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MORPHOLOGICAL AND BIOCHEMICAL DIVERSITY IN RECENT COLLECTIONS OF BARLEY (*Hordeum vulgare* L.) FROM VARIOUS REGIONS OF ETHIOPIA

SHAMBEL KUMBI FAYISSO

Addis Ababa, June 2001

MORPHOLOGICAL AND BIOCHEMICAL DIVERSITY IN RECENT COLLECTIONS OF BARLEY (*Hordeum vulgare* L.) FROM VARIOUS REGIONS OF ETHIOPIA

SHAMBEL KUMBI FAYISSO

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DEDICATION

THIS WORK IS DEDICATED TO THE FARMING COMMUNITY OF THE OROMO PEOPLE.

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ABSTRACT

Morphological and isozyme diversity study of 47 barley (Hordeum vulgare L.) populations that were collected from 11 regions in Ethiopia was undertaken for this study. The populations with number of individuals ranging from 43 to 45 per population, which make up a total of 2057 individual plants were evaluated for 16 quantitative and 14 qualitative morphological characters. Isozyme analysis was conducted using three enzyme systems: esterase (EST), asparatase aminotransferase (AAT) and leucine aminopeptidase (LAP) for seven barley populations selected after clustering them on morphological characters. Protein analysis was determined for 47 barley populations based on the Kjeldahl standard procedure. Several statistical methods such as mean, coefficient of variations, estimates of heritability, genetic advance, correlation coefficients, cluster analysis, chi-square test, principal component analysis, discriminant analysis, correspondence analysis and Shannon-Weaver diversity index (H') were used to estimate the morphological variation. Analysis of variance showed that there were highly significant differences between populations. In principal component analysis the first five principal components were contributed 85.5% of the total variation. Discriminant analysis revealed that 45 populations out of 47 (95.7%) were correctly classified to their respective cluster. Correspondence analysis result indicated that the first five components or variables accounted for 89.15% of the total inertia while the first two axes retained 67.79% of the total inertia. The Shannon-Weaver diversity index (H') for individual characters, populations, altitudinal classes and regions were estimated. Based on this, the H' value ranged from 0 (monomorphic) for awn colour and kernel cover to 1.0 (highly polymorphic) for flag leaf area. Region wise comparison displayed higher magnitude of differences. The variation ranged from H'=0.56 for Hararge to H'=0.71 for Shewa, Arsi

and Gojam. Among 11 regions, Shewa, Arsi and Gojam showed highest diversity index. On altitudinal base the highest diversity index (H'=0.74) was observed in altitudinal class of 2501-3000 m asl. The overall mean diversity index for the present Ethiopian barley was estimated to be (H'=0.75). Phenotypic polymorphism was observed for the esterase (EST) enzyme and there was no variation observed for Aspartate aminotransferase (AAT) and leucine aminopeptidase (LAP). Correlation between Shannon- Weaver diversity index of qualitative morphological characters and isozymes variations showed significant positive and negative association. When $P < 0.05$ is used, the following character combinations showed significance. EST-1 and Kernel colour; EST-3 and glume hairiness; EST-4 and kernel colour; EST-2 and lodging; awn colour and glume hairiness; and flag leaf area and lodging. Est-3 and glume hairiness, and flag leaf area and lodging are the character combinations that showed a negative significant correlation. Protein analysis of 47 barley populations was undertaken and the result showed highly significant differences between populations, altitudinal classes and regions. The present study indicated that regions with high diversity index (H') are more important for germplasm collection and for identification of suitable sites for in-situ conservation.

Key words: Hordeum vulgare; Barley genetic diversity; Morphological character; Isozyme; Protein

1. INTRODUCTION

Barley (*Hordeum vulgare* L.) is a member of the tribe Triticeae of the grass family Graminae (Poaceae) and genus *Hordeum* L. (Berrie, 1977). It is a diploid species with 2n=14 (Purseglove, 1972).

It is one of the major world cereal crops ranking fourth in the world coverage and production following wheat, corn and rice (Zohary and Hopf, 1988). And in Ethiopia, it ranks only third in terms of acreage after tef (*Eragrostics tef*) and sorghum (*Sorghum bicolour*) (Engels and Hawkes 1991).

Barley is an economically important and genetically well-studied crop. It is one of the oldest crops probably as old as agriculture. It is widely grown in many different climates. Major production areas are Europe, the Mediterranean fringe of North Africa, Ethiopia, Near East, Russia, China, India, Canada, the United States and Australia (Nevo, 1992).

Barley grows where other cereals cannot grow any more due to frost, salinity, and is for this reason important for traditional farmers that dwell in the marginal areas. It can be used for animal or human nutrition, but it is also of vital importance for malt, i.e. beer and whisky production. Barley is popular as an experimental crop since it is easy to grow and autogamous, with many well-known specific gene effects (Harlan, 1976).

Barley is a crop grown widely all over the world. Its worldwide acreage is over 76 million ha. which is 5.6% of the world area of arable land and 10.6% of the area occupied by cereals

(FAO, 1991). In Ethiopia, the total area under crops was estimated to be 7. 56 million hectares. Out of which 5.6 million hectares are under cereal crops, among these, barley occupies an area of about 0.68 million hectares (CSA, 1998).

Ethiopian barley possesses a wide range of diversity and there unique features. It has been investigated using morphological (Asfaw, 1988; Negassa, 1985a; Demissie and Bjørstand, 1996a), isozymes (Bekele, 1983b; Demissie and Bjørstand, 1997), hordein (Asfaw, 1989a) polymorphism and Random Amplified Length Polymorphism (RFLP) markers (Demissie and Bjørstand, 1996b). All of these studies show that the diversity index is very high. This is one of the indications that Ethiopia is undoubtedly rich in diversity of barley that have great importance for many purposes.

Although much of the diversity in agricultural crops is still in the hands of farmers, a lot of it has already been lost. The impact of the threat has also been extended to the traditional management systems of varieties of crops developed and used by the local people through generations.

Generally, genetic erosion is caused due to diffusion of exotic seed varieties that has been displacing farmers' varieties. The pace of genetic erosion was tremendously increased after the seventies (Worede, 1988).

The loss of genetic diversity may lead to increasing crop susceptibility to diseases, pests and environmental stresses. Gene banks have been established to provide plant breeders with the

genetic resources for developing more resistant and tolerant crops that will maintain stable and high yield (Plucknett *et al.*, 1987; Brown *et al.*, 1989).

To over come such erosion, collection, characterization, and conservation of germplasm are highly important. The Ethiopian region, often referred to as a major Vavilovian gene center and characterized by wider range of agro-climatic conditions, would provide a considerable number of appropriate conservation sites.

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. To achieve a more efficient and comprehensive utilization of the conserved wild gene pool, it is essential to be able to predict, screen and evaluate promising genetic resources in natural populations and genotypes, thereby optimizing breeding.

More genes for better agronomic characteristics, disease resistance, earliness, good quality, and higher biological yield are necessary for further progress in barley improvement. The availability of such genes depends on the identification of areas of concentration for various characters of agronomic value. The identification of these sites has paramount importance for the collection mission and appropriate *in-situ* conservation site selection. Choice of sites for *in-situ* conservation may depend on high diversity estimates based on markers and on knowledge of adaptive traits linked to certain ecological conditions (Negassa, 1986; Demissie and Bjørstand, 1996a)

In addition, prior knowledge of the nature and extent of genetic variation is crucial since successful conservation and utilization of germplasm depends on the proper assessment of variation within and among populations (Tesemma and Belay, 1991).

However, most recently collected and preserved barley landraces, at the Institute of Biodiversity Conservation and Research (IBCR) are not yet studied for their genetic diversity. Hence, this study was carried out with the following objectives.

OBJECTIVES

The general objective of the study was to generate information on diversity covering various morphological and biochemical characters of barley landrace of Ethiopia.

Specific objectives

- to evaluate the diversity of recent collections of barley (since 1980) based on morphological characters and biochemical characters (isozymes)
- to study the distribution of morphological and biochemical characters with respect to agro ecology, provenance, etc.
- to identify sites of high genetic diversity which could help in the identification of additional on-farm *in-situ* conservation sites.
- to study the relation between morphological and biochemical characters and compare them with the results obtained by previous studies.

2. LITERATURE REVIEW

2.1. Origin and taxonomy of barley (*Hordeum vulgare* L.)

Barley belongs to the genus *Hordeum* L. in the tribe *Tritceae* of the family *Poaceae* (*Gramineae*). The genus *Hordeum* is a distinct genus in the tribe, well distinguished by its three one- flowered spikelets at each rachis node. The genus, *Hordeum*, is a relatively small genus with about 28 species distributed over wide geographical areas and diverse ecological habitats (Bothmer *et al.*, 1981).

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). Archaeological evidence indicates that hulled two- rowed barley was found to be one of the first crops brought to domestication. According to Harlan and De wet (1971), the earliest archaeological findings of barley date back to 8000 to 7500 B.C. in Mureybit, Syria.

According to the archeological findings, fully domesticated two-rowed barley appeared around 7000 B.C., whereas both six-rowed and naked types appeared by 6000 B.C. The sites of early cultivation do not completely match with the present distribution of the wild type, *H. valgare subsp. spontaneum*, but this might have been the case during the times of domestication due to climatic change (Holm and Frost, 1981). The introduction of barley in

Ethiopia, where it evolved to a secondary center of diversity is considered to be from the Middle East about 5000-6000 years ago (Harlan, 1971). Since then, the crop has diversified and formed unique morphotypes including the irregular and deficiens types.

2. 2. Distribution and importance of barley (*Hordeum vulgare* L.) in Ethiopia

Barley (*H. vulgare* L.) is one of the oldest cultivated plants that has been grown in Ethiopia for at least 5000 years (Harlan, 1968). Barley is cultivated in every region of Ethiopia and demonstrates a wide ecological plasticity and physiological amplitude throughout the country (Asfaw, 1988; 1989b; Lakew *et al.*, 1996).

The most important barley producing regions are Shewa, Arsi, Gojam, Gonder, Wollo, Bale, and Tigray, which account for more than 85% of the total production (Yirga *et al.*, 1998). Barley is produced twice annually, i.e. during the main season, "meher" and the short rainy season, "belg". "Belg" barley is important in Wollo, Bale and North Shewa (Yirga *et al.* 1998). Barley can be cultivated at altitudes between 1500 and 3600 meters above sea level, but is predominantly grown between altitudes of 2000 and 3000 meters above sea level (Lakew *et al.*, 1996). The higher preponderance of some morphotypes (six- rows, naked caryopsis types, dense spikes at higher altitudes, other types (two-rows, lax types), and some hordein polypeptide patterns at lower elevations are documented (Asfaw, 1988; 1989b). Differential distribution, including abundance of primitive flavonoid patterns (Bekele, 1983a), resistant genes (Negassa, 1985a) and phenotypes and diverse molecular markers has been

reported (Asfaw, 1988; Demissie and Bjørstand, 1996a, 1997). Difference in frequency of isozyme phenotypes were observed among populations within regions and between regions and the distributions of alleles varied from region to region and from locality to locality (Bekele, 1983b). Within the general barley growing areas, there are pockets where some morphological and chemical groups concentrated. While some barley morphotypes are widely distributed, others are restricted to narrow ranges (Asfaw, 1990).

2. 3. Genetic diversity

Ethiopia is a major center of genetic diversity for many important domesticated crop species such as sorghum, barley, tef, chickpea and coffee. The existence of genetic diversity has special significance for the maintenance and enhancement of productivity in agricultural crops in a country like Ethiopia, which is characterized by highly varied agro-climates and diverse growing conditions. Such diversity provides security for the farmers against diseases, pests, drought and other stresses (Worede *et al.*, 2000).

Genetic diversity is not evenly distributed within the geographical range of the species gene pool. This has been recognized since Vavilov work in the 1920s and has been demonstrated in many studies. Some unique alleles occur in some regions but not in others. Some of the heterogeneity in the distribution of variation is explained by migration or chance factors such as founder effects and genetic drift (Demissie, 1996a). Within the general barley growing areas there are pocket areas as shown for Jibat and Mecha (Shewa), where some

morphological and chemical groups that can guide the development of conservation strategies are concentrated (Asfaw, 1990).

N.I. Vavilov was one of the early plant collectors who is credited for setting the phyto-geographic basis for plant exploration and collection. It was after him that plant breeders became aware of the fact that variations were not evenly distributed throughout the world but rather confined to relatively restricted geographic regions or centers. Vavilov proposed that the centers of diversity are the centers of origin of cultivated plants. In general, the place of origin of a species of cultivated plant is to be located in areas, which contain the largest variation of the plant in question. This concept is based on the assumption that selective forces of the environment are uniform and of equal intensity throughout evolutionary history of a given plant. Thus the longer the plant occupies an area, the greater would be the variation (Zohary, 1970).

Orlov (1929) recognized the uniqueness of Ethiopian barley as a marked ecological type significantly differing from the European and Asiatic types. Owing to the conspicuously large morphological variations, it was thought that Ethiopia was the center of origin of cultivated barley (Vavilov, 1926). Vavilov initially considered Ethiopia as the center of origin for barley but later as a secondary center of diversity due to the absence of the wild progenitor in the region.

According Bekele (1985), there are different forces that maintain barley polymorphism in Ethiopia such as balance between mutation and selection, balance between selection and migration, the heterogeneous environment, natural polymorphism, frequency dependent

selection, and transient polymorphism among the forces. It appeared that the question of the center of origin of barley had been settled some how (Zohary, 1964; Harlan, 1971), based on the simultaneous occurrence of the progenitor or rather, wild relative (*Hordeum spontaneum* C. Koch) of barley with the cultivated barley and archeological evidence of antiquity in the fertile crescent. Ethiopia was considered as a center of diversity of barley, but not of origin, because of the absence of the wild relatives of barley, *H. spontaneum* C. Koch (Schiemann, 1951; Harlan 1971). It also appears, according to Harlan (1971) that the occurrence of a wild relative, *per se*, is not enough to define a center of origin for a crop if the wild relatives are widely spread and there is a lack of variability in the cultivated crop.

However, in recent studies some evidence has been presented to suggest that Ethiopia might be a center of origin. Bekele (1983a) challenged the single origin hypothesis, arguing that based on barley flavonoid data, it is highly plausible that *spontaneum* gene was introgressed and gradually swamped up into *vulgare* gene pool in Ethiopia. Based on the clinal variation and concentrations of some characters including genes for disease resistance, a particular restricted area can be defined as a center of diversity and origin of barley in southern Ethiopia in accordance with the current concept of the center of origin and in the sense of the word 'Center' itself. This is the Arsi-Bale highland, where barley grows from an altitude of 1600 m asl to above the timber-line. The important roles played by barley in the cultural and religious aspects of the local population, the Oromo people have been discussed by several authors (Gugsa, 1975; Hassan 1983 both cited in Negassa, (1985a).

For the genetic variation of cultivated barley (*Hordeum vulgare* L.) the wild progenitor (*Hordeum spontaneum*) has significant contributions that are economically important. Some

of the traits include earliness, high yield, biomass, resistant genes for abundant genetic variation against physiological stress such as drought and salinity (Nevo *et al.*, 1984). Ethiopian barley genetic diversity is not evenly distributed within the geographical range of the species gene pool. The genetic diversity of this crop exists in many environments but, its continuity is particularly observed in marginal areas (mountains) due to the well-marked environmental change over small distance, seasonally and in isolation between the production zone.

The theory of fitness suggests that the amount of genetic variation could be an indication of adaptation to environmental heterogeneity, and uncertainty giving rise to habitat generalist populations (Bekele, 1983b). The numbers of alleles in a population could be used to estimate the relative potential of the population, as a high number of alleles increase the potential for the production of genetically diverse and adapted phenotypes, allowing their durability in changing environments (Bekele, 1983b). He also showed that there is genetic variation in Ethiopian barley landraces within localities (44.85%), between localities (20.13%), within areas (18.7%) and within region (16.31%). He also indicated that the contribution of such variation is different from locus to locus. The magnitude of contribution of different components of the hierarchy to the total gene diversity varies from region to region and indicates differences in conservation strategy to be developed.

The genetic diversity has been proposed as a common factor on different environmental response in greater release of variability from biparental crosses and in the heterosis of F_1 hybrids. The practical value of genotype-environment interactions in cross hybrid can be tested concerning genetic relationships of parent cultivars (Habgoods, 1983).

Genetically, cultivated barley and its wild relatives have no barrier between them and hybrids are fertile, and this close relationship makes them the same species *Hordeum vulgare* L. (Bothmer *et al.*, 1981). This is supported by the fact that Ethiopian barley landraces hybridize with the wild barley relative (*Hordeum spontaneum* C. Koch), and that the resulting hybrids develop normally with full variability and high seed production with average seed set of 37% and 24 % with two-rowed barley and with the six-rowed barley, respectively (Asfaw and Bothmer, 1990).

In fact, the large genetic diversity of Ethiopian barley landraces could be due to the diversity in soils, climate, altitude and topography together with geographical isolation for longer periods (Harlan, 1968). These environmental stresses play significant role for the diversification through evolutionary process (Harlan, 1968).

The relation of morphological diversity of barley with biochemical components like hordein polypeptide, flavonoid are some of the important indications of Ethiopian barley diversity. The distribution and pattern of arrangements of hordeins were different. Based on this the chemical analysis using hordein polypeptide variation has been applied to the systematic of Ethiopian barley (Asfaw, 1989a).

Furthermore, the spike row numbers have shown significant correlation with flavonoid "B" pattern that is more common in six-rowed than two-rowed barley (Frost *et al.*, 1975). The correlation between frequencies of flavonoid patterns and morphological means showed that the "C" pattern is positively correlated with number of seeds but negatively correlated with

sterile florets. This advantageous relationship might be the reason for prevalence of "C" patterns in Ethiopian barley (Bekele 1984). Therefore, the implication of environmental influence on the number of morphological characters and correlation between morphological characters and biochemical components, clearly indicate the presence of diversity in Ethiopian Barley.

Morphology is significantly associated with altitude for individual characters such as rachilla hair type, spike length, and spike density, spike row number and caryopsis type (Asfaw, 1989b). Some characters are strongly associated with high altitude and others with low altitude. In high altitude of southeast part of Ethiopia, the spike density, short spike length and six-row type are highly diverse (Asfaw, 1989b). According to Negassa (1985a), the six-row type is concentrated in Arsi-Bale highlands because of their genes for frost resistance, it also appears that the short spike phenotypic class (<10cm) is frequent at higher altitudes (>2400 m asl) in Arsi and of this about 12% are dense.

Furthermore, the major assessment of DNA polymorphism in Ethiopian landraces of barley shows the amount of variations in different genotypes, populations, geographical regions and altitudinal classes and agro-ecological zones (Demissie and Bjørstand, 1996a).

Estimates of genetic diversity of Ethiopian barley using biochemical analysis give more reliable information about the genetic variability. Bekele (1983a) studied esterase (four loci) and acid phosphatase (one locus) of the genetic relation of 158 landrace populations of the barley from 19 regions of Ethiopia and showed the presence of genetic diversity between different regions that can be used for *in-situ* conservation. Protein markers, such as isozyme

variants of the esterase) have been employed for this purpose in barley (*Hardeum vulgare*) population studies (Havid and Nieisen, 1977. In addition, the analysis of variability of genes indicating six isozyme system (15 loci) and two storage proteins (2 loci) of landrace barley from Ethiopia also indicated that the genetic variability is concentrated at some geographical regions (Demissie and Bjørstand, 1997).

The estimate of genetic diversity using the level of DNA diversity were weakly correlated with morphological and isozyme variations more with population than with region. The DNA variation was the highest in (2500-3000 m asl) altitude areas especially in Arsi-Bale, Shewa and Gamo Gofa regions suggesting the importance of these regions based on DNA polymorphism (Demissie and Bjorstand, 1996b). Furthermore, these authors showed that the RFLP level of polymorphism (84%) suggested the presence of large DNA variation in Ethiopian landrace barley. In general the results obtained from estimation by using marker systems such as morphological, isozyme and adaptive traits provide valuable database for creating a core collection for determining sites for intensive germplasm sampling and *in-situ* conservation for Ethiopian barely.

According Demissie and Bjørstand (1997), the relationship between the morphological diversity and the genetic diversity measured by isozymes was examined. A correlation between morphological diversity estimates (Shannon-Weaver diversity index, H') and expected heterozygosity estimates (H) at population level is low (r = 0.006), indicating the absence of biochemical and morphological data associations. On the other hand, Bekele (1984) reported that number of tillers and plant height; number of tiller and kernel weight; plant height and spike length; spike length and number of seeds; spike length and acid

phosphatase locus 1; number of seeds and kernel weight; kernel weight and rachilla hairiness; kernel weight and esterase D locus; row number and esterase A locus; glume hairiness and rachilla hairiness; glume hairiness and esterase C locus; and esterase A locus and esterase C locus showed significance by spearman's rank correlation coefficients. Kernel weight and rachilla hairiness are the only character combinations that showed a negative significant correlation ($p \leq 0.05$).

The relationships of morphological diversity of barley with biochemical components like hordein polypeptide and flavonoid variation are some of the important indications of Ethiopian barley diversity. The distribution and pattern of arrangements of hordein were different. Based on this the chemical analysis of hordein polypeptide variation has been used for systematics of Ethiopian barley (Asfaw, 1989b). The different analyses indicated that the variation in barley spike morphology and hordein polypeptide patterns share some features. However, the magnitude of association varied among characters, the highest being for rachilla hair type (46%) and the lowest for the caryopsis type (34%) (Asfaw, 1989b). Therefore, the observed association could have resulted from linkages between genes for certain morphological features and a given hordein pattern or from accumulation either randomly or by selection of genes for a certain morphological feature to a given hordein pattern.

2. 4. Importance of genetic diversity

Plant genetic resources are the total genetic diversity of cultivated species and their wild relatives. Plant genetic resources constitute the building blocks of all modern plant breeding:

they form the raw material from which new, more resistant and high yielding varieties have been systematically bred to meet the growing need for more food. These traditional gene pools are individual assets to the welfare of mankind and should be preserved, both for current use and posterity (Worede, 1988; Engles and Hawkes, 1991).

It is highly prized for their potential value as sources of important variations for crop improvement programs. Populations of these various forms of plant species also represent sources having the greatest potential for genetic diversity and can, therefore, serve as invaluable means to fill the gaps that still exist in the available base of genetic diversity in the world collection of many major crop species. Among the most important traits, which are believed to exist in these materials are earliness, disease and pest resistance, nutritional quality, resistance to drought and other stress conditions and characteristics especially useful in low input agriculture. Other important sources of germplasm include obsolete cultivars, varieties or cultivars in current use, breeding lines and special genetic stocks normally maintained by breeders (Worede, 1988).

Genetic diversity also allows farmers to exploit the full range of the countries highly varied microenvironments differing in characteristics such as soil, water, temperature, altitude, slope, and fertility. Diversity among species is especially significant to Ethiopia as it represents important resource to subsistence farming communities throughout the country (Worede *et al.*, 2000).

2. 5. Sources of diversity

Variation is just another way of saying differences existing among individuals. It is generally defined according to the population of individual characters. The large variation in Ethiopian barley (*Hordeum vulgare* L. *subsp. vulgare*) has been reported by different researchers. The country's heterogeneous environment coupled with the high altitude and its low latitude tropical position are frequently cited as main reasons for barley diversification in the Ethiopian region (Simoons 1965 cited in Asfaw, 1989b). These heterogeneous environments are believed to provide ample microniches favorable to new recombination, compared to more uniform environments. In addition to this, the increased mutation rate on highlands because of ultraviolet rays and temperature shocks (Schiemann, 1951), frequent hybridization, accumulated mutations and disruptive selection (Negassa, 1985a) are considered as the most probable causes of genetic diversity of barely in Ethiopia. Furthermore, the anthropogenic factors which are involved in the past and present history of agricultural systems, socio-cultural factors, and the process of domestication play a significant role for the diversification (Asfaw, 1989c).

The variation of Ethiopian barley is caused by several factors that bring significant diversification. The processes of hybridization caused somatic and meiotic chromosome number variations in the hybrids that were obtained from the cross between *Hordeum vulgare* with wild *Hordeum* species (Bothmer *et al.*, 1983). The heritability estimates suggested that effectiveness of single plant selection would be greatly based on grain number per ear (Habgoods, 1983). Therefore Ethiopian barley today is a cumulative result of natural process

superimposed by conscious selection. Thus the accumulated long-term effect of mutations, hybridization, gene recombination and natural selection have ultimately led to the proliferation of morphological, genetic and biochemical groups that prevail in barley forms (Asfaw, 1989c). Accordingly, Asfaw (1989c) categorized the main factors that are believed to have been responsible for diversification of barley into three:

1. Conducive environment: - heterogeneous, favorable for mutations
2. Biological process: - selfing and outcrossing, disruptive selection, outcrossing and predominant and combined selfing
3. Anthropogenic factors such as domestication process (primitive agricultural system, agglomeration of types in a field and deliberate selection) and social factor (social values as criteria for selection, ethnic diversification, diversified uses and association between barley types and uses).

It is further commented that, of the above, anthropogenic factors probably claim more contribution to the morphological variation as they are easily influenced by cultivators. In addition, Harlan *et al.* (1973) showed that landraces evolve and adapt to particular ecological zones and geographical regions.

2. 6. Importance and utilization of barley in Ethiopia

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 (1990). According to this report in Ethiopia the highest level of barley consumption is in highland areas where it is widely cultivated, accounting for the bulk of the total crop harvest. 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, blow away the fragments of the rachis and glume, and consume the tasty raw green grains in the field in limited quantities. Such unripe spikes may 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 "waiting" food. As for the type of total food preparations, it is shown that different kinds of bread, dough balls, porridges, 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. It is also used for cultural purpose. The Oromo people, for instance, consider it the holiest of all crops; their songs and sayings often feature this "King of grains". It has magnificent relationship with the culture, such as the *gada* system, *Atete* and marriage traditions. The barley straw is used in the construction of traditional huts and grain stores either as thatching or as a mud plaster. The barley crop residue is used as fodder mainly for bovine cattle and equine. The small grain that fail to fill up and those crushed in the process of threshing and consequently mix with the chaff are kept aside for chicken feed (and sometimes small ruminants and riding horses or mules) by some families. Some barley types are purposely cultivated for their special uses (e.g., partially naked type for roasted grain) while many other are more of multipurpose types (Asfaw, 2000).

2. 7. Some features of Ethiopian barley

The Ethiopian barley has many distinctive features. They are mostly two rowed (including the deficient type) with lax to intermediate spike with mainly white to brown and black colour kernel although the six-row type is predominant type mainly in the highland areas. The Ethiopian landrace barley is distinctive in the short-rachilla hair and long barbed awn. Some landrace populations have shown useful traits, especially for resistance to powdery mildew (Negassa, 1985b), barley yellow dwarf virus, leaf rust, septoria, scald, spot blotch, loose smut, barely septoria mosaic virus (Qualset, 1975) and quality traits such as high protein content and high lysine (Munck *et al.*, 1971). Another example supporting the remarkable status of the Ethiopian barley is given by Frost, *et al.* (1975); Bekele (1984) found a pattern C of flavoniods that was almost confined to Ethiopia. Useful characters of Ethiopian barley, includes high tillering capacity, tolerance to marginal soil conditions and resistance to barley shoot fly, aphids and frost. Vigorous seedling establishment and quick grain filling period have also been observed (Gebre and Alemayehu, 1991). The crop tends to show sensitivity to lodging because of lack of straw strength and excessive straw length. Due to undesirable straw characteristics, and in some fragile rachis, they tend to display low grain to straw ratio (Gebre and Alemayehu, 1991).

2. 8. Genetic erosion

Genetic erosion has been variously defined as the loss of genetic variation like so many tangible and intangible assets that make up the framework of our life on the earth. Genetic variation is the foundation of evolution. Evolution is the basis for continuation of all life forms. However, variation is being narrowed as a result of various agents of genetic erosion. Human activity is the most important factor threatening biological diversity.

At present the existing broad range of genetic diversity, particularly that of primitive and wild gene pools is being rapidly depleted, displaced or abandoned due to many and complicated reasons. These include displacement of indigenous landraces by new genetically uniform varieties; change and displacement in agriculture or land use, problems like drought, displacement or loss of wild gene pool, habitat disappearance, genetic vulnerability and various factors related to the national breeding program and other related activity also directly or indirectly attributed to persistent loss of genetic resources, narrowing down of genetic base or even genetic wipe out (Worede, 1988).

The extent to which the displacement of native seeds by exotic or improved materials occurs in Ethiopia has not been fully documented. Rates of displacement vary depending on regions and crops. In many cases, farmers still plant both native and exotic types interchangeably or along side each other, at times in mixtures, depending on their particular needs, market demand, or other prevailing factors (Worede *et al.*, 2000).

In general, native barley and durum wheat are among the crops most threatened by new varieties and/or by other crop species such as tef and bread wheat, which are expanding with the cereal growing highlands of the Shewa, Arsi, and Bale regions, largely because of greater market demand (Worede *et al.*, 2000). Broadly speaking factors of genetic erosion can be grouped into two categories, man-made and natural factors.

2. 8. 1. Natural factors

Droughts contribute to the genetic erosion of both cultivated and wild species. In recent years recurrent droughts had led to complete crop failure and subsequently a displacement of local cultivars. The reduced morphological diversity and isozyme polymorphism recorded could partly by associated with this particular natural phenomenon, although other factors (founder effect/genetic drift, etc) might be involved. Furthermore, natural selection as a result of repeated moisture stress would undoubtedly eliminate non-drought tolerant genotypes that might have other potentially desirable attributes (Demissie, 1996a).

Insect pests especially locusts and armyworm can cause considerable crop losses. Several regions have had problems associated with this in the past, floods and other natural calamites have been cited as possible causes of genetic depletion. Forest fire can also be a cause of genetic erosion primarily when followed by other stress factors (Demsisie, 1996a). In Bale the major constraints to barley production are barley shoot fly (*Delia arambourgi*), frost, moisture stress, net blotch (*Helminthosporium teres*), rust (*Puccinia hordei*), smuts (*Ustilago hordei*), and scald (*Rhynchosporium secalis*) (Gebre *et al.*, 1996).

2. 8. 2. Man-made factors

The transformation of agriculture from traditional farming systems to modern types has been instrumental in genetic replacement of old and diverse cultigenes. Although this process is not widespread at present, substantial replacement is recorded in many regions in the country. The large state farms that utilized highly uniform varieties have directly or indirectly contributed to genetic losses (Demissie, 1996a).

Barley growing areas gradually diminish due to the expansion of wheat cultivation and oats in some places. Presently the crop is pushed to marginal areas (to high altitudes and where frost prevails) and threatened by genetic erosion. Therefore, rare morphotypes are declining in frequency of occurrence through time. According to Asfaw (1988), some morphotypes which were reported by Orlov in the 1920s to occur in abundance in a given region or locality were either never encountered or found only as rare admixtures.

2. 9. Maintenance of genetic diversity

The objective of conservation is to conserve maximum diversity within each species to ensure that its genetic potential will be available in the future. Ideally all plants should be conserved as evolving populations in their natural ecosystem (Demissie, 1996b).

The importance of crop genetic resources and threats to them has led to the creation of conservation programs to preserve crop resources for future generations. One type of crop genetic conservation is *ex-situ* maintenance of genetic resources in gene banks, botanical gardens, and agricultural research stations (Plucknett *et al.*, 1987). Another type is *in-situ* maintenance of genetic resources on farm in natural habitats (Brush 1991; Maxted *et al.*, 1997). In actuality, two types of *in-situ* conservation can be distinguished. First, *in-situ* conservation refers to the persistence of genetic resources in their natural habitats, including areas where everyday practices of farmers maintain genetic diversity on their farms. This type is a historic phenomenon, but it is now especially visible in regions where farmers maintain local, diverse crop varieties (landraces), even through modern, broadly adapted or higher yielding varieties are available (Asfaw, 2000).

Second, *in-situ* conservation refers to specific projects and programs to support and promote the maintenance of crop diversity, sponsored by national governments, international programs, and private organizations. *In-situ* conservation programs may draw on the existence and experience of the first type but they are designed to influence farmers in the direction of maintaining local crops by employing techniques that may not be local. This type of conservation faces daunting tasks. It must cope with continued social, technological, and biological change while preserving the critical elements of crop evolution-genetic diversity, farmer knowledge and selection, and exchange of crop varieties (Brush, 2000). *In-situ* gene conservation also is used to provide information on how a population of plants becomes adapted to different regions. This in turn could be used to utilize the new derivatives of existing variability in the improvement of crops (Bekele, 1985).

In- situ conservation of domesticated plants is defined as the maintenance of traditional cultivars or landraces in the surroundings to which they have adapted, or in the farming systems where they have acquired their distinctive characteristics. *In-situ* conservation of landraces is seen as " evolutionary conservation". *In-situ* conservation will help sustain the evolutionary systems that are responsible for generating genetic variability and provide, therefore, available option for conserving crop diversity (Worede, 1991). For genetic conservation work, measures of genetic variability and its pattern of distribution are essential. At present the genetics of resistance is studied together with that of the parasite and pathogen in order to explore more fully the genetic basis of the interactions in which host and parasite as well as pathogens are involved. This should be taken up in relation with the problems of genotype-environment interactions. The complex genetic build-up should be the basis for plant breeding procedures and also suggest the value of *in-situ* gene conservation (Bekele, 1985). Maintaining this dynamic process is especially significant in regions of the country subject to drought and other stresses, because it is under such environmental extremes that variations useful for stress- resistance breeding are generated. In the case of diseases and pests, this would allow for on going host parasite co evolution. The ability of landraces to survive under stress is conditioned by a broad genetic base, inherent to landrace populations (Worede *et al.*, 2000).

Both *in-situ* and *ex-situ* methods of conservation have their own problems and drawbacks. The drawback in *ex-situ* conservation strategy is that seed storage freezes the evolutionary process by preventing the evolution of new types or levels of adaptations. There may be loss of the material due to degeneration and it is associated with high cost, which is not affordable by developing countries.

2. 10. Marker systems used in the present study

2. 10. 1. Morphological marker

Morphological characters have traditionally been used as a basis for classification since the early days of taxonomy and overwhelming reliance is still placed on morphological traits to produce practical classifications. Practical consideration so far demanded that any genetic constituent to be expressed morphologically for identification and characterization of plant germplasm. There are two sets of data generated in gene banks: Characterization and evaluation data. Characterization includes those traits that are highly heritable, can easily be seen by the eye and equally expressed in all environments. Furthermore, a limited number of additional traits that are thought desirable can be included in the characterization work. On the other hand, evaluation involves traits that are susceptible to environmental differences but generally useful in crop improvement (Demissie, 1996a).

2. 10. 2. Biochemical marker (Isozyme)

Isozymes are excellent tools for biochemical and genetical studies. The characterization and identification of isozymes is carried out on the basis of the phenotype of electrophoretic band patterns and these patterns can usually be interpreted in terms of loci and alleles. These qualities make isozymes one of the most ideal marker systems in population studies, especially in assessing variation within and among populations (Demissie, 1996a). The

capacity to see allelic variation at isozyme loci has revolutionized research in the field of biochemical genetics and evolution. This variation, called allozymic polymorphism, has been used in plants to examine genetic processes at every stage of the life cycle and ascertain genetic diversity in all major crops as well as many other species (Wendel and Weeden, 1990). Allelic variants of genes that encode isozymes are useful in many basic and applied investigations of crop species.

Isozyme variations have been used as markers to detect and map genes controlling quantitative traits. The relationships between characters determined by major and minor genes on the one hand, and morphological variation and enzyme variations on the other hand, are not always clear. This is partly due to the genetic backgrounds and the gene expression of the two classes of genes being confounded with various ecological variables (Bekele, 1984).

This marker system has advantage in that it is not affected by environmental condition, allelic differences are always reflected in terms of mobility difference. Like other marker systems, isozymes have some shortcomings. Not all genetic changes occurring at DNA level is detected at protein (isozyme) level and polyploidy is quite a common phenomenon in plants and caution is required.

3. MATERIAL AND METHODS

3. 1. Morphological study

3. 1.1. The study area

The study was carried out at Sinana Agricultural Research Center, which is located 7^0 7' N latitude and 40^0 10'E longitude in the Oromia Regional State of Bale Zone. Sinana Agricultural Research Center is specifically located at 463 Km South east of Addis Ababa and 33 Km from Robe Capital of Bale Zone, on the way to Goro and Sofumer Cave. The Elevation is 2400 m asl and characterized by gentle slope. The soil type is vertisol and is slightly acidic (pH 6.2). As far as chemical composition of the soil is concerned a total N content of 0.243%, available P 30 PPM, K value of 240 mg/kg soil and CEC of 64 Meq/kg of soil has been reported (Ethicha, *et al.*, 1998). The experimental site is located in the area where barley is important.

3. 1.2. Weather condition of the season

Sinana Research Center represents a high altitude with high rainfall distributed bimodaly. Total annual rainfall amounts over ten years ranges from 750-1000mm (average 860mm). The average annual maximum temperature is 21^0C while that of minimum temperature is 9^0C (Ethicha *et al.*, 1998). In the experimental season (August 2000-January 2001), the total rainfall amount was 421 mm and ranged from 0.0 to 116.8 mm per month. The average minimum and maximum temperatures were 9.4 ^{O}C and 20.3 ^{O}C, respectively (Appendix 1).

3. 1. 3. Germplasm Acquisition

Barley germplasm materials for this study were provided by the Institute of Biodiversity Conservation and Research. The accessions were collected from 11 regions of Ethiopia, which have altitudinal ranges between 1610 to 3600 m asl, collected from 1980 to 1993. A total of 47 barley populations were used for the study. Selection of the populations for the study was based on altitude and region. Genetic variability of barley collected from different parts of Ethiopia has been studied by several investigators (Bekele, 1984, Negassa, 1985c, Demissie and Bjørstand, 1996a). Some of the materials that are used in this study were also collected from the regions and altitudinal ranges covered by previous studies. Appendix 2 shows the list of accessions, origin and altitude of the populations used for this study.

3. 1. 4. Experimental procedures

The experiment was laid out in randomized complete block design (RCBD) with three replications in single row of 3 m length. The spacing between plants was 0.1m and the spacing between rows and replication were 4 m and 1.5 m, respectively. Twelve to 15 individual plants per population, and a total of 2057 individual plants were evaluated for the following agronomic characters using the International Board of Plant Genetic Resources Descriptors for barley (IPGRI, 1994).

1. **Days to heading (DH)**: - the number of days from planting up to heading
2. **Grain filling (GF)**: - the number of days from heading up to physiological maturity
3. **Days to maturity (DM)**: - the number of days from planting up to physiological maturity

4. **Tiller number (TN)**: - the actual count of the fertile number of tillers (spike bearing)
5. **Node number (NN)**: - the actual count of the number of nodes of the mother plant
6. **Plant height (PH)**: - height of mother plant before maturity, the distance between the ground level to the tip of the terminal spikelet in cm.
7. **Spike length (SL)**: - distance from the base of the spike to the tip of the highest spikelet (excluding own) in cm.
8. **Awn length (AL)**: - distance from the tip of the spike to the end of the awn.
9. **Number of spikelet per spike (NSPS)**: - the actual count of the number of spikelet of the mother spike
10. **Number of kernel per spike (NKPS)**: - the actual count of the number of seeds (kernels) of mother plant
11. **Yield per spike (Y/S)**. The dried weight of seeds harvested from one spike.
12. **Seed yield weight per plant (g/plant) (SYW)**: - the dried weight of seeds from each individual plant in gram per plant.
13. **Thousand-grain weight (TGW)**: - 1000 seeds were randomly taken from each single plant and weighted.
14. **Biomass (BM)**: - the weight of the individual plant (with its tillers) material above the ground (biological yield).
15. **Flag leaf length (FLL)**: - the length of the flag leaf from its bottom to tip.
16. **Harvest index (HI)**: - the ratio of seed yield per plant to the biological yield per plant multiplied by 100.
17. **Spike density (SD)**: - visual measurement according to the key given in IBPGR descriptor list and this was rated as 1 for lax, 2 for medium dense and 3 for dense.

18. **Glume hairiness (GH)**: - measured on outer side of sterile glume and this was rated as 1 for glabrous and 2 for hairy.
19. **Glume colour (GC)**: - observed on the outer glume and this was rated as 1 for white, 2 for yellow, 3 brown red and 4 for black gray
20. **Kernel colour (KC)**: - observed on the harvested seeds of each individual plant and recorded as 1 for white 2 for black 3 for blue and 4 for purple
21. **Awn colour (AC)**: - observed on the awn and rated as 1 for white, 2 for yellow, 3 for brown and 4 for black
22. **Ear shape (ES)**: - the shape of the mother spike was taken and recorded as 1 for parallel, 2 for broad and 3 for triangle.
23. **Kernel covering (KCO)**: - rated as 1- naked grain (grain without glume, 2-covered grain (grain with usually persistent glumes.
24. **Spike bending tendency (SBT)**: - angle of ears during maturity. 1 for erect, 2 for intermediate and 3 for bend.
25. **Awn type (AT)**: - 1 for smooth and 2 for rough.
26. **Kernel row number (KRN)**: - the number of spike rows found on the mother plant. 1 for two-row type, 2 for six row types and 3 for irregular type.
27. **Length of barley awns (LBA)**: - the length of the awns which extend beyond the ear, compared with the actual length of the ear itself. It can be rated 1 for awns which extend beyond the ears for a distance which is longer than the length of the ears, 2 for the distance by which the awns extend beyond the ear is more or less equal to the ear length and 3 is for the distance by which the awns extend beyond the ear is shorter than the length of the ear.

28. **Length of rachilla hair (LRH)**: - is a structure laying in the crease or furrow on the ventral side of a barley grain. The rachilla hairs may be 1 short hair rachilla 2 long hair rachilla.

29. **Flag leave Area (FLA)**: - the width of flag leaves on its side to side horizontally and can be rated 1 for narrow, 2 for intimidate and 3 for broad.

30. **Lodging (LOD)**: - the ability of the crop to resist lodging and can be rated 1 as poor, 2 for intermediate and 3 for good

3. 2. Biochemical study

3.2. 1. Isozymes

Isozyme analysis was carried out on seven populations of barley selected from seven clusters that were grouped based on quantitative and qualitative morphological characters. One population from each cluster was selected for the study. Selection of one population from each cluster was made randomly. Thirty-six individual plants per population were used.

The method of extraction and starch gel electrophoresis procedure were according to Chamberlain (1998). The enzyme systems studied were Aspartate aminotransferase (AAT; EC 2.6.1.1), Esterase (EST; EC 3.1.1-) and Leucine aminopeptidase (LAP; EC 3.4.11.1). The gel staining procedure followed was that of Chamberlain (1998). An assessment of isozyme phenotype polymorphism was made using the overall banding patterns. Phenotypic polymorphism, genetic distance and heterozyosisty (Nie, 1978) were determined using BIOSYS computer software (Swofford and Selander, 1981). A genetic interpretation of the banding patterns was made based on the reported structure of each enzyme in different plant species (Wendel and Weeden, 1990).

3. 2. 2. Protein analysis

All forty-seven populations grown at Sinana were also used for protein analysis (Appendix 2). Protein analysis was carried out using the Kjeldahl method (Ogbai and Sereke berhan, 1997). Organic nitrogen is converted into ammonium ions by digestion with concentrated sulphuric

acid in the presence of a catalyst sulphate. Following Kjeldahl digestion, the digests were made alkaline and ammonium was determined by steam distillation of ammonia, which involved trapping in boric acid and titration (AOAC, 1990). When using the steam distillation method the distillate was titrated against a standard acid (0.1N HCl). From this titration the amount of nitrogen was determined, and multiplied by 6.25 to convert to crude protein (AOAC, 1990).

3. 3. Statistical analysis

Analysis of variance (ANOVA) for each quantitative trait data was carried out in a RCBD model with three replications using the MSTATC computer program (MSTATC, 1991). Components of variance and coefficients of variance were computed by substituting the appropriate mean squares (MS) from the ANOVA. They were in turn used to estimate broad sense heritability and the expected genetic advance.

The following steps were followed to calculate phenotypic and genotypic coefficients of variation, broad sense heritability and expected genetic advance.

Phenotypic variance (Vp)= Genotype MS/r

Error variance (Ve)= Error MS/r

Genotypic variance (Vg)= Vp-Ve

Where r is number of replications and MS is mean square

Phenotypic coefficient of variance (PCV)= $100(\sqrt{Vp}/M)$

Genotypic coefficient of variance (GCV)= $100(\sqrt{Vg}/M)$

Where M is the mean value

Heritability (h^2 (broad)) = Vg/Vp

Genetic advance (GA)= (I) (h^2) ($\sqrt{Vp}$)

I= Selection differential (2.06) for selecting 5% of the genotypes

GA (% of the mean) = (GA/M) 100

Simple correlation analysis between all possible traits was carried out using SPSS MS Windows 7.5 (SPSS, 1998). The percentages of the phenotypic classes of character populations, regions and altitude classes were calculated for the qualitative characters by crosstabs. Phenotypic frequency distributions of the characters were worked out for all the populations, the regions of origin and the altitudes for a particular character state and were tested with chi-square using the MSTATC computer program (MSTATC, 1991).

The percentage frequencies of phenotypic classes of each character, populations and altitudinal groups were calculated for the qualitative characters. The regional frequency distribution and respective chi- square values for the thirteen qualitative characters also calculated by SPSS (1998).

Correspondence analysis using the simple correspondence procedures as devised by MINITAB (1998) was used to group barely growing regions through an ordination technique using the qualitative traits. The region contingency table was assessed in terms of row profiles (regions) and column profiles (traits).

The Shannon–Weaver diversity index (H') was computed using the phenotypic frequencies to assess the phenotypic diversity for each character for the entire sample and the sample grouped for each region and altitude. The Shannon- Weaver diversity index H', which has been widely used in measuring the diversity of germplasm collections (Bekele, 1984; Negassa, 1985a; Engles, 1994; Demissie, 1996a). The Shannon-Weaver diversity index (H') is defined as follows (Hutchenson, 1970).

$$H = -\sum_{i=1}^{n} P_i \ln P_i$$

Where pi is the proportion of accessions in the i^{th} class of an n class character and n is the number of phenotypic classes for a character. Each value of H' was divided by its maximum value, $\log_e n$, and normalized in order to keep the values between zero and one. A one-way analysis of variance of H' was carried out for each trait using region and altitudinal classes. The Hierarchical ANOVA of the H' index over characters was carried out to assess differences between regions and among populations within regions for the level of phenotypic diversity. Chi-square analysis was performed on the frequency data to assess the distribution of characters. It is also to estimate the contribution of the different variance components like region and altitude to the level of diversity.

The partitioning of the phenotypic diversity into within and between regions of origin and within and between altitudes was made following the methods given by WACHIRA *et al.* 1995 as cited by Ayana and Bekele (1998).

Principal component analysis was performed using correlation matrix to define the patterns of variation both between accessions and between their regions of origin. First and second principal component scores were plotted to aid visualization of group differences.

Hierarchical agglomerative clustering method was performed with Ward criterion minimum variance method using MINITAB computer software. The agglomerative distances among group means produced a dendrogram showing successive fusions of individuals that belong to the same cluster. The distance between two clusters is the sum of squares of the distances between the two clusters added over all the variables and is expressed as semi partial (R^2) sum of squares between clusters just joined divided by the corrected sum of square.

Clustering was computed based on mean quantitative and qualitative data and by using Rogers *et al.* (1997), a novel statistical method that allows quantitative and qualitative data to be combined by statistical analysis. Data were analyzed using MINITAB Release 12.22 (MINITAB, 1998), STATISTICA Release 4.5 (STATISTICA, 1993), and SPSS for Windows Release 7.5 (SPSS, 1998).

Canonical discriminant analysis as used in germplasm evaluation by Pecetti and Damania (1996) was undertaken to assess the distinctiveness between the different barley growing regions. Distance matrix, i.e. square Euclidean distances, computed from means of sixteen quantitative characters of the 47 populations were used.

Analysis of isozyme data was performed using BIOSYS-1 (Swofford and Selander, 1981). Average number of alleles per loci (A), percent of polymorphic loci (P) (95 % criterion) and

average hertrozygosity (H) were estimated (Nei, 1978). Wright's fixation indices (F_{ST}) (Wright, 1978) were also determined.

Allele frequencies at loci were used to compute unbiased genetic identity (I), and genetic distance (D) coefficients (Nei, 1978). Cluster analysis was performed with Ward criterion minimum variance method using MINITAB based on a genetic identity matrix.

4. RESULTS

4. 1. Morphological diversity

4. 1. 1. Mean and coefficient of variance for quantitative characters

Summary statistics, including [mean (M) and coefficient of variance (CV)] of the morphological characters and of the geographic and altitude collecting variables for the landrace populations from the eleven Ethiopian regions are reported in table 1 and 2. The data for each population and each character is presented in Appendix 3.

The mean and coefficient of variation by region and altitude over the entire individuals of 47 barley populations that were considered in this study are presented in Tables 1 and 2. The result showed that there were differences in coefficient of variations in all altitudinal classes and regions. The highest CV was obtained in tiller number, biomass weight, yield per spike, harvest index, seed weight per plant and flag leaf length in most of the regions and altitudinal

classes. In all altitudinal classes, number of spikelet per spike and harvest index showed highest coefficient of variation.

The highest CV for tiller number was recorded from populations collected from Gamo Gofa (34.38%), Gonder (30.44%), and Tigray (26.58%). And that of biomass (BM) was observed in the materials from Bale (58.8%), Welega (56.4%) and Gonder (55.08%). Highest seed yield per plant was recorded on materials from Bale (12.49%), Welega and Hararge (11.81%). Populations collected from Arsi, Hararge, Gamo Gofa and Bale showed 5.52 g, 1.4g, 1.48g and 1.4 g seed yield per spike, respectively.

Variation on morphological traits was also observed on altitude groups (Table 2). Based on this, tiller number showed highest CV for populations collected from altitudinal classes < 2000 m asl (16.82%), 2001-2500 m asl (15.95%)and 2501-3000 m asl (15.12%). Biomass weight also showed highest CV in altitude range 2001-2500 m asl (30.65), <2000m asl (25.47) and 2500-3000 (21.50). Yield per spike, seed yield weight, harvest index and flag leaf length also showed highest CV in most altitudinal classes.

Analysis of variance for 16 quantitative morphological traits showed highly significant differences between populations in all recorded characters, except harvest index, which showed a significant difference between populations (Table 3).

There was also a highly significant difference that was observed between replications in tiller number and awn length. Significant differences were observed for days to heading, days to maturity, grain-filling period, number of kernels per spike, biomass weight, thousand-grain

weight and harvest index were also noted. In the rest of the characters differences were non-significant (Table 3).

Table 1. Mean (M) and

Region	DH		DM		GF		NN	
	M	CV	M	CV	M	CV	M	CV
Arsi	84.3	2.3	120.7	2	37.2	8.2	5	9.7
Bale	78.9	5.3	116.1	1.5	37.3	10.5	4.7	13
Gamo Gofa	79.7	4.6	116.2	0.9	36.3	8.9	4.8	8.5
Gojam	87.9	3.3	125.8	4.4	38	13.5	5.1	6.5
Gonder	73.3	10.9	118	4.2	45.7	13.3	4.6	15
Hararge	82.7	1.8	118.7	5.9	35.8	18.3	5	0
Shewa	88.9	3.3	125.6	2.2	37.2	5.9	4.5	7
Sidamo	82.1	3.8	117	1.5	34.6	9.4	4.7	12
Tigray	80.8	2.6	120.7	2.4	39.5	7.1	4.8	9.9
Welega	72	3.6	115.6	2.5	42.3	6	4.3	16
Wollo	82.6	2.9	119.3	2.7	36.9	9.5	4.7	8.2

DH: Days to heading, DM: days

spikelet per spike, NKPS: Numl

grain weight, HI: Harvest index,

Table 2. Mean (M) and

Altitude range	DH		DM		GF		NN	
	M	CV	M	CV	M	CV	M	C
<2000	76.3	4	115.7	2.5	39.4	9.4	4.5	1
2001 - 2500	82.5	5.7	120.7	3.3	37.6	9.5	5	8.
2501 - 3000	81.8	4.8	119.8	2.4	38.8	11.4	4.8	1
3001 - 3500	89.5	3	125.4	2.6	36.4	6.5	4.8	8
>3500	78	-	116	-	38	-	4	-

- = No CV since it has only on

DH: Days to heading, DM: days

spikelet per spike, NKPS: Numb

grain weight, HI: Harvest index,

Table 3. Mean squares for 16 quantitative morphological traits of barley populations

Character	Mean Squares			CV (%)	SE
	Replication (2)	Landrace (46)	Error (92)		
AL	6.01**	1.50**	0.67	7.62	0.47
BM	519.31*	457.75**	154.16	26.01	7.17
DH	178.18**	189.06**	14.12	6.40	2.17
DM	38.06*	109.93**	11.22	2.80	1.93
FLL	10.91ns	20.67**	10.73	16.68	1.89
GF	51.28*	47.79**	14.51	9.97	2.19
HI	46.20*	17.99*	10.39	16.19	1.86
NKPS	61.68*	332.99**	19.65	11.61	2.56
NN	0.305ns	0.738**	0.225	10.02	0.27
NSPS	60.62ns	333.99**	19.66	11.61	2.56
PH	24.97ns	182.02**	35.66	6.79	3.45
SL	0.45ns	5.25**	0.92	10.52	0.55
SYW	7.98ns	24.75**	8.21	30.02	1.65
TGW	40.66*	80.53**	8.58	10.03	1.69
TN	62.32**	29.40**	11.22	21.83	1.93
Y/S	0.03ns	0.15**	0.04	16.01	0.12

*: $P < 0.05$, **: $P < 0.01$, ns: non significant

AL= Awn length; BM= Biomass; DH= Days to heading; DM= Days to maturity; FLL= Flag leaf length; GF= Grain filling ; HI= Harvest index; NKPS= Number of spike per spike; NN= Node number; NSPS= Number of spike per spike; PH= Plant height; SL= Spike length; SYW= Seed yield weight; TGW= Thousand grain weight; TN= Tiller number and YS yield per spike.

4.1. 2. Heritability (broad sense), Genetic advance and Estimates for components of variance.

Phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV), estimates of the components of variance, broad sense heritability (h^2), expected genetic advance and genetic advance as percent of mean are presented in (Table 4).

A phenotypic coefficient of variation (PCV) was high for grain yield weight (30.33%), number of kernel per spike (27.61%), number of spikelet per spike (27.58%). The genotypic coefficient of variation (GCV) was high for number of spikelet per spike (26.77%) and number of kernel per spike (26.75%).

Some of the characters, such as days to heading, days to maturity, number of kernel per spike, number of spikelet per spike, thousand grain weight, exhibited high heritability (>80%). Grain filling period, node number, yield per spike, plant height and spike length showed moderate heritability.

Biomass weight, seed yield weight, tiller number, awn length, flag leaf length and harvest index showed 66%, 66%, 62%, 60%, 48% and 42% heritability, respectively.

Expected genetic advance was also high for all characters except for days to maturity, awn length and yield per spike. The value ranged between 8.09 for grain filling period and 53.49 for number of kernel per spike. The highest estimates of genetic advance were observed in

spike length (23.62%), tiller number (26.24%), thousand grain weight (32.71%), biomass weight (35.38%), seed yield weight (41.40%) and number of kernel per spike (53.49%) and number of spikelet per spike (53.44%),

Generally, in this study, intermediate to high estimate of PCV, GCV and genetic advance percentage of the mean were observed for characters, such as tiller number, spike length, number of kernels per spike and number of spikelets per spike, biomass weight, seed yield weight and thousand grain weight.

4. 1. 3. Correlation analysis

Correlation coefficients between characters could be used to measure the similarity in the patterns of variation of characters. Correlation was calculated for each trait in all accessions and regions (Table 5) and (Appendix 4-12, respectively). The strongest correlation ($r = 0.93^{**}$) was observed between seed yield weight and biomass weight. However, number of spikelet showed highly significant negative association with most of the characters except grain filling days and days to heading. Where as kernel per spike showed significant positive association with most of the characters except grain filling days and days to heading.

Seed yield weight was highly significantly and positively correlated with biomass weight, harvest index, spike length, plant height and tiller number. It significantly associated with flag leaf length, grain filling period, thousand grain weight and yield per spike. Thousand-grain weight was highly significantly and positively correlated with awn length, biomass weight,

grain filling periods and tiller number. Yield per spike was highly significant and positively correlated with number of spikelets per spike, number of kernels per spike and node number. In general, about 66% of the characters are significantly correlated to each other.

Tiller number was positively associated with awn length, biomass weight, grain filling periods, plant height, seed yield weight and thousand grain weight. It is also negatively associated with days to heading, days to maturity, number of kernels per spike, number of spikelets per spike and node number.

Table 4. Summary statistics and estimate of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV), broad sense heritability (h^2) and genetic advance (GA) in 47 barley populations for 16 quantitative morphological traits

Traits	M	Range	Ms	Vp	Ems	Ve	Vg	Pcv	Gcv	h^2	GA	GA% of the M
AL	10.8	9.1 - 11.9	1.5	0.5	0.7	0.2	0.3	6.55	5.07	0.60	0.87	8.09
BM	47.7	21.7 - 68.9	457.8	152.6	154.2	51.4	101.2	25.90	21.09	0.66	16.88	35.38
DH	81.7	62.7 - 95.33	187.1	62.4	14.1	4.7	57.7	9.67	9.30	0.92	15.05	18.42
DM	119.8	110.67 - 136.7	109.9	36.6	11.2	3.7	32.9	5.05	4.79	0.90	11.20	9.35
FLL	19.6	13.0 - 31.4	20.7	6.9	10.7	3.6	3.3	13.40	9.27	0.48	2.59	13.20
GF	38.1	31.7 - 47.7	47.8	15.9	14.5	4.8	11.1	10.47	8.74	0.70	5.73	15.05
HI	19.9	14.3 - 24.2	18	6	10.4	3.5	2.5	12.31	7.95	0.42	2.10	10.57
NKPS	38.2	22.7 - 65.0	333.5	111.2	19.7	6.6	104.6	27.61	26.77	0.94	20.43	53.49
NN	4.7	3.7 - 6.0	0.74	0.25	0.2	0.06	0.2	10.64	9.52	0.80	0.82	17.53
NSPS	38.2	22.7 - 65.0	333	111	19.7	6.6	104.4	27.58	26.75	0.94	20.41	53.44
PH	88	67.9 - 100.7	182	60.7	35.7	11.9	48.8	8.85	7.94	0.80	12.90	14.66
SL	9.1	5.1 - 11.9	5.3	1.8	1	0.3	1.4	14.74	13.00	0.78	2.15	23.62
SYW	9.5	3.2 - 14.6	24.8	8.3	8.2	2.7	5.5	30.33	24.69	0.66	3.93	41.40
TGW	29.2	18.6 - 40.2	80.5	26.8	8.6	2.9	24	17.73	16.78	0.90	9.55	32.71
TN	15.3	10.0 - 22.0	29.4	9.8	11.2	3.7	6.1	20.46	16.14	0.62	4.01	26.24
Y/S	1.3	0.9 - 2.0	0.1	0.05	0.04	0.013	0.04	15.35	8.77	0.80	0.36	28.34

M-Mean, Ms-Genotypes mean square, Ems- Error mean square, Ve- Error variance, Vg- Genotypic variance

AL= Awn length; BM= Biomass; DH= Days to heading; DM= Days to maturity; FLL= Flag leaf length; GF= Grain filling ; HI= Harvest index; NKPS= Number of spike per spike; NN= Node number; NSPS= Number of spike per spike; PH= Plant height; SL= Spike length; SYW= Seed yield weight;TGW= Thousand grain weight; TN= Tiller number and YS yield per spike.

Table 5. Correlation among 16 different quantitative characters of barley populations in Ethiopia

	BM	DH	DM	FLL	GF	HI	NKPS	NN	NSPS	PH	SL	SYW	TGW	TN	YS
AL	.47**	-.54**	-.56**	.33*	0.28	0.35*	-0.24	-0.32*	-0.25	0.33*	0.42**	0.47**	0.40**	0.42**	-0.05
BM		-.62**	-.55**	0.25	.37**	0.46**	-0.07	-0.06	-0.07	0.66**	0.44**	0.93**	0.40**	0.55**	0.22
DH			.87**	-.44**	-.65**	-0.50**	0.37*	0.54**	0.37*	-0.61**	-0.72**	-0.64**	-0.51**	-0.63**	0.20
DM				-.68**	-0.2	-0.62**	0.13	0.48**	0.13	-0.67**	-0.61**	-0.62**	-0.24	-0.40**	0.12
FLL					-0.18	0.42**	0.12	-0.28	0.12	0.42**	0.31*	0.31*	-0.10	0.04	0.01
GF						0.04	-0.53**	-0.34*	-0.53**	0.15	0.48**	0.31*	0.65**	0.62**	-0.20
HI							0.09	-0.17	0.09	0.45**	0.28	0.71**	0.12	0.22	0.23
NKPS								0.49**	1.00	0.16	-0.52**	0.01	-0.73**	-0.61**	0.83**
NN									0.49	-0.01	-0.54**	-0.08	-0.30*	-0.32*	0.50**
NSPS										0.16	-0.52**	0.01	-0.73**	-0.61**	0.83**
PH											0.49**	0.68**	0.14	0.30*	0.27
SL												0.43**	0.52**	0.66**	-0.35**
SYW													0.33*	0.51**	0.29**
TGW														0.57**	-0.32**
TN															-0.38**
YS															

*: - P< 0.05, **: - P< 0.01

AL= Awn length; BM= Biomass; DH= Days to heading; DM= Days to maturity; FLL= Flag leaf length; GF= Grain filling ; HI= Harvest index; NKPS= Number of spike per spike; NN= Node number; NSPS= Number of spike per spike; PH= Plant height; SL= Spike length; SYW= Seed yield weight; TGW= Thousand grain weight; TN= Tiller number and YS yield per spike.

4. 1. 4. Cluster analysis

In Figure 1, seven clusters are formed on the basis of quantitative and qualitative morphological characters. The results showed that cluster A consisted of eleven populations seven of them were collected from Bale, two from Sidamo and the other two from Welega and Hararge. Out of eleven, five of them came from altitudinal class of < 2000 m asl and four from 2001-2500 m asl. Cluster B grouped three populations collected from Gonder and Welega. All of the populations were from altitudinal class of < 2500 m asl. Cluster C had five populations of diverse origins including Arsi, Tigray, Welega and Wollo. But three of them from altitudinal class of < 2000 m asl and the other two from 2501-3000 m asl. Cluster D consisted of seven populations collected from Tigray, Gojam and Shewa. The populations of this cluster were collected from all altitudinal classes. Cluster E grouped four populations from Shewa, Gojam and Gonder. Shewa populations were collected from altitudinal class of 3001-3500 m asl, whereas populations from Gojam and Gonder were from altitudinal class of 2001-2500 m asl. Cluster F was an outlier that comprised only one population collected from Arsi at an elevation of 2200 m asl. Cluster G consisted the maximum number of populations (sixteen) collected from Arsi (25%), Wollo (25%), Tigray (12.5%), Shewa (12.5%), Hararge (6.25%) and Sidamo (6.25%). When we consider the populations of this cluster altitude wise, they belong to in altitudinal classes of <2000, 2001-2500, 2501-3000, 3001-3500 and >3500 m asl with the percentage of 12.5, 25, 25, 31, and 6.25 of each class, respectively. The distributions of 47 barley populations over seven clusters by regions and altitudes based on both quantitative and qualitative characters were presented in (Appendix 13).

Based on quantitative morphological characters, the populations were grouped into seven clusters. Mean and standard deviation of quantitative characters of each cluster is shown in Table 6. The number of clusters varied from 3 populations in cluster C to 12 populations in cluster E. Cluster A grouped nine populations of diverse origin, but six of the accessions in this cluster originated from Bale and the rest three were collected from Welega, Sidamo and Hararge. Four populations were from the altitudinal class of less than 2000 m asl and five populations from altitudinal class of 2001-2500 m asl. Populations of this cluster were characterized by earliness (maturity and heading), short grain filling period, high thousand-grain weight, tall plant height, high yield per spike and high grain yield weight. Cluster B grouped eight populations, which were collected from various regions such as Welega, Gonder, Wollo, Arsi and Tigray. Four populations were colleted from the altitudinal class of less than 2000 m asl and the rest four were from altitudinal class of 2001-2500 m asl. The main feature of this cluster is short days to heading, short days to maturity, long grain filling periods, long plant height, small number of spikelets per spike, small number of kernels per spike and large amount of thousand grains weigh. Cluster C grouped three populations mainly from Tigray and Gonder. The main characters of this cluster are short days to maturity, high tiller number, high biomass weight and low yield weight, small number of spikelets and kernels per spike. Cluster D grouped six populations that were with high number of spikelets, number of kernels per spike and high yield per spike. These populations were collected from Arsi and some of them were from Hararge, Sidamo and Gamo Gofa. Cluster E, which was closest to cluster D, had twelve populations and the largest in number of populations with long flag leaf, low grain yield weight and thousand grain weight. Cluster F had four populations. These populations had long days to heading and maturity, short plant height and flag leaf length, low grain yield weight and high thousand grain weight. The populations were

collected from Shewa, Gojam and Gonder. Cluster G consisted of five populations, which mostly originated from Gojam, Shewa, Tigray and Bale. Most of the populations had long days to heading and maturity, short plant height, small number of spikelets and kernels per spike and thousand-grain weight.

Table 6. Summary of m

Cluster		No.	DH
A	M	9	76.78
	SD		3.23
B	M	8	70.25
	SD		5.04
C	M	3	73.67
	SD		0.57
D	M	6	83.50
	SD		2.88
E	M	12	86.17
	SD		3.81
F	M	4	90.40
	SD		2.63
G	M	5	92.75
	SD		3.36

AL= Awn length; BM= Biomass; I
spike; NN= Node number; NSPS=
and YS yield per spike.

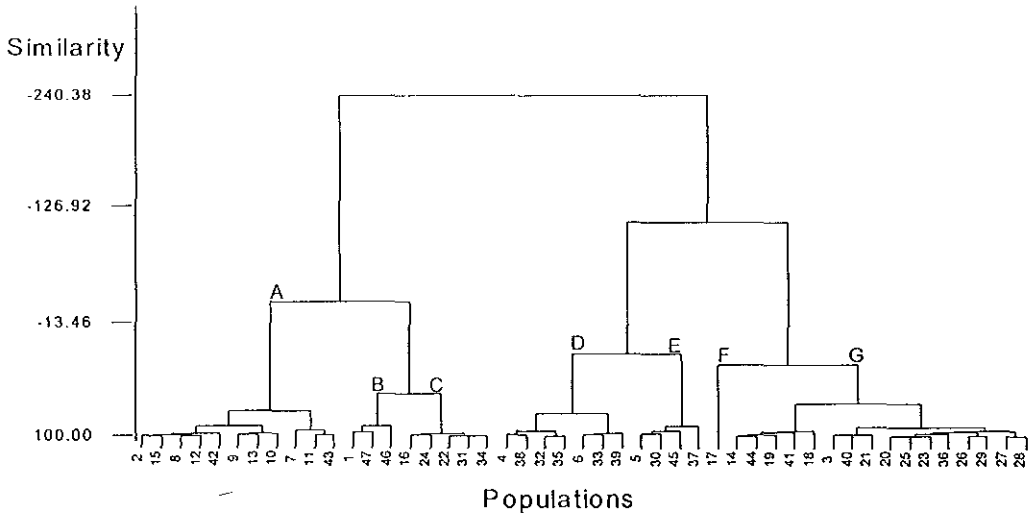


Fig.1. Dendrogram constructed using ward methods on 47 barley populations based on quantitative and qualitative characters

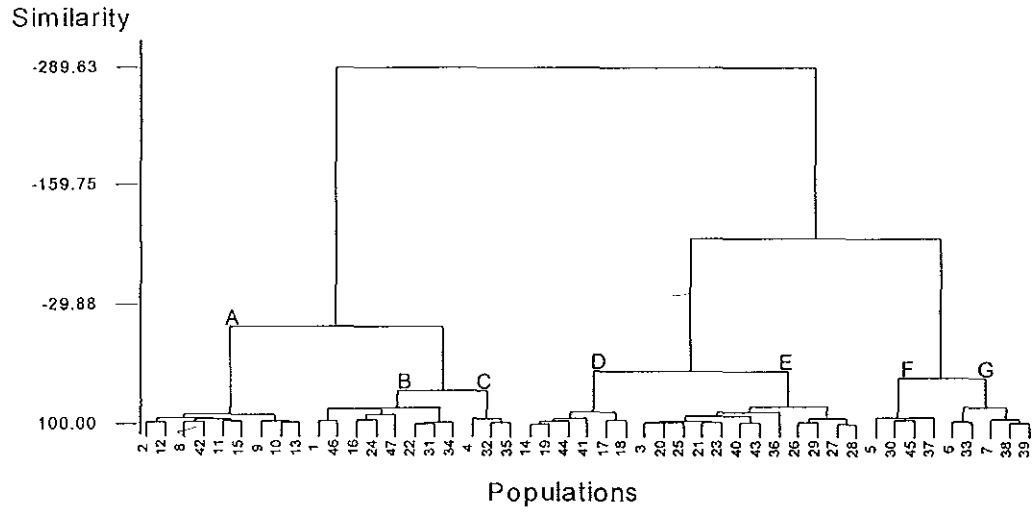


Fig . 2. Dendrogram constructed using ward methods on 47 barley populations based on sixteen quantitative morphological characters

4. 1. 5. Discriminant analysis

Discriminant analysis was done by taking cluster of accessions as a grouping variable. The result revealed that 45 populations out of 47 (95.7%) were correctly classified to their respective cluster. The percent of populations correctly classified was higher for clusters A, B, C and G whereas clusters D and E had certain degree of overlapping.

Further analysis of the patterns of diversity among entries from different geographic origins was done by canonical discriminant analysis. Eigenvalues, percentage and cumulative proportion of total variance and canonical correlation were calculated. The first two canonical variables accounted for 95.3% of the total variance (Table 7). The first canonical variable explains 67.5% of the total variance and this axis, which maximized the differentiation among populations, was closely related (mainly positively correlated) with tiller number and flag leaf length. The second canonical variable accounting for 27.8% of the total variance was mainly positively correlated with number of spikelets and kernels per spike, yield per spike and plant height. The third canonical variable is mainly correlated with spike length, awn length, and grain filling periods and accounting for 2.7% of the total variance. The fourth canonical variable accounting for 1.9 % of the total variance and was mainly positively correlated with seed yield weight, biomass weight, days to heading, days to maturity, harvest index, thousand grain weight and node number.

Table. 7. Correlation between four canonical (CAN) variables with eigenvalues, percent of variance and cumulative variance of the first four functions of 16 quantitative traits in barley landraces

Character	Function			
	1	2	3	4
TN	0.459*	0.027	-0.008	0.117
FLL	0.324*	0.103	-0.218	-0.224
NKPS	-0.603	0.772*	0.201	-0.014
NSPS	-0.603	0.772*	0.202	-0.015
YS	-0.300	0.549*	0.188	0.334
PH	0.134	0.373*	0.084	-0.264
SL	0.434	0.107	0.665*	-0.598
AWL	-0.069	-0.004	-0.390*	-0.049
GF	0.004	0.124	-0.161*	-0.144
SYW	0.308	0.556	-0.033	0.771*
BM	0.261	0.343	-0.006	0.609*
DH	-0.471	-0.356	0.552	0.589*
DM	-0.406	-0.219	0.416	0.423*
HI	0.247	0.331	-0.102	0.398*
TGW	0.107	0.137	0.143	0.371*
NN	-0.177	0.099	0.130	0.353*
Eigenvalues	16.50	6.79	0.66	0.47
% of total variance	67.50	27.80	2.70	1.90
Canonical correlation	0.97	0.93	0.63	0.57
% of cumulative variance	67.50	95.30	98.10	100.00

*: - $P < 0.05$

AL= Awn length; BM= Biomass; DH= Days to heading; DM= Days to maturity; FLL= Flag leaf length; GF= Grain filling ; HI= Harvest index; NKPS= Number of spike per spike; NN= Node number; NSPS= Number of spike per spike; PH= Plant height; SL= Spike length; SYW= Seed yield weight; TGW= Thousand grain weight; TN= Tiller number and YS yield per spike.

4.1. 6. Principal Component analysis

The principal components loadings, eigenvalues, variance proportion and cumulative proportion are shown in Table 8. The first five principal components contributed 85.5% of the total variation among 47 barley populations for the 16 quantitative characters. The first three components accounted for 76.7 % of the total variation. The first and the second principal components explained 42.1 % and 24.3 % of the total variation, respectively (Table 8).

The first principal component was strongly associated with traits such as days to heading and tiller number. Characters like number of kernels per spike and number of spikelets per spike, yield per spike and seed yield per spike were important for the second principal component. In the third principal component days to maturity, biomass weight and flag leaf length were the most important character. Fourth principal component known by plant height, spike length, awn length and harvest index, whereas in the fifth principal component grain filling days and node number were the important characters. Distributions of 11 regions two axes were plotted for 16 quantitative characters based on the populations (Fig 3).

Principal component analysis was further made for the 11 regions of origin based on the mean of the 16 quantitative characters in order to study the regional pattern of variation. Apparently three principal components were extracted. The analysis was effective in that the first three principal components, explained 85.6% of the total variation. The first and second principal components accounted for 41.9% and 35.9% of the total variation, respectively (Table 9).

Table 8. Eigenvalues, percent of variance and commutative variance of the first five of principal component of 16 quantitative traits of barley populations

Variable	PC1	PC2	PC3
DH	0.355	0.076	-0.076
DM	0.299	0.206	-0.306
GF	-0.244	0.162	-0.331
NN	0.215	-0.154	-0.403
TN	-0.303	0.113	-0.203
PH	-0.212	-0.317	-0.053
SL	-0.317	0.046	0.093
AL	-0.246	-0.079	0.047
FLL	-0.140	-0.236	0.502
NKPS	0.223	-0.396	-0.004
NSPS	0.223	-0.396	-0.004
BM	-0.266	-0.232	-0.310
Y/S	0.125	-0.391	-0.298
SYW	-0.266	-0.290	-0.240
HI	-0.188	-0.283	0.079
TKW	-0.263	0.201	-0.270
Eigenvalues	6.735	3.882	1.665
Proportion	0.421	0.243	0.104
Commutative	0.421	0.664	0.767

AL= Awn length; BM= Biomass; DH= Days to heading; DM= Days to maturity; FLL= Flag leaf length; GF= Grain filling ; HI= Harvest index; NKPS= Number of spike per spike; NN= Node number; NSPS=Number of spike per spike; PH= Plant height; SL= Spike length; SYW= Seed yield weight; TGW= Thousand grain weight; TN= Tiller number and YS yield per spike.

Characters like days to heading, and thousand-grain weight were the most important characters, contributing to the first principal component. Days to maturity, biomass weight, harvest index, number of kernels per spike, number of spikelets per spike and seed yield weight were important characters in the second principal component. Awn length, flag leaf length and plant height were important in the third principal component. In the fourth principal component, node number, spike length and tiller number were the most important. In the fifth principal component grain filling period was an important character.

Figure 4 shows the distribution of 11 regions of origin of the populations along the first three axes of the dimensional plotting. The regions of origin of the populations were dispersed in all four quadrants. The second principal component was much more important than the first one in separating the regions of origin of the accessions. The regions of origin of the populations were dispersed in the three dimensional representation of the component scores (Fig. 4).

The extremes of the first axis were occupied by Gojam, Shewa, Gamo Gofa, Wollo and Tigray and Gonder materials with positive principal scores and on the other hand, by Bale, Welega, Hararge, Arsi and Sidamo with negative scores (Fig. 4). Apparently, the first principal component differentiated accessions that were long days to heading and thousand grain weight. On the second axis, Shewa, Gojam, Arsi had relatively high principal positive scores, while Welega and Bale had low negative scores. This axis differentiated populations mainly based on biomass weight, days to maturity, harvest index, number of kernels per spike, number of spikelets per spike, seed yield weight and yield per spike.

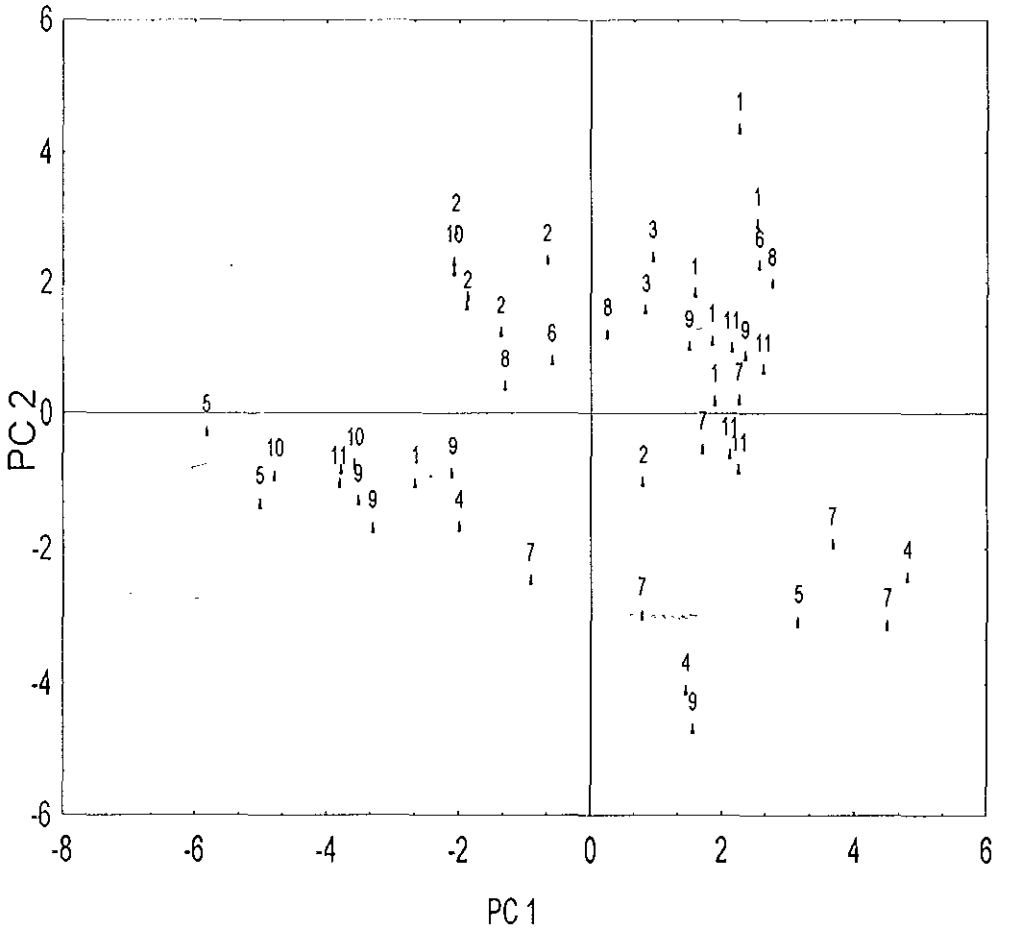


Fig. 3. Plots of principal component of 47 barley populations for 16 quantitative characters based on their regions of origin (1-Arsi, 2-Bale, 3-Gamo Gofa, 4 - Gojam, 5- Gonder, 6- Hararge, 7- Shewa, 8- Sidamo, 9- Tigray, 10- Welega, 11- Wollo).

Table 9. Principal component co-ordinate scores, eigenvalues, total variance and cumulative variance for the first three principal components for the eleven regions of 47 barley populations (based on the mean for the regions)

Region	PC1	PC2	PC3
Arsi	2.184	-2.258	1.055
Bale	-1.907	-2.296	-0.241
Gamo Gofa	1.146	-2.714	-1.139
Gojam	2.052	4.157	1.239
Gonder	-3.796	2.125	0.767
Hararge	1.690	-2.208	1.578
Shewa	2.849	2.828	-1.246
Sidamo	0.910	-1.506	0.103
Tigray	-1.348	1.457	0.241
Welega	-4.882	-0.293	-0.438
Wollo	1.101	0.708	-1.921
Eigenvalues	6.703	5.742	1.259
Proportion	0.419	0.359	0.079
Cumulative	0.419	0.778	0.856

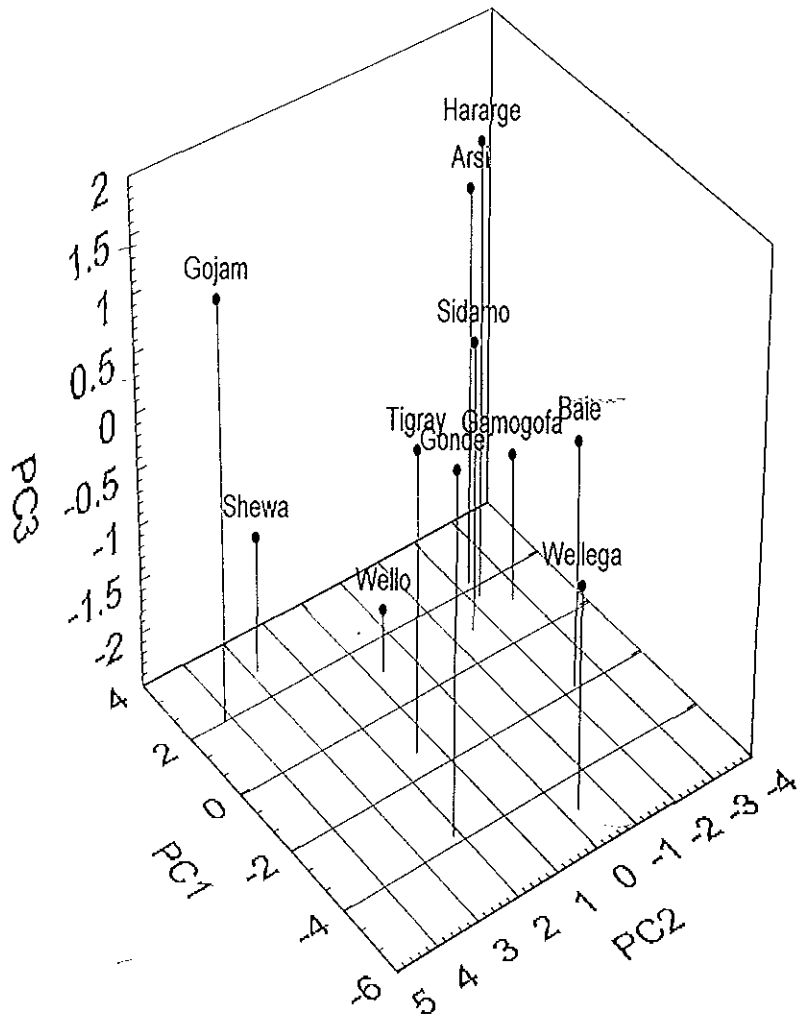


Fig.4. Three dimensional representation of the inter region distances resulting from principal component (PC) analysis of region means for 16 quantitative morphological traits

4. 1. 7. Frequency distribution and Genetic diversity

4.1. 7. 1. Regional trait distribution

From analysis of the percentage of frequency of phenotypic classes, white and yellow awn colour occurred in all regions at higher frequencies (Table 10). It is observed that populations with high frequencies of white awn colour originated from Hararge, Gojam, Arsi and Bale. Whereas yellow awn colour was recorded for populations collected from Sidamo, Bale and Tigray. Brown and black awn colours were rare in all regions. However, populations collected from Gonder, Welega have shown high frequencies in brown awn colour. Black awn colour was observed in populations collected from Gonder and Shewa in a higher frequency than the other regions. In all regions the chi-square value showed no significant variation for awn colour except Gonder and Welega where highly significant variation were observed.

Populations originating from Tigray (74%) and Gonder (79%) showed higher frequencies of triangular type ear shape. Parallel ear shape was observed in all regions. However, the distribution of this character was very low.

Flag leaf area did not show significant variation. However, higher frequency was observed in broad leaf area for all regions. Populations collected from Arsi (67%), Hararge (75%) and Shewa (66%) showed higher frequencies for this character.

All glume colour character states were found in all regions except in Gamo Gofa where brown and black colours were totally absent. Populations with higher frequencies for white

glume colour originated from Bale, Sidamo, Hararge and Gamo Gofa. Populations collected from Gonder showed highly significant deviation in glume colour.

Both two-rowed and six-rowed barley were observed in all the regions. Two- rowed was more frequent in Gojam, Welega, Gonder and Tigray, with the frequencies of 49%, 67%, 68% and 75%, respectively. The highest frequency of six row type were recorded in Gamo Gofa, Arsi, Shewa, Wollo and Hararge with the frequencies of 80%, 74%, 62%, 62%, and 61%, respectively. The highest frequency of irregular type barley was observed in Bale (61%), followed by Sidamo (42%) and Hararge (35%).

Chi- square values showed highly significant deviation in kernel row number in all regions with the exception of Gojam, Shewa, Sidamo and Welega regions.

Hairiness and glabrous glume characters were observed in all regions. Gojam and Tigray showed highly significant difference and significant difference in glume hairiness, respectively. Higher frequencies for glume hairiness were observed in populations collected from Tigray (95%), Bale (90%), Hararge (90%), Welega (90%), Gonder (87%), Arsi (78%) and Sidamo (70%). However, the glabrous type was more frequent on populations from Gamo Gofa (71%) and Gojam (43%).

Among kernel colour character states, black kernel colour was the predominant character observed in all regions. Blue and purple kernels were found in low frequencies. However, populations from Gonder showed highly significant deviation in purple colour. White kernel colour was more frequent on populations from Gamo Gofa and Arsi. Among the 11 regions

the highest frequency for white kernel colour was observed in populations collected from Gamo Gofa (34%) and Arsi (21%) respectively.

Even distribution of awn and spike length of barley awn was observed in all regions. Populations collected from Gonder (72%), Wollo (71%) and Bale (62%) showed even distribution with respect to length of barley awn and spike length. Significant deviation was observed in populations collected from Gamo Gofa in length of barley awn distributions.

Although most of the populations collected from all regions were poor in lodging resistance, populations collected from Bale showed higher frequencies in lodging whereas populations from Gonder were intermediate. Populations samples collected from Hararge and Shewa showed higher frequencies in loading toleranee. In general, populations from Bale, Gamo Gofa, Welega and Wollo were susceptible to lodging, whereas populations collected from Hararge and Shewa showed higher frequency in lodging tolerance.

Both short and long rachilla hair types were occurred in all regions. Populations collected from Gamo Gofa (91%), Sidamo (81%), Gojam (76%) and Shewa (77%) showed higher frequencies of short rachilla hair. Whereas populations from Bale, Hararge, Tigray, Bale and Welega showed higher frequencies of 94%, 91%, 89% and 87% in longer rachilla hair, respectively. Highly significant chi- square deviation was observed in long rachilla hair type in all regions, except in Arsi, Gonder and Wollo.

Among the character states recorded for spike bending tendency, erect spike showed higher frequencies as compared with intermediate and bending type. Populations from Gamo Gofa

showed higher frequencies in erect spike bending tendency type. Bending type of spike was more frequent in populations from Welega and Wollo.

Lax, intermediate and dense spike types were found in all regions, with higher frequencies of dense types. Populations from Gamo Gofa, Gonder and Welega showed highly significant chi- square deviation in spike density. Lax spike type was observed in populations from Gonder and Welega with the frequencies of 57% and 51%, respectively. Dense spike type from Gamo Gofa, Gonder and Arsi had frequencies of 80%, 69 % and 57%, respectively. Medium dense spikes of intermediate diversity were more frequent in populations from Gojam 53% and Bale 46%.

Table 10. Percentage o

Region	Kernel Cover			Awn colour				
	N	C	X*	W	Y	Bn	Bk	X^2
Arsi	0	100	0	47	41	3	9	4.3
Bale	0	100	0	44	54	2	0	8.1
G/Gofa	0	100	0	47	52	1	0	9.6
Gojam	0	100	0	52	45	2	1	8.3
Gonder	0	100	0	24	44	12	20	54.7
Hararge	0	100	0	60	40	0	0	20.4
Shewa	0	100	0	38	42	5	15	16
Sidamo	0	100	0	41	55	3	1	5.1
Tigray	0	100	0	43	51	1	5	3.2
Welega	0	100	0	23	47	21	9	67.8
Wollo	0	100	0	37	59	1	3	7.06

N- Naked; C-covered; X^2- Chi-s

Gl-Glabrous; Hi- Hairy; Bu- Blu

Table 10. Continued.

Region	Awn type			Kernel row number			
	R	S	X²	Two	Six	Irr	X
Arsi	100	0	0	17	74	9	36.
Bale	100	0	0	14	25	61	91.
G/Gofa	100	0	0	18	80	2	55.
Gojam	100	0	0	49	32	19	9.8
Gonder	100	0	0	68	21	11	50.
Hararge	100	0	0	4	61	35	41.
Shewa	100	0	0	33	62	5	20
Sidamo	100	0	0	16	42	42	28
Tigray	100	0	0	71	20	9	59.
Welega	100	0	0	67	5	28	67.
Wollo	100	0	0	21	62	18	12

Two -two rowed; Six -six rowed;

awn; Po- Poor; In- intermediate;

4 .1. 7. 2. Correspondence analysis

Correspondence analysis was done for the qualitative characters, and calculated inertia, percentage of variance and cumulative variance are indicated in Table 11. The regions contingency table was further assessed in terms of row profiles (regions) and column (traits) by a correspondence analysis using the simple corresponding procedure devised by MINITAB (1998). The first two axes retained 67.79 % of the total inertia (Table 11).

In the first component, traits such as glabrous glume type, two-row barley, six-rowed barley, and spike density were important. In component two, traits like black glume colour, purple kernel colour, irregular kernel row number, short and long length of rachilla hair had high contribution for the separation of regions. Fig 5 shows the distribution of regions of origin of the populations along the two axes of component variables. The presence of lax spike type in Gonder separated this region into the top edge of the negative scores of the first component. The preponderance of six-row type barley and short rachilla hair type in Gamo Gofa separated this region into the top of the first positive axis.

The preponderance of black glume colour and purple kernel colour in Gonder distinguished the region into an extreme category from the second component of first positive axis. The predominance of irregular kernel row number and long rachilla hair in Bale and Hararge according to the order of importance distinguished the regions in the negative direction of the second axis.

The distinctiveness of Gamo Gofa, Gonder and Welega on component one was due to the lower frequencies of white glume colour, high frequencies of glabrous glume and lower frequencies of white kernel colour.

Table 11. Correspondence analysis ordinate scores, inertial, proportion of variance and cumulative variance for the first two components for barley populations by eleven regions of origin

Region	C_1	C_2
Arsi	0.087	0.001
Bale	-0.49	-0.329
Gamo Gofa	0.495	0.192
Gojam	0.109	0.061
Gonder	-0.448	0.349
Hararge	0.105	-0.329
Shewa	0.141	0.225
Sidamo	0.162	-0.031
Tigray	-0.280	-0.101
Welega	-0.441	-0.041
Wollo	0.121	0.005
Inertia	0.073	0.040
Proportion of variance	0.438	0.239
Cumulative variance	0.438	0.677

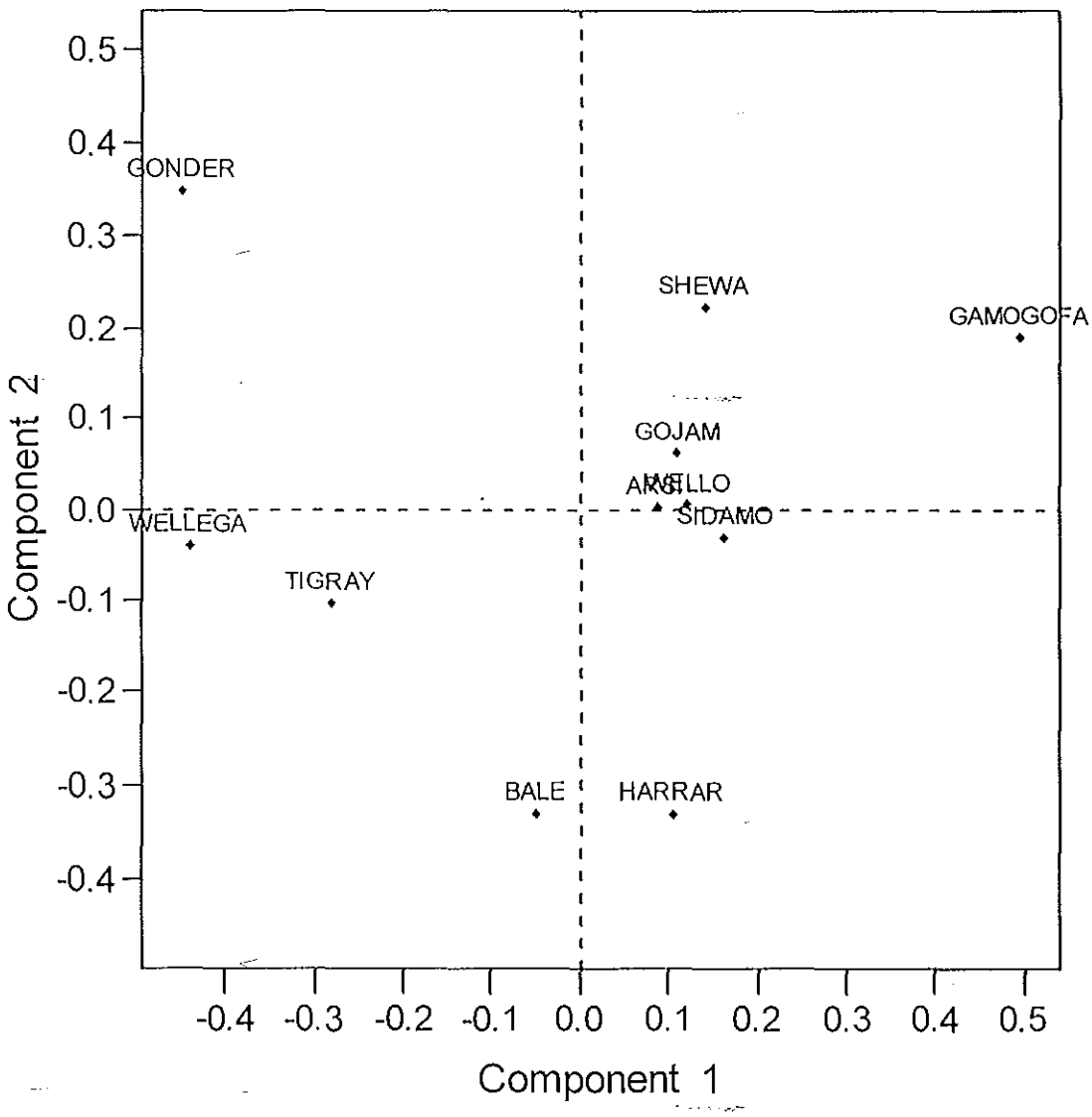


Fig 5. Distribution of the eleven regions of origin of the populations of barley along the two axes of the component variables using qualitative characters

4. 1. 7. 3. Altitude trait distribution

Table 12 summarizes the phenotypic frequencies for individual characters and altitudinal classes as percentage of the number of genotypes from each altitudinal class by pooling populations together. Moreover, it shows the result of chi square tests for the traits examined. Each altitudinal class is well represented, except the upper most altitudinal class that has only one population.

All the awn colour traits, white, yellow, brown and black occurred in all altitudinal classes except white awn colour which was not observed in altitudinal class > 3500 m asl. Yellow awn colour showed higher frequencies in altitude > 3500 m asl (80%). Highly significant chi-square deviation was found for awn colour at upper altitudinal class (> 3500 m asl).

Among the traits recorded for ear shape, a triangular type predominantly occurred in all altitude classes. The frequency of triangular ear shape showed a clinal variation and tends to decrease from lower to higher altitudinal classes and a significant deviation was obtained in the higher altitude (>3500 m asl). However, the frequency of broad type ear shape showed an increasing trend with increasing altitude. In general, the triangular type ear shape was predominantly occurred in lower altitudes, while the broad type was frequent in higher altitudes.

All the characters recorded for kernel row number were found in all altitudinal classes. Among these, two–rowed barley was predominantly appeared in lower altitude, < 2000 m asl. Whereas six-rowed type was predominantly found in areas above 3000 m asl with the

frequencies of 74% and 87% in altitudinal class 3001-3500 and >3500 m asl, respectively. The frequency of irregular type barley was low in all altitudinal classes. There is a similar pattern for the rachilla hair type and spike density.

The frequency of long rachilla hair in the lower altitude < 2500 m asl was high, whereas in the higher altitude classes, > 3000 m asl, the frequency of short rachilla hair was predominant. In general, long rachilla hair, two rowed and lax spike type predominantly occurred in lower altitudinal classes. Similarly, short rachilla hair, six-rowed and dense spikes were more frequent in the higher altitudes.

Glume hairiness also goes with altitude. The hairy type glume occurred with about 80 % frequencies in the lower altitude. This result is related with long rachilla hair and two–row type barley that were predominantly found in lower altitudes.

Among the different seed colours considered, black colour was the most frequent (43%) in altitudinal class of < 3000 m asl, whereas in altitudinal classes of 3001-2500 m asl and >3500 m asl blue and purple appeared with a frequency of 34% and 42%, respectively.

Among the different flag leaf characters, broad type was found predominantly in all altitudinal of classes. Its frequency showed a clinal variation in that, as altitude increased its frequency increased.

Glume colour was distributed in all altitudinal classes with predominance of white and yellow colours. White colour was more frequent in altitudinal class, 2001-2500 m asl. However, brown and black glume colours were observed in low frequencies in all altitudinal classes.

Spike bending tendency and spike density were observed in altitudinal class of 2001-2500 m asl. Highly significant chi-square deviation was observed in spike bending tendency in altitudinal class of > 3500 m asl. Significant deviation was also observed in spike density in altitude < 2000 m asl, 3001-3500 m asl and > 3500 m asl. Among the characters recorded for spike bending tendency, erect spike type was found to be the dominant of all the characters. Among the traits recorded for spike density, dense spike occurred in higher frequency, followed by medium type. Lax spike occurred in lower altitude, < 2000 m asl, with a frequency of 37%.

Different lengths of barley awn were found in all altitudes. However, equal length type of awn and spike occurred in higher frequencies. As altitude increases the frequency of equal length awn and spike increased. This shows that in higher altitudes the length of awn and spike are more or less the same. In higher altitude, > 3500 m asl, the frequency of lodging was low. Smooth awn type, naked kernel and hooded type of barley were not observed in all altitude classes for this study.

Table 12. Percentage of

Altitude	Awn type			Kernel row number			
	R	S	X^2	Two	Six	In	X^2
1	100	0	0	52	20	28	48.02**
2	100	0	0	25	46	29	5.97
3	100	0	0	44	41	15	14.66
4	100	0	0	14	74	12	16.84*
5	100	0	0	0	87	13	19.92*

1-<2000, 2- 2001-2500, 3- 2501

Two-two rowed; Six-six rowed;

awn; Po- Poor; In- intermediate;

Table 12.Continued.

Altiu	Kernel Cover				Awn colour			
de	C	N	X^2	W	Y	Bn	Bk	
1	100	0	0	35	57	3	5	
2	100	0	0	45	48	4	3	
3	100	0	0	46	43	6	5	
4	100	0	0	4	38	3	16	
5	100	0	0	0	80	9	11	5

1-<2000, 2- 2001-2500, 3- 2501

N- Naked; C-covered; X^2- Chi-s

Globlous; Hi- Hairy; Bu- Blue; F

4. 1. 7. 4. Estimates of diversity

The estimates of Shannon-Weaver diversity index, H', for individual characters, populations, regions and altitudinal classes are shown in Appendix 14 and Tables 13 and 14, respectively. Polymorphism was common in varying degree for most characters, thus, implying the existence of a wide range of variation in the barley populations. The H' value ranged from 0.0 (monomorphic) for awn type and kernel covering to 1.0 (highly polymorphic) for flag leaf area in one population. The highest mean diversity index (H') pooled over traits was shown by populations collected from Arsi (PGRC/E 232224, H'= 0.69 $\pm$ 0.08) and (PGRC/E 232223, H'= 0.68 $\pm$ 0.09), Sidamo (PGRC/E 235651, H'= 0.66 $\pm$ 0.09) and Welega (PGRC/E 64339, H'=0.66 $\pm$ 0.08). The lowest H' was found for individual populations from Shewa (PGRC/E 235541, 0.40 $\pm$ 0.08) and Tigray (PGRC/E 235239, H' =0.40 $\pm$ 0.09). Among the characters recorded, length of rachilla hair, lodging, kernel row number, spike density, spike bending tendency and kernel colour showed diversity index (H') of 0.99, 0.98, 0.97, 0.97, 0.96 and 0.95, respectively.

Unlike the individual population estimates, the regionwise comparison displayed a higher magnitude of differences. Individual characters showed different levels of diversity index in different regions. They ranged from H'= 0.56 $\pm$ 0. 076 for Hararge to H'=0.71 $\pm$ 0. 083 for Shewa. Most regions showed higher level of polymorphism H' > .60. Overall, Gamo Gofa (H'= 0.58 $\pm$ 0. 082) and Hararge (H'=0.56 $\pm$ 0. 076) showed relatively lower average diversity estimates. Among the eleven regions Shewa (0.71 $\pm$ 0. 083), Arsi (0.71 $\pm$ 0.086) and Gojam (0.71 $\pm$ 0. 081) showed highest diversity index.

Table 13. Diversity in

Region	AC	AT	
Arsi	0.75	0	
Bale	0.55	0	
Gamo Gofa	0.53	0	
Gojam	0.56	0	
Gonder	0.92	0	
Hararge	0.48	0	
Shawa	0.84	0	
Sidamo	0.58	0	
Tigray	0.65	0	
Welega	0.89	0	
Wollo	0.6	0	
Entire data	0.73	0	

AC= Awn colour; AT=Awn typ

KRN= Kernel row number; LB

On altitudinal basis, all the traits showed diversity estimates from H'= 0.00 for kernel cover and awn type to H'=0.99 for length of rachilla hair, spike bending tendency and spike density. Higher diversity estimates were recorded for spike bending tendency in all altitudinal classes. Highest diversity index (H') = 0.74 $\pm$ 0.088 was observed in altitudinal class of 2501-3000 m asl, whereas the lowest diversity index (H') = 0.59 $\pm$ 0.082 was recorded in altitudinal class of >3500 m asl.

In general altitudinal class of 2501-3000 m asl (H' =0.74 $\pm$ 0.088) showed highest diversity estimates followed by altitudinal class of < 2000 m asl (H' =0.72 $\pm$ 0.089). The overall mean diversity index for Ethiopia was estimated to be H'= 0.75 $\pm$ 0.089

One-way analysis of variance of diversity (H') for individual traits was performed and the result showed that much of the variation was due to variations between populations rather than regions and altitude classes. However, kernel row number showed significant variation among regions ($P< 0.05$) (Table 15). The hierarchical analysis of variance also revealed that, most of the total variance was due to the lowest hierarchy, the "within-population" component. Significant and highly significant differences were detected only for the "populations within region" and populations within altitude" group variances, respectively (Table 16).

Table 14, Diversity ind

Altitude range	AC	AT
1	0.68	0.00
2	0.68	0.00
3	0.75	0.00
4	0.81	0.00
5	0.46	0.00
Entire data	0.73	0.00

1-<2000, 2- 2001-2500, 3- 2501

AC= Awn colour; AT=Awn type

KRN= Kernel row number; LBA

Table 15. Mean squares for the different levels of grouping involving regions and altitudes from the ANOVA of H for individual characters

Character	Between		Between population within	
	Region (df=10)	Altitude (df=4)	Region (df=35)	Altitude (df = 42)
AC	0.059	0.060	0.037	0.039
AT	0.00	0.00	0.00	0.00
ES	0.064	0.127	0.061	0.054
FLA	0.028	0.041	0.029	0.028
GC	0.081	0.139	0.062	0.057
GH	0.120	0.119	0.061	0.071
KC	0.103	0.051	0.053	0.064
KCO	0.00	0.00	0.00	0.00
KRN	0.153*	0.027	0.072	0.097
LBA	0.027	0.029	0.015	0.016
LOD	0.096	0.057	0.073	0.081
LRH	0.072	0.133	0.056	0.053
SBT	0.100	0.047	0.058	0.069
SD	0.112	0.089	0.055	0.064

* = $P < 0.05$

AC= Awn colour; AT=Awn type; ES= Ear shape; FLA= Flag leaf area; GC= Glume colour; GH= Glume hairness; KC= Kernel colour; KCO= Kernel cover; KRN= Kernel row number; LBA= Length of barley awn; LOG= Lodging; LRA= Length og rachilla hair; SBT= Spike bending tendency; SD= Spike density

Table 16. Hierarchical analysis of variance of diversity H' overall characters

Source of variation	Df	Sum square	Mean square	F-ratio
Between				
Regions	10	1.3971	0.1397	0.8975^{ns}
Populations within region	36	2.6264	0.1730	1.5915^{*}
Altitude groups	4	0.2057	0.0514	0.2692^{ns}
Population within altitude group	42	3.8178	0.1909	1.7562^{**}
Character within populations	657	71.3881	0.1087	

Df = degree freedom, * $p < 0.05$, ** $P < 0.01$, ^{ns} = non significant

Partitioning of the phenotypic diversity into within and between regions of origin and altitudinal classes are given in (Table 17). Ninety percent of the total variation was found within regions of origin, while only ten percent was found between regions. Length of rachilla hair and kernel row number contributed relatively more than 31% and 22%, respectively, to the regional differentiation. Similarly, ninety-five percent of the variation was found within altitudes, while only five percent was found between altitudes (Table 17). Though the differentiation among the altitudes was weak, length of rachilla hair and kernel row number contributed relatively more to the observed differentiation. There is more differentiation on the regional level than on the altitudinal class. In general, length of rachilla hair, kernel row number, spike density, glume hairiness and glume colour are useful in discriminating accessions of different regions or altitudes.

Table 17. Partitioning of the qualitative phenotype diversity into within and between regions of origin and altitudinal classes

Characters	H_{sp}	H_r	H_r/H_{sp}	$(H_{sp}-H_r)/H_{sp}$	H_{ar}	H_a/H_{sp}	$(H_{sp}-H_{ar})/H_{sp}$
AC	0.73	0.67	0.92	0.08	0.67	0.92	0.08
AT	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ES	0.78	0.77	0.99	0.01	0.78	1.00	0.00
FLA	0.87	0.84	0.97	0.03	0.87	1.00	0.00
GC	0.67	0.60	0.90	0.10	0.69	1.03	-0.03
GH	0.78	0.69	0.88	0.12	0.80	1.03	-0.03
KC	0.95	0.87	0.92	0.08	0.89	0.94	0.06
KCO	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KRN	0.97	0.76	0.78	0.22	0.77	0.79	0.21
AT	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LBA	0.82	0.77	0.94	0.06	0.77	0.94	0.06
LOD	0.98	0.89	0.91	0.09	0.89	0.91	0.09
LRH	0.99	0.67	0.68	0.32	0.78	0.79	0.21
SBT	0.96	0.88	0.92	0.08	0.92	0.96	0.04
SD	0.97	0.85	0.88	0.12	0.85	0.88	0.12
MEAN	0.73	0.66	0.90	0.10	0.69	0.95	0.05

H_{sp} = Diversity index for each character calculated from the entire data set; H_r and H_a = Average diversity index of each character for eleven regions and the five altitudes, respectively. H_r/H_{sp} and H_a/H_{sp} = Proportion of diversity within regions and within altitudes, respectively; $(H_{sp}-H_r)/H_{sp}$ and $(H_{sp}-H_{ar})/H_{sp}$ = Proportion of diversity between regions and between altitudes, respectively, in relation to the total variation.

AC= Awn colour; AT=Awn type; ES= Ear shape; FLA= Flag leaf area; GC= Glume colour; GH= Glume hairness; KC= Kernel colour; KCO= Kernel cover; KRN= Kernel row number; LBA= Length of barley awn; LOG= Lodging; LRA= Length og rachilla hair; SBT= Spike bending tendency; SD= Spike density

4. 2. Biochemical diversity

4. 2. 1. Isozymes

The allele frequency data by populations are summarized in Table 18. The allele frequencies of the loci considered showed various levels of polymorphism in different populations. The most polymorphic loci were EST-6 and EST-3 where the monomorphic loci included AAT-1, AAT-2, AAT-3 and LAP-1, when the 95 % criterion of polymorphism is applied. A locus was considered polymorphic if the frequency of the most common allele in that locus was less than 0.95. Genetic variability measures for seven populations at the nine loci are presented in Table 19. The mean number of alleles per locus (A) ranged from 1.1 for PGRC/E 232216 and PGRC/E 234307 to 1.6 for PGRC/E 229790 and PGRC/E 235744 and averaged 1.33. The percentage of polymorphic loci (P) ranged from 11.1 % (PGRC/E 232216 and PGRC/E 234307) to 55.6% (PGRC/E 229790) and averaged 31.7%. Estimates of expected Heterozygosity ranged from 0.014 to 0.114 with a mean of 0.066. The average diversity index (H) across populations ranged from 0.082 (PGRC/E 232216) to 0.383 (PGRC/E 235744) and averaged 0.236. The two barley populations with the greatest diversity were PGRC/E 235744 (H= 0.383) and PGRC/E 230006 (H= 0.307). Over the populations, the EST-6 and EST-3 isozyme loci showed the highest genetic diversity (H= 0.452) and (H = 0.356), respectively.

Table 18. Summary of allele frequencies for 9 isozyme loci among 7 populations

Locus	Population						
	1	2	3	4	5	6	7
AAT-1A	1.00	1.00	1.00	1.00	1.00	1.00	1.00
AAT-2A	1.00	1.00	1.00	1.00	1.00	1.00	1.00
AAT-3A	1.00	1.00	1.00	1.00	1.0	1.00	1.00
LAP-1A	1.00	1.00	1.00	1.00	1.00	1.00	1.00
EST-1A	0.97	1.00	1.00	1.00	1.00	.94	0.94
EST-1B	0.03	0.00	0.00	0.00	0.00	0.06	0.006
EST-2A	0.97	1.000	1.00	0.94	1.00	1.00	1.00
EST-2B	0.03	0.00	0.00	0.06	0.00	0.00	0.00
EST-3A	0.94	0.97	0.93	0.67	1.00	0.92	0.90
EST-3B	0.06	0.00	0.07	0.00	0.00	0.08	0.09
EST-3C	0.00	0.03	0.00	0.33	0.00	0.00	0.00
EST-4A	0.83	0.97	1.000	0.89	1.00	0.94	0.81
EST-4B	0.17	0.00	0.00	0.11	0.00	0.00	0.19
EST-4C	0.00	0.03	0.00	0.00	0.00	0.06	0.00
EST-6A	0.83	0.52	1.00	1.00	0.46	1.00	0.72
EST-6B	0.17	0.48	0.00	0.00	0.54	0.00	0.25
EST-6C	0.00	0.00	0.00	0.00	0.00	0.00	0.03

1- PGRC/E 229790, 2- PGRC/E 230006, 3- PGRC/E 232216, 4- PGRC/E 232217, 5- PGRC/E 234307, 6- PGRC/E 234307, 7- PGRC/E 235546

Table 19. Summary of diversity data for isozyme traits in barley populations over 9 loci in seven populations (standard errors in parentheses)

Population	N^a	A	P	H	Mean heterozygosity	
					H_o	H_e**
PGRC/E 229790	35.7(0.2)	1.6(0.2)	55.6	0.307(0.073)	0.006(0 .006)	0.087(0.039)
PGRC/E 230006	35.1(0.5)	1.3(0.2)	33.3	0.24(0.175)	0.010(0 .010)	0.069(0.055)
PGRC/E 232216	36.1(0.1)	1.1(0.1)	11.1	0.082 (0.082)	0.009(0 .009)	0.014(0 .014)
PGRC/E 232217	36.0(0.0)	1.30.2)	33.3	0.241(0.119)	0.074(0 .074)	0.084(0 .051)
PGRC/E 234307	36.0(0.0)	1.1(0.1)	11.1	0.218 (0.218)	0.040(0 .040)	0.056(0 .056)
PGRC/E 235546	36.0(0.0)	1.3(0.2)	33.3	0.181 (0.085)	0.019(0 .019)	0.041(0 .021)
PGRC/E 235744	36.0(0.0)	1.6(0.2)	44.4	0.383 (0.121)	0.022(0 .022)	0.114(0 .053)
Mean	35.843	1.329	31.73	0.236	0.023	0.066

** Unbiased estimate (Nei, 1978)

The (N^a) estimates of mean sample size per locus; (A) average number of alleles per locus; (P) percent of polymorphic loci; (H) genetic diversity; (H_O) mean observed heterozygosity and (H_e) mean expected heterzygosity

A comparison of F statistics for 7 populations is given in Table 20. The value of F_{ST} indicates differentiation between populations (Biosys, 1998). The degree of differentiation (F_{ST}) of the individual loci ranged from 0.031 for EST-1 to 0.277 for EST-6. The mean value for F_{ST} indicates a moderate level of differentiation between populations. F_{ST} compares the ratio of the between population component of diversity to the total diversity. In this study, F_{ST} for all populations was estimated as 0.183, which indicates that between population component accounts for about 18 % of the total variation. Marked differences in the extent of differentiation (F_{ST}) were shown between two loci. The populations differentiated markedly

among themselves for some loci via. EST-3 and EST-6, and the levels of differentiation were low for EST-1, EST-2 and EST-4.

Table 20. Summary of F-statistics at 5 polymorphic loci among 7 populations of barley

Locus	F (IS)	F (IT)	F (ST)
EST-1	1.000	1.000	.031
EST-2	1.000	1.000	.035
EST-3	-.096	.060	.142
EST-4	1.000	1.000	.084
EST-6	.731	.805	.277
Mean	.607	.679	.183

F (IS)- Indicates variability within populations, F (IT)- total genetic variability
F (ST)- variability among populations

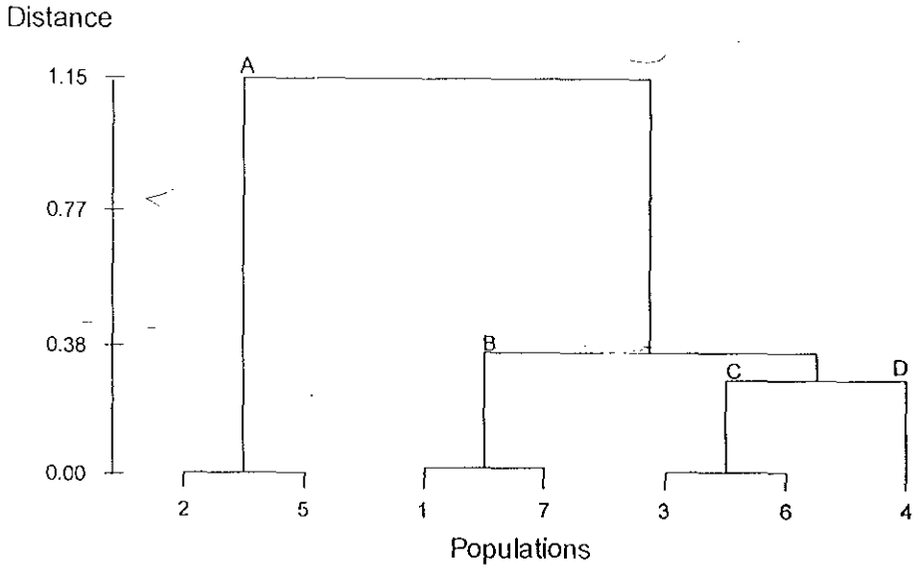
Unbiased genetic identity and genetic distance estimates between the populations ranged from 0.964 to 0.999 and 0.000 to 0.046, respectively (Table 21). The highest genetic distance was observed between populations (PGRC/E 232217 and PGRC/E 234307), which were collected from Arsi and Tigray, respectively. These two populations were collected from different corners of the country and altitudinal classes. The highest genetic identity was observed between populations collected from Bale (PGRC/E 230006) and Tigray (PGRC/E 234307), Arsi (PGRC/E 232216) and Shewa (PGRC/E 235546) and between populations from Gojam (PGRC/E 229790) and Gonder (PGRC/E 235744).

A dendrogram based on the allelic frequency data is shown in Fig 6. The dendrogram defines four main clusters. Group 1 was composed of two populations, which were collected from Bale and Tigray. Group 2 was also composed of two populations collected from Gojam and Gonder. Group 3 consisted of two populations from Arsi and Shewa. Group 4 was composed of only one population. This population was collected from Arsi at an elevation of 2200 m asl and it was an outlier both on morphological character and isozyme study.

Table 21. Nei's (1978) genetic similarity (above diagonal) and distance (below diagonal) for pairwise comparisons of populations of barley over 9 loci

Population [@]	1	2	3	4	5	6	7
PGRC/E 229790	*****	0.985	0.994	0.984	0.979	0.994	0.998
PGRC/E 230006	0.013	*****	0.973	0.959	0.999	0.971	0.989
PGRC/E 232216	0.006	0.026	****	0.988	0.966	0.999	0.988
PGRC/E 232217	0.013	0.037	0.011	****	0.950	0.988	0.979
PGRC/E 234307	0.018	0.000	0.033	0.046	****	0.964	0.985
PGRC/E 235546	0.005	0.026	0.000	0.011	0.033	*****	0.989
PGRC/E 235744	0.000	0.009	0.012	0.018	0.013	0.010	****

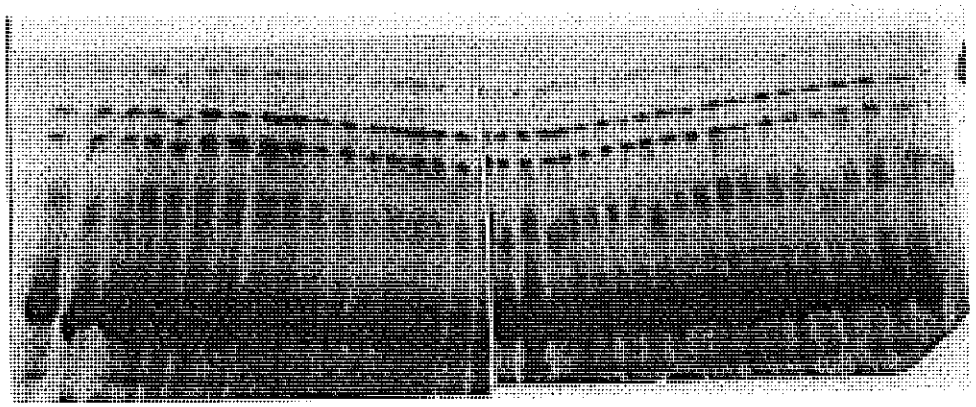
[@] 1 is PGRC/E 229790, 2- PGRC/E 230006, 3- PGRC/E 232216, 4- PGRC/E 232217, 5- PGRC/E 234307, 6- PGRC/E 235546, 7- PGRC/E 235744



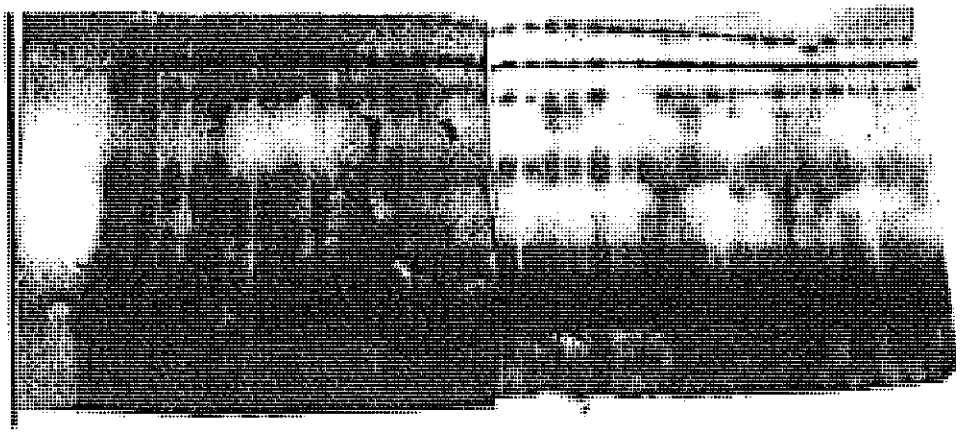
Where 1 is PGRC/E 229790, 2- PGRC/E 230006, 3- PGRC/E 232216, 4- PGRC/E 232217, 5- PGRC/E 234307, 6- PGRC/E 235546, 7- PGRC/E 235744

Fig 6. Dendrogram showing the cluster of 7 barley populations based on isozyme data

Below is given the zymogram banding pattern of the different barley populations studied. The sequence of loading (each gel) was (each represented by thirty six plants per population) 1 is PGRC/E 229790 (Gojam), 2- PGRC/E 230006 (Bale), 3- PGRC/E 232216 (Arsi), 4- PGRC/E 232217 (Arsi), 5- PGRC/E 234307 (Tigray), 6- PGRC/E 235546 (Shewa), 7- PGRC/E 235744 (Gonder) (Appendix 16).

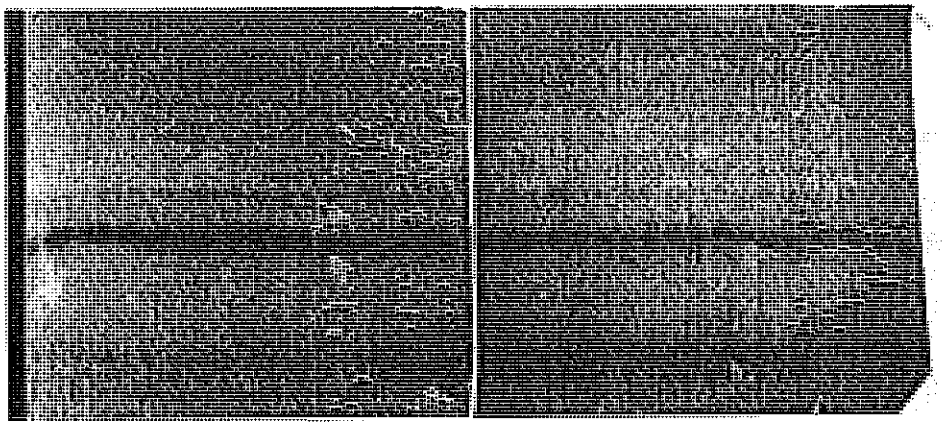


EST, PGRC/E 229790



EST, PGRC/E 235744

Fig. 7. Zymogram banding pattern of barley population for enzyme system EST (Appendix 16)



LAP, PGRC/E 235546

Fig. 8. Zymogram banding pattern of barley population for enzyme system LAP (Appendix 16)

AAT, PGRC/E 229790

Fig.9. Zymogram banding pattern of barley population for enzyme system AAT (Appendix 16)

4. 2. 2. Protein analysis

Determination of total nitrogen (crude protein) of 47 barley populations collected from 11 regions was carried out and the result shown on (Table 22). To examine the differences in crude protein content of the populations a one-sample t-test was used (Table 23). The result revealed that highly significant differences were observed between populations, regions of origin and altitudes. The highest protein mean scored was observed in population from Gamo Gofa (17.87) followed by Gojam (17.50) and Shewa (17.14). The lowest crude protein was scored in population from Wollo (15.65) (Table 24). The highest crude protein content (16.99) was observed in altitudinal class 2001-2500 m asl and followed in altitude class 3001-3500 m asl (16.57) was recorded. Lower protein content (15.94) (Table 25) in altitude greater than 3500 m asl. On population basis, the highest protein score was found in population collected from Gojam PGRC/E 229791 (19.75) and followed by population from Shewa (PGRC/E 235552 (18.87) (Table 23).

Table 22. Protein contents of 47 barley populations

Landrace		% Protein	Landrace		% Protein
PGRC/E	64335	17.44	"	235079	16.06
"	64339	16.62	"	235084	16.44
"	223208	16.81	"	235096	15.94
"	229789	15.81	"	235115	15.81
"	229790	16.94	"	235239	15.13
"	229791	19.75	"	235243	15.88
"	230003	15.94	"	235254	16.81
"	230006	17.37	"	235255	15.13
"	230591	17.25	"	235261	16.37
"	230594	15.19	"	235529	16.50
"	230595	16.69	"	235541	17.50
"	230614	16.81	"	235546	18.19

Table 22. Continued.

Landrace	% Protein		Landrace	% Protein
"	230653	17.50	"	235552	18.87
"	230814	16.69	"	235647	17.37
"	231455	17.12	"	235648	18.37
"	232216	16.25	"	235651	17.19
"	232217	16.06	"	235653	17.69
"	232219	16.25	"	235654	16.31
"	232221	15.25	"	235731	14.63
"	232223	17.75	"	235744	16.37
"	232224	15.69	"	235745	16.00
"	234293	15.69			
"	234307	17.00			
"	234896	16.56			
"	235066	15.13			
"	235074	14.94			

Table 23. T- test for crude protein content of barley populations with altitudes and regions

Sources	df	Mean Difference	t	Standard error	95% Confidence Interval of the Difference		Sig. (2-tailed)
					Lower	Upper	
Altitude	4	16.44	93.19	0.17	15.95	16.93	.000
Region	10	16.70	77.56	0.21	16.22	17.18	.000
Population	46	16.57	108.09	0.15	16.26	16.88	.000

Table 24. Mean Protein content of the population by region of origin

Region	Mean ± SE
Arsi	16.208 ±0.35
Bale	16.678 ± 0.32
Gamo Gofa	17.870 ± 0.5
Gojam	17.500 ± 1.17
Gonder	15.666 ±0.53
Hararge	16.905 ± 0.22
Shewa	17.135 ±0.51
Sidamo	17.063 ±0.40
Tigray	16.161 ±0.40
Welega	16.873 ±0.28
Wollo	15.652 ±0.28
Mean	16.575 ± 0.15

Table 25. Mean Protein content of the populations by altitudinal classes

Altitude classes	Mean ± SE
< 2000 m asl	16.451 ± 0.21
2001-2500 m asl	16.999 ±0.29
2501-3000 m asl	16.239 ±0.40
3001 –3500 m asl.	16.578 ± 0.34
>3500 m asl	15.940 ± --
Mean	16.576 ± 0.15

- = It has only one population

4.3.Comparison between morphological and biochemical polymorphism

The relationship between the morphological diversity and the genetic diversity measured by isozymes was worked out in these populations with their respect to the classification variables. The correspondence between the enzymatic variation and qualitative morphological variation is studied by Spearman's rank correlation coefficients (Table 26). When the level of $P<-0.05$ is used, correlation between morphological diversity elements (Shannon–Weaver diversity index H'), and Shannon diversity index (H) of isozyme diversity estimates at population level, the following character combination showed significance: EST-1 and kernel colour; EST-3 and glume hairiness; EST-4 and kernel colour; EST-2 and lodging; awn colour glume hairiness; and flag leaf area and lodging. EST-3 and glume hairiness, and flag leaf area and lodging are the character combinations that showed a negative significant correlation. On the other hand, there were significant negative correlations between the presently studied isozymes and quantitative morphological characters (Appendix 15).

Table 26. Rank correla

between cha

	EST1	EST
EST1		-0.1
EST2		
EST3		
EST4		
EST5		
AC		
ES		
FLA		
GC		
GH		
KC		
KRN		
LBA		
LOD		
LRH		
SBT		

*:- P < 0.05

AC= Awn colour; AT=Aw
cover; KRN= Kernel row n
SD= Spike density

5. DISCUSSION

5. 1. Morphological characters

5. 1. 1. Character distribution

The highest mean grain yield per plant was recorded from populations collected from Arsi, Hararge, Bale and Gamo Gofa. Most of these areas are characterized by receiving high rainfall with relatively even distribution. Plant height was also very high in populations from Arsi and Bale. This is also related to the amount of precipitation in the region studied thereby leading to long maturity period and long grain filling days.

When the quantitative morphological characters are examined in terms of altitude, the highest yield per plant was recorded in the altitudinal class of 2001-2500 m asl. In this altitude class, plant height, number of spikelets and kernels per spike and biomass weight were also high. In higher altitude >3000 m asl, days to heading and days to maturity were high. This may be due to the fact that at higher altitudes the duration and amount of rainfall is very high that leads to the extending days of plant to heading and maturity. Alemayehu (1995) studied the variations in 18 landrace accessions and noted a significantly delayed maturity and an increased resistance to scald (*Rhynchosporium secalis*) with increasing altitude. His hypothesis was that increased rainfalls in the cooler highlands allowed a longer growing season, but also better conditions for foliar diseases. Net blotch (*Helminthosprium* spp.) disease severity was recorded on the materials collected from higher altitudes while high barley leaf rust (*Puccinia hordei*) severity was recoded on populations from the lower altitudinal classes < 2000 m asl.

Mean square of quantitative morphological characters showed highly significant difference between 47 barley populations for 16 different quantitative characters. This shows that there were significant variations between populations rather than between regions and altitudes.

Discriminant analysis showed that the materials from cluster E and F have certain degree of overlapping, however, materials grouped in clusters A, B, C, D and G were correctly classified. For most of the regions, the populations were distributed in a patchily pattern over many clusters, reflecting wide variation among populations within a particular region.

Analysis of variance for 16 quantitative morphological traits showed a highly significant difference for all recorded characters, thus confirming for the presence of adequate genetic variability.

Principal component analysis resulted in the distribution of 11 regions of origin of the populations along the first three-dimension. In the first principal axis, Gojam, Shewa, Wollo, Hararge, Arsi, Sidamo and Gamo Gofa with positive principal components, and Gonder, Bale, Tigray and Welega were with negative principal components. In the second principal component, Gojam, Gonder, Shewa, Wollo and Tigray were categorized in the negative part, whereas Arsi, Bale, Hararge, Wollo, Sidamo and Gamo Gofa were grouped in the positive principal component. The grouping system may have been based on the proximity of the regions to each other, perhaps indicating the possibility of being from the same original seed stock and on going seed exchange between these regions than between the rest.

Barley populations from northern part of the country (Gonder, Gojam and Tigray) are predominantly two-rowed. Its distribution increases from north to south. Most of the two rowed barley populations are found in lower altitudes, whereas six-rowed barley are more concentrated in Gamo Gofa, Arsi, Shewa, Wollo and Sidamo in the altitude class >3000. This result is concordant with previous report (Asfaw, 1989). Demissie *et al.* (1996a) has also reported that six-rowed barley concentrated in Gamo Gofa. According to Negassa (1985a), six- rowed types are concentrated on the highlands because of environmental selection of genes for resistance to frost and the high amount and distribution of rainfall of the area.

Rachilla hair type (long and short) was also related with kernel row number and altitude (Asfaw, 1988; Demissie *et al.* 1996a). The rachilla hair character has been considered selectively neutral and with no selective significance (Takahashi, 1955). The association of six-row and short rachilla in the highlands, two-row and long rachilla in the lowlands may probably due to linkage with adaptive traits on their respective chromosomes (Demissie, 1996a). The present study also showed similar pattern. Asfaw (1989) also reported that the concentration of some traits at higher and others at lower altitudes resulted from random or selective accumulation of certain genes at high or low elevations, which could result partly from farmers' selection of morphotypes based on their selection criteria of suitability to the prevailing climatic and edaphic conditions of the areas. Irregular type barley is predominantly found in Bale, followed by Sidamo and Hararge, it is to be noted that Ethiopia is a center of concentration for this trait.

All kernel colour (white, black, blue and purple) were observed in all regions with different frequencies. However, black colour was found predominantly in all regions in lower altitudes

< 3000m asl. Demissie (1996a) reported that the pattern of kernel colour was not evenly distributed because it has association with phenotype and utility for human consumption. Ceccarelli *et al.* (1987) suggested that the black seeded types are prime choice of local farmers in drier areas due to specific characters of adaptation to low moisture conditions. Some rare characters such as smooth awn type, naked kernel covers and hooded types of barley reported in previous studies are not observed in the present study. This may be due to insufficient sample size or the declining of rare morphtypes in frequency of occurrence through time (Asfaw, 1988).

Correspondence analysis of qualitative traits of 47 barley populations distantly separated the region gene pools into different parts of the axis. Based on this, populations from Gonder, Gamo Gofa, Bale, Hararge, Welega and Tigray were distributed in two different corners, while populations from Gojam, Wollo, Arsi and Sidamo were concentrated at the center indicating the similarity of the materials. This could be due to germplasm exchange or the impact of similarity in agro ecology on the proportion of gene pools.

4. 1. 2. Diversity index

Summary of mean diversity indeces for individual characters, populations, regions of origin and altitudes are shown in appendix 16 and Tables 13 and 14. The mean diversity index of altitude class > 3500 m asl was low as compared to other classes. This could be due to selection pressures such as frost at higher altitude. The highest mean diversity index (H') pooled over traits was obtained for populations for Arsi (H'=0.69 and H'= 0.68), Sidamo (H'

= 0.66) and Welega (H' = 0.66) populations, whereas the lowest is for populations from Shewa (H =0.40) and Tigray (H = 0.40). Demissie *et al.* (1996a) also reported that the highest mean diversity for individual populations were from Arsi (H= 0.64) and Welega (H=0.62) and the lowest was for individual populations from Bale, Shewa, Tigray and Gamo Gofa. In both studies the regions that showed lowest and highest diversity indices for individual populations are the same, though with different magnitudes.

The diversity of Ethiopian barley in all regions were estimated. The result showed that high diversity index was observed in Shewa (H' = 0.71), Arsi (H' = 0.71) and Gojam (H' = 0.71) and the lowest diversity index was obtained for populations from Hararge (H' = 0.56) and Gamo Gofa (H' = 0.58). This may be due to small number of samples taken from the two regions (two populations from each region). However, Negassa (1985a) reported that the highest estimate of diversity was from Gamo Gofa (H'=0.71) and the lowest from Begemidir (Gonder) (H'=0.54). Dimissie *et al.* (1996a) also showed that regionwise comparison displayed diversity study for regions ranged from H=0.43 for Gojam to H'=0.86 for Welega. This discrepancy in regional diversity index (H') might be due to inadequate sampling and/or dissimilar and insufficient number of characters for the study.

The genetic diversity of barley altitude wise is also high. Altitude class 2501-3000 m asl showed high diversity index (H'=0.74) followed by altitude class <2000 m asl that showed diversity index (H'=0.72). As compared to others the highest altitude class >3500 m asl showed lower diversity index (H'=0.59). This is probably associated with the sample size, natural selection pressures of frost operating at higher altitude (Negassa, 1985a) and anthropogenic factors such as farmer selection pressure (Asfaw, 1989c).

Quantitative estimate of diversity value for Ethiopian barley has been reported by Tolbert *et al.* (1979), who estimated a diversity value of H' = 0.51; Negassa (1985a), who estimated H' = 0.68; Engels (1991), who estimated H' = 0.70 and Demissie *et al.* (1996a) who estimated H'= 0.71. In the present study, a diversity index H = 0.75 was recorded for Ethiopia, which is a little bit higher than the previous studies. This may be due to the high number of characters used for this study.

Analysis of variance revealed that the level of diversity of Ethiopia barely is about the same in all the regions and altitudes. These results are further supported by partitioning of the phenotypic diversity into within and between the regions and altitude and the result showed that there were no significant variations between and within regions and altitudes and much of the variance of the estimate of the diversity is due to the difference among populations. According to Demissie *et al* (1996a), this could be due to the absence of pronounced geographical types, may be explained in terms of the environmental heterogeneity of regions, irrelevance of administrative and regional boundaries in maintaining diversity and the significant degree of seed migration among regions. Negassa (1985a) reported that much of the variation was due to differences within regions rather than among regions.

Hierarchical analysis of variance based on normalized data showed that much of the variation is due to the variation within region and within altitude rather than between region and altitude. The highest polymorphism was concentrated in areas between altitudinal classes of 2500-3000 m asl. The major barley growing areas in the country with maximum diversity

have been reported by Demissie *et al.* (1996a) and Engels (1994) in areas between 2000-3000 m asl. The present result was also found in this range.

5. 2. Biochemical characters

5. 2. 1. Isozymes

Isozyme data allow quantification of similarity, or differences, between populations, groups of populations and/or species (Gottlieb, 1981). Populations and /or species can be characterized on the bases of differences in allele frequencies.

Isozyme analysis was done for three-enzyme systems Esterase (EST), Leucine aminopeptidase (LAP) and Aspartate aminotransferase (AAT). The allelic frequencies of the loci considered showed various levels of polymorphism in different populations. The most polymorphic loci were EST-1, EST-2, EST-3, EST-4 and EST-6 whereas the monomorphic loci were AAT-1, AAT-2, AAT-3 and LAP-1. Demissie and Bjørnstad (1997) also reported monomorphic loci for the AAT-1, AAT-2 and AAT-3.

The number of alleles for the EST locus is fewer than the one reported by Bekele (1983a) and Demissie and Bjørnstad (1997) who included large number of populations and covered different geographical regions. The mean number of alleles per locus and percentage of polymorphic loci for EST were comparable to the result obtained by Demissie and Bjørnstad (1997).

The degree of differentiation (F_{ST}) of the individuals loci ranged from 0.031 for EST-1 to 0.227 for EST-6 (Table 20). The value 0.183 for the mean F_{ST} is low as compared to the average F_{ST} value for inbreeding species (0.51) reported by Hamrick and Godt (1990). According to Demissie *et al.* (1997), the highest F_{ST} value may reflect adaptation to the strong environmental dissimilarities or high levels of genetic drift maintained by restricted gene flow between populations. Genetic diversity was calculated for each population and the result revealed that high Shannon diversity index in PGRC/E 235744 (H= 0.383), PGRC/E 229790 (H= 0.307) and PGRC/E 232217 (H= 0.241) as compared to other populations.

Nei's genetic distances among seven populations ranged from 0.00 to 0.46. The highest genetic distance estimate was recorded between PGRC/E 232217 and PGRC/E 224307. This may be due to the fact that the two populations were collected from different region (Arsi and Tigray) that are widely separated geographically and found on different altitude classes, and probably also less human interaction between the two regions.

Genetic identity values were calculated and it ranged from 0.950 between PGRC/E 232217 and PGRC/E 234307 to 0.999 between (PGRC/E 230006 and PGRC/E 234307) and (PGRC/E 232216 and PGRC/E235546). The highest genetic identity was observed between populations collected from Gojam - Gonder and Arsi - Shewa. These regions are adjacent to each other. Therefore, there may be an exchange of seed between the regions in addition to agro ecological similarities. Cultural uniformity may also explain at least part of the reason for the similarities.

Cluster analysis and subsequent construction of dendrogram suggested two populations that were collected from distinct regions may share more biochemical genotypes than geographically adjacent ones for the markers used in the present study. This is especially true for populations from Bale and Tigray that are widely separated geographically.

Correlation between Shannon–Weaver diversity index of qualitative morphological characters and isozyme analysis showed some significant negative and positive associations. However, the values of the quantitative characters showed negative correlations with enzymes variations. Similarly, Bekele (1984) reported that number of tillers and plant height; number of tiller and kernel weight; plant height and spike length; spike length and number of seeds; spike length and acid phosphatase locus 1; number of seeds and kernel weight; kernel weight and rachilla hairiness; kernel weight and esterase D locus; row number and esterase A locus; glume hairiness and rachilla hairiness; glume hairiness and esterase C locus; and esterase A locus and esterase C locus showed significance by Spearman's rank correlation coefficients. Kernel weight and rachilla hairiness are the only character combinations that showed a negative significant correlation when the level of $p \leq 0.05$ is used. On the other hand, Demissie and Bjørnstad (1997) indicated the absence of biochemical and morphological data associations. He suggested that the reason for the absence of correlation between biochemical and morphological characters may be due to: i) direct human or natural selection for some morphological traits, whereas isozymes tend to be neutral or subject to natural selection alone, or ii) the morphological and isozyme markers reflect different parts of the genome, with different level of associations with adaptive traits.

5. 2. 2. Proteins

Determination of the total nitrogen (crude protein) for 47 barley population collected from 11 regions showed highly significant differences between populations, altitudinal classes and regions. The amount of protein content of populations varies from 14.63 to 19.75 with the overall mean of 16.57. Wondimu (1996) reported that the protein content of food barley ranged from 14.8- 15.6. However, Kerssie and Goitom (1996) showed that barley protein is composed of 19 amino acids, but low in lysine and methionine. They further noted that the average protein of barley is about 9.3 %, which is very low as compared to the present study. Breeding/crossing program was initiated at Holetta Agricultural Research Center for improving the protein content of the Ethiopian barley and apparently lines having high protein content (9.7% to 16.5%) were identified (Gebre *et al.*, 1996). Protein quality is dependent upon the concentration of essential amino acids. However, it is affected by genotype, environment and the genotype-environment interaction.

6. CONCLUSSION

Morphological and biochemical diversity study on recent collections of barley populations were undertaken in this study. Plant species of economic importance have never been equally distributed through out the world. The Ethiopian region has been recognized as an important center of diversity for many crops such as barley. The country's wide altitudinal range, substantial temperature and rainfall differences with diverse edaphic conditions create a wide range of agro-ecological conditions and microenvironments. Therefore, Ethiopian barley is distributed in different regions, agro ecological zones and altitudinal ranges.

Measurement and characterization of genetic variation in germplasm of crop plants are primary concern for genetic resource conservation and utilization. Analysis of variance for quantitative morphological character showed highly significant differences between populations in all recorded characters in the present study.

Genetic erosion of barley germplasm in the country is high, thus, specific collecting operations have to be carried out to fill gaps in terms of geographical coverage of the existing collections. From this study it can be recognized that there is still abundant genetic variability within the studied populations. This suggests the potential of local landraces for breeding programs.

The present study indicates that the amounts of variability are not uniformly distributed in all geographic regions and altitudes. The overall diversity index of the recently collected (i.e, since 1980) barley landraces used in this and other similar studies support the conclusion that

Ethiopia is still an important center of diversity for barley. This diversity may indicate the presence of important genes for breeding purposes. It was also observed that some correlations between morphological characters and isozymes variations suggesting further studies in this area. From a genetic point of view, regions with highest diversity index (Shewa, Arsi and Gojam) are suitable for germplasm collection and *in-situ* conservation sites. Further study with large sample size and additional biochemical markers to estimate fuller picture about the genetic variation in Ethiopian landrace barley is required.

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8. APPENDICES

Appendix 1. Sinana Agricultural Research Center temperature and rainfall data during the 2000/2001 crop-growing season (July 2000- February 2001).

Month	Date range	Temperature (° C)		Rainfall	Total RF
		Min	Max.	(mm)	(mm)
July 2000	1-10	10.5	21.3	0.9	68.8
	11-20	10.8	22.3	53.1	
	21-31	10.1	19.7	14.8	
August 2000	1-10	9.8	20.0	12.2	64.2
	11-20	10.2	20.7	15.8	
	21-31	10.4	19.9	36.2	
September 2000	1-10	10.2	20.8	46.0	121.8
	11-20	9.6	20.1	22.8	
	21-31	10.0	18.7	53.0	
October 2000	1-10	11.2	18.4	86.8	116.8
	11-20	10.3	18.8	12.4	
	21-31	10.0	18.8	17.6	
November 2000	1-10	8.3	19.9	2.4	26.6
	11-20	9.1	19.7	15.1	
	21-31	7.2	19.9	9.1	
December 2000	1-10	7.8	21.0	1.7	10.7
	11-20	8.2	20.9	9.0	
	21-31	6.8	22.0	0.0	
January 2001	1-10	7.9	22.7	0.0	0.0
	11-20	8.3	22.9	0.0	
	21-31	8.05	23.0	0.0	
February 2001	1-10	8.1	22.8	0.0	12.1
	11-20	8.8	23.9	0.0	
	21-31	8.6	23.9	0.0	
Mean		9.39	20. 3	21.5	421

Appendix 2. Accessions with their localities and Altitude

No.	Accession No.	Region	Worada	Altitude	Year of collection
1	PGRC/E 64335	Welega	Arjo	2160	1982
2	" 64339	Welega	Dambidolo	1680	1982
3	" 223208	Tigray	Agame	2380	1986
4	" 229789	Gojam	Mota	2250	1988
5	" 229790	Gojam	Mota	2500	1988
6	" 229791	Gojam	Mota	2500	1988
7	" 230003	Bale	Dinsho	2730	1989
8	" 230006	Bale	Goro	2120	1989
9	" 230591	Bale	Ababa	2400	1989
10	" 230594	Bale	Sinana	2240	1989
11	" 230595	Bale	Sinana	2230	1989
12	" 230614	Bale	Goro	1870	1989
13	" 230653	Bale	Dinsho	2610	1989
14	" 230814	Hararge	Gara Mulata	2050	1989
15	" 231455	Hararge	Jijiga	1900	1990
16	" 232216	Arsi	Abomsa	1690	1990
17	" 232217	Arsi	Jeju	2220	1990
18	" 232219	Arsi	Guna	2360	1990
19	" 232221	Arsi	Guna	2550	1990
20	" 232223	Arsi	Guna	2760	1990
21	" 232224	Arsi	Arba gugu	2780	1991
22	" 234293	Wollo	Kerem	2140	1991
23	" 234307	Tigray	Agame	3100	1984
24	" 234896	Welega	Arjo	2350	1992
25	" 235066	Wollo	Yeju	3010	1992
26	" 235074	Wollo	Dese Zuriya	2950	1992
27	" 235079	Wollo	Lagambo	3500	1992
28	" 235084	Wollo	Were Himeno	3300	1992

Appendix 2. Continued.

No.	Accession No.	Region	Worada	Altitude	Year of collection
29	" 235096	Shewa	Mehalmeda	3600	1992
30	" 235115	Shewa	Ankober	3320	1992
31	" 235239	Tigray	Chercher	1750	1992
32	" 235243	Tigray	Inda mehone	1610	1992
33	" 235254	Tigray	Maychew	2300	1992
34	" 235255	Tigray	Inda Mohone	2720	1992
35	" 235261	Tigray	Ambalage	2750	1992
36	" 235529	Shewa	Sebat bet	3250	1993
37	" 235541	Shewa	Sebat bet	3260	1993
38	" 235546	Shewa	Chabo	3050	1993
39	" 235552	Shewa	Chabo	2900	1993
40	" 235647	Gamo Gofa	Bako Gazer	1810	1993
41	" 235648	Gamo Gofa	Bako Gazer	2080	1993
42	" 235651	Sidamo	Yabelo	1780	1993
43	" 235653	Sidamo	Mega	1610	1993
44	" 235654	Sidamo	Arero	1880	1993
45	" 235731	Gonder	Debark	2840	1993
46	" 235744	Gonder	Chilga	2200	1993
47	" 235745	Gonder	Chilga	2000	1993

Appendix 3. Total mean

Plot No.	Accession No.	DH
1	PGRC/E 64335	71
2	" 64339	74
3	" 223208	88
4	" 229789	73
5	" 229790	95
6	" 229791	95
7	" 230003	88
8	" 230006	75
9	" 230591	81
10	" 230594	74
11	" 230595	81
12	" 230614	73
13	" 230653	75
14	" 230814	85
15	" 231455	80
16	" 232216	78
17	" 232217	83
18	" 232219	86
19	" 232221	84
20	" 232223	87
21	" 232224	88
22	" 234293	71
23	" 234307	91
24	" 234896	71
25	" 235066	87

Appendix 3. Continued

Plot No.	Accession No.	DH
26	PGRC/E 235074	84
27	" 235079	86
28	" 235084	87
29	" 235096	78
30	" 235115	93
31	" 235239	73
32	" 235243	74
33	" 235254	93
34	" 235255	72
35	" 235261	74
36	" 235529	92
37	" 235541	94
38	" 235546	88
39	" 235552	88
40	" 235647	82
41	" 235648	78
42	" 235651	78
43	" 235653	84
44	" 235654	85
45	" 235731	89
46	" 235744	63
47	" 235745	63
	Mean	**81.5**

AL= Awn length; BM= Bio
Number of spike per spike; N
Thousand grain weight; TN=

Appendix 4. Correlati

	AL	BM	DH	DM
AL		0.45	0.00	-0.22
BM			-0.77	-0.50
DH				0.80
DM				
FLL				
GF				
HI				
NN				
NKPS				
PH				
SL				
NSPS				
SYW				
TN				
TGW				
YS				

*- $P < 0.05$, **- $P < 0.01$

AL= Awn length; BM= Bi
Number of spike per spike
Thousand grain weight; T

Appendix 5. Corre

	AL	BM	DH	DM	F
AL		0.12	-0.02	-0.225	0
BM			-0.73	-0.751	0
DH				0.894**	-(
DM					-(
FLL					
GF					
HI					
NN					
NKPS					
PH					
SL					
NSPS					
SYW					
TN					
TGW					
YS					

*- $P < 0.05$, **- $P < 0.01$

AL= Awn length; BM= Bio
Number of spike per spike;
Thousand grain weight; TN

Appendix 6. Correlatic

	AL	BM	DH	DM
AL		0.83	-0.5	-0.208
BM			0.07	0.376
DH				0.951
DM				
FLL				
GF				
HI				
NN				
NKPS				
PH				
SL				
NSPS				
SYW				
TN				
TGW				
YS				

*- $P < 0.05$, **- $P < 0.01$

AL= Awn length; BM= Bio
Number of spike per spike;
Thousand grain weight; TN

Appendix 7. Correlation

	AL	BM	DH	DM
AL		0.76	-1**	-0.993
BM			-0.76	-0.833
DH				0.993
DM				
FLL				
GF				
HI				
NN				
NKPS				
PH				
SL				
NSPS				
SYW				
TN				
TGW				
YS				

*- $P < 0.05$, **- $P < 0.01$

AL= Awn length; BM= Bio
Number of spike per spike;
Thousand grain weight; TN

Appendix 8. Correlati

	AL	BM	DH	DM
AL		0.71	-0.01	-0.141
BM			0	0.131
DH				0.919*
DM				
FLL				
GF				
HI				
NN				
NKPS				
PH				
SL				
NSPS				
SYW				
TN				
TGW				
YS				

*- $P < 0.05$, **- $P < 0.01$

AL= Awn length; BM= Bi
Number of spike per spike
Thousand grain weight; T

Appendix 9. Corr

	AL	BM	DH
AL		1*	-0.99
BM			-0.99
DH			
DM			
FLL			
GF			
HI			
NKPS			
PH			
SL			
NSPS			
SYW			
TN			
TGW			
YS			

*- $P < 0.05$, **- $P < 0.01$

AL= Awn length; BM= Bio
Number of spike per spike;
Thousand grain weight; TN

Appendix 10. Correlatio

	AL	BM	DH	DM	F
AL		0.54	-0.91**	-0.856*	0.
BM			-0.64	-0.458	0.
DH				0.869*	-0
DM					-0
FLL					
GF					
HI					
NN					
NKPS					
PH					
SL					
NSPS					
SYW					
TN					
TGW					
YS					

*- $P < 0.05$, **- $P < 0.01$

AL= Awn length; BM= Bio
Number of spike per spike;
Thousand grain weight; TN

Appendix 11. Correla

	AL	BM	DH
BM			-0.13
DH			
DM			
FLL			
GF			
HI			
NKPS			
PH			
SL			
NSPS			
SYW			
TN			
TGW			
YS			

*- $P < 0.05$, **- $P < 0.0$

AL= Awn length; BM= Bio

Number of spike per spike;

Thousand grain weight; TN

Appendix 12. Correlati

	BM	DH	DM
BM		-0.88	-0.7
DH			0.98
DM			
FLL			
GF			
HI			
NN			
NKPS			
PH			
SL			
NSPS			
SYW			
TN			
TGW			
YS			

*- P < 0.05, **- P < 0.01

AL= Awn length; BM= Bio
Number of spike per spike;
Thousand grain weight; TN

Appendix 13. Distributions of 47 barley populations over seven clusters by regions and altitudes based on quantitative and qualitative characters.

Regions	Altitude class	Clusters							Total for Altitude	Total for Region
		A	B	C	D	E	F	G		
Arsi	1			1					1	6
	2						1	1	2	
	3							3	3	
	4									
	5									
Bale	1	1							1	7
	2	4							4	
	3	2							2	
	4									
	5									
Gamo Gofa	1							1	1	2
	2							1	1	
	3									
	4									
	5									
Gojam	1				1				1	3
	2				1	1			2	
	3									
	4									
	5									
Gonder	1		1						1	3
	2		1						1	
	3					1			1	
	4									
	5									
Hararge	1	1							1	2
	2							1	1	
	3									
	4									
	5									
Shewa	1									6
	2									
	3				1				1	
	4				1		1	2	4	
	5							1	1	
Sidamo	1	2						1	3	3
	2									
	3									
	4									
	5									
Tigray	1			1	1				2	7

Appendix 13. Continued.

Regions	Altitude class	Clusters							Total for Altitude	Total for Region
	2				1			1	2	
	3			1	1				2	
	4							1	1	
	5									
Welega	1	1							1	3
	2		1						1	
	3									
	4							1	1	
	5									
Wollo	1			1					1	5
	2									
	3			1				1	2	
	4							2	2	
	5									
Total		11	3	5	7	2	2	17	47	47

Altitude class: 1= < 200 m asl, 2= 2001-2500 m asl, 3= 2501-3000 m asl, 4 = 3001-3500 m asl and 5= > 3500 m asl

Appendix 14. Diversity

No.	Accession number		A
1	PGRC/E	64335	0.
2	"	64339	0.
3	"	223208	0.
4	"	229789	0.
5	"	229790	0.
6	"	229791	0.
7	"	230003	0.
8	"	230006	0.
9	"	230591	0.
10	"	230594	0.
11	"	230595	0.
12	"	230614	0.
13	"	230653	0.
14	"	230814	0.
15	"	231455	0.
16	"	232216	0.

Appendix 14. Continued.

No.	Accession number		A
17	"	232217	0
18	"	232219	0
19	"	232221	0
20	"	232223	0
21	"	232224	0
22	"	234293	0
23	"	234307	0
24	"	234896	0
25	"	235066	0
26	"	235074	0
27	"	235079	0
28	"	235084	0
29	"	235096	0
30	"	235115	0
31	"	235239	0
32	"	235243	0
33	"	235254	0

Appendix 14. Continued.

No.	Accession number		A
34	"	235255	0
35	"	235261	0
36	"	235529	0
37	"	235541	0
38	"	235546	0
39	"	235552	0
40	"	235647	0
41	"	235648	0
42	"	235651	0
43	"	235653	0
44	"	235654	0
45	"	235731	0
46	"	235744	0
47	"	235745	0
Entire data			0

AC= Awn colour; AT=Awn typ

KRN= Kernel row number; LBA

Appendix 15. Rank co

Char. @	EST1	EST2	EST3	EST4	EST6	AL
EST1		-0.2	0.38	0.68	-0.17	0.51
EST2			0.4	0.45	-0.37	-0.32
EST3				0.59	-0.7	0.57
EST4					-0.13	0.33
EST6						-0.15
AL						
BM						
DH						
DM						
FFL						
GF						
HI						
NN						
NOKS						
NOSS						
PH						
SL						
SYW						
TGW						
TN						
YS						

** $P < 0.01$, * $P < 0.05$ @= see the footnote under Table

Appendix 16. Number of alleles in each locus for the seven barley populations

PGRC/E 229790

AAT-1 AA: 36
AAT-2 AA: 36
AAT-3 AA: 36
LAP-1 AA: 36
EST-1 AA: 34 BB: 01
EST-2 AA: 34 BB:01
EST-3 AA: 32 AB:02 BB:01
EST-4 AA: 30 BB:06
EST-6 AA: 30 BB:06

PGRC/E 230006

AAT-1 AA: 36
AAT-2 AA: 36
AAT-3 AA: 36
LAP-1 AA: 36
EST-1 AA: 36
EST-2 AA: 34
EST-3 AA: 33 CC:01
EST-4 AA: 35: CC: 01
EST-6 AA: 15 AB; 03 BB: 14

PGRC/E232216

AAT-1 AA: 36
AAT-2 AA: 36
AAT-3 AA: 36
LAP-1 AA: 36
EST-1 AA: 36
EST-2 AA: 36
EST-3 AA: 32 AB:03 BB:01
EST-4 AA: 36
EST-6 AA: 36

PGRC/E 232217

AAT-1 AA: 36
AAT-2 AA: 36
AAT-3 AA: 36
LAP-1 AA: 36
EST-1 AA: 36
EST-2 AA: 34 BB:02
EST-3 AA: 12 AC: 24
EST-4 AA: 32 BB:04
EST-6 AA: 10 AB: 13 BB: 13

PGRC/E234307

AAT-1 AA: 36
AAT-2 AA: 36
AAT-3 AA: 36
LAP-1 AA: 36
EST-1 AA: 36
EST-2 AA: 36
EST-3 AA: 36
EST-4 AA: 36
EST-6 AA: 15 AB: 03 BB: 14

PGRC/E 235546

AAT-1 AA: 36
AAT-2 AA: 36
AAT-3 AA: 36
LAP-1 AA: 36
EST-1 AA: 34 BB:02
EST-2 AA: 36
EST-3 AA: 30 AB:06
EST-4 AA: 34 CC:02
EST-6 AA: 36

PGRC/E235744

AAT-1 AA: 36
AAT-2 AA: 36
AAT-3 AA: 36
LAP-1 AA: 36
EST-1 AA: 34 BB: 02
EST-2 AA: 36
EST-3 AA: 29 AB: 07
EST-4 AA: 29 BB:07
EST-6 AA: 26 BB:09 CC:01