: Variance among regions, Vb: Variance within regions

As on table 22 indicated pairwise F_{ST} for altitude classes showed no significant population differentiation and the differentiation increases with increase in altitude. The closer the altitude classes the lower the population differentiation observed.

Table 22: Pairwise F_{ST} among altitude classes (below diagonal) and significance test level (above diagonal)

	Altitude classes				
	I	II	III	IV	F_{ST}^
I		-	-	-	0.01187
II	0.00057		-	-	0.01108
III	0.0195	0.0114		-	0.01082
IV	0.03059	0.01361	0.0064		0.01070

Where: Significance levels: - P>0.05,*0.01<P<0.05,**0.001<P<0.01,***P<0.001,^ Population specific fixation indices; Altitude Class I= < 2000m, Altitude Class II=2001-2500m, Altitude Class III=2501-3000m, Altitude Class IV= >3000m

4.2.3 Diversity of accessions

To estimate variation exists between and within accessions, two accessions from each region with 10 spikes from each accession were analyzed using 15 polymorphic SSR markers but analysis was conducted only for 13 polymorphic markers since two of the markers were monomorphic they were not included for this analysis. As the result indicated in Table 23 high variation observed within accession (65.65%) as compared to among accessions (34.35%). Very great differentiation among accessions (F_{ST}=0.34) was observed which were significantly different from zero (P<0.0001) and gene flow indicated that 0.48 immigrants per generation.

Table 23: Analysis of variance for accessions based on 13 polymorphic SSR markers

Source of Variation	DF	Sum of Squares	Variance component	Percent of variation (%)
Among accession	19	339.49	0.83	34.35
Within accessions	374	590.97	1.58	65.65
Total	393	930.45	2.41	100
F_{ST}=0.34(P<0.0001)				
Nm=0.48				

Where: Nm=Gene Flow

4.2.4 Population differentiation for quantitative traits (Q_{ST})

4.2.4.1 Regional differentiation

Population differentiation for quantitative traits was conducted using the variance between regions and within regions with similar pattern to F_{ST} analysis. Q_{ST} was calculated for days to heading, days to maturity, thousand seed weight and glucan content, traits which had high variance among population as compared to within regions. Population Q_{ST} values ranged from 0.013 (days to heading) to thousand seed weight (0.054) with an average Q_{ST} estimate of 0.030 (Table 24). To determine if observed levels of quantitative trait differentiation were significantly different from neutral traits, bootstrap with 1000 samples were used. Judging from 95% bootstrap confidence intervals all Q_{ST} estimates were significantly different from neutral expectation (F_{ST}=0.074, 95% CI 0.055-0.095) while glucan (Q_{ST}=0.027, 95% CI 0.013-0.063) was not significantly different from F_{ST}. Even though the Q_{ST} value was smaller than average F_{ST}, days to heading, days to maturity and thousand seed were significantly different from F_{ST} estimates. Over all mean quantitative traits estimates (Q_{ST}=0.030, 95% CI 0.017-0.047) were significantly different from neutral trait estimates (F_{ST}=0.074, 95% CI 0.055-0.095).

Table 24: Quantitative traits (Q_{ST}) and SSR Markers (F_{ST}) differentiation of regions and altitude classes

Regions				Altitude classes			
Traits	Q_{ST}	95% CI		Q_{ST}		95% CI	
		Lower	Upper			Lower	Upper
DH	0.013*	0.007	0.050	DH	0.037***	0.016	0.095
DM	0.028**	0.019	0.091	DM	0.075***	0.030	0.161
TSW	0.054**	0.034	0.123	TSW	0.119***	0.057	0.162
Glucan	0.027**	0.013	0.063	Glucan	0.065**	0.000	0.115
Average Q_{ST}	0.030*	0.017	0.047	ANL	0.019**	0.000	0.155
F_{ST}	0.074	0.055	0.095	FLL	0.424***	0.088	0.385
				FTP	0.201**	0.000	0.279
				Average Q_{ST}	0.135***	0.058	0.241
				F_{ST}	0.011	0.007	0.015

*↓ Q_{ST} was significantly smaller than F_{ST} at P=0.05, ** Q_{ST} was not significantly different from F_{ST} at P=0.05, ***↑Q_{ST} was significantly larger than F_{ST} at P=0.05; DH=Days to Heading, DM=Days to Maturity, TSW=Thousand Seed Weight, FLL=Flag Leaf Length, AL=Awn Length, NFTP=Number of Fertile Tiller per Plant, BG=Beta Glucan

4.2.4.2 Altitudinal differentiation

Quantitative trait differentiation based on altitude class showed wide divergence as compared to neutral traits estimate F_{ST}. For altitude classes most of the quantitative traits showed wide divergence with small value for awn length (0.019), days to heading (0.37) and days to maturity (0.075) (Table 24) as compared to regional differentiation. Since the confidence interval of F_{ST} overlap with the confidence interval of glucan (Q_{ST}=0.065, 95% CI 0.-0.115), awn length (Q_{ST}=0.019, 95% CI 0.0-0.155) and fertile tiller per plant (Q_{ST}=0.201, 95% CI 0.0-0.279), there was no significant difference between Q_{ST} of each trait and F_{ST} estimates based on 15 SSR loci. But overall quantitative trait (Q_{ST}=0.135, 95% CI 0.058-0.241) estimate were high and significantly different from neutral trait estimate (F_{ST}=0.011, 95% CI 0.007-0.015).

4.2.5 Clustering

On the basis of allele frequencies genotypes were assigned to populations in STRUCTURE analyses, assuming that populations were admixed and allele frequencies were correlated as a consequence of shared ancestry and/or migration. Bayesian clustering based on model with admixture assumes each individual have inherited some proportion of its ancestry from each of K populations (Pritchard et al 2000). As defined by (Evanno et al.2005) analysis to determine number of K clusters using STRUCTURE were resulted in K=10 (Fig 7).

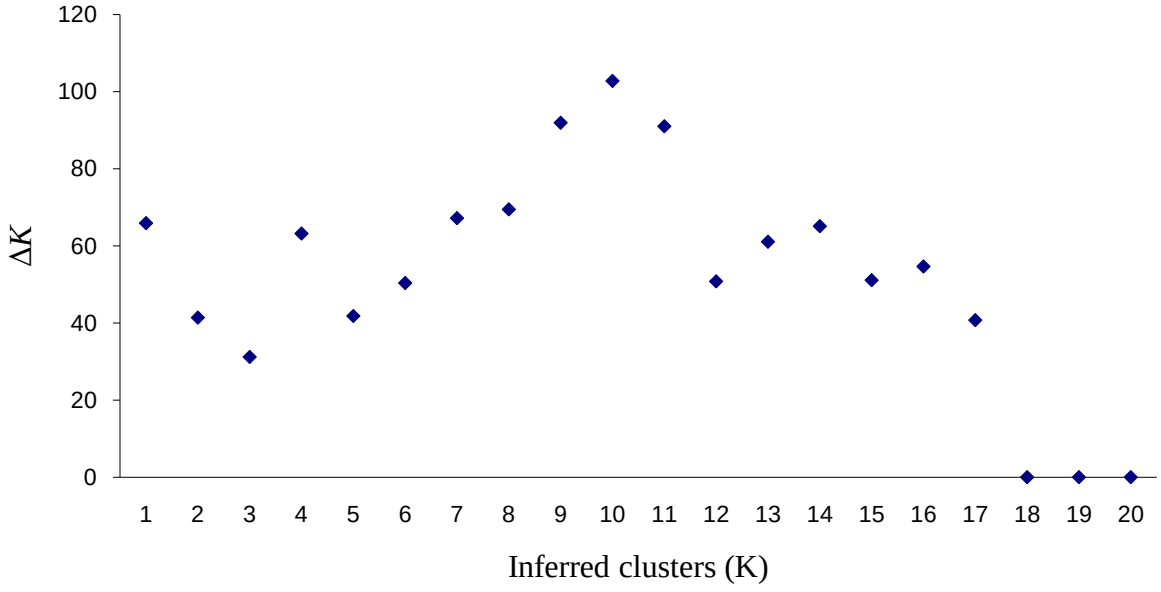

Fig. 7: Diagram showing the number of inferred K clusters based on Likelihood plot from STRUCTURE analysis

A graphic representation of the estimated membership coefficients to the K=2 and K=10 clusters for each individual obtained running structure was shown in Fig 8 and it showed no distinct structure among populations and revealed admixture of populations. Each accessions in the graph was represented by a single vertical line broken into K colored segments, with lengths proportional to each of the K inferred clusters. Each color represents the proportion of membership of each accession, represented by a vertical line, to the 10 clusters. From K=2 graph it was possible to observe that Tigray was the region which was less admixture as compared to

others while Arsi and Bale showed the opposite and this analysis supported the result obtained from Neighbor Joining tree.

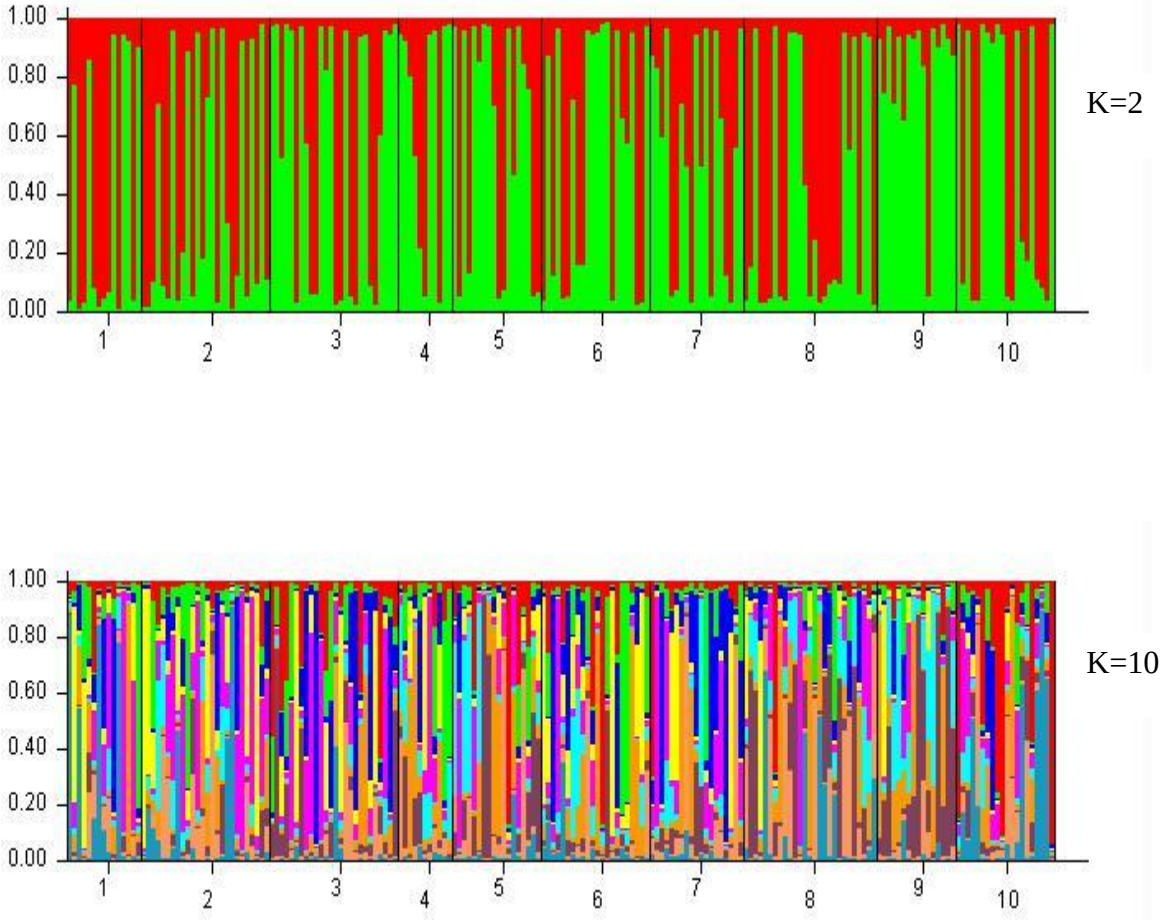

Fig. 8: Model based ancestry of accessions with cluster numbers K=2 and K=10, Horizontal numbers correspond to the population numbers (Code for Population (regions) given in Appendix 1), membership coefficients (Q) are depicted vertically for each individual

Table 25: Proportion of membership of analyzed 199 barley accessions in each of the 10 clusters (K=10)

	Inferred clusters									
Region	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Arsi	0.043	0.112	0.103	0.130	0.165	0.032	0.106	0.023	0.056	0.23
Bale	0.036	0.088	0.079	0.196	0.214	0.078	0.064	0.045	0.102	0.096
Gamo Gofa	0.145	0.086	0.200	0.125	0.148	0.029	0.062	0.113	0.064	0.028
Gojam	0.042	0.186	0.082	0.119	0.107	0.128	0.175	0.077	0.068	0.017
Gonder	0.124	0.051	0.041	0.053	0.117	0.149	0.144	0.18	0.109	0.032
Harerge	0.111	0.15	0.131	0.178	0.076	0.076	0.135	0.05	0.054	0.039
Shewa	0.064	0.074	0.239	0.114	0.152	0.076	0.088	0.057	0.115	0.021
Sidamo	0.041	0.051	0.066	0.064	0.103	0.094	0.119	0.188	0.17	0.104
Tigray	0.034	0.034	0.040	0.071	0.051	0.211	0.194	0.305	0.038	0.021
Welo	0.263	0.054	0.08	0.026	0.092	0.047	0.08	0.049	0.11	0.199

Table 25 demonstrated the proportion of membership of accessions from each region in each of the 10 clusters. As compared to other regions Tigray was the most differentiated region, with 30.5% of the accessions assigned to a single cluster 8 followed by Welo in which 26.3% of the accessions grouped in first cluster. While accessions from Arsi and Gonder were highly differentiated and showed high admixture among clusters.

The clustering pattern based on Dice similarity coefficient of the SSR data showed a degree of ‘chaining ‘ (Romesburg 1990) as the clusters tended to become progressively larger as the similarity coefficient increased (Fig 9). As a result, it was not possible to find a set of clusters with roughly equal numbers accessions. Cutting the denderogram with a similarity coefficient of 0.54 resulted in 10 clusters (Table 26) in which the first cluster consisted 79% of the accessions. The rest 42 accessions grouped into nine clusters and cluster II contained the highest number of 10 accessions while cluster VII contained single accession collected from Arsi of altitude class III. Five pairs of accessions were genetically similar with similarity coefficient of one. The first accession pair which showed 100% genetic similarity was accession number 238847 from Arsi

and accession number 236139 from Gamo Gofa; the second pair was accessions number 215362 from Arsi and accession number 238839 from Bale; the third pair was accession number 225986 from Gonder and accession number 241120 from Harerge; the fourth accession number was 217107 from Sidamo and accession number 224988 from Bale; and finally accession number 217059 from Gojam and accession number 237856 Bale. Region based distribution of accessions showed all accessions collected from Shewa grouped under cluster I followed by Gamo Gofa and Tigray where all the accessions grouped in two clusters only. While accessions from Sidamo appeared in six of the total clusters. Arsi and Welo formed Cluster XI and X each with two accessions collected from altitude class IV and III respectively. All the 10 accessions in cluster II were late matured accessions and collected from altitude class II, III and IV in contrast to six accessions from cluster III which were collected from altitude I and II and matured relatively early.

Table 26: Distribution of accessions by cluster for regions and altitude classes based on SSR markers

Regions	Cluster										
	I	II	III	IV	V	VI	VII	VIII	XI	X	Total
Arsi	11			1			1		2		15
Bale	20		3	1	1	1					26
Gamo Gofa	21	4									25
Gojam	9		1		1						11
Gonder	14				1	2		1			18
Harerge	17	1	2					2			22
Shewa	19										19
Sidamo	22	1	1	1		1		1			27
Tigray	12				2						16
Welo	12	4		2						2	20
Total	157	10	7	5	5	6	1	4	2	2	199
Altitude Classes											
I	19		1	1							21
II	76	4	6	2	2	3		2			95
III	49	4		1	3	3	1	1		2	64
IV	13	2		1				1	2		19

Where: Altitude Class I= < 2000m, Altitude Class II=2001-2500m, Altitude Class III=2501-3000m, Altitude Class IV= >3000m

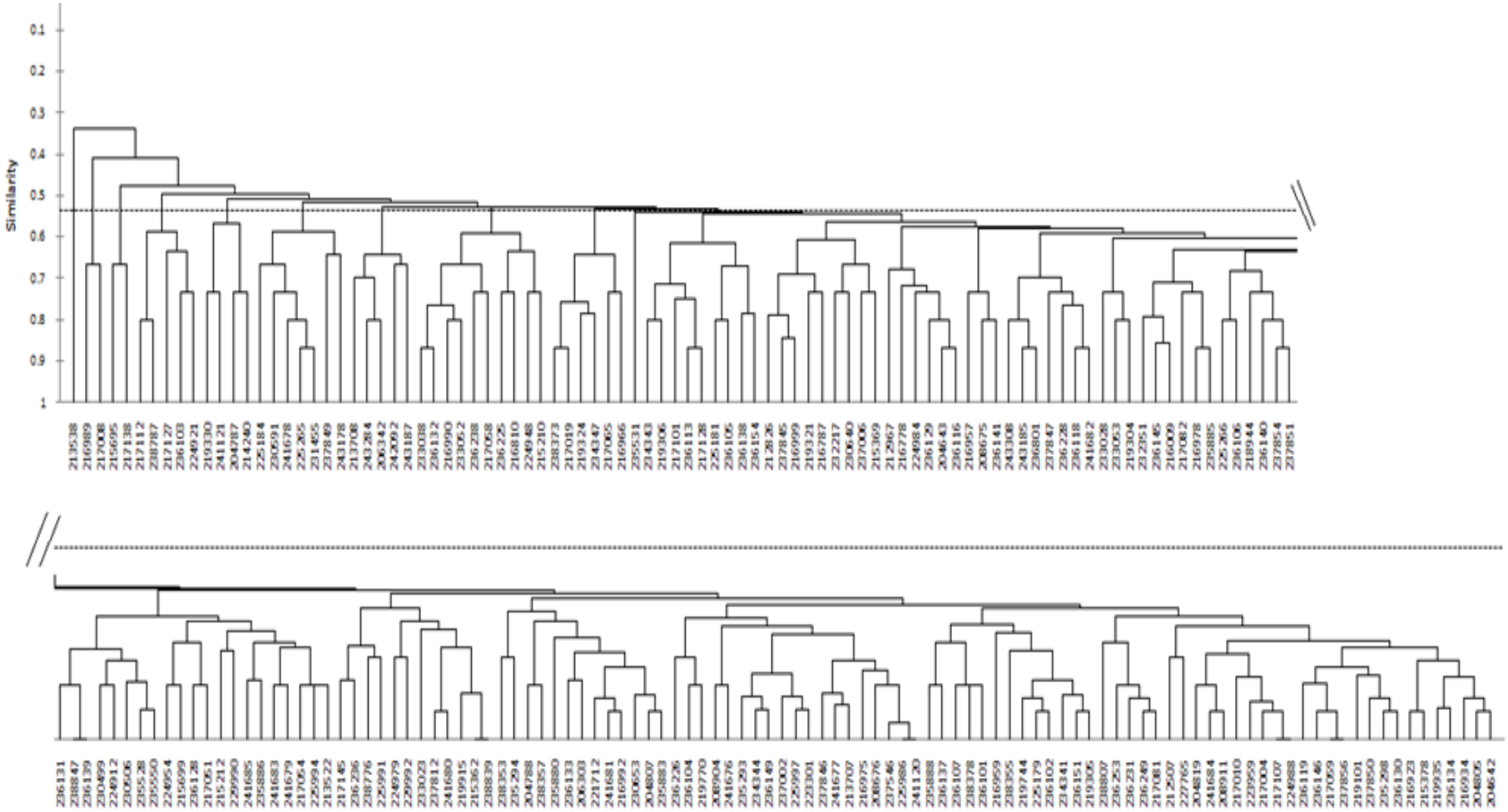

Fig. 9: Unweighted Pair Group Method with Arithmetic Average (UPGMA) dendrogram of 199 barley accession based on SSR data. The dendrogram was constructed from the Dice's similarity coefficients matrix.

To affirm the genetic relationships among 199 barley accessions revealed by cluster analysis, principal coordinate analysis (Fig 9) was generated. The first and second axis explained 3.84% and 3.37% of total variation respectively. Accessions which grouped in other clusters appeared scattered on the outer part while accessions from cluster I appeared aggregated in the center. These results corresponded with the cluster analysis obtained through UPGMA.

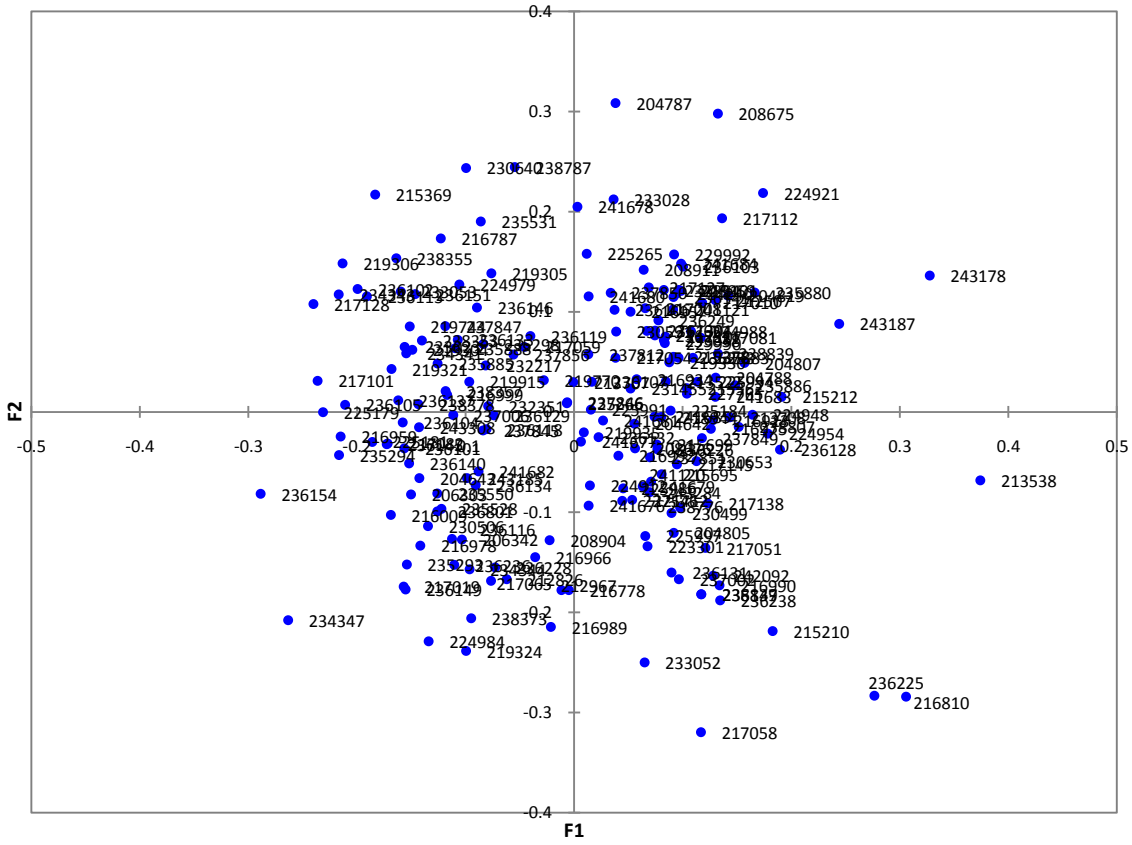

Fig. 10: Plot of first two principal coordinate axes for 199 barley accessions revealed by using the Dice's similarity coefficients based on the SSR data

The result obtained from correlation analysis between genetic similarity values and morphological distances showed a non-significant and weak correlation (r=0.027, P=0.13) so the null hypothesis which stated as there is no correlation between genetic similarity values and morphological distance was accepted.

The measure of goodness-of fit analyses suggest that UPGMA was not a greatly distorted representation of the similarity matrix, with cophentic r =-0.63. To see if genetic similarity distance varied with geographic distance a mantel correlation analysis between geographic distance and dice similarity matrix conducted and the result showed weak and non significant correlation value (r=-0.016, P=0.22) which indicated the presence of negligible association.

The negative r value for mantel test result when correlation for distance matrix and similarity matrix are employed (Johnson et al. 2002).

5. Discussion

In this study 199 Ethiopian barley accessions collected from 10 different regions of Ethiopia from different altitude ranges were used to analyze the diversity. Morphological data from field experiments were run for multivariate analysis and SSR markers were also used to generate valuable information on the traits, on the regions and altitudes. The results obtained will be discussed below briefly and in detail.

5.1 Phenotypic diversity

5.1.1 Regional diversity

Current study detected high morphological variation for regions and different altitude ranges based on quantitative characters, which suggested that the structure of morphological variation in Ethiopian barley landraces strongly influenced by environmental factors so that the degree of variation of characters differ with regions and altitudes from where the accessions collected. Phenotypic diversity in Ethiopia barley was also reported by different authors (Negassa 1985; Asfaw 1988; Cross 1994; Engels 1994; Kebebew et al. 2001).

Since heritability is the degree to which a trait is genetically determined as the ratio of the total genetic variation to the phenotypic, the heritability estimates of region varied depending on the variation of traits involved. Arsi, Bale, Shewa and Sidamo are main contributors for barley production and showed high heritability estimates for all traits unlike other regions. The lowest heritability for all traits except plant height and spike length and absence of estimates for flag leaf length in Harerge could associate with the moisture stress and drought prone nature of the region which resulted in weak competence of barley as compared to lowland crops like sorghum, millet and sweet potato in the region. The relationship between low heritability and low input or stressed environment reported in different studies (Ud-Din et al. 1992; Bertin and Gallais 2000; Sinebo et al. 2002; Brancourt-Hulmel et al. 2005; Eid Manal 2009). It was possible to speculate that not the stress directly resulted in low heritability but the very different stress condition and the reaction of plants.

The same assumption was drawn for Moroccan barley in which limited-irrigated conditions resulted in lower heritability estimates for the majority of characters compared with those observed in well-irrigated conditions (El Madidi et al. 2005).

Generally the heritability pattern showed high estimate for the traits, which are important from farmer's point of view in regions suitable for agricultural production and diversified culture.
Since the administrative regions are political sub divisions and not based on ecological aspects there was no significant variation among them. Engels (1994), Negassa (1985) and Ayana and Bekele (1999) reported the same result where no significant variation was observed among different administrative regions for barley and sorghum respectively. Kebebew et al. (2001) reported the absence of significant difference between Bale and North Shewa for genetic diversity in barley landraces. The same result was revealed by Tadesse and Bekele (2003) for grass pea collected from different regions of Ethiopia. The presence of ample genetic diversity within each region could be because of the nature of selections forces operating in similar manner across geographic regions which resulted in no differences between regions. Less genetic variation in Harerge and Tigray could be related with warm and dry weather conditions which increase high environmental influence and increases the frequency of specific phenotypes adapted to the prevailing climatic and edaphic conditions (Jaradat et al. 2004). Regions with high levels of stress factors (e.g. drought, frost) tend to exhibit more homogenous genotypes and less degree of variations (Demissie and Bjørnstad 1996) which could be because of selection of the genotypes which adapt to particular environment.

5.1.2 Altitudinal diversity

High level of diversity in respect to different altitude classes in Ethiopian barley landraces and high broad sense heritability indicated presence of substantial variation in the germplasm and possibility of selection response in these traits. Generally high heritability values was observed for all studied traits except number of tiller per plant and flag leaf length, which showed relative ease with which selection can be made based on phenotype. The same result was reported for low heritability of fertile tiller per plant in barley (Chand et al. 2008) and high heritability for days to heading (Esparza Martínez and Foster 1998).

High genetic variation was observed in an altitude class II and III, which included the major barley growing areas in the country. Similar result was reported by Demisse and Bjørnstad (1996) and Engles (1994), where they found high variation concentration in areas between 2000-3000 and 2400 and 3000 m.a.s.l. respectively. Kebebew et al. (2001) also reported the reduction of diversity at altitude beyond 2600 m.a.s.l for barley landraces. Positive and significant association between diversity index and altitude as well low temperature reported by Abay et al. (2009). This high variation attributed to mixed farming system, which is typically found in areas of higher elevation usually above 2000 m.a.s.l. (Tanto et al. 2009) also reported the reduction of area of cultivation for barley as altitude decreased which indicated that barley is cool climate crop. Crop production under this farming system is diverse for food and as a source of cash income. It is highly varied being influenced by diversified agro-climates, and diverse social and cultural nature of the people (IPGR 1996).

To see if there was association between morphological traits, especially heading and maturity date, and longitudes and latitudes correlation analysis was conducted. But the result showed no correlation between any of the traits and geographic coordinates.

5.1.3 Principal component

Generally the principal component analysis confirmed diversity since the entire variation cannot be explained in terms of few PCs. This, in turn indicated the involvement of a number of traits in contributing towards the overall observed diversity. In line with the present findings, Demissie and Bjørnstad (1996) also employed principal component analysis for detecting variation in 49 barley populations in which the first four PCs contributed 63% of total variation. Similarly in highland maize accessions of Ethiopia 71.8% of total variation was accounted by first four PCs (Beyene et al. 2005). The well adapted traits like days to heading, days to maturity, plant height and awn length, played a role in differentiating accessions collected from different regions and altitude class in to principal components.

5.1.4 Clustering and discriminant analysis

Grouping accessions into morphologically similar cluster of different groups is useful for selecting parents for crossing. Clustering of accessions based on the agronomic traits under study

revealed no distinct regional grouping patterns in which accessions from same or adjacent regions appeared in different clusters. Same result was reported by (Assefa et al. 2003) for tef germpalsm. In general accessions which matured earlier and tended to have smaller seed size, short plant height and short spike length clustered together where as large seeded and taller accessions which matured relatively late clustered together. As compared with accessions of other regions, Arsi, Bale, Gamo Gofa and Sidamo appeared in all clusters, suggesting that accessions from these region was relatively variable than those regions which appeared rarely.

Based on discriminant analysis around 83.98% of studied accessions are misclassified from their respective regions. This finding may be explained by long distance seed exchange, continuous seed introduction and gene flow between agro ecologies. This result agreed with the hypothesis made by Holcomb et al. (1977) and Pecetti and Damania (1996) that the higher the diversity of the group, the higher the probability of misclassification and vice versa. Same result was reported by Ayana and Bekele (1999) where lack of strong regional differentiation observed by the cluster and discriminant analysis could be partly ascribed to gene flow between regions (Teshome et al. 1997; Doggett 1988). This indicated that clustering pattern of accessions did not follow their geographic origin and more emphasis should has to be given to population from different agro ecology than to geographic origins alone as source of diversity (Alemayehu and Becker 2002).

5.2. Genotypic diversity

Genetic diversity estimated based on allelic richness as a total number of detected alleles per locus and gene diversity also called PIC, showed the existence of high variation. According to (Hildebrand et al. 1994) 47% of the markers used in this study were moderately (0.44-0.7) to highly (above 0.7) informative. Among the markers *Bmac0040* was the richest in terms of alleles and same result was reported by (Malysheva-Otto et al. 2006; Malysheva-Otto 2007). *Bmag0007* (0.79) and *Bmag0211* (0.70) were the highly informative SSRs markers for this study (Hildebrand et al. 1992).

5.2.1. Genetic population differentiation

The population of most, if not all, species show some levels of genetic structuring, which may be due to a variety of exclusive agents. Environmental barriers, historical processes and life histories (e.g. mating system) may all, to some extent, shape the genetic structure of populations (Donnelly and Townson 2000; Gerlach and Musolf 2000).

5.2.1.1 Genetic diversity and differentiation among regions

In cultivated crop species, geographical distribution patterns reflect both the specific selection pressures prevailing in a particular environment as well as history of selection and production (Hawtin et al. 1997). Hence in diversity study, the inclusion of genotypes collected from different geographic areas has been adopted as a strategy to capture all sort of allelic diversity of a particular crop plant.

AMOVA was conducted for regions with the assumption of existence of high variation between regions as compared to within regions. But in contrast the result showed high variation within regions. The result obtained from analysis of phenotypic data also showed same trend with no significant difference between regions while statistically significant difference observed within regions (Abebe et al. 2010). Similarly fairly low contribution of regions to total variation as compared to within region was reported for barley of Ethiopia based on isozyme analysis (Demissie and Bjørnstad 1997) and in same pattern for barley collected from Tunisia 95% of total variation resided withinregion (Ould Med Mahmouda and Hamzaa 2009). Birmeta et al. (2004) reported high genetic diversity within population than among population for enset (*Ensete ventricosum*) collected from Southern part of Ethiopia, using RAPD markers. (Geleta et al. 2007) revealed high variation within region for niger (*Guizotia abyssinica)* landraces collected from different parts of Ethiopia based on AFLP analysis. Partitioning of genetic variation by AMOVA showed very low variability among regions of collection for Ethiopian yam (*Dioscorea* spp.) as compared to within regions (Tamiru et al. 2007).

Fixation indices, which describe the level of heterozygosity in a population, showed medium genetic differentiation among populations accompanied with high gene flow of two migrants per

generation. According to (Wright 1931) no random differentation among populations could be expected if the average number of migrants (gene flow) per generation, (Nm) exceeds 1; where *N* is the effective population size and *m* is the fraction of immgration. For barley High variation among farmers' varieties was observed as compared to variation within farmers' varieties among farmers field (van Leur and Gebre 2003) which indicated high gene flow between farmers fields. Accordingly, the medium F_{ST} value reflects adaptation to strong environments or high level of genetic drift maintained by restricted gene flow among populations. Generally high differentiation within population resulted in less gene flow among population and low fixation index. Wright (1951) found an inverse relationship between Nm and population differentiation, F_{ST}. Pairwise population differentiation showed Tigray and Welo were the two regions which were significantly differentiated from the rest of the regions but in the later case the value of differentiation was smaller. Results from Neighbor Joining tree confirmed that Tigray was the highly differentiated region while Arsi and Bale, geographically closer regions, were the less differentiated. STRUCUTRE analysis at K=2 also showed the same result for Tigray and Welo as differentiated and fewer admixtures. Meanwhile the discriminant analysis from phenotypic data also showed that 56.25% of accessions collected from Tigray grouped in its respective region, which indicated low gene flow and relatively differentiated population. With same fashion Sidamo and Bale differentiated significantly from all regions except Gojam and Arsi too in later case, in which 29.62% and 23.08% of accessions from Sidamo and Bale classified under Gojam with discriminant analysis of phenotypic data. This result indicated that regions which share high percentage of accessions were not different from each other. Determining the level of variation within, and among, barley populations is an essential step towards conserving genetic resources and developing future strategies for plant improvement (Abay et al. 2009).

As locus by locus analysis of variance showed 60% of the markers were significantly different from zero and were able to differentiate among populations. According to Beaumont (2005) loci that are subject to strong balancing selection should have a lower level of genetic differentiation and the significant result also indicated the presence of selection.

5.2.1.2 Genetic diversity and differentiation among altitude classes

With similar pattern as regions, partitioning of total genetic variation was conducted for altitude classes and resulted in high genetic variation within altitude classes. The same result was reported for niger (*Guizotia abyssinica*) of Ethiopia based on altitudes as adaption zone (Geleta et al. 2007). As altitudes within a region cover a wide range (Table 2) there was no population differentiation observed among altitude classes which could be associated with the ease of movement along altitude class within regions and there were possibilities to cover all altitude classes with short distance accompanied by high gene flow as compared to regions. High seed exchange among farmers and using seed from sources of own preference have different reasons for the later case mainly depends on the quality and price of the seed (Almekinders and Louwaars 1999). Furthermore, the need to get a new variety, inability to save seed, the need to replace farmers' own diseased or 'degenerated' seed, unfavourable seed production conditions, inability to produce a variety, inability to store quality seed for long period, the need to specialize own production for market are reasons why farmers use seed from other sources. Such reasons as quality and price of seed seem to affect farmer's sources of seed preference and seed flow within farmers in a community (Hunduma 2006). Farmers in Ethiopia claimed travelling as far as 20 km to buy seed locally. In one incident the farmer had sourced seed of the new variety from a distance of over 100 km through family acquaintances (Bishaw 2004). There were no significant differences between populations in relation to the extent and altitude of cultivation was observed for niger (*Guizotia abyssinica)* landraces of Ethiopia (Geleta et al. 2007). High allelic variation observed in altitude class II and III which holds same with the morphological analysis in which high and significant variation for all studied traits were observed. As barley is highland crop, the presence of less allelic variation in altitude class I could be related with high temperature and less cultivation of barley in low altitudes. Tanto et al. (2009) reported the reduction of area of cultivation for barley as altitude decreased. The same result was reported by Demissie and Bjørnstad (1997) for the presence of less polymorphism in altitudes less than 2000 m.a.s.l and above 3500 m.a.s.l where as high polymorphism observed in altitudes 2001-3000 for isozyme analysis of Ethiopian barley.

5.2.2 Genetic diversity of accessions

Analysis of genetic relationships in crops species is an important component of crop improvement. It helps to analyze genetic variability of cultivars, select parental materials for hybridization for making new genetic recombination select inbred parents or tester for maximizing heterotic response and identify materials that should be maintained to preserve maximum genetic diversity in germplasm.

Diversity among accessions and within accessions showed the potential of genetic variation within accessions which is a source material for barley improving purpose. The exploitation of within accession variation through pure line selection has proven to provide superior germplasm for disease resistance and yield characteristics (Lakew et al. 1997; Semeane et al. 1998). Exotic cultivars out yield local landraces under good management practices, but local landraces usually out yield the exotic material under the low input conditions which predominate among peasant farms. For such conditions, native germplasm should be exploited to improve productivity (Lakew et al. 1997). In addition the presence of genetic diversity among local germplasm and use as source of breeding materials was reported for barley and other crops (Lakew et al. 1997; Semeane et al. 1998; Adugna 2008; Marame et al. 2009).

In general the diversity observed within accessions indicated that it is possible to accommodate large proportion of variation with collection of small number of samples. So it is possible to suggest that during sampling concentrating on the accessions is more worth, time saver and costly effective than to give more attention to regions. However because of some traits which are region specific still regions are important depending on target traits of selection. Regions like Harerge where drought prevails heading and maturity date was important from farmers' point of view to select for early matured landraces to produce some grains while for Shewa and Sidamo thousand seed weight plays major role since farmers need barley for market and no problem of moisture stress for barley growing areas.

5.2.3 Quantitative *vs* genetic population differentiation

Theory suggests that heterogeneous distribution of genetic variation within populations and among populations is due to mutation, genetic drift, due to finite population size, and heterogeneous natural selection, whereas gene flow and homogenous directional selection tend to produce genetic homogeneity (Slatkin 1987; Chan and Arcese 2003). On broad scale, the factors affecting diversity at the ecological level also affects or shape genetic differentiation at the molecular level (Tilman 1999). Comparing patterns of population genetic differentiation at quantitative traits (Q_{ST}) and molecular markers (F_{ST}) permits inferences about the relative role of selection in population divergence, by contrasting the degree of adaptive change with that of differentiation due to solely to drift (Spitze 1993; Zhan et al. 2005). There are three possible outcomes from comparison of molecular F_{ST} and quantitative Q_{ST} differentiation. If molecular differentiation in neutral molecular markers among populations F_{ST} is of the same magnitude as that for Q_{ST} or significantly more than Q_{ST}, then the hypothesis that among population variance in quantitative traits is due to random drift cannot be rejected, or uniform selection maybe involved as a cause for the reduced differentiation. The third case where Q_{ST} is greater than F_{ST}, suggests a prominent role for natural selection in accounting for patterns of quantitative traits differentiation among populations. With the same pattern in this study three patterns of population differentiation were observed. The result obtained showed the importance of the among population variance in determining quantitative differentiation. From morphological data it was possible to detect little or no significant difference among population. Since among regions variance was small for many of the traits only days to heading, days to maturity thousand seed weight and glucan content had Q_{ST} values. However from the Q_{ST} values of regions it was observed that only heading date was significantly smaller and different from F_{ST} which showed that uniform selection among regions while days to maturity, thousand seed weight and glucan content displayed no significant difference between Q_{ST} and F_{ST}, indicating that the effects of drift and selection are indistinguishable, probably of same magnitude (Chan and Arcese 2003; Luttikhuizen et al. 2003). Overall mean Q_{ST} for regions showed homogenized selection which resulted in no or small variation among regions.

In contrast to regions high Q_{ST} was observed in altitude classes with low F_{ST}, it probably means that selective pressure was heterogeneous with high gene flow and it inhibited local adaptation.

Lenormand (2002) and Hendry and Taylor (2005) reported negative correlation between the magnitude of adaptive traits and gene flow in stickleback. Gene flow into a population can counteract gene frequency changes because of selection, imposing a limit on local adaptation (Lenormand 2002). The stronger the local adaptation, the more Q_{ST} differs from F_{ST} and the weaker the association (Latta and McKay 2002).

Generally the contrast between population differentiation estimated from quantitative traits (Q_{ST}) and molecular markers (F_{ST}) was used to detect whether selection was acting on the traits under study and it was also possible to identify traits which were under pressure of selection from farmers point view. Glucan content was the only trait which was not under selection pressure in respect to regions and altitude classes.

5.2.4 Hierarchal clustering

Since the populations were not strongly differentiated the cluster pattern was not clear enough and there were no distinct groups. This is due to the fact that a large portion of the variation was found among accessions within region of origin and within the altitude classes, which confirms that differentiation of the barley accessions on the basis of region of origin and altitude was weak. With the same pattern the absence of coherent grouping, which matched regional, altitudinal or agro ecological zones, was observed for barley landraces collected from Ethiopia. Demissie et al. (1998); Ould Med Mahmouda and Hamzaa (2009) reported lack of geographical differentiation which failed to indicate clear pattern of division among barley accessions based on geographic origin. These results may reflect the impact of seed exchange between farmers which is likely to limit highlighting favorable alleles due to local adaptation.

Instead of molecular markers, genetic variation in populations can be investigated by assessing quantitative variation that is under polygenetic control where many loci and the environmental effects on those loci contribute to the quantitative variation in the traits being investigated. Yet analyzing patterns of genetic variation from molecular markers has become increasingly popular as molecular techniques become more cost effective and less invasive. Unfortunately evidence for concordance in these two measures of genetic diversity is ambiguous with a number of

studies suggesting it is poor (Reed and Frankham 2001; McKay and Latta 2002). The present study resulted in weak correlation between morphological and SSR markers which is suggested that the molecular markers may not necessarily track quantitative genetic variation due to non-additive effects, differential selection, different mutation rates, environmental effects on quantitative variation, and the influence of genetic variation on gene regulation (Lynch et al. 1999; Reed and Frankham 2001). Meta analysis indicated absence of correlation between molecular markers and quantitative traits for many studies (Reed and Frankham 2001). With the same trend weak correlation observed among genetic similarity and geographic distance which indicated the genetic distances between populations were independent of the corresponding geographical distances (George et al. 2009). It was possible to suggest that there was no isolation by distance because of the presence of high gene flow among population which was not dependent of the distance. Isolation by distance is observed in natural plant species where the likelihood of gene flow is inversely related to distance (Pusadeea et al. 2009).

The cophentic correlation indicated that the goodness of fit of the cluster analysis to genetic distance estimates is not good, as described in Rohlf (Rohlf 2000) However, it does not mean that clustering is not possible, but only indicates that some distortion might have occurred (Mohammadi and Prasanna 2003).

6. Summary and conclusions

Ethiopia as a region with wide altitudinal range, substantial temperature, and rainfall differences with diverse edaphic conditions create a wide range of agro-ecological conditions and microenvironments. For this study results obtained from analysis of morphological traits and SSR analysis will be summarized as follows.

In general there was no significant variation observed among regions while results obtained from analysis of regions of origin and different altitude class showed wide variation within regions of high altitude, humid and cooler temperature. So morphological traits which are under direct influence of both human and natural selections are strongly associated with altitude. From this finding it was suggested that to exploit the available genetic variation potential in barley landraces one should concentrate with an altitude range of 2001-3000.The concentration of some morphological traits at high or low altitudes and in different sites could result from farmer's selection activity based on their selection criteria to the prevailing climatic and edaphic conditions. And traits which are adapted well like days to heading, days to maturity, plant height and awn length, played a role in differentiating accessions collected from different regions and altitude class in to principal components. Results obtained from clustering of accessions into distinct group showed no consistent pattern and accessions did not follow their geographic origin and more emphasis should has to be given to population from different agro ecology than to geographic origins alone as source of diversity.

Although genetic differentiation was not great both on regional and altitudinal bases, regional differentiation was greater than differentiation on the basis of altitudes. However, applying discriminative analysis to quantitative morphological and agronomic data, it is found that altitude was more discriminative than regions of origin.

The presence of high gene flow among altitudes as compared to regions of origin inhibited the adaptation of traits by counteracting the gene frequency created by selection and there was diversified selection against altitudes. Glucan was traits which were not under selection pressure of farmers.

Generally because of environmental factors on the observed morphological variation future germplasm collection should consider to explore wide geographical and climatic differences within the country. Results of the present study can help to define the strategies for further collection. In general *in-situ* and *ex-situ* conservation strategies shall be implemented to complement each other for sustainable conservation and utilization of crop genetic resources. No single method is adequate for assessing genetic variation in germplasm collections because different methods sample genetic variation at different levels and differ in their power of genetic resolution as well as the quality of information content.

The size of this study limits the conclusions that can be drawn, but the patterns of variation described here can be a basis for studies with a higher number of markers. The conservation of such locally common variation is important, since it may represent genotypes adapted to specific environments. The patterns of variation described in this study may be useful for researchers designing larger studies of barley germplasm.

6. References

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7. Appendix

Appendix 1: List of 199 barley accessions collected from 10 regions of Ethiopia with detail passport data

Region code*	Accession No.	Region	Latitude	Longitude	Altitude	Collection year
1	213538	Arsi	07° 27' N	39° 15' E	2980	1985
1	215362	Arsi			2540	1985
1	216989	Arsi	07° 29' N	39° 11' E	2600	1986
1	232217	Arsi	08° 30' N	39° 39' E	2220	1989
1	237006	Arsi	07° 19' N	39° 16' E	2810	1988
1	229990	Arsi	07° 38' N	39° 19' E	3120	1989
1	217112	Arsi	07° 29' N	39° 15' E	2920	1986
1	218944	Arsi	07° 55' N	39° 08' E	2260	1986
1	224979	Arsi			2510	1988
1	238847	Arsi			2500	1997
1	217008	Arsi	08° 07' N	39° 35' E	2690	1986
1	223959	Arsi	07° 33' N	39° 22' E	3150	1988
1	237002	Arsi	07° 19' N	39° 16' E	2350	1988
1	229992	Arsi	07° 35' N	39° 32' E	2400	1989
1	213707	Arsi	07° 06' N	38° 44' E	2640	1985
2	237845	Bale			2440	1995
2	230591	Bale	07° 00' N	39° 23' E	2400	1989
2	237849	Bale			1960	1995
2	243187	Bale	07° 00' N	40° 23' E	2150	2004
2	217065	Bale	07° 23' N	39° 32' E	2650	1986
2	238776	Bale			2420	1997
2	217081	Bale	07° 07' N	40° 02' E	2450	1986
2	238807	Bale			2300	1997
2	237854	Bale			2420	1995

2	230640	Bale	07° 12' N	39° 57' E	2240	1989
2	215369	Bale			2390	1985
2	215378	Bale			2150	1985
2	217082	Bale	07° 06' N	40° 44' E	2470	1986
2	212826	Bale	07° 22' N	40° 12' E	2390	1985
2	243185	Bale	07° 01' N	40° 23' E	1990	2004
2	224984	Bale			2410	1988
2	237850	Bale			2070	1995
2	243178	Bale	07° 01' N	40° 19' E	2200	2004
2	238787	Bale			2580	1997
2	237851	Bale			2080	1995
2	224988	Bale			2150	1988
2	237847	Bale			1810	1995
2	237856	Bale			2450	1995
2	238839	Bale			2420	1997
2	230653	Bale	07° 07' N	39° 53' E	2610	1989
2	237846	Bale			2330	1995
3	216990	Gamo Gofa	06° 16' N	37° 35' E	2960	1986
3	236133	Gamo Gofa			2990	1994
3	241685	Gamo Gofa	06° 01' N	37° 29' E	2160	2002
3	233038	Gamo Gofa	06° 05' N	37° 15' E	2380	1990
3	241684	Gamo Gofa	06° 21' N	37° 30' E	2200	2002
3	216999	Gamo Gofa	06° 17' N	37° 35' E	3030	1986
3	236128	Gamo Gofa			2560	1994
3	217004	Gamo Gofa	06° 32' N	37° 45' E	2830	1986
3	236131	Gamo Gofa			2990	1994
3	204807	Gamo Gofa	06° 14' N	37° 34' E	2590	1983
3	236141	Gamo Gofa			2830	1994
3	236132	Gamo Gofa			2990	1994
3	233028	Gamo Gofa	05° 55' N	37° 20' E	2050	1990
3	236129	Gamo Gofa			2540	1994
3	236137	Gamo Gofa			2960	1994

3	236140	Gamo Gofa			1950	1994
3	217051	Gamo Gofa	06° 17' N	37° 28' E	2900	1986
3	212967	Gamo Gofa	36° 51' N	06° 18' E	2350	1985
3	236134	Gamo Gofa			2990	1994
3	217054	Gamo Gofa	06° 21' N	37° 36' E	2700	1986
3	233023	Gamo Gofa	06° 15' N	37° 33' E	2800	1990
3	217058	Gamo Gofa			2350	1986
3	236130	Gamo Gofa			2650	1994
3	236139	Gamo Gofa			1950	1994
3	216992	Gamo Gofa	06° 16' N	37° 35' E	2960	1986
4	238378	Gojam	10° 20' N	37° 09' E	2530	1996
4	219770	Gojam	11° 33' N	37° 16' E	2450	1986
4	225997	Gojam	11° 32' N	37° 22' E	2000	1988
4	216978	Gojam	11° 04' N	37° 51' E	2345	1986
4	225266	Gojam	10° 16' N	37° 26' E	2260	1988
4	225265	Gojam	10° 16' N	37° 26' E	2260	1988
4	217059	Gojam	10° 15' N	37° 50' E	2550	1986
4	216975	Gojam	10° 50' N	37° 36' E	3090	1986
4	216009	Gojam	10° 16' N	37° 26' E	2260	1986
4	216957	Gojam	10° 58' N	37° 13' E	2880	1986
4	213708	Gojam	10° 58' N	37° 13' E	2780	1985
5	243284	Gonder	11° 48' N	38° 12' E	2990	2004
5	243308	Gonder	11° 35' N	38° 03' E	2358	2004
5	204788	Gonder	11° 36' E	38° 34' E	2350	1983
5	225994	Gonder	12° 32' N	37° 16' E	2010	1988
5	235888	Gonder	13° 20' N	37° 38' E	2800	1993
5	216966	Gonder	11° 49' N	38° 08' E	2810	1986
5	235883	Gonder	12° 30' N	37° 46' E	2900	1993
5	217019	Gonder	11° 44' E	38° 25' E	3000	1986
5	206303	Gonder			2500	1983
5	225986	Gonder	11° 36' N	38° 11' E	2890	1988

5	235885	Gonder	13° 38' N	37° 44' E	2260	1993
5	225991	Gonder	11° 25' N	37° 58' E	2385	1988
5	235886	Gonder	13° 19' N	37° 38' E	2940	1993
5	217010	Gonder	12° 38' N	37° 06' E	2090	1986
5	235880	Gonder	12° 28' N	37° 38' E	2280	1993
5	214240	Gonder			3009	1985
5	216959	Gonder	11° 50' N	38° 00' E	2730	1986
5	219744	Gonder	11° 35' N	37° 28' E	2400	1986
6	241678	Harerge	08° 54' N	40° 46' E	2350	2002
6	208675	Harerge	09° 14' N	41° 50' E	2600	1983
6	230499	Harerge	09° 24' N	42° 17' E	2250	1989
6	219101	Harerge	09° 20' N	40° 54' E	2090	1986
6	216778	Harerge	09° 37' N	42° 24' E	2330	1986
6	230506	Harerge	09° 29' N	42° 15' E	2530	1989
6	216787	Harerge	09° 15' N	41° 46' E	2510	1986
6	232351	Harerge	09° 37' N	42° 24' E	2380	1989
6	241677	Harerge	08° 50' N	40° 47' E	1990	2002
6	241682	Harerge	09° 06' N	41° 54' E	2440	2002
6	241683	Harerge	09° 06' N	41° 54' E	2220	2002
6	223301	Harerge	08° 41' N	40° 19' E	2030	1987
6	216810	Harerge	09° 21' N	41° 26' E	2430	1986
6	241681	Harerge	06° 21' N	41° 54' E	2230	2002
6	241680	Harerge	08° 54' N	40° 46' E	2340	2002
6	241679	Harerge	08° 54' N	40° 46' E	2350	2002
6	241121	Harerge	09° 59' N	40° 51' E	2080	2002
6	204787	Harerge	09° 24' N	41° 35' E	2200	1983
6	241120	Harerge	09° 02' N	40° 54' E	2200	2002
6	241676	Harerge	08° 50' N	40° 47' E	1990	2002
6	231455	Harerge			1900	1990
6	208676	Harerge	09° 12' N	41° 45' E	2420	1983

7	224954	Shewa	09° 50' N	39° 45' E	2830	1988
7	236119	Shewa			2200	1994
7	235528	Shewa	08° 07' N	38° 11' E	3250	1993
7	212507	Shewa	10° 03' N	39° 35 ' E	3130	1985
7	204643	Shewa			3120	1983
7	237812	Shewa			2120	1995
7	237546	Shewa			1740	1995
7	236118	Shewa			2190	1994
7	208904	Shewa	09° 00' N	37° 30' E	2450	1984
7	235550	Shewa	08° 02' N	38° 02' E	2910	1993
7	235531	Shewa	08° 07' N	38° 12' E	3200	1993
7	208911	Shewa	09° 12' N	37 °12' E	2900	1984
7	213522	Shewa	07° 21' N	37° 47' E	2600	1985
7	216934	Shewa	09° 06' N	38° 12' E	2900	1986
7	216923	Shewa	09° 07' N	38° 36' E	2730	1986
7	224912	Shewa	09° 19' N	39° 31' E	3280	1988
7	236116	Shewa			2240	1994
7	236801	Shewa	39° 51' N	39° 44' E	3200	1995
7	204642	Shewa			2950	1983
8	219321	Sidamo	05° 19' N	39° 35' E	2520	1986
8	225179	Sidamo	06° 57' N	37° 51' E	2100	1988
8	236104	Sidamo			2200	1994
8	236145	Sidamo			2200	1994
8	236103	Sidamo			2170	1994
8	233053	Sidamo	06° 18' N	38° 14' E	1850	1990
8	233052	Sidamo	06° 18' N	38° 14' E	1850	1990
8	236149	Sidamo			2420	1994
8	219304	Sidamo	06° 20' N	38° 16' E	1930	1986
8	236101	Sidamo			2190	1994
8	236151	Sidamo			2200	1994
8	217107	Sidamo	06° 50' N	37° 41' E	1880	1986

8	219330	Sidamo	07° 00' N	37° 44' E	1950	1986
8	219324	Sidamo			2020	1986
8	236113	Sidamo			1910	1994
8	225184	Sidamo	06° 57' N	37° 51' E	2100	1988
8	225181	Sidamo	06 °57' N	37° 51' E	2100	1988
8	236105	Sidamo			2200	1994
8	236106	Sidamo			1980	1994
8	236102	Sidamo			2150	1994
8	236107	Sidamo			2200	1994
8	217101	Sidamo	06° 55' N	37° 49' E	2100	1986
8	219305	Sidamo	06° 03' N	38° 11' E	2220	1986
8	236154	Sidamo			2200	1994
8	236146	Sidamo			2110	1994
8	227765	Sidamo			2400	1988
8	219306	Sidamo	05° 41' N	38° 13' E	2940	1986
9	234344	Tigray	14° 08' N	38° 33' E	2200	1991
9	219915	Tigray	14° 10' N	38° 55' E	2600	1986
9	238357	Tigray	14° 30' N	39° 50' E	2920	1996
9	242092	Tigray	13° 00' N	39° 32' E	2950	2002
9	238373	Tigray	13° 52' N	39° 43' E	2130	1996
9	219935	Tigray	14° 05' N	38° 14' E	1940	1986
9	235298	Tigray	13° 24' N	39° 23' E	2000	1992
9	235293	Tigray	13° 27' N	31° 26' E	2060	1992
9	234341	Tigray	14° 07' N	38° 51' E	2100	1991
9	234343	Tigray	14° 08' N	38° 30' E	2180	1991
9	234347	Tigray	14° 08' N	38° 34' E	2100	1991
9	206342	Tigray			2198	1983
9	238353	Tigray	13° 04' N	38° 04' E	1970	1996
9	221712	Tigray	13° 31' N	39° 28' E	1990	1986
9	235294	Tigray	13° 26' N	39° 25' E	2060	1992
9	238355	Tigray	14° 30' N	39° 50' E	2910	1996

10	215212	Welo	10° 54' N	39° 31' E	2950	1985
10	236249	Welo			2650	1994
10	236231	Welo			3340	1994
10	217127	Welo			2860	1986
10	236228	Welo			3430	1994
10	236226	Welo			3180	1994
10	204805	Welo	11° 20' N	39° 46' E	2350	1983
10	215699	Welo	11° 22' N	39° 51' E	2240	1985
10	224948	Welo	11° 48' N	39° 22' E	2860	1988
10	236225	Welo			3200	1994
10	217145	Welo			2960	1986
10	236236	Welo			3150	1994
10	204819	Welo	10° 54' N	39° 21' E	2650	1983
10	215695	Welo	11° 20' N	39° 47' E	2540	1985
10	217128	Welo			3050	1986
10	236238	Welo			3360	1994
10	236253	Welo			2410	1994
10	224921	Welo	10° 57' N	39° 33' E	2920	1988
10	217138	Welo			3080	1986
10	215210	Welo	11° 00' N	39° 33' E	2900	1985

*Codes used for the regions on the diagram derived from STRUCTURE