

MORPHOLOGICAL AND MOLECULAR DIVERSITY OF BARLEY (*Hordeum vulgare* L.)
GENOTYPES AND ASSESSMENT OF GENETIC IMPROVEMENT IN ETHIOPIA



Mihret Yirgu W/Semayat

A Dissertation Submitted to
The Department of Applied Biology
School of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree of Doctor of Philosophy in
Biotechnology (Specialization in Plant Biotechnology)

Office of Graduate Studies
Adama Science and Technology University

June, 2023
Adama, Ethiopia

Morphological and Molecular Diversity of Barley (*Hordeum vulgare* L.) Genotypes and
Assessment of Genetic Improvement in Ethiopia

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DECLARATION

I hereby declare that this dissertation entitled “**Morphological and Molecular Diversity of Barley (*Hordeum vulgare* L.) Genotypes and Assessment of Genetic Improvement in Ethiopia**” is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials used for this thesis have been duly acknowledged through appropriate citations.

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RECOMMENDATION

I/we, the supervisor(s) of this dissertation, hereby certify that I/we have read and revised the dissertation entitled “**Morphological and Molecular Diversity of Barley (*Hordeum vulgare* L.) Genotypes and Assessment of Genetic Improvement in Ethiopia**” prepared under my/our guidance by **Mihret Yirgu** submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biotechnology (Plant Biotechnology). Therefore, I/we recommend the submission of the dissertation to the department for further review and defense.

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APPROVAL SHEET

I/we hereby certify that the recommendations and suggestions made by the board of examiners are appropriately incorporated into the final version of the dissertation entitled **“Morphological and Molecular Diversity of Barley (*Hordeum vulgare* L.) Genotypes and Assessment of Genetic Improvement in Ethiopia”** by **Mihret Yirgu**.

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LIST OF ACRONYMS AND ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphisms
AMOVA	Analysis of molecular variance
ANOVA	Analysis of Variance
DArT	Diversity Array Technology
DBARC	Debre Birhan Agricultural Research Center
DzARC	Debre Zeit Agricultural Research Center
EBI	Ethiopia Biodiversity Institute
EIRC	Ethiopia Institute of Agricultural Research Center
GBS	Genotyping by Sequencing
HARC	Holetta Agricultural Research Center
IAR	Institute of Agricultural Research
Indels	Insertions and deletions
ISSR	Inter simple Sequence Repeats
KARC	Kulumsa Agricultural Research Center
KASPar	KBioscience Competitive Allele-Specific PCR
MAS	Marker Assisted Selection
MoARD	Ministry of Agriculture and Rural Development
MU	Mekelle University
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SARC	Sinana Agricultural Research Center
SNNPR	Southern Nations, Nationalities, and Peoples' Region
SNPs	Single Nucleotide Polymorphisms
SRARC	Sirinka Agricultural Research Center
SSRs	Simple Sequence Repeats

ABSTRACT

The effect of climate change together with the expected future demand represents a huge challenge for barley improvement in Ethiopia. Ethiopian barley germplasm is a potential source of useful traits to alleviate the production challenges of barley farming and to enhance yield productivity in both favorable and marginal environments. Performing morphological and molecular evaluation of barley genetic resources can provide an opportunity to develop improved cultivars that help to support production challenges and enhance its productivity. The overall objectives of the current study were to assess the morphological and molecular (SNP markers) diversity of barley genotypes and assess the genetic improvement of released food barley varieties in Ethiopia. Seventeen qualitative and twenty three quantitative traits of 125 barley genotypes were addressed using an alpha lattice design with two replications at Kulumsa Agricultural Research Center. The Shannon-Weaver diversity (H') index was used to estimate qualitative traits of diversity. Principal component and cluster analyses were also applied to assess the qualitative and quantitative traits of diversity. For the molecular diversity study, genotyping using 10K single nucleotide polymorphism (SNP) marker was used to assess genetic diversity and population structure. Analysis of molecular variance (AMOVA) was carried out to address the total genetic variation that occurred within accessions and among populations. Fifteen qualitative traits of barley accessions originating from various regions and altitude ranges revealed high morphological variations. However, two traits namely stem branching and lemma awns were monomorphic. The highest (0.94) overall mean of H' was obtained for glumes color, kernel row and kernel shape. The estimated H' ranged from 0.41 to 0.99 across regions, and 0.52 to 0.99 across altitude ranges with an overall mean of 0.76. Heat map analysis based on qualitative traits of barley has grouped the genotypes into three distinct clusters. Estimates of variance (ANOVA) in the quantitative traits showed a significant variability that the majority of the measured traits had a wide range of variances. The heritability ranged from 16% for the number of tillers per plant to 94% for days to heading and flowering. The average gene diversity was 0.253, polymorphism information content (PIC) of 0.216, and minor allelic frequency (MAF) of 0.118 which revealed a high genetic variation in barley genotypes. The genetic differentiation also showed the existence of variations, ranging from 0.019 to 0.117, indicating moderate genetic differentiation between barley populations. The heat map, principal components and population structure analysis further confirmed the presence of four distinct clusters. Thus, this finding confirmed that the Ethiopian barley genotypes showed substantial genetic variations across the regions and altitude ranges. In the genetic improvement study, the yield of barley over the breeding periods showed consistent improvement. Based on regression analysis, grain yield has increased linearly at the rate of $0.021 \text{ tons ha}^{-1} \text{ yr}^{-1}$. Over the past years, barley yield has significantly increased as a result of grain yield improvement. This improvement comes through consequent rounds of breeding involving the introduction of exotic and local collections in the barley breeding programs in Ethiopia. We recommend carefully considering this material as a source of genetic material for direct selection, the crossing of targeted traits to enhance productivity, tolerance to abiotic and abiotic stresses, and the quality of barley products using conventional programs and modern tools.

Keywords: Genetic differentiation, Heritability, Population structure, Qualitative traits, Shannon diversity index, Single nucleotide polymorphism

CHAPTER ONE

1. INTRODUCTION

1.1. Background of the study

Barley (*Hordeum vulgare* L.) is originated from the Eastern Mediterranean in an area known as the Fertile Crescent (Badr *et al.*, 2000; Pourkheirandish and Komatsuda, 2007; Azhaguvel and Komatsuda, 2007). It was first domesticated about 10,000 years ago from its wild relative, *Hordeum vulgare* ssp *spontaneum*, in the Middle East (Grando and Helena, 2005; Dai *et al.*, 2012). Barley (*Hordeum vulgare* L., $2n=2x=14$ chromosomes, 5.3 Gbp genome size) is one of the important cereal crops cultivated worldwide in a wide range of environments (Sato, 2020; FAOSTAT, 2023). It has a long evolutionary history and still exists in centres of diversity throughout its wide geographic and ecological ranges (Ceccarelli, 1994). Barley ranks fifth in world cereal cultivation after wheat, rice, maize and soybeans in terms of both area coverage and production. The worldwide annual production of barley has been estimated as 145.79 million metric tons with an area coverage of 49.17 million hectares with a per hectare yield of 2.96 metric tons (USDA, 2023). The Russian Federation is the first-largest barley producer in the world followed by France and Germany (FAOSTAT, 2023). Barley has been part of a sustainable food source for humans since pre-historic times. It is mainly used for human food, animal feed, malting and brewing (Newman and Newman, 2006; Baik and Ullrich, 2008). The crop is a major component of staple food in China, India, Morocco, Ethiopia and Eritrea, the mountainous regions of Bolivia, Ecuador, Colombia and Peru (Newman and Newman, 2006).

Ethiopia is one of the eight Vavilovian gene centers of the world and is recognized as a secondary center of diversity for barley crops due to a lack of wild relatives (Vavilov, 1951; Harlan, 1969), different sources agree that it has been grown for at least 5000 years. Though others signify the country is considered as a center of origin due to its wide and distinctive morphological diversity (Negassa, 1985). The long history of barley cultivation and the great agroecological and cultural diversity in the country has resulted in a huge number of barley landraces, primitive cultivars and endemic forms (Lakew *et al.*, 1997; Worede *et al.*, 2000). Besides, mutations, hybridization, gene recombination and natural selection in varied

environments play an important part in the diversification of Ethiopian barley (Lakew and Alemayehu, 2011). Barley is grown in a broad range of agro-climatic regions under several production systems. It can be cultivated in the highland of Ethiopia (Oromia, Amhara, Tigray and part of Southern Nations, Nationalities, and Peoples' regions) at altitude ranges of 1800 and 3500 meters above sea level, but the crop is most widely grown at altitudes between 2000 and 3000 masl (Lakew *et al.*, 1997; Mohammed *et al.*, 2016). It is cultivated both in Meher which relies on the main long rainy seasons from June to September and Belg which relies on the short rainfall season from March to May (Bekele *et al.*, 2005; Gebeyehu *et al.*, 2021). Barley is one of the earliest cultivated and the most essential staple food crop in Ethiopia (Bekele *et al.*, 2020; MoARD, 2021). It is used in numerous traditional foods such as *injera*, *kinche*, bread, *kolo*, *basso*, porridge and in making local drinks such as *tela*, *borde* and *areki* (Hailemichael and Sopade, 2011; Mohammed *et al.*, 2016). Barley is also used as feed for animals, as a thatching of roofs and as a source of income generation for many smallholder farmers in Ethiopia (Bekele *et al.*, 2020). It constitutes one of the major crops (Kifle, 2016) for food and industrial crops used as raw material for malting and brewing industries in Ethiopia (Assefa *et al.*, 2021; MoARD, 2021).

Barley ranks fifth, after teff (*Eragrostis tef*), wheat (*Triticum aestivum*), maize (*Zea mays*) and sorghum (*Sorghum bicolor*) both in area coverage and quantity of production in Ethiopia (CSA, 2021). The main producing regions are Oromia, Amhara, Tigray and Southern Nations Nationalities and Peoples (SNNP), which account for about 99.5% of the total annual barley production (Bakana *et al.*, 2018; MoARD, 2021). The total area covered by barley in the country is 0.96 million hectares and their annual production of about 2.35 million metric tons (USDA, 2023). The country is the second-largest barley producer in Africa after Morocco (FAOSTAT, 2023), accounting for about 25% of the total barley production in Africa. However, the average yield of barley in the country is 2.45 tons ha⁻¹ (USDA, 2023), which is less than the world average of 2.96 tons ha⁻¹ (USDA, 2023). This low productivity of barley in Ethiopia is due to various production challenges such as abiotic factors (water logging, poor soil fertility, drought and frost) and biotic stress such as net blotch (*Pyrenophora teres*), scald (*Rhynchosporium secalis*) and leaf rust (*Puccinia hordei*) (Mashilla, 2019; Gardi *et al.*, 2022). The low production is also attributed to several and interrelated factors such as extensive adoption of introduced cultivars, climate change that resulted in habitat destruction and

frequent drought, and development in agricultural technology including a shift towards using mechanized farming that thoroughly favored crops such as wheat that are mainly produced in bulk for commercial and manufacturing purposes (Wosene *et al.*, 2015; Tofu and Wolka, 2023; Gadissa *et al.*, 2021b). A huge annual rainfall irregularity and unpredictable drought stress during the rainy season are severe constraints to barley production. Besides, the low yield of barley is partially attributed to the cultivation of low-yielding farmer varieties (landraces), the poor level of soil nutrient contents mainly nitrogen and phosphorus and the low yield stability of improved barley varieties (Mulatu and Lakew, 2011; Bekele *et al.*, 2020). Hence, the use of genetic diversity is one of the main approaches to enhancing crop productivity and achieving food security (Hernandez *et al.*, 2020; Temesgen, 2021). A diverse collection of barley germplasm is collected from various geographical regions to identify desirable genetic traits that can be valuable for endorsement to maximize barley crop productivity (Dido *et al.*, 2021; Gadissa *et al.*, 2021a).

Barley germplasm is a potential source of useful traits to alleviate the production challenges of barley farming and enhance yield productivity. To cite the case, Ethiopian barley accessions are the source of valuable traits for Ethiopia and world agriculture, such as resistance and tolerance to disease and insect pests (Yitbarek *et al.*, 1998; Bonman *et al.*, 2005), high lysine and protein content (Munck *et al.*, 1970), and malting and brewing quality (Lance and Nilan, 1980). Thus, barley germplasm preserved in the Ethiopian Biodiversity Institute (EBI), with more than 17,000 accessions (www.ebi.gov.et), is a useful resource for genetic diversity and can play an important role in developing new crop varieties having desirable traits that help to support resilience and enhance barley yield potential.

The combined use of morphological and molecular diversity studies allows a more accurate study of the genetic diversity of crops (Mohammadi and Prasanna, 2003). Morphological diversity study is a very useful step before advanced tools such as molecular and genomic studies. Understanding the existing agro-morphological diversity in barley germplasm is an important step in enhancing the selection of breeding materials with varied genetic backgrounds and efficient management of crop genetic resources (Demissie and Bjørnstad, 1996; Nyiraguhirwa *et al.*, 2021). Moreover, molecular diversity study is a powerful tool to

detect the genetic diversity of plant genetic resources (Mondini *et al.*, 2009). It can provide an effective tool for the efficient selection of desired traits and for developing conservation.

Previous research reports showed high morphological (Demissie and Bjornstad 1996; Kebebew *et al.*, 2001; Assefa and Labuschagne, 2004; Abay *et al.*, 2009; Hadado *et al.*, 2009; Derbew *et al.*, 2013; Shumet and Tesema, 2014; Mekonnen *et al.*, 2015; Addisu *et al.*, 2018; Gadissa *et al.*, 2021a) and genetic (Assefa *et al.*, 2007; Demissie *et al.*, 1998; Hadado *et al.*, 2010; Abebe and Léon, 2013; Misganaw *et al.*, 2017; Dido *et al.*, 2021; Teklemariam *et al.*, 2022) variation of barley germplasm in Ethiopia. These diversity studies and the relationship encompassed crop genetic resources are prerequisites for designing efficient selection in crop breeding programs and for developing conservation strategies. However, the genetic diversity of Ethiopia's diverse barley germplasm collection has not been completely characterized and exploited using morphological and advanced molecular tools such as SNPs markers, there is still a limitation. Most of the early studies were based on either collection from distinct geographical regions only or a few samples collected from wider geographical ranges (Dido *et al.*, 2022). Besides, the large number of preserved barley germplasm at EBI (gene bank) has not been fully and widely exploited yet for their diversity (Fantahun *et al.*, 2023). Previously, Abdi (2011) indicated that there is limited information about the diversity and practically useful morphological traits in barley breeding in Ethiopia. Therefore, continuous characterization and evaluation of barley germplasm for morphological and molecular diversity can be an alternative approach to generate directly new adaptable varieties for smallholder farmers under both favorable and marginal production environments.

The evaluation of released varieties for associated changes in morphological traits can also provide a major desirable indication for future breeding programs (Yadav *et al.*, 2021). The estimation of the genetic improvement impact on morphological traits is determining crop yield that helped to identify traits of either limited or potential value for future improvement. Abeledo *et al.* (2003) and Waddington *et al.* (1987) noted that studies related to genetic improvement have an immense contribution to plant breeding, for crop yield improvement and evaluation of past works. Genetic improvement in yield potential and associated attributes produced by breeding and benefits obtained thereof have been documented in different crops in different countries by comparing old and recent varieties (Austin *et al.*, 1989). In Ethiopia,

genetic improvement studies have been reported in a few cereal crops including bread wheat (Amsal *et al.*, 1995), barley (Wondimu *et al.*, 2011), teff (Teklu and Tefera, 2005; Dargo *et al.*, 2016) and durum wheat (Temtme *et al.*, 2018). The genetic improvement of barley using the introduction of exotic germplasm and the local collections to advance yield potential and grain quality in Ethiopia (Hailu *et al.*, 1996). Several institutions and groups such as regional and national Agriculture Research Centers, higher learning institutes, Ethiopian Biodiversity Institute and Agricultural Transformation Agency are involved in barley improvement, aiming to enhance the productivity, quality, and sustainability of barley crops. The major aim of the improvement program is to develop new varieties with high yield potential and resistance to various abiotic and biotic stresses (drought, frost, poor soil fertility, salinity, diseases, insects and pests) (Yirga *et al.*, 1998; Bekele *et al.*, 2005; Mulatu and Lakew, 2011; MoARD, 2021). To achieve these, the barley program has developed numerous breeding strategies such as the selection of local and introduction of exotic materials in field trials and exploiting the best genetic materials. Due to this several lines, accessions and introductions have been evaluated annually at different locations representing major barley production systems to improve the productivity of barley. Currently, barley breeders developed several food barley varieties (MoARD, 2021). However, the genetic improvement in grain yield and associated changes in morphological traits of released food barley varieties have not been properly evaluated. Therefore, the present study was to assess the genetic improvement in grain yield and associated traits of food barley varieties in Ethiopia.

1.2. Statement of the problem

The highest genetic diversity of barley genetic resources with various useful traits exists in Ethiopia (Harlan, 1969; Lakew *et al.*, 1997; Abebe *et al.*, 2010). According to MoARD (2021), the highest variability of barley in terms of morphology, genetic races, disease-resistant lines, and endemic morphotypes presents in Ethiopia. This is due to diverse agro-ecologies, wide ranges of altitude, soil variability, climate and farming systems and topography together with geographical isolation (Eticha *et al.*, 2010; Mekonnen *et al.*, 2015).

In the past decades, the Ethiopian Biodiversity Institute (EBI) has collected and conserved more than 17,000 accessions sampled from various agro-ecologies across the country (EBI,

2018; www.ebi.gov.et). Due to this large number of barley accessions preserved *ex-situ* at the EBI, the genetic diversity study in barley germplasm is enormously vital. The world's large barley collection is approximately 23% of the total collection in the EBI, and 93% of the barley collection originated from Ethiopia (Konopka, 2007). The author states the Ethiopian barley collection is one of the world's ten largest barley collections and is used as a source of elite breeding material for national as well as global breeding programs. However, most of the collected and preserved barley collections at EBI have not been entirely studied yet for their morphological diversity (Abdi, 2011).

Earlier research reports showed the high morphological variation of barley germplasm in Ethiopia. Twenty two accessions for nine qualitative and quantitative traits (Kebebew *et al.*, 2001), forty four landraces for four qualitative and three quantitative traits (Assefa and Labuschagne, 2004), one hundred six landraces for eight qualitative traits (Hadado *et al.*, 2009), forty three landraces for eight qualitative traits (Shumet and Tesema, 2014), one hundred two accessions for six qualitative and ten quantitative traits (Mekonnen *et al.*, 2015), thirty-six landraces for eleven qualitative traits (Addisu *et al.*, 2018), and one hundred twenty landraces for seven qualitative and fourteen quantitative traits (Gadissa *et al.*, 2021a) were reported. While these reports realized the role of morphological diversity in past decades, in general, there is still a gap in the characterization and evaluation of the preserved germplasm and their valorization in barley breeding. These diversity studies on barley germplasm have evaluated a limited number of qualitative and quantitative traits, some accessions from specific collection periods, and specific regions of origin. Recently, Fantahun *et al.* (2023) indicated that the earlier research efforts made so far to uncover the characterization of barley focusing on the barley germplasm of the country were not much compared to the vast germplasm in Ethiopia. Delessa and Jemal (2021) also revealed that the EBI collected a huge number of accessions from barley-growing regions of Ethiopia which covers a broad range of agro-ecological conditions and is preserved in the national gene bank while the magnitude of the variation has not been yet completely studied and documented.

DNA markers such as amplified fragment length polymorphism (AFLP) (Assefa *et al.*, 2007), restriction fragment length polymorphisms (RFLPs) (Demissie *et al.*, 1998), simple sequence repeats (SSR) (Hadado *et al.*, 2010; Abebe and Léon, 2013; Misganaw *et al.*, 2017; Dido *et*

al., 2021 and 2022) have been employed and provided crucial information for barely breeding programs. A recent study by Teklemariam *et al.* (2022) has indicated a weak correlation between geographic distance and genetic differentiation of some Ethiopian barely germplasm collections using SNP markers. Currently, single nucleotide polymorphisms (SNPs) markers are the markers of choice for genetic diversity studies (Nantongo *et al.*, 2022). SNPs are the most abundant and robust, feasible for automated high-throughput genotyping (Alkan *et al.*, 2011), and highly reproducible and can be used to identify variants (Qiu *et al.*, 2012), replacing the earlier markers due to high throughput, efficient and cost-effective (Muñoz-Amatriaín *et al.*, 2014; Alam *et al.*, 2018). However, the genetic diversity of Ethiopia's diverse barley germplasm collection has not been adequately characterized and exploited using SNP markers. Therefore, assessment of the genetic diversity and characterization of barley genotypes can be an important source of information in order to discover and exploit novel alleles to be used in breeding programs.

Barley yield is a complex trait (Wang *et al.*, 2021) and variety yield increment depends on genetic progress and the combination of yield traits (Hu *et al.*, 2018; Rodrigues *et al.*, 2020). Thus, tracing back barley varieties from different years of their release in common environments is the most inclusive and direct way that has been used to estimate progress in yield improvement (Dargo *et al.*, 2016). Genetic improvement made in terms of yield potential and related traits produced by diverse crop improvement program, and the benefits obtained has been evaluated and reported in different countries (Donmez *et al.*, 2001; Abeledo *et al.*, 2003; Yadav *et al.*, 2021; Cossani *et al.*, 2022) including Ethiopia (Amsal *et al.*, 1995; Teklu and Tefera, 2005; Dargo *et al.*, 2016; Temtme *et al.*, 2018). A previous study by Wondimu *et al.* (2011) conducted a genetic improvement study using only eight released barley varieties during the period from 1970 to 2006. However, this study on Ethiopian barley has evaluated a limited number of improved barley varieties in a single season. Presently, more than forty-five food barley varieties have been released and registered by the regional and national Agriculture Research Centers and higher learning institutes in Ethiopia (MoARD, 2021). However, the so far morphological and molecular diversity studies on barley in Ethiopia are relatively low compared to the huge germplasm collection the country is endowed with. Thus, the present study was carried out to investigate the morphological and molecular diversity and genetic improvement of barley germplasm in Ethiopia.

1.3. Objectives

This study was conducted with the following objectives:

General Objective

- The general objective of this research was to study the diversity and relationship of barley genotypes using morphological and SNP markers and genetic improvement of released food barley varieties in Ethiopia.

❖ Specific objectives

- To assess the extent and patterns of qualitative traits variation in Ethiopia's barley germplasm.
- To study distribution and patterns of quantitative trait variations in Ethiopian barley germplasm.
- To determine the genetic diversity and population structure of Ethiopian barley germplasm using Single Nucleotide Polymorphism (SNP) markers.
- To assess the genetic improvement of grain yield and the associated changes in morphological traits of released food barley varieties in Ethiopia.

1.4. Significance of the study

Ethiopia is recognized as a center of diversity for many cereal crops such as barley (Harlan, 1969). Hence, studies on diversity and the pattern of variation of this crop are essential for the appropriate management and better exploitation of the gene pool (EBI) (Assefa, 2003). The knowledge of existing genetic diversity is important for the efficient management of crop genetic resources (Bekele and Endashaw, 2014). Besides, assessment of the extent of genetic variations within barley is of the most important for breeding programs and the conservation of genetic resources and is mainly useful as a general guide in the choice of parents for the development of hybrids (Ahmad *et al.*, 2008). Earlier studies have shown that the highest genetic diversity within the Ethiopian barley genetic resource for various useful traits can be valorized for barley breeding (Harlan, 1969; Lakew *et al.*, 1997; Abebe *et al.*, 2010). Hence, analyzing the morphological and molecular diversity and its genetic relationship encompassed in barley crop genetic resources is a prerequisite for designing efficient selection in crop breeding programs and for developing conservation and valorization strategies. It is valuable

for the identification of novel alleles and provides a good opportunity for plant breeders to develop new cultivars with desirable traits.

Studies on the genetic improvement achieved over time from a breeding program are also indispensable (Abeledo *et al.*, 2003; Lange and Federerizzi, 2009), to develop effective and efficient breeding strategies by assessing the effort of past works in genetic yield potential. Knowledge about genetic improvement over the years due to related changes in morphological traits is very important for a critical understanding of breeding challenging factors (Yadav *et al.*, 2021) and helps for breeders to redefine and adopt appropriate breeding strategies (Lange and Federerizzi, 2009). Besides, the understanding of the past achieved work of yield in the previously developed cultivars has practical worth for the use of consistent improvement of new varieties (Wondimu *et al.*, 2011; Miroslavljević *et al.*, 2016). A good understanding of past improvement is markedly important for an effective barley crop research program. Therefore, the current study of grain yield improvement within released food barley varieties is fundamental for the future breeding of barley crops in Ethiopia.

1.5. Scope of the study

The scope of the study is to characterize the barley genotypes using both morphological and molecular (SNP) markers to determine the diversity and relationship as well as the genetic improvement of released food barley varieties in Ethiopia. The study was limited to two consecutive main cropping seasons in 2020 and 2021 under rain-fed conditions at Kulumsa Agricultural Research Center. One hundred twenty seven barely panels comprised of eighty-five barley accessions and thirty-eight improved varieties including two local checks and two wild crosses of barley were used in this study.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Origin and domestication of barley

Barley is first domesticated about 10,000 years ago from its wild relative, *Hordeum vulgare* ssp. *spontaneum*, in the area of the Middle East known as the Fertile Crescent (Grando and Helena, 2005; Dai *et al.*, 2012). It is believed to be among the oldest crop species and it was domesticated from the large-seeded wild barley (*Hordeum vulgare* ssp. *spontaneum*) (Pourkheirandish and Komatsuda, 2007). The genus *Hordeum* has the centre of diversity in central and southwestern Asia, Western North America, Southern America, and the Mediterranean (Von Bothmer, 1992).

Barley has a long evolutionary history and still exists in centers of diversity throughout its wide geographic and ecological range (Ceccarelli, 1994). Cultivated barley is grown in a range of diverse environments that vary from sub-arctic to subtropical, with greater concentration in temperate areas and high altitudes of the tropics and subtropics (Leino and Jenny, 2010; Hadado *et al.*, 2010). Barley is one of the oldest cultivated crops and has been grown in Ethiopia for at least 5000 years (Harlan, 1969). The country is recognized as a center of diversity for barley crops (Harlan, 1969; Asfaw, 1988; Worede *et al.*, 2000). Many explanations and views strengthen the argument that barley originated independently in Ethiopia (Negassa, 1985). It is grown in a broad range of agro-ecology for centuries and it has a vast diversity in Ethiopia with several distinctive morphological appearances from the rest of the world barley collections (Milner *et al.*, 2019)

2.2. Taxonomy of barley

Barley belongs to the genus *Hordeum* in the tribe *Triticeae* of the grass family, Poaceae/*Gramineae*. The *Triticeae* tribe is a temperate plant group including numerous economically important cereals and forages (Von Bothmer, 1992). The genus *Hordeum* is among the *Triticeae* as it includes both annual species (*H.vulgare* and *H. marinum*) and perennial species (*H. bulbosum*). The cultivated barley (*Hordeum vulgare* L. ssp.) is self-

pollinating, with a diploid ($2n=2x=14$ chromosomes) species. Besides, they exist in tetraploid ($2n=4x=28$) and hexaploid ($2n=6x=42$) forms. Its wild progenitor is *Hordeum vulgare* L. ssp. *spontaneum* species (Komatsuda *et al.*, 1999).

2.3. Botanical description of Barley

Barley is highly variable and differs greatly in their morphological traits (Briggs, 1978). The stems are erect, separated by the nodes and grow to 60 to 120 cm tall. The stem of the barley crops has on average five to seven joints separated by the nodes, which grow the leaves. It has few alternate leaves, which alternate and the upper leaf is close to the spike (Fettell *et al.*, 2010). The leaf consists of a sheath, which squashes around the lately emerging leaf. Tillers are shoots or lateral branches that arise from buds in the axil of the leaves at the base of the main stem. The primary tillers are produced from the leaves of the main stem and can form their own, secondary tillers (Newman and Newman, 1991; Fettell *et al.*, 2010).

The inflorescence of barley is known as the head or spike. The flowering units called the spikelets, are attached to the middle axis, or rachis, which is the extension of the stem that supports the spike (Williams-Carrier *et al.*, 1997; Bothmer *et al.*, 2003). Barley comes in two forms, determined by the number of rows of flowers on its flower spike. Two-rowed barley has central florets that produce kernels and lateral florets that are normally sterile. However, six-rowed barley has its spike notched on opposite sides, with three spikelets at each notch, each containing a small individual flower, or floret, that develops a kernel (Bothmer *et al.*, 2003; Komatsuda *et al.*, 2007). The flowering glumes (husk) adhere to the kernel. The kernel is the reproductive part of barley crops (Fettell *et al.*, 2010).

Overall, barley has three distinctive development phases, namely the vegetative, reproductive and grain-filling phases. The vegetative phase hosts the germination of the seed, the growth and emergence of seedlings and the tillering phase where the roots commence establishing in the soil. The second phase, the reproductive phase includes the start of budding flowers, the stems elongate and the plant starts to stand large. The flowers begin to open and pollen grains begin to germinate. The final phase is grain filling, where the grain physiologically matures with cellular divisions (Pins and Kaur, 2006).

2.4. Global barley distribution and production

Barley is grown in broad geographic and ecological ranges around the world (Ceccarelli, 1994, Ceccarelli *et al.*, 2008). It is one of the best-adapted crops to diverse cultivation conditions. It successfully grows in the arid climates of the Sahara, the Tibetan plateaus, the highlands of the Himalayas, and the Andean countries (Grando and Helena, 2005). It has substantial economic importance in agriculture and industry in several countries (Baik and Ullrich, 2008; Newman and Newman, 2006). The worldwide production of barley accounted for 145.9 million metric tons in 2021/2022 (FAOSTAT, 2023). Barley production in the world over the years has increased (Figure 1). Currently, barley has become the fifth most important produced cereal crop in the world after wheat, maize, rice and soybean. The Russian Federation is the first-largest barley producer in the world followed by Germany and France (FAOSTAT, 2023). Spain, Ukraine, Canada, Australia, the United Kingdom, Turkey and Argentina are also the major barley producers in the world.

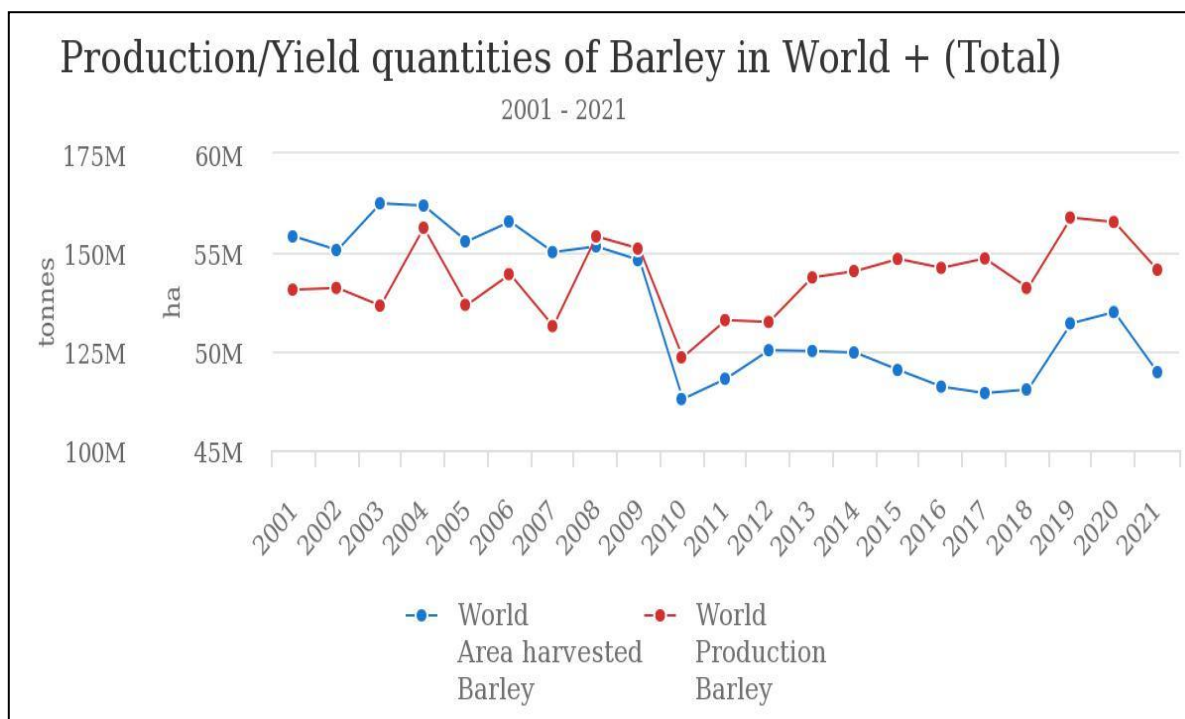


Figure 1. Production/yield quantities (in million tons) of barley in the World

Source: FAOSTAT, 2023

2.5. Barley cultivation and production in Ethiopia

Barley ranks fifth, after teff (*Eragrostis tef*), wheat (*Triticum aestivum*), maize (*Zea mays*) and sorghum (*Sorghum bicolor*) both in the area and quantity of production in Ethiopia (CSA, 2021). It is grown in a broad range of agro-climatic regions under several production systems, on steep slopes, in degraded soils, and in stressed areas with occasional drought and frost where other cereals fail to grow (Bekele *et al.*, 2005). It can be cultivated in the highland of Ethiopia at altitude ranges of 1800 and 3500 meters above sea level, but the crop is most widely grown at altitudes between 2000 and 3000 masl (Lakew *et al.*, 1997; Mohammed *et al.*, 2016). The annual production is better distributed across seasons, agro-ecologies, and production systems than any other cereal crop (Bekele *et al.*, 2005). The main producing regions are Oromia, Amhara, Tigray and Southern Nations Nationalities and Peoples (SNNP), which account for about 99.5% of the total annual barley production (Bakana *et al.*, 2018; MoARD, 2021). The area devoted to barley production in Ethiopia over the past years has increased in rate (Mulatu and Lakew, 2011), but below that of other cereals such as teff, wheat, maize and sorghum (Figure 2).

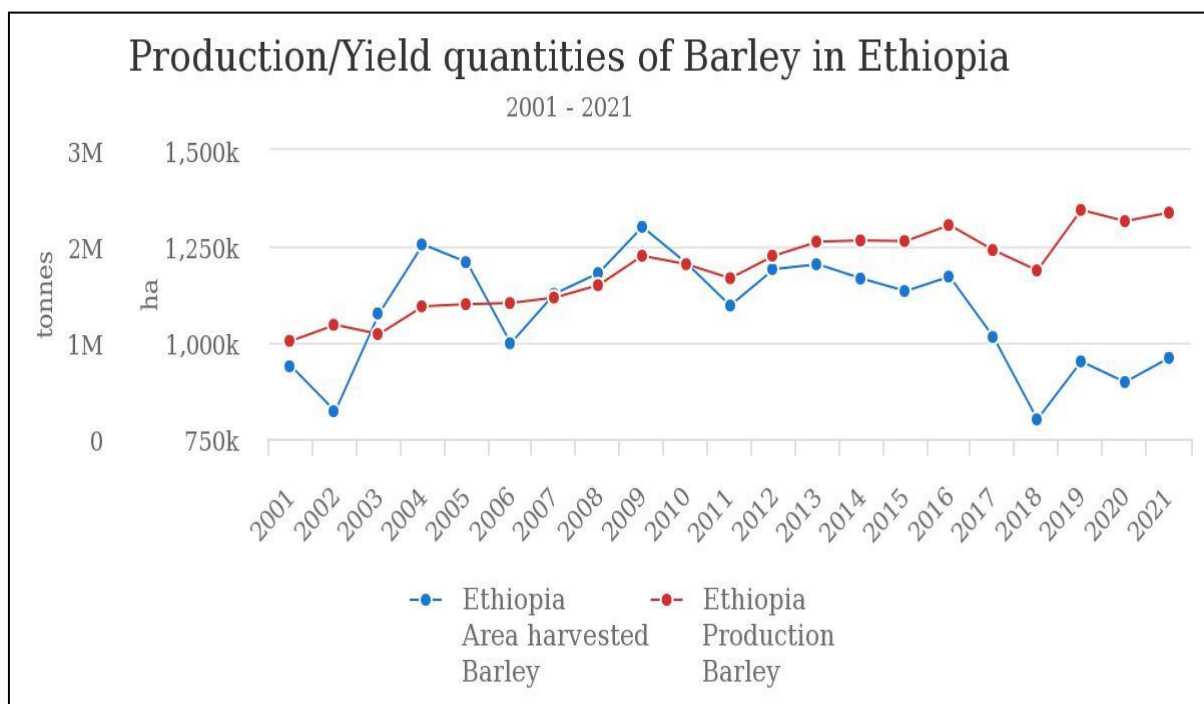


Figure 2. Production/yield quantities (in million tons) of barley in Ethiopia

Source: FAOSTAT, 2023

In the 2020/21 cropping season, the national level was 926, 106.90 ha of land covered with barley and over 2.34 million tons are produced (MoARD, 2021). This indicates Ethiopia is the second-largest barley producer in Africa after Morocco (FAOSTAT, 2023), accounting for about 25% of the total barley production in Africa. However, the average yield of barley in the country is 2.11 tons ha⁻¹ (CSA, 2021), which is lower than the world average of 3.1 tons ha⁻¹ (FAOSTAT, 2020).

2.6. Importance of barley

Barley is mainly used in making alcoholic beverages such as barley wine in Babylonia in 2800 BC and the staple diet of those living in ancient Egypt, Greece and China (Mulatu and Lakew, 2011). It is an important food grain in several regions of the world including Ethiopia. According to MoARD (2021), barley is one of the earliest cultivated and the most essential staple food crop in Ethiopia. Based on their uses mainly categorized as food and malting barley while the major proportion of barley production area is allocated for food barley (Wondimu *et al.*, 2011). It is grown mostly for local food and beverage utilization. Barley is used in numerous traditional foods such as *injera*, *kinche*, bread, *kolo*, *basso*, porridge and in making local drinks including *tela*, *borde* and *areki* (Hailemichael and Sopade, 2011; Mohammed *et al.*, 2016). It is also used as feed for animals, as a thatching of roots and as a source of income generation for many smallholder farmers in Ethiopia (Bekele *et al.*, 2020). Barley constitutes one of the major crops (Kifle, 2016) for food and industrial crops, used as raw material for malting and brewing industries in Ethiopia (CSA, 2021).

2.7. Barley production constraints in Ethiopia

Many field crops were introduced centuries ago and have since adapted and developed wide genetic diversity in Ethiopia. However, this wide range of genetic diversity has been eroded due to various biotic and abiotic factors (Abdi, 2011; Mulatu and Lakew, 2011). The productivity of barley in production fields has remained low due to a lack of improved varieties, disease, insect and pest problems, weed competition, and poor soil fertility as major production constraints in barley improvement in the country (Mulatu and Lakew, 2011). Numerous factors including water logging, drought, frost and poor crop management practices have also contributed to the low productivity of barley in Ethiopia (Yirga *et al.*, 1998). Many

insect pests that attack barley have been reported in Ethiopia, among these Russian wheat aphids (RWA) and barley shoot fly is the major ones in barley production (Tafa *et al.*, 2004). Diseases such as net blotch (*Pyrenophora teres*), scald (*Rhynchosporium secalis*) and leaf rust (*Puccinia hordei*) are major biotic constraints on barley production in Ethiopia (Yitbarek *et al.*, 1998; Mashilla, 2019).

2.8. Barley breeding in Ethiopia

Barley improvement was started at Debre Zeit Agricultural Research center in the 1950s through the introduction of exotic germplasm and collections from local landraces (Hailu *et al.*, 1996), to improve yield potential and constancy with high-quality. In 1966 with the establishment of the Holetta Agricultural Research Center (HARC), then the Institute of Agricultural Research (IAR) currently the Ethiopian Institute of Agricultural Research (EIAR), was responsible for managing the national crop improvement efforts on barley from 1967 to 2005 (Mulatu and Lakew, 2011). At present, barley research is coordinated by Holetta Agricultural Research Center in strong collaboration with various governmental and non-governmental organizations (Bekele *et al.*, 2005). A comprehensive research scheme was set up with the bulk of the work being conducted at Holetta such as hybridization (selection from huge collections of local and introduced genetic materials), variety trials and seed multiplication (Mulatu and Lakew, 2011). Besides, a hybridization and selection program has been carried out on barley by aiming to improve grain yield, lodging resistance, disease and insect pest resistance, stress tolerance (drought, water logging and frost) and grain production in major barley-growing quality (food, malting or feed).

Ethiopia is known for having a huge amount of barley genetic diversity (Bekele *et al.*, 2005). The existence of a wide genetic diversity has special significance for barley breeding in the country (Worede *et al.*, 2000). The phenotypic variations among barley accessions are important to identify the genetic potential of parental lines and to increase the efficiency of the barley breeding programs (Mekonnen *et al.*, 2015). The major objective of the barley breeding program in Ethiopia is the selection for high grain yield with resistance and or tolerance to biotic and abiotic stresses among exotic and landrace genotypes (Yirga *et al.*, 1998). Due to this several lines, accessions and introductions have been evaluated annually at different sites

representing major barley production systems to improve the productivity of barley in Ethiopia. Besides, the existence of genetic variability and favorable access to germplasm exchange, barley breeders have to date developed many barley varieties (Bekele *et al.*, 2005). Currently, forty nine food and thirty malt barley varieties have been released and registered by the Federal and Regional Agriculture Research Centers and higher learning institutes in Ethiopia from 1973 to 2020/21 (MoARD, 2021).

2.9. Genetic diversity of barley in Ethiopia

Ethiopia is well known for being one of the Vavilovian centers of genetic diversity (Vavilov, 1951). It is considered as one of the richest genetic diversification centers of many crop species and their wild relatives (Hancock, 2004). Earlier studies brought to confirm the existence of a huge number of botanical forms and morphological types of Ethiopian barley germplasm. There is high diversity and several distinctive forms of hulled (covered) and hull-less (naked) types with six-rowed, two-rowed, and irregular morphologies, and varied spike shapes, density and grain colors (Harlan, 1969; Asfaw, 1988). The authors showed that the *deficiens* and *labile* botanical forms are endemic and the most common in Ethiopian barley.

The Ethiopian barley germplasm is also a source of valuable traits for Ethiopia and world agriculture (Vavilov, 1951; Harlan, 1969; EBI, 2018). Its desirable traits such as resistance to disease and insect pests (Yitbarek *et al.*, 1998; Bonman *et al.*, 2005), high lysine and protein content (Munck *et al.*, 1970), and malting and brewing quality (Lance and Nilan, 1980). Besides, traits such as disease resistance to barley yellow dwarf virus, powdery mildew, leaf rust, loose smut, and barley stripe mosaic virus, and content are unique to Ethiopia (Bekele *et al.*, 2019). This is due to diverse agroecologies, wide ranges of altitude, soil variability, climate and farming systems, and topography together with geographical isolation (Eticha *et al.*, 2010; Mekonnen *et al.*, 2015). This diversity provides security for the farmer against biotic and abiotic stress factors. Moreover, the diversity of germplasm is the greatest way to achieve proficient management of crop genetic resources to improve breeding value (Al-Abdallat *et al.*, 2017; Ketema *et al.*, 2020).

More than 90% of the barley grown in the country is from farmers' landraces, rather than improved or breeder-produced varieties (Kebebew *et al.*, 2001). Ethiopia contributed a major share of accessions to the world's ten largest collections (Konopka, 2007). Of the world's total of 129, 000 accessions, 51% are considered to be landraces, mostly originating from developing countries, of which Ethiopia accounts for 23.3% (Konopka, 2007). The world's large barley collection is approximately 23% of the total landraces in the genebank, and 93% of the barley collection originated from Ethiopia. Ethiopian landraces are the backbone of agricultural systems in Ethiopia and as a potential source of novel alleles (Ceccarelli and Grando, 1996; Hadado *et al.*, 2010). They are a key component of agro-biodiversity and they represent a crucial reservoir of genetic diversity for crop improvement (Hadado *et al.*, 2010). Barley is therefore a good nominee crop to study genetic diversity from its wild relatives to locally adapted germplasm (Russell *et al.*, 2004).

2.10. Genetic markers

2.10.1. Morphological markers

The morphological marker provides a simple, direct rapid and inexpensive way of characterizing germplasm (Mutengwa, 2004). It is a conventional approach and is viewed as the best determinant of the agronomic value of crop plants (Cholastova and Knotova, 2012). These markers used mostly contain visible traits (Jiang, 2010), and they can visually distinguish qualities and other important phenotypic traits. They are easy to use, no need for sophisticated instruments (Missihoun *et al.*, 2015). It is useful in estimating genetic diversity because of its visually differentiated and ease of achieving the variation of the agronomic traits (Liu *et al.*, 2011). It is highly recommended at the beginning before biochemical and molecular studies and markers allow the assessment of genetic variability based on individual phenotypic differentiation (Bekele and Endashaw, 2014). However, there are limitations associated with these markers such as limited in number, influenced by the plant growth stages and various environmental factors (Eagles *et al.*, 2001; Bekele and Endashaw, 2014).

Abebe *et al.* (2010) indicated the high morphological diversity of barley landraces from North Shewa, Oromia and Amhara regions. Likewise, Mekonnen *et al.* (2015) revealed the existence of morphological diversity of traits in Ethiopian food barley landraces in relation to regions of

origin and altitude ranges. In the same way, the high diversity of barley landraces in the highlands of Ethiopia was revealed by Addisu *et al.* (2018) and Gadissa *et al.* (2021a). Derbew *et al.* (2013) showed the presence of high genetic variability in the morpho-agronomic traits of barley in the southern region of Ethiopia. The author revealed that most of the barley landraces were amber or white in awn color. In contrast, Bouhaouel *et al.* (2019) showed yellow awn color was dominant in Tunisian barley accessions.

Spike-related traits are useful traits of smallholder farmers in Ethiopia (Mancini *et al.*, 2017) and key traits for yield enhancement in breeding (Gao *et al.*, 2015; Allel *et al.*, 2017). Kebebew *et al.* (2001) reported that intermediate and lax types of spike density were dominantly found in Ethiopian barley. Bouhaouel *et al.* (2019) and Tolbert *et al.* (1979) also indicated the predominance of the six-rowed in the Tunisian barley collection and the world barley collection, respectively. High variations in plant height, days to heading, days to maturity and thousand kernels weight existed in Ethiopian barley (Abebe *et al.*, 2010). Ahmad *et al.* (2008) found greater variations for grains per spike, thousand kernels weight and plant height in barley genotypes. The highest diversity is found for tiller number and grain yield per plant while a thousand kernels weight has shown moderate diversity among the genotypes of barley in India (Pawan *et al.*, 2017). Demissie and Bjørnstad, (1996) reported that white kernel color was dominant in Ethiopian barley accessions, which may be associated with Ethiopian smallholder farmer selection pressures operating for the white phenotypic class. Similarly, the majority of Ethiopian barley landraces have white kernel color as reported by Assefa and Labuschagne (2004) and Hadado *et al.* (2009). On the contrary, the barley accessions were largely characterized by the grey kernel color in Tunisia (Bouhaouel *et al.*, 2019).

2.10.1.1. Estimates of heritability

Heritability is a measure of the value of selection for particular traits and an index of their transmissibility (Johnson *et al.*, 1955; Wills, 2007). It is a good index of the level of transmission of the traits from parents to their progeny. Gregorio *et al.* (2016), the estimated heritability provides good insight into the quality of a breeding program for traits. It plays a predictive role in improvement, expressing the consistency of phenotype as a guide to its breeding value (Tazeen *et al.*, 2009). There is a direct relationship between heritability and

response to selection, which is referred to as genetic advance (GA) (Johnson *et al.*, 1955; Larik *et al.*, 2000). Heritability is classified into broad sense and narrow sense heritability. Narrow sense heritability is a ratio of additive genetic variance to the total phenotypic variance and gives the best estimate of heritable variance, which can be fixed by selection (Sleper and Poehlman, 2006).

Broad sense heritability is the proportion of the total genetic variance to the phenotypic variance of individuals (Wray and Visscher, 2008). Moreover, genetic variance namely additive, dominance and epistatic genetic variances for traits are essential to estimate breeding values and for making maximum selection decisions (Monnahan and Kelly, 2015; Vitezica *et al.*, 2018). The additive genetic effect is part of an individual's entirety genetic effect that is transmissible across generations from parents to progeny. The additive genetic effects are the major source of genetic differences exploited by most crop breeding programs. However, non-additive genetic effects (dominance and epistasis) can be distinguished as the residual genetic effect after fitting additive substitution effects (Mäki-Tanila *et al.*, 2014; Vitezica *et al.*, 2018). The interactions among alleles at the same locus (dominance) and the interactions of alleles between loci (epistasis) might play a vital role in heterosis, assortment, and natural selection (evolution) (Yu *et al.*, 1997; Berlin *et al.*, 2019).

Robinson (1966) categorized broad sense heritability into high heritability (>75%), moderate heritability (50-75%) and low heritability (<50%). Hence, selection for traits with a heritability of more than 75% should be practically more efficient than for low heritability (<50%). Traits with high heritability have genetic variations accounting mainly for the variation due to genetic factors. However, traits with low heritability have phenotypic differences that are greatly influenced by environmental effects (Abinasa *et al.*, 2011; Xu *et al.*, 2017). Low heritability and selection could be challenged due to the masking effect of the environment on genetic effects. Shegaw *et al.* (2013) and Azeb *et al.* (2016) reported high heritability values together with relatively high genetic advances in barley. This high genetic advance coupled with high heritability values offers the most useful condition for selection (Dinsa *et al.*, 2018). Derbew *et al.* (2013) revealed that high heritability and high genetic advance as a percent of mean were studied for susceptibility to lodging, spikelet per spike and grain yield per plant. Dinsa *et al.* (2018) reported the highest heritability for days to heading and thousand kernels

weight. In the same way, the greatest heritability estimates in days to heading, thousand kernels weight, plant height, biomass yield, awn length, and harvest index were studied in barley genotypes by Haile *et al.* (2015). High heritability values for days to 50% flowering, days to 75% maturity, number of tillers per plant, spike length, spikelet per spike, number of kernels per spike, thousand kernels weight and grain yield were also reported by Delessa (2021). In addition, Addisu and Shumet (2015) revealed high heritability for biomass per plant, plant height, maturity date, thousand seed weight, grain filling period, number of grains per spike and grain yield.

2.10.1.2. Correlation coefficients of variation

Correlation among traits has resulted from the association between genes effect, environmental effects and/or both. According to Allard (1960), there are two types of correlations; genetic correlation is a measure of the degree to which the same gene or strongly linked genes cause concurrent variation in two diverse traits. Phenotypic correlation measures the extent to which two observed traits are linearly related. Traits of cereal crops are commonly associated with either positive or negative and relationships among such traits are frequent features in crop improvement. The information on their coefficients offers a measure of inherited correlation among traits to determine the essential traits to be considered in an improvement program (Yadav *et al.*, 2011). Azeb *et al.* (2016) revealed that significant positive phenotypic and genotypic relationships were found between grain yield with biomass yield and thousand kernels weight. Geleta *et al.* (2019) showed significant positive genotypic and phenotypic correlations of thousand kernels weight, biomass yield, awn length, and plant height with grain yield and a negative significant correlation of yield with days to heading and physiological maturity. In contrast, Gadissa *et al.* (2021a) indicated that the phenotypic and genotypic correlations of grain yield showed a strong correlation with the number of grains per plant, thousand kernels weight, and effective fertile tillers.

2.10.1.3. Estimating genetic improvement of barley

The genetic improvement achieved over time from a breeding program point of view is extremely essential to build up effective and efficient breeding strategies by assessing the efficiency of past breeding works in yield potential (Abeledo *et al.*, 2003). When the breeder

intends to know the level of genetic improvement achieved not from a single or a few cycles of selection but from a long-term breeding effort made over time by a breeding program. Hence, the estimation of genetic improvement from a breeding program enables to know the cumulative impacts of long-term breeding efforts. Based on the result of such estimation, the whole breeding program may be revised and more effective and efficient breeding strategies may be developed (Ustun *et al.*, 2001).

A genetic improvement study serves to determine the success of a breeding program. Lange and Federerizzi (2009) indicated that genetic improvement study enables breeders to compare breeding enrichment realized from different breeding strategies applied at different times as well as diverse environments. Accordingly, a good understanding of past improvement is useful and important. So, the information helps breeders to redefine, elaborate and adopt appropriate breeding strategies. However, the lack of such valuable information may adversely affect future breeding advancement (Lange and Federerizzi, 2009). The performance of cultivars in common environments regressed over years of release as a continuous quantitative variable provided the most direct estimate of genetic progress from breeding and has widely been used in different crops (Cox *et al.*, 1988).

The genetic improvement achieved over time from the breeding of various crops in Ethiopia has been studied. Teklu and Tefera (2005) evaluated using one local check and ten tef varieties released in the period from 1960 to 1995. In the same way, Dargo *et al.* (2016) conducted thirty three tef varieties released over the period 1970-2012 to estimate the progress made in improving grain yield potential and associated agronomic traits. Wondimu (2010) evaluated grain yield improvement using one local check and eight released barley varieties during the period from 1970 to 2006. The author revealed an increment in barley grain yield of modern varieties over the local check, *Balami*, and the oldest improved variety. Rodrigues *et al.* (2020) studied the genetic improvement in barley grain yield from 1968 to 2008 in Brazil to determine the physiological traits related with the increase in grain yield.

2.10.1.4. Yield potential and improvement through breeding

Yield is the fundamental trait characterizing the economic value of any commercial variety (Hudzenko *et al.*, 2022). Yield potential is the yield of a cultivar when grown in environments to which it is adapted, with nutrients and water non-limiting (Evans and Fischer, 1999). In measuring improvement in grain yield potential, complications can arise as a result of the possibility of interactions between cultivar and environment or growing conditions. Grain yield is a polygenically controlled complex trait and is dependent on several component traits, which are quantitatively inherited (Singh, 2001). It is controlled by multiple genes and is mainly influenced by environmental conditions (Shi *et al.*, 2009).

Genetic gain of barley yield potential was maintained nearly constant and since then it increased at a rate of 41 kg/ ha year⁻¹ until the 1970s (Abeledo *et al.*, 2003). The grain yield of tef was estimated to have risen from 3425 to 4599 kg/ha for 35 years of breeding and a substantial increase of modern teff varieties over the older ones (Teklu and Tefera, 2005). Similarly, Wondimu *et al.* (2011) found that grain yield increased from 3314.8 kg/ha to 5088.6 kg/ha during the period from 1970 to 2006 in Ethiopia barley. The analysis of dry land data revealed an average yield increase of about 46 kg ha⁻¹ yr⁻¹ between 1957 and 2008 (Yared and Staggenborg, 2009). Likewise, teff grain yield increased from 3848.68 kg ha⁻¹ to 4934.4kg ha⁻¹ over the past 42 years (Dargo *et al.*, 2016). The yield potential of bread wheat varieties increased from 2523.5 kg ha⁻¹ in 1960 to 3294.8 kg ha⁻¹ in 1990. Donmez *et al.* (2001) showed the grain yield improvement ranged from 2718 kg ha⁻¹ for older wheat variety to 4987 kg/ha for recently released varieties.

2.10.2. DNA markers

Molecular markers are defined as a fragment of DNA revealing variations, which can be used to detect polymorphism among different genotypes (Jiang, 2010). It is described as a fragment of DNA that is linked to a certain location within the genome. They offer advantages for comparison over morphological and biochemical markers with respect to cost-effectiveness, testing at any stages of development, rapidity, environment-independent expression and easy detection of polymorphism (Ghosh and Sarkar, 2001; Brumlop and Finckh, 2011). It became possible to select desirable traits with more direct and easily detectable DNA markers. It

represents one of the most powerful tools for the analysis of genomes and enables the association of heritable traits (Mishra *et al.*, 2014). Molecular marker has proven to be powerful tools in the assessment of genetic variation and the explanation of genetic relationships within and among species (Chakravarthi and Naravaneni, 2006). Earlier studies have used DNA markers to determine the genetic diversity of various crops. Markers developed such as amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), diversity array technology (DArT), and single nucleotide polymorphisms (SNPs) have been used to study the genetics of crops (Kesawat and Das, 2009). Besides, marker systems are classified into low-throughput marker systems (RFLPs), medium-throughput marker systems (RAPDs, SSRs and AFLPs), high-throughput marker systems (SNPs, DArT, KASPar) and Ultra high throughput marker/assay (like Infinium assays, GBS) (Figure 3).

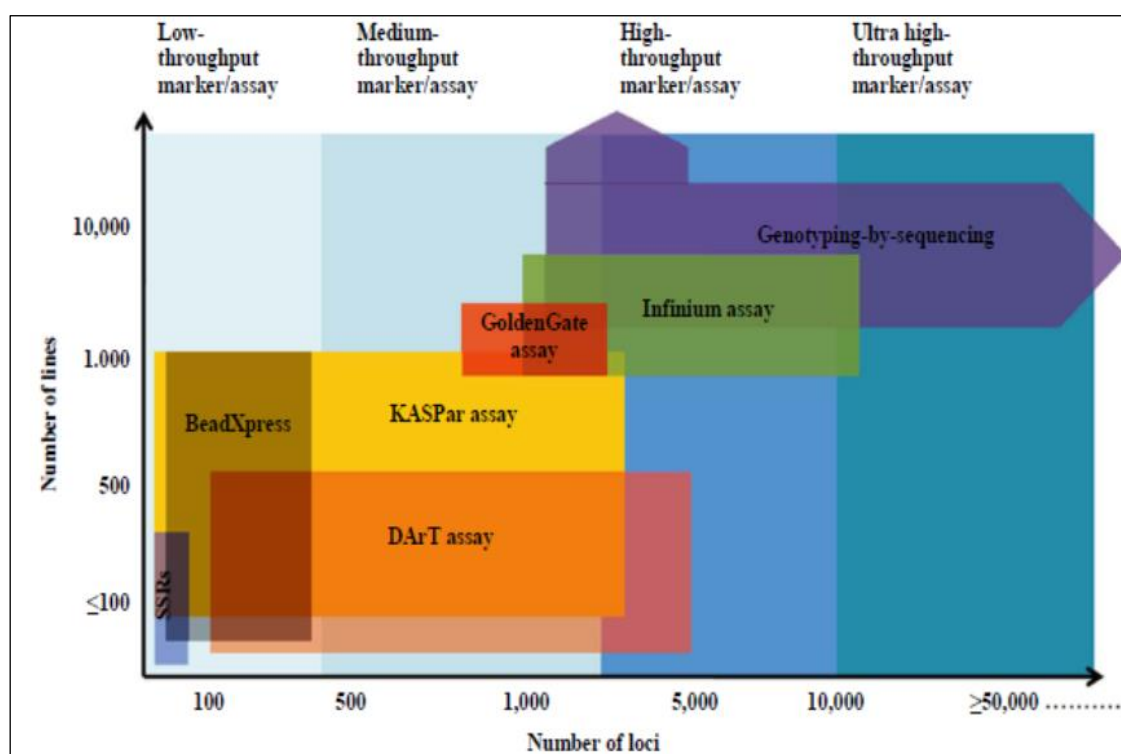


Figure 3. Low to ultra-high-throughput cost-effective marker assay platforms for genetic diversity study. Source: Mir *et al.* (2013)

2.10.2.1. Single nucleotide polymorphism marker

Several DNA markers are used to determine the genetic diversity study of various crops while the most common ones are single nucleotide polymorphisms (SNPs). Recently, SNP markers are effective markers in genetic diversity studies (Close *et al.*, 2009), genome-wide association mapping (Li *et al.*, 2020), phylogenetic relationships and population evolutionary history studies (Amdadul *et al.*, 2016), and varieties registrations and certification (Jamali *et al.*, 2017; Li *et al.*, 2020).

SNPs are the most abundant and robust, feasible for automated high-throughput genotyping (Alkan *et al.*, 2011), and highly reproducible and can be used to identify variants (Qiu *et al.*, 2012; Muñoz-Amatriaín *et al.*, 2014). SNPs have become widely used DNA markers because they are high throughput, efficient, stable and cost-effective (Table 1). However, SNP discovery and genotyping require expensive and sophisticated platforms (Zhu *et al.*, 2003; Mammadov *et al.*, 2012). Relatively, high initial costs for marker development, high-quality DNA required and high technical/equipment demands were the major limitations of SNP identification and discovery (Batley *et al.*, 2003; Semagn *et al.*, 2006). In addition, SNP detection a challenge of computational remains the differentiation between true biological variation and the often more abundant errors within the sequence data. This is particularly true for data generated from next-generation sequencing platforms, which often sacrifice data quality for quantity (Semagn *et al.*, 2006).

SNPs are rapidly replacing SSRs as the DNA marker of choice for applications in plant breeding and genetics (Edwards and Batley, 2010). The exploitation of high SNP genotyping platforms has provided important insights into population structure in barley germplasm arrays that, in turn, generate clues regarding the geographical origin and allelic diversity (Bayer *et al.*, 2017; Milner *et al.*, 2019). Early studies of diversity with different cereal crops in various countries generally indicated genetic variation using SNPs markers. A recent study by Teklemariam *et al.* (2022) has shown the relationship between geographic distance and genetic differentiation of Ethiopian barely germplasm collections. Genetic diversity studies using SNP markers have been also reported on European barley (Russell *et al.*, 2004), in

ICARDA spring barley collections (Amezrou *et al.*, 2018) and in Nordic spring barley collections (Bengtsson *et al.*, 2017).

Table 1. Comparison of most widely used DNA markers

Feature and Description	RFLP	RAPD	AFLP	SSR	SNP
Genomic abundance	High	High	High	High	High
Level of polymorphism	Moderate	High	High	High	High
Type of polymorphism	Single base changes, indels	Single base changes, indels	Single base changes, indels	Changes in length of repeats	Single base changes, indels
Number of loci	Small (<1,000)	Small (<1,000)	Moderate (1,000s)	High(1,000s-10,000s)	Very high (>100,000)
Cloning and/or Sequencing	Yes	No	No	Yes	Yes
PCR-based	Usually no	Yes	Yes	Yes	Yes
Ease/Amenable to automation	Low	Moderate	Moderate	High	High
Reproducibility/ Reliability	High	Low	High	High	High
Genotyping Throughput	Low	Low	High	High	High
Amount of DNA Required	Large (5 -50 µg)	Small (0.01-0.1µg)	Moderate (0.5 -1.0µg)	Small (0.05 - 0.12 µg)	Small (≥ 0.05 µg)
Quality of DNA Required	High	Moderate	High	Moderate to High	High
Timedemanding	High	Low	Moderate	Low	Low
Ease of use	Not easy	Easy	Moderate	Easy	Easy
Cost per analysis	High	Low	Moderate	Low	Low

Source: Muhammad *et al.* (2018)

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Morphological study

The study was intended to analyze the genetic diversity among barley genotypes using morphological markers. The area of the region of origins and altitude ranges of barley collection was used as the basis for this study.

3.1.1. Description of the experimental sites

The experiment was conducted at Kulumsa Agricultural Research Center during the two consecutive main cropping seasons in 2020 and 2021 under rain-fed conditions. Kulumsa Agricultural Research Center is located 160 km southeast of Addis Ababa a latitude of 8°1'7" N and a longitude of 39°9'35" E with an altitude of 2200 masl. It is characterized by an annual rainfall of 832 mm and with means maximum and minimum temperatures of 22.8 and 10.4°C, respectively. The monthly mean minimum and maximum temperature values and average monthly rainfall data of Kulumsa Agricultural Research Center for eleven years are presented in Appendix 1 (from 2011-2021). The soil is classified as clay loam with a pH of 6 (Abebele *et al.*, 2020).

3.1.2. Experimental plant materials

A total of 125 barely genotypes consisting of eighty five accessions and thirty eight improved varieties including two local checks (Aruso and Muga) (Table 2). The accessions were obtained from the Ethiopian Biodiversity Institute along with their passport data. The random sampling procedure was modified to allow the equal representation of barley accessions from 1976 to 2018 collection periods from four regions of the country (Oromia, Amhara, Tigray, and SNNPR) as well as at four major altitude ranges (less or equal to 2000, between 2001 to 2500, from 2501 to 3000, and above 3001 masl) (Table 2). The improved varieties including local checks were obtained from Mekelle University, different regional and national agricultural research centers in the country (Table 2). The geographical distribution of the barley genotypes for this study of the collection and testing sites is indicated in Figure 4.

Table 2. List of 125 barley genotypes obtained from the Ethiopian Biodiversity Institute, Mekelle University, and research centers

S/N	Accession Number	Collection Date	Regions	Zone/state	Altitudes
1	15397	22/11/2011	Oromia	Arssi	2100
2	1697	20/1/1978	Oromia	Arssi	2440
3	208815	3/1/1983	Oromia	Arssi	2680
4	64265	18/8/1981	Oromia	Arssi	2920
5	16863	3/1/2012	Oromia	Arssi	3070
6	239538	28/8/1998	Oromia	Bale	2480
7	237839	22/12/1995	Oromia	Bale	2930
8	237838	22/12/1995	Oromia	Bale	3300
9	3248	1/1/1979	Oromia	Bale	3570
10	208857	15/1/1984	Oromia	Borena	2500
11	29523	9/1/2017	Oromia	East Hararghe	2512
12	29524	9/1/2017	Oromia	East Hararghe	2412
13	18871	16/12/2011	Oromia	East Hararghe	2541
14	18319	7/5/2004	Oromia	East Shewa	2199
15	15366	16/11/2011	Oromia	East Shewa	2431
16	4496	25/1/1980	Oromia	East Wellega	2210
17	17672	19/4/2004	Oromia	East Wellega	1510
18	244890	22/12/2007	Oromia	East Wellega	2502
19	17685	19/4/2004	Oromia	East Wellega	2516
20	244944	12/1/2008	Oromia	Illubabor	1993
21	3611	16/11/1978	Oromia	Jimma	1940
22	239077	16/1/1997	Oromia	North Shewa	2550
23	24237	31/12/2014	Amhara	Agew Awi	1707
24	3489	4/1/1979	Amhara	Agew Awi	2150
25	17255	26/1/2012	Amhara	Agew Awi	2519
26	26514	15/3/2014	Amhara	East Gojam	2308
27	9950	8/1/2011	Amhara	East Gojam	2360
28	26519	15/3/2016	Amhara	East Gojam	2489
29	17220	7/5/2004	Amhara	North Gondar	2416
30	243605	14/1/2004	Amhara	North Gondar	2605
31	243598	14/1/2004	Amhara	North Gondar	3120
32	24126	12/11/2014	Amhara	North Gondar	3632
33	17231	17/1/2012	Amhara	South Gondar	2997

34	242067	14/12/2002	Amhara	South Gondar	2280
35	1625	15/12/1977	Amhara	North Shewa	2680
36	30228	19/5/2017	Amhara	North Shewa	2988
37	1652	4/1/1978	Amhara	North Shewa	3010
38	1661	5/1/1978	Amhara	North Shewa	3020
39	15291	2/1/2011	Amhara	North Shewa	3079
40	236811	2/2/1995	Amhara	North Shewa	3100
41	30230	19/5/2017	Amhara	North Shewa	3117
42	242096	26/12/2002	Amhara	North Wello	1940
43	235059	13/6/1992	Amhara	North Wello	2410
44	243574	4/1/2004	Amhara	North Wello	3460
45	236254	29/7/1994	Amhara	South Wello	2100
46	235057	13/6/1992	Amhara	South Wello	2540
47	3470	2/1/1979	Amhara	West Gojam	1920
48	3473	2/1/1979	Amhara	West Gojam	2430
49	3509	11/1/1979	Amhara	West Gojam	2770
50	29689	18/3/2017	SNNPR	Gamo-gofa	2196
51	29682	16/3/2017	SNNPR	Gamo-gofa	2521
52	244774	3/1/2008	SNNP	Gedeo	2315
53	16857	3/1/2012	SNNP	Gurage	2718
54	16853	2/1/2012	SNNP	Gurage	2865
55	16737	15/12/2011	SNNP	Gurage	2957
56	16739	15/12/2011	SNNP	Gurage	3089
57	16861	3/1/2012	SNNP	Gurage	3091
58	235530	13/1/1993	SNNP	Gurage	3200
59	16862	3/1/2012	SNNP	Gurage	3258
60	1637	21/12/1977	SNNP	Hadiya	2280
61	16804	29/12/2011	SNNP	Kembata Alabana Tembaro	2198
62	244771	25/12/2007	SNNP	Kembata Alabana Tembaro	2498
63	244772	25/12/2007	SNNP	Kembata Alabana Tembaro	2553
64	64255	16/8/1981	SNNP	North Omo	1500
65	240795	26/12/2000	SNNP	North Omo	2450
66	208843	12/1/1983	SNNP	North Omo	2780
67	208845	12/1/1984	SNNP	North Omo	2850
68	27503	7/1/2015	SNNP	Segen	1601
69	27507	7/2/2015	SNNP	Segen	1995

70	29705	25/3/2017	SNNP	Sidama	2563	
71	242583	18/10/2003	Tigray	Central/Mehakelegnaw	1720	
72	242584	23/10/2003	Tigray	Central/Mehakelegnaw	1950	
73	17190	24/4/2004	Tigray	Central/Mehakelegnaw	2085	
74	25919	12/2/2015	Tigray	Central/Mehakelegnaw	2151	
75	25915	11/2/2015	Tigray	Central/Mehakelegnaw	2508	
76	243586	8/1/2004	Tigray	Central/Mehakelegnaw	2580	
77	234311	9/11/1991	Tigray	East/Misrakawi	2090	
78	15255	28/12/2010	Tigray	East/Misrakawi	2386	
79	15260	28/12/2010	Tigray	East/Misrakawi	2559	
80	15277	29/12/2010	Tigray	East/Misrakawi	2596	
81	234305	3/11/1991	Tigray	East/Misrakawi	2980	
82	235252	24/11/1992	Tigray	South/Debubawi	2250	
83	242094	25/12/2002	Tigray	South/Debubawi	2455	
84	238349	28/11/1996	Tigray	South/Debubawi	2900	
85	242092	24/12/2002	Tigray	South/Debubawi	2950	
Improved food barley varieties						
Genotype name		Year of release	Regions	Zone/State	Maintaining centers	Altitudes
86	Aruso	Local check	Oromia	Arssi	-	2200
87	Muga	Local check	Oromia	Arssi	-	2200
88	IAR-485	1975	Oromia	West Shewa	Holetta	EIAR/HARC 2400
89	FBHB-42	1984	Oromia	West Shewa	Holetta	EIAR/HARC 2400
90	Shege	1995	Oromia	West Shewa	Holetta	EIAR/HARC 2400
91	Dimtu (3369-19)	2001	Oromia	West Shewa	Holetta	EIAR/HARC 2400
92	Harbu (Aruso Bale# 10-1)	2004	Oromia	Bale	Sinana	ORARI/SARC 2400
93	Dinsho (Wadago-4)	2004	Oromia	Bale	Sinana	ORARI/SARC 2400
94	Defo (Aruso (42) 4 (Sn 99G)	2005	Oromia	Bale	Sinana	ORARI/SARC 2400
95	Biftu (Shasho # 22 GO-1 (Sn 98B)	2005	Oromia	Bale	Sinana	ORARI/SARC 2400
96	HB-1307 (EH-1700/F7,B,63)	2006	Oromia	West Shewa	Holetta	EIAR/HARC 2400
97	Bentu (EMBSN 5th 2/95-3-3-3)	2006	Oromia	Arssi	Kulumsa	EIAR/KARC 2200
98	Guta (Acc. 3260-18)	2007	Oromia	Bale	Sinana	ORARI/SARC 2400
99	Dribie (7th EMBSN 19/98)	2010	Oromia	Arssi	Kulumsa	EIAR/KARC 2200
100	Abdane (Aruso/EH956/F2-8H-6-4SNR FBC99G0003-21)	2011	Oromia	Bale	Sinana	ORARI/SARC 2400
101	EH 1493/F6.32H.3	2012	Oromia	West Shewa	Holetta	EIAR/HARC 2400

102	Cross #41/98	2012	Oromia	West Shewa	Holetta	EIAR/HARC	2400
	Gobe (CBSS96M00487TD-1M-1Y-2M-OY)	2012	Oromia				
103				Arssi	Kulumsa	EIAR/KARC	2200
104	Robera	2016	Oromia	Bale	Sinana	ORARI/SARC	2400
105	HB1965(SCFBRVT P#3/11)	2017	Oromia	West Shewa	Holetta	EIAR/HARC	2400
106	HB1966(FBRVTB P#24/11)	2017	Oromia	West Shewa	Holetta	EIAR/HARC	2400
107	Adoshe (QUINA/MJA//SCARRLETT)	2018	Oromia	Bale	Sinana	ORARI/SARC	2400
108	Abay	1998	Amhara	Western Gojjam	Adet	ARARI/ADARC	2240
109	Meserach (Kulumsa 1/88/)	1998	Amhara	Northeast	Sirinka	ARARI/SRARC	1850
110	Mezezo (4748-16)	2004	Amhara	North Shewa	D. Birhan	ARARI/DBARC	2828
111	Basso (4731-7)	2004	Amhara	North Shewa	D. Birhan	ARARI/DBARC	2828
112	Setegn (3369-17)	2004	Amhara	Western Gojjam	Adet	ARARI/ADARC	2828
113	Estayish (218963-4)	2004	Amhara	Northeast	Sirinka	ARARI/SRARC	1850
114	Yedogit (BI 95 IN 198)	2005	Amhara	Northeast	Sirinka	ARARI/SRARC	1850
115	Agegnehu (218950-08)	2007	Amhara	Northeast	Sirinka	ARARI/SRARC	1850
116	Tilla (EMBSN 14/98)	2007	Amhara	Western Gojjam	Adet	ARARI/ADARC	2240
117	Debark-1(Acc. 221300)	2017	Amhara	North Gonder	Gonder	ARARI/GARC	2300
118	Hagere (Feres gama)	2018	Amhara	North Shewa	D. Birhan	ARARI/DBARC	2828
119	Felamit	2011	Tigray	Mekelle	Mekelle	M.University	2214
120	Hriti (SXH, S I06)	2012	Tigray	Mekelle	Mekelle	M.University	2214
121	Fetina (SXH,T182)	2012	Tigray	Mekelle	Mekelle	M.University	2214
	Illala-01(ERETH07-51/ Saesea/Cl	2016				TARI /MARC	
122	11458)		Tigray	Central Tigray	Mekelle		2254
123	IllaIa-02 (ERETH07-80/ Saesea/Atsa)	2016	Tigray	Central Tigray	Mekelle	TARI /MARC	2254
124	Adena (SXH-08-F4-S-143)	2016	Tigray	Mekelle	Mekelle	M.University	2214
125	Wolelay (SXH-08-F4-T-10)	2016	Tigray	Central Tigray	Mekelle	TARI /MARC	2254

Abbreviations: ARARI=Amhara Regional Agricultural Research Institute, DBARC=Debre Birhan Agricultural Research Center, ADARC= Adet Agricultural Research Center, HARC= Holetta Agricultural Research Center, GARC= Gonder Agricultural Research Center, EIAR=Ethiopian Institute of Agricultural Research, KARC=Kulumsa Agricultural Research Center, MoARD=Ministry of Agriculture and Rural Development, M. University=Mekelle University, MARC=Mekelle Agricultural Research Center, ORARI=Oromia Regional Agricultural Research Institute, SARC=Sinana Agricultural Research Center, TARI=Tigray Agricultural Research Institute, SRARC=Sirinka Agricultural Research Center.

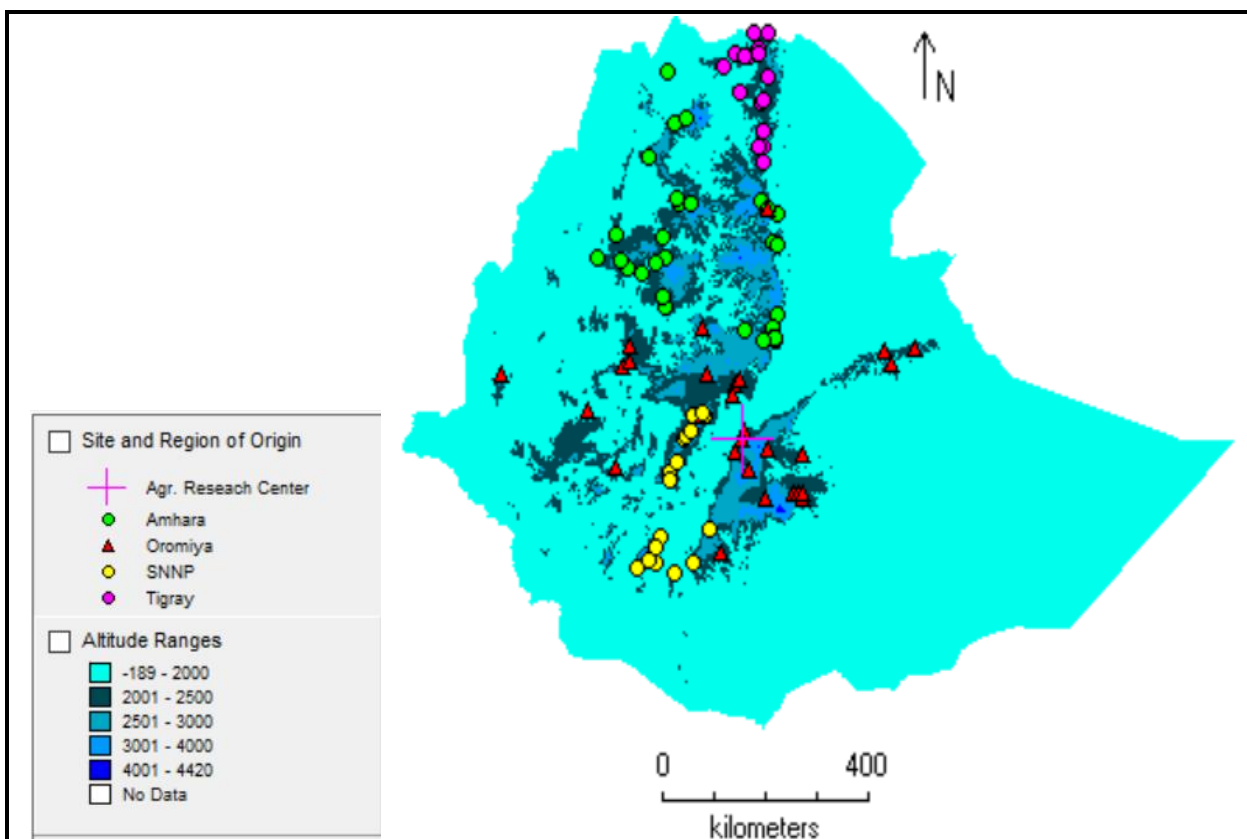


Figure 4. Map of Ethiopia showing the experimental sites, collection regions, and their altitudinal ranges. Dots represent barley genotypes in different colors depending on the region of origins

3.1.3. Experimental design and field management

The experiment was laid out in an alpha lattice design with two replications. The trial was arranged in 25 blocks, with 5 entries in each block. The gross plot area was 0.4m^2 which accommodates two rows having a length of 1m. The spacing between plots and rows was 0.5m and 0.2m, respectively. Ten grams of seed for each genotype were sown manually at the rate of 125kg ha^{-1} , on 11 July 2020 and 19 July 2021. Nitrogen and phosphorous fertilizers were used at the recommended rate for the area, 50kg ha^{-1} Urea and 120 kg ha^{-1} DAP at planting, respectively. Maximum care was taken during the experiment to minimize the possible confounding factors which could affect the expected result of the study. All other management practices such as hand weeding (three times weeding at seedling and vegetative stages) were uniformly applied.

3.1.4. Data collection

3.1.4.1. Qualitative traits

Each barley accession was morphologically characterized, and observations on different qualitative traits were recorded on ten randomly selected plants from the most frequent variant. Barley standard descriptor lists developed by International Plant Genetic Resource Institute (IPGR, 1994) were used in this study (Table 3). Color traits were also recorded based on the Munsell color Company manual (1957).

Table 3. Seventeen qualitative traits and their classes were used for the study

No	Traits	Classes
1	Stem pigmentation(Immature)	Green-1, Purple (basal only)-2, Purple (half or more)-3
2	Leaf color (LfC)	Light green-1, Medium green-2, Dark green-3
3	Stem branching (StBr)	Opposite-1, Alternate-2, Ternate-3, Mixed-4
4	Glume color at the hard dough stage (GIC)	White-1, Yellow-2, Brown-3, Black-4, Purple-5
5	Glume color at physiological maturity (GICM)	White-1, Yellow-2, Brown-3, Black-4, Purple-5, Grey-6
6	Lemma color at the hard dough stage (LmC)	Amber/normal-1, Red-2, Purple-3, Black-4, Grey-5, Yellow-6, Brown-7
7	Lemma color at maturity (LmCM)	
8	Glume hairiness(GIH)	Glabrous-1, Hairy-2
9	Spike density (SpDs)	Lax-3, Intermediate-5, Dense-7
10	Kernel row number (KrRw)	Two row-1, Irregular-3, Six row-5
11	Awn color (AwC)	Amber/White-1, Yellow-2, Brown-3, Reddish-4, Black-5
12	Lemma awn (LmA)	Awnless-1, Awnleted-2, Awned-3, Sessile-4, Elevated-5
13	Lemma awn barbs (LmAB)	Smooth-3, intermediate-5, Rough-7
14	Beak length (BkLn)	Very short-1, Short-2, Medium-3, Long-4, Very long-5
15	Beak shape(BkSh)	Straight-1, slightly curved-2, Medium curved-3, Strongly Curved-4, Geniculated-5
16	Kernel shape (KrSh)	Spherical-1,Spherical-Flattened-2,Elongated-3, Elongated-Flattened-4
17	Kernel color (KrC)	White-1, Red-2, Purple-3, Black-4, Grey-5, Amber-6, Yellow-7, Brown-8

3.1.4.2. Quantitative traits

The quantitative traits data were measured based on the International Plant Genetic Resource Institute (IPGR, 1994) descriptor for barley. The number of split variants (degrees of heterogeneity) was recorded for each plot; the most frequent variant in each plot was considered the dominant phenotype for each genotype, and tagged middle plants and used for quantitative traits diversity study. For other morphological traits, the recording was done on plot bases (Table 4).

Table 4. Twenty-three morphological traits were recorded for the study

No	Quantitative traits	Measured
1	Days to 50% emergency (DE, the day when more than 50% of the barley plants in a plot started seedling emergence)	On a plot basis
2	Days to 50% heading (DH, the number of days when more than 50% of the barley plants in a plot initiated heading)	
3	Days to 50% flowering (DF, the number of days from sowing to the days when 50% of the heads fully flower)	
4	Days to 75% maturity (DPM, the number of days from days to planting up to 75% of the head in a plot showed maturity)	
5	Plant height (PH, the height of the mother plant during maturity, starting from the ground level to the tip of the terminal spikelet in cm)	Ten plants were taken randomly from each harvestable plot
6	Flag leaf length (FLL, the length flag leaf of the mother plant before maturity from its bottom to tip in cm)	
7	Spike length (SpL, the length from the base of the spike to the tip of the highest spikelet in cm, excluding awn)	
8	Awn length (AwL, the distance from the tip of the spike to the end of the awn in cm)	
9	Number of tillers per plant (NTPP, the number of tillers per plant, counted on five randomly selected plants from each plot)	Five plants were taken randomly from each harvestable plot
10	Number of fertile tillers per plant (NFTP, the average number of fertile tillers per plant, counted on five randomly selected plants)	

11	Thousand kernels weight (TKW, a thousand kernels were randomly taken from each harvestable plot and weighted, by adjusting to 12.5% standard grain moisture content of crops	On a plot basis
12	Number of kernels per plant (NKP, count of the number of kernels of the mother plant)	Ten plants were taken randomly from each harvestable plot
13	Spike weight (SpW, the dried weight of the spike from each plant in grams per plant)	
14	Grain yield per plant (GYPP, the dried weight of kernels from each plant in grams)	
15	Biomass yield per plant (BMYPP, the dried weight of the above-ground biomass of the mother plant in grams)	
16	Lodging(Ldg, the visual scoring, measures the ability of the crop to resist lodging and can be rated 1 as very poor, 2 for poor, 3 for intermediate, 4 for good and 5 for very good or no visible susceptible sign to lodging)	On a plot basis
17	Grain yield (GYHa, grain produced per plot in grams, converted in tons/ha)	
18	Biomass yield (BMYHa, the dried weight of the above-ground biomass yield per plot in grams, converted in ton/ha)	
19	Grain filling period (GFP, the number of days from days to flowering up to days to physiological maturity)	Were calculated from the measured parameters
20	Grain filling rate (GFR, the ratio of grain yield to grain filling period in kg/ha/day)	
21	Biomass production rate (BPR, the ratio of above-ground biomass yield to days to physiological maturity in kg/ha/day)	
22	Harvest index per plant (HIP, the ratio of dried grain yield to the above-ground biomass yield of an individual plant multiplied by 100)	
23	Harvest index per plot (HI, the ratio of dry grain yield to the above-ground biomass yield per hectare multiplied by 100)	

3.1.5. Data analysis

3.1.5.1. Qualitative data analysis

The percentage of frequency of the morphological traits for classes of each trait, region, and altitudinal range were calculated using GenStat (GenStat, 2015) for the seventeen qualitative traits. The chi-square (X^2) was analyzed to test deviation from the pool mean of qualitative traits using the PROC FREQ procedure in SAS software for the region of origins and altitude ranges (SAS, 2008). The Shannon-Weiner evenness index (H') was estimated for the region of origins and altitude ranges using GenStat (GenStat, 2015) with bootstrapping. A principal component analysis (PCA) was conducted using the *autoplot.prcomp()* function to summarize the variation in morphological traits. An ANOVA was performed on morphological data collected including the region of origins and altitude ranges in R using the *aov()* function. The R program was also used to make a plot using *heatmap()* function using R.4.2 statistical software (R Core Team, 2022).

3.1.5.2. Quantitative data analysis

The morphological data were computed using the best linear unbiased (BLUP) method, which is a standard method for measuring the random effects of a mixed model compared to other procedures. Bartlett's test for homogeneity of variance was carried out to determine the validity of the individual experiment, and combined analysis of variance (ANOVA) using META-R software (Gregorio *et al.*, 2016). Combined data analysis of environments/seasons using a mixed linear model with all factors set as random effects. PROC MIXED was conducted to estimate genotypes as well as residual variance components. The analysis of the variance of quantitative traits was done using the following general linear model:

$$Y_{ijk} = \mu + g_i + r_j + bk_j + \epsilon_{ijk}$$

Where the observed phenotypic value Y_{ijk} = the response of Y trait from the i^{th} genotype, grown in the k^{th} alpha lattice design of j^{th} replicate μ = general mean, g_i = random effect of the i^{th} genotype, r_j = random effect of the j^{th} replicate, bk_j = random effect of k^{th} alpha lattice design in a j^{th} replicate, ϵ_{ijk} = is the effect of the error associated with the i^{th} replication, j^{th} incomplete block, and k^{th} genotype.

The total phenotypic variance of each of the traits was partitioned into contributions due to genetic and non-genetic factors using the analysis of variance components method according to Singh and Chaudhury (1996) was calculated as:

$$\text{Genotypic variance } (\sigma^2g) = \frac{MSg - MSei}{rl} \text{ and phenotypic variance } (\sigma^2p) = \sigma^2g + \sigma^2gs + \sigma^2e$$

Where MSg=mean squares of genotypes, MSei=mean squares of experimental error of interaction, σ^2g is genotypic variance, σ^2gs is genotype by seasons interactions, σ^2e is environmental variance and r is the number of replication and l is the number of location or seasons.

The broad-sense heritability of the measured traits was taken as the proportion of total genetic variance to total phenotypic variance. Broad-sense heritability (H^2) was calculated as the equations given by Gregorio *et al.* (2016):

Broad-sense heritability (H^2) = $\frac{\sigma^2g}{\sigma^2p} \times 100$, Where, σ^2g is genotype variance and σ^2p is the phenotypic variance component.

Computed phenotypic and genotypic coefficient of variation also suggested by Singh and Chaudhury (1996) using the following formula:

$$PCV = (\sqrt{\sigma^2p}/X) \times 100 \text{ and } GCV = (\sqrt{\sigma^2g}/X) \times 100$$

Where, PCV is the phenotypic coefficient of variation, σ^2ph is phenotypic variance, GCV is the genotypic coefficient of variation, σ^2g is genotypic variance, and X is the grand mean for the trait measured.

In addition, the genetic advance for all traits, as suggested by Allard (1960), and Singh and Chaudhary (1985) were computed as follows:

$$\text{Expected genetic advance (GA)} = K * (\sqrt{\sigma^2ph} * H^2)$$

Where GA is genetic advance, 'K' is constant with the value of 2.06 at 5% selection intensity, $\sqrt{\sigma^2ph}$ are the square root of phenotypic variance and H^2 is broad sense heritability.

Genetic advance as a percent of the mean was also calculated as follows:

$$GA (\% \text{ of mean}) = \frac{GA}{X} \times 100\%$$

Where GA is genetic advance and X is sample mean for the trait considered.

Based on the pooled data, genotypic and phenotypic coefficient correlations were performed using the META-R software (Gregorio *et al.*, 2016). The correlation coefficient measures the mutual relationship between various traits and determines the component traits on which selection can be based for improvement in yield (Singh, 2007). These correlations of the traits measured in the study were estimated using Pearson Simple Correlation which deals with the association between any two traits. Phenotypic correlation is the observable correlation between two variables, which includes both genotypic and environmental effects. Genotypic correlation is the inherent association between two variables. Phenotypic and genotypic coefficients of correlation between two traits were estimated by using the variance and covariance components (Singh, 2007).

$$r_{pxy} = \frac{\text{Covp (X,Y)}}{\sqrt{\sigma^2_{px} \cdot \sigma^2_{py}}}$$

Where r_{pxy} is the phenotypic correlation coefficient between traits X and Y, Covp (x, y) is the phenotypic covariance between traits X and Y, σ^2_{px} is the phenotypic variance of trait X, σ^2_{py} is the phenotypic variance of trait Y

$$r_{gxy} = \frac{\text{Covg (X,Y)}}{\sqrt{\sigma^2_{gx} \cdot \sigma^2_{gy}}}$$

Where, Covg (x, y) is the genotypic covariance between traits X and Y, r_{gxy} is the genotypic correlation coefficient between traits X and Y, σ^2_{gx} = Genotypic variance of trait X, σ^2_{gy} is the genotypic variance of trait Y.

To quantify the variation between barley genotypes, we also performed principal component analysis (PCA) in R software using the *autoplot.prcomp()* function, to estimate the contribution of each trait for the total observed variations in the barley genotypes (R Core Team, 2022). This permits us to make out the major traits accounting for the greater share of the observed variations. Cluster analysis was also performed in R software version 4.2 using the *heatmap()* function (R Core Team, 2022) and the META-R software (Gregorio *et al.*, 2016).

3.2. Molecular study using SNP markers

3.2.1. Plant materials

One hundred five barely panels comprised of eighty-five barley accessions and sixteen improved varieties, two local checks (Aruso and Muga) and two wild crosses were used in this study (Table 5). These lines were chosen based on cultivars regional passport information and the breeding merit they have for subsequent germplasm enhancement. Additionally, two wild crosses of barley were obtained from Debre Zeit Agricultural Research Center (DzARC) primarily introduced by the International Center for Agricultural Research in the Dry Areas (ICARDA) (Table 5).

Table 5. Number of barley genotypes used in the molecular study

No	Regions	Accessions	Improved varieties	Sub total
1	Oromia	15397, 1697, 208815, 64265, 16863, 239538, 237839, 237838, 3248, 208857, 18319, 15366, 4496, 17672, 244890, 17685, 29523, 29524, 18871, 3611, 244944 and 239077	Aruso and Muga (local checks), Shesge, HB-1307, Guta, Cross #41/98, Gobe, HB1965, HB1966 and Adoshe	32
2	Amhara	24237, 3489, 17255, 26514, 9950, 26519, 242067, 17220, 243605, 17231, 243598, 24126, 1625, 30228, 1652, 1661, 15291, 236811, 30230, 242096, 236254, 235059, 243574, 235057, 3470, 3473, and 3509	Mezezo, Yedogit, Debark-1 and Hagere	31
3	Tigray	242583, 242584, 17190, 25919, 25915, 243586, 234311, 15255, 15260, 15277, 234305, 235252, 242094, 238349 and 242092	Felamit, Illala-01, Adena and Wolelay	19
4	Southern Nations (SNNP)	29689, 29682, 244774, 16857, 16853, 16737, 16739, 16861, 235530, 16862, 16804, 244771, 244772, 1637, 64255, 240795, 208843, 208845, 27503, 27507 and 29705	-	21

No	Wild crosses						
1	DzARC/ ICARDA	SLB15-05/11	Spont	96-3/3	Roho//Alger/ceres	362-1-1/5/Roho/4/Zanbaka/3/ER/APm//Lignee 131	2
2	DzARC/ ICARDA	Soufara-02/3/	RM	1508/	Por//W12269/4/HM1-02/Arabi	Abiad//ER/APM/5/Sl-B15-05/4/H-Spont.96.3/3/Roho//Alger/Ceres 362-1-1	
Total							105

Abbreviations: DzARC= Debre Zeit Agricultural Research Center, ICARDA=International Centre for Agricultural Research in the Dry Areas, SNNP =Southern Nations, Nationalities and Peoples.

3.2.2. Planting, leaf sampling and genomic DNA extraction

Each barley genotype was sown on 02 August 2021 in a seedling tray, five seedlings each, in a greenhouse at National Agricultural Biotechnology Research Center (NABRC), Holetta. After two weeks, old barley leaf samples were collected and pooled from the same genotype in equal amounts (Figure 5). Then samples were placed into 96 collection plates (96-well plates). During the sample preparation scissors were sterilized with 70% alcohol before cutting the next genotype to prevent cross-contamination.

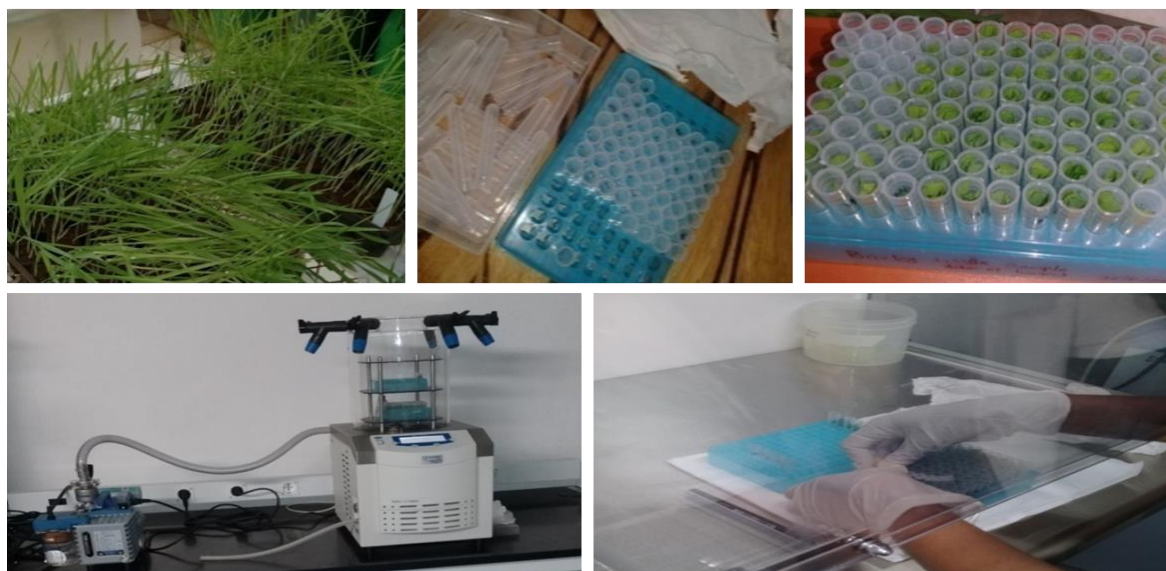


Figure 5. Sample preparation and freeze-dried at the National Agricultural Biotechnology Research Center

The collected leaf samples were first freeze-dried at -20°C for 24h (Figure 5). Then the freeze-dried leaf samples were shipped for genotyping to the Integrated Genotyping Service and Support (IGSS) platform located at Biosciences Eastern and Central Africa- International Livestock Research Institute Hub (BecA-ILRI Hub) based in Nairobi. Genomic DNA from 105 barley genotypes was extracted using the Nucleomag Plant Genomic DNA extraction kit following the manufacturer's instructions. The genomic DNA concentration was used in the range of 50-100 ng/μl. The quality and quantity of extracted DNA in each sample were determined by electrophoresis on 0.8% agarose gel.

3.2.3. SNP Genotyping

Genomic libraries were constructed according to Kilian *et al.* (2012), complexity reduction method through the digestion of genomic DNA using a combination of PstI and HpaII enzymes and ligation of bar-coded adapters followed by PCR amplification of adapter-ligated fragments. Libraries were sequenced using single-read sequencing runs for 77 bases. Next-generation sequencing technology was carried out using HiSeq2500 (Illumina). Marker scoring was achieved using DArTsoft14, which is an in-house marker scoring pipeline based on algorithms. Two types of markers were scored, SilicoDArT and SNP markers which were both scored '1' for presence, '0' for absence, and '-' for calls with non-zero count. However, too low counts to score certainly as '1' for the SilicoDArT whereas the sequences SNPs were scored '0' for reference allele homozygote, '1' for SNP allele homozygote and '2' for heterozygote. Totalities of 31,646 silicoDArTs and SNP markers were employed to genotype the materials. Both SilicoDArT and SNP markers were aligned to the reference genome of *Hordeum vulgare_v2.0* (Mayer *et al.*, 2012), to identify chromosome positions. Our study which included only SNP markers data was used for this analysis after SNP calling and imputation.

3.2.4. SNP calling and data filtering

An initial set of 14,454 single nucleotide polymorphisms (SNPs) containing monomorphic and undefined chromosomes or positions were removed from the raw dataset. Then further quality control was performed, and SNP markers with >95% call rate, minor allele frequency (>5%), and missing rate per sample (<10%) and per SNP (<30%) were retained for downstream

analysis using R software version 4.1.3 (R Core Team, 2022). The diversity analysis was performed using complete (non-missing) data; therefore, SNP with missing loci was imputed using the R package *snpReady* (Granato *et al.*, 2018). Finally, 10,103 (69.9%) SNP markers were retained for further analysis.

3.2.5. Data analysis

Genetic parameters such as polymorphism information content (PIC), genetic diversity, heterozygosity (observed and expected), minor allele frequency (MAF), genetic differentiation, gene flow, and genetic distance were computed using the R package *snpReady* (Granato *et al.*, 2018). The molecular variance analysis (AMOVA) was performed at different hierarchical levels (among and within the populations and accessions) in the stats R package using the *aov* function. To explore the genetic structure of the study population, a cluster analysis was performed using the agglomerative hierarchical algorithm (wards method, Euclidean distance; Anderberg, 1972) of the accession-by-locus reference allele count table for the SNP markers that passed the filtration process using R software. The incidence of accessions in the dendrogram was plotted against the testing location. The silhouette method (implemented in the R package *factoextra*) was used to determine the optimum number of clusters (k) (Lengyel and Botta-Dukát, 2019). Briefly, a matrix of silhouette lengths was estimated as Euclidian distances among individual pairs of observations for a cluster. Then the level of cohesiveness within neighboring accessions in a cluster was estimated as the average distance within the cluster. For each level of k , the individual silhouette lengths were averaged among the accessions between clusters and the minimum value was considered as a point of separation. Finally, silhouette width for accessions was calculated as the ratio of (i) the difference between cohesiveness and separation to (ii) the maximum values of cohesiveness and separation. The optimal number of clusters maximizes the average silhouette width over a range of possible values for k . A principal coordinate analysis (PCoA) of the SNP markers was conducted using the *prcomp* function to summarise the contributions of each part to the variation that existed in the population. The genetic diversity pattern among the population was visualized using the *pheatmap* R package (Granato *et al.*, 2018).

3.3. Assessment of genetic improvement

3.3.1. Planting materials

Thirty eight released barley varieties including two local checks (Aruso and Muga) were evaluated at Kulumsa Agricultural Research Center during the two consecutive main cropping seasons in 2020 and 2021 under rain-fed conditions. The barley varieties including two local checks were obtained from national and regional agricultural research centers, and Mekelle University (Table 2).

3.3.2. Experimental design and field management

The experiment was laid according to alpha lattice design with two replications and twenty five blocks. The gross plot area was 0.4m^2 which accommodates two rows having a length of 1m. The spacing between plots and rows was 0.5m and 0.2m, respectively. Five grams of seed (a total of 10 g for two replications) for each genotype were sown manually at the recommended rate for the area (125kg ha^{-1}), on 11 July 2020 and 19 July 2021. Nitrogen and phosphorous fertilizers were also used at the recommended rate for the area, 50kg ha^{-1} Urea and 120 kg ha^{-1} DAP at planting, respectively. The management practices such as hand weeding were uniformly applied.

3.3.3. Data collection

In this study, barley standard descriptor lists developed by International Plant Genetic Resource Institute (IPGR, 1994) were used for data recording. For plant height, flag leaf length, spike length and weight, awn length, number of kernels per spike, grain and biomass yield per plant, data were recorded from ten randomly selected and tagged middle plants for each plot, and the average was considered as data analysis. However, the number of fertile tillers was determined from five middle plants in each harvestable plot. Other phenotypic traits, days to 50% heading and flowering, days to 75% physiological maturity, grain filling period and lodging were collected on a plot basis. Grain yield and biomass yield were also recorded on a plot basis in grams and then converted into tons per hectare. A thousand kernels weight was recorded by weighing one thousand randomly taken kernels from each harvestable plot and by adjusting its moisture content to 12.5%. Biomass production and grain filling rate,

grain filling period, and harvest index per plant and hectare were performed from the measured parameters.

3.3.4. Data analysis

The data were analyzed using a mixed linear model with all factors set as random effects performed using the META-R software (Gregorio *et al.*, 2016). Bartlett's test for homogeneity of variance was carried out to ensure the validity of the individual trial and then, combined analyses of variance (ANOVA) were done following the best linear unbiased (BLUP) method (Gregorio *et al.*, 2016). Linear regression analysis was used to calculate the genetic improvement for selection traits considered in the study. The breeding effects were estimated as a genetic enhancement for grain yield and associated morphological traits in barley improvement through regressing the mean of each trait for each variety against the year of release of that variety using *lm()* function in R software 4.2.1 (R Core Team. 2022) and PROC REG procedure in SAS software version 9.2 (SAS, 2008). The genetic improvement achieved over the year of the released period for traits under consideration was assessed as a ratio of genetic improvement to the corresponding mean value of local checks. Correlation coefficients between all traits were computed using the mean of each variety in each year using the PROC CORR procedure. Stepwise regression analysis was also carried out on the varietal mean using PROC STEPWISE procedure using SAS software (SAS, 2008), to determine those variables that contributed more to yield variation between varieties.

The annual rate of progress/improvement (b) = $\frac{Cov\ XY}{Var\ X}$, from which the annual rate of improvement was computed (Singh and Chaudhary, 1996).

Where Cov is the covariance, Var is the variance, X is the year of variety release, and Y is the mean value of each trait for each variety. The relative annual progress achieved over the years was determined as a ratio of genetic improvement to the corresponding mean value of local checks and expressed as a percentage.

Correlation between traits using means of each variety was calculated as follows:

Correlation coefficient between X and Y (r_{xy}) = $\frac{Cov(X,Y)}{\sqrt{Var(X) Var(Y)}}$ Where r_{xy} is the correlation coefficient between X and Y, Cov (X, Y) is the covariance between X and Y, Var (X) is the variance of X, and Var (Y) is the variance of Y.

CHAPTER FOUR

4. RESULTS

4.1. Qualitative traits variations in Ethiopian barley germplasm

4.1.1. Distribution of qualitative traits of barley accessions across the region of origins

The frequency distribution of each qualitative trait of barley accessions across the region of origins and showed high morphological diversity (Table 6). Chi-square(X^2) values are also displayed to understand the degree of deviation of the observed frequency distribution of qualitative traits from expected values. The result indicated that the highest percentage frequency distribution of green and purple stems (basal only) was recorded in Oromia and Tigray regions with values of 90.9 and 53.3%, respectively. Light green leaf (66.7%) in the Tigray region followed by medium green leaf (51.9%) in the Amhara region as a dark-green leaf was the least abundant (9.5%) in the SNNP region. Awn colors of barley accessions showed different distributions among the region of origin (Table 6). Among the awn colors, amber or white was the most frequent (95.5%) color in the Oromia region, however, reddish (20.0%), yellow (13.3%), brown (4.8%) and black (4.8%) colors were with low frequencies.

White GLC was highly variable with a percentage frequency distribution of 60.0% in the Tigray region followed by purple color (55.6%) in the Amhara region (Table 6, Appendix 2A and B). Besides, white (86.7%) and black (28.6%) glume colors were commonly obtained in Tigray and SNNP regions at the maturity stage (Table 6, Appendix 3A). The amber or normal (66.7%) in the Tigray and purple (59.3%) in the Amhara regions were the most prevalent LmCs (Table 6). On the other hand, both amber or normal (52.2%) and black (28.6%) colors were predominantly obtained from the SNNP regions whereas grey (33.3%) color was mainly recorded in the Tigray region at maturity time (Table 6, Appendix 3A). Awned LmA was monomorphic in all regions, while awnless, awnleted, sessile and elevated traits were absent (Table 6).

Table 6. Frequency distribution of 17 qualitative traits of barley accessions by region of origins and altitude ranges

Traits	Classes	Regions				Chi-square (X^2)	Altitudes				Chi-square (X^2)
		Oromia	Amhara	Tigray	SNNP		≤ 2000	2001-2500	2501-3000	≥ 3001	
StP	1	90.9	74.1	20.0	85.7	149**	63.6	65.5	73.3	86.7	63.5**
	2	4.5	22.2	53.3	9.5		9.1	31.0	16.7	13.3	
	3	4.5	3.7	26.7	4.8		27.3	3.4	10.0	0.0	
LfC	1	45.5	48.1	66.7	52.4	28.4**	63.6	58.6	50.0	33.3	40.6**
	2	45.5	51.9	33.3	38.1		27.3	34.5	46.7	66.7	
	3	9.1	0.0	0.0	9.5		9.1	6.9	3.3	0.0	
GlH	1	86.4	81.5	73.3	71.4	8.6*	54.5	75.9	80.0	100.0	60**
	2	13.6	18.5	26.7	28.6		45.5	24.1	20.0	0.0	
StBr	1	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0	0
	2	100.0	100.0	100.0	100.0		100.0	100.0	100.0	100.0	
AwC	1	95.5	85.2	66.7	66.7	45**	90.9	75.9	76.7	86.7	50**
	2	0.0	3.7	13.3	9.5		9.1	6.9	6.7	0.0	
	3	0.0	0.0	0.0	4.8		0.0	3.4	0.0	0.0	
	4	4.5	7.4	20.0	14.3		0.0	10.3	16.7	6.7	
	5	0.0	3.7	0.0	4.8		0.0	3.4	0.0	6.7	
SpDs	3	9.1	22.2	40.0	33.3	40.4**	54.5	27.6	13.3	20.0	48.7*
	5	63.3	66.7	53.3	47.6		36.4	58.6	66.7	60.0	
	7	27.3	11.1	6.7	19.0		9.1	13.8	20.0	20.0	
GIC	1	50.0	44.4	60.0	57.1	23.8**	63.6	44.8	43.3	73.3	45**
	2	9.1	0.0	0.0	4.8		9.1	0.0	6.7	0.0	
	3	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
	4	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
	5	40.9	55.6	40.0	38.1		27.3	55.2	50.0	26.7	
GICM	1	77.3	59.3	86.7	66.7	57**	72.7	62.1	76.7	73.3	74.4**
	2	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
	3	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
	4	4.5	14.8	6.7	28.6		0.0	13.8	13.3	26.7	
	5	13.6	25.9	6.7	4.8		27.3	24.1	6.7	0.0	
	6	4.5	0.0	0.0	0.0		0.0	0.0	3.3	0.0	
LmC	1	54.5	37.0	66.7	52.4	70.1**	54.5	44.8	46.7	66.7	87.6**
	2	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
	3	36.4	59.3	33.3	42.9		27.3	55.2	50.0	26.7	
	4	0.0	3.7	0.0	0.0		0.0	0.0	0.0	6.7	
	5	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
	6	9.1	0.0	0.0	0.0		9.1	0.0	3.3	0.0	
	7	0.0	0.0	0.0	4.8		9.1	0.0	0.0	0.0	
LmC M	1	50.0	48.4	38.0	52.4	117.2*	27.3	37.9	60.0	60.0	118**
	2	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
	3	4.5	21.0	22.0	4.8		27.3	17.2	10.0	0.0	
	4	4.5	15.8	6.7	28.6		0.0	13.8	13.3	26.7	
	5	22.7	14.8	33.3	9.5		27.3	27.2	13.3	6.7	
	6	13.6	0.0	0.0	0.0		9.1	3.4	3.3	0.0	
	7	4.5	0.0	0.0	4.8		9.1	0.0	0.0	6.7	

Abbreviations: StP=Stem pigmentation, LfC=Leaf color, GlH=Glume hairiness, StBr=Stem branching, AwC=Awn color, SpDs=Spike density, GIC=Glume color at hard dough stage,

GLCM=Glume color at physiological maturity stage, LmC=Lemma color at hard dough stage and LmCM=Lemma color at physiological maturity stage. **, significant at the 1% level; * significant at the 5 % level; n.s, not significant.

Table 6. *Continued*

Traits	Classes	Regions				Chi-square (X^2)	Altitudes				Chi-square (X^2)
		Oromia	Amhara	Tigray	SNNP		≤ 2000	2001-2500	2501-3000	≥3001	
KrRw	1	18.2	37.0	80.0	47.0	97.8**	63.6	58.6	26.7	26.7	79.1**
	3	18.2	7.4	6.7	0.0		0.0	6.9	16.7	0.0	
	5	63.6	55.6	13.3	52.4		36.4	34.5	56.7	73.3	
LmA B	3	81.8	81.5	80.0	57.1	23.1**	72.7	72.4	73.3	86.7	8.1**
	5	18.2	18.5	20.0	42.9		27.3	27.6	26.7	13.3	
	7	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
LmA	1	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0	0
	2	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
	3	100.0	100.0	100.0	100.0		100.0	100.0	100.0	100.0	
	4	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
	5	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
BkLn	1	0.0	7.4	6.7	4.8	46.3**	0.0	6.9	3.3	6.7	60.1**
	2	4.5	3.7	13.3	0.0		9.1	6.9	3.3	0.0	
	3	27.3	33.3	46.7	42.9		27.3	41.4	26.7	53.3	
	4	59.1	51.9	26.7	47.6		45.5	44.8	60.0	33.3	
	5	9.1	3.7	6.7	4.8		18.2	0.0	6.7	6.7	
BkSh	1	18.2	40.7	40.0	28.6	19.4**	36.4	37.9	23.3	33.3	22.8**
	2	63.6	44.4	40.0	47.6		45.5	41.4	56.7	53.3	
	3	18.2	14.8	20.0	23.8		18.2	20.7	20.0	13.3	
	4	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
	5	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
KrSh	1	0.0	0.0	0.0	0.0	45**	0.0	0.0	0.0	0.0	30.8**
	2	4.5	3.7	20.0	0.0		0.0	10.3	3.3	6.7	
	3	59.1	44.4	53.3	52.4		36.4	44.8	63.7	53.3	
	4	36.4	51.9	26.7	47.6		63.5	44.8	33.1	40.0	
KrC	1	54.5	40.7	26.7	57.1	132.5* *	36.4	34.5	56.7	53.3	114.8* *
	2	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
	3	4.5	22.2	20.0	4.8		27.3	17.2	10.0	0.0	
	4	4.5	14.8	6.7	28.6		0.0	13.8	13.3	26.7	
	5	18.2	14.8	33.3	4.8		18.2	24.1	13.3	6.7	
	6	0.0	7.4	6.7	0.0		0.0	6.9	0.0	6.7	
	7	13.6	0.0	6.7	0.0		9.1	3.4	6.7	0.0	
	8	4.5	0.0	0.0	4.8		9.1	0.0	0.0	6.7	

Abbreviations: KrRw=Kernel rows, LmAB=Lemma awn barbs, LmA=Lemma awn, BkLn=Beak length, BkSh=Beak shape, KrSh=Kernel shape and KrC=Kernel color. Significant codes: 0 '****' 0.001, '**' 0.01, '*' 0.05, 'ns' 0.1, 'ns' 1, ns-non significant.

Spike related traits of barley accessions showed different distributions among regions (Table 6). Intermediate SpDs (66.7%) was commonly found in the Amhara region followed by lax type (40.0%) in the Tigray region and dense type (27.3%) in the Oromia region. Two-rowed

(80.0%) is the predominant type of KrRw in the Tigray region while six-rowed (63.6% and 55.6%) was frequently found in the Oromia and Amhara regions, respectively (Table 6).

The high percentage distribution of long (59.1%) and medium (46.7%) types of BkLn was recorded in Oromia and Tigray regions, respectively (Table 6). The BkSh of slightly curved (63.6%) in Oromia and straight (40.7%) types in Amhara regions showed the highest frequency (Table 6). Correspondingly, the elongated (59.1%) and elongated-flattened (51.9%) kernel shapes primarily originated in Oromia and Amhara regions, respectively (Table 6). Kernel colors (KrC) of barley accessions showed different distributions among regions (Table 6, Figure 6A-E), and KrC was variable with 1 to 8 classes (Table 6). White color was predominantly observed in the SNNP region (57.1%) followed by Oromia (54.5%), Amhara (40.7%) and Tigray (26.7%) regions (Table 6, Figure 6A). The highest frequency of black kernel accessions was observed from the region of SNNP and Amhara regions (Table 6, Figure 6C). The brown kernel was only sourced from Oromia and SNNP regions while the red kernel was absent in all regions. The purple kernel was frequent in Amhara and Tigray regions (Table 6, Figure 6D). Grey was dominant in the Tigray region (Table 6, Figure 6B).



Figure 6. Diversity of kernel color of barley accessions (A) White (B) Grey (C) Black (D) Purple and (E) Amber colors. The kernel colors of barley accessions are measured with a scale

of 1 to 8 classes (white-1, red-2, purple-3, black-4, grey-5, amber-6, yellow-7, and brown-8) following the barley descriptors.

4.1.2. Distribution of qualitative traits in the four altitude ranges

The frequency distribution of the 17 qualitative traits of barley accessions was observed in relation with four altitude ranges (Table 6). Chi-square(X^2) values are also indicated to understand the degree of deviation of the observed frequency distribution of qualitative traits from expected values. High percentage frequency distribution was obtained in the green stem (86.7%) at higher altitudes followed by the purple stem (31.0%) (basal only) at medium altitudes. Consecutively, medium (66.7%) and light green (63.6%) leaves were observed at higher and lower altitudinal ranges, respectively. The distribution of the two classes of GLH, glabrous and hairy, showed a strong trend in the four altitude ranges (Table 6). Glabrous increased its frequency of distribution from 54.5% in lowlands (≤ 2000) to 100% in highlands (≥ 3001), while hairy type decreased its distribution from 45.5% in low altitude (≤ 2000) and absent at greater than 3001 masl.

The most abundant GLC at the hard dough stage was white color (73.6%) at higher altitudes (≥ 3001 masl) followed by purple color (55.2%) at medium altitudes (2001-2500 masl) (Table 6, Appendix 2A and B). At the GLCM stage, white (76.7%) and purple (27.3%) colors were also mainly occurred at altitudes 2501-3000 and ≤ 2000 masl, respectively (Table 6). The LmC of amber or normal (66.7%) in higher altitudes and purple (55.2%) in medium altitudes had abundantly occurred while yellow (9.1%), brown (9.1%) and black (6.7%) colors were the least abundant. Moreover, white or normal color was predominately found at higher altitudes (60%) followed by purple (27.3%) and grey (27.3%) colors recorded at lower altitudes during maturity.

The distribution of spike density (SpDs) in barley accessions showed different distributions in the four altitude ranges (Table 6). Intermediate SpDs (66.7%) was the most abundant class for all altitude ranges except in lower altitudes (≤ 2000). Lax SpDs was found in higher frequency at lower altitude while the dense type had a relatively higher frequency at higher altitude (Table 6). The distribution of KrRw types also varied in the four altitude ranges (Table 6). Six-rowed type in barley accessions (73.3%) was the most dominant in the higher altitude range

(≥ 3001 masl) while two-rowed barley (63.6%) was more dominant in the lower altitude (≤ 2000 masl).

Kernel related traits of barley accessions including beak length (BkLns), beak shape (BkSh) and kernel shape (KrSh) showed different distributions in the four altitude ranges (Table 6). High frequency of long (60%) and medium (53%) BkLns was common in higher altitudes. The slightly curved (56.7%) BkSh was mainly observed at 2501-3000 masl followed by the straight (37.9%) BkSh at the medium altitude ranges. However, KrSh of the elongated-flattened and elongated were mainly found at altitudes ≤ 2000 and 2501-3000 masl, respectively. The dominant traits of BkLn and BkSh largely originated from higher altitudes except for KrSh which was concentrated at lower and higher altitudes (Table 6). The barley accessions showed a wide range of kernel color (KrC) distribution in the four altitude ranges (Table 6, Figure 6A-E). Among the KrC, the high frequency was observed in white color (56.7%) followed by purple (27.3%), black (26.7%) and grey (24.1%) colors. Besides, the white color showed an increasing trend from low to high altitude, while the brown color showed decreasing trend from low to high altitude ranges (Table 6).

4.1.3. Diversity index of qualitative traits

The level of barley diversity was analyzed using the Shannon-Weaver diversity index (H') in relation to region of origins and altitude ranges (Table 7). Across the region of origins, the overall mean values for H' in Oromia, Amhara, SNNP and Tigray regions were 0.71, 0.76, 0.77 and 0.79, respectively (Table 7). High H' values were estimated across the regions with the highest (0.99) values for LfC and GlC in Amhara, GlC in Tigray and KrRw, KrSh and LmAB in SNNP regions. However, very low to low H' values were estimated for StBr ($H'=0.00$) and LmA ($H'=0.00$) in all regions, AwC ($H'=0.41$) and StP ($H'=0.46$) in the Oromia region, and StP ($H'=0.58$) in SNNP regions. In addition, the H' values for each qualitative trait, over regions and altitude ranges, varied from 0.68 for AwC to 0.94 for KrRw, GlC and KrSh with an overall mean of 0.76 (Table 7, Figure 7). The diversity index of barley accessions was also estimated across four altitude ranges. Across the altitude ranges, the overall mean value for H' was 0.74 for altitude ≤ 2000 , 0.79 for altitude ranges 2001-2500, 0.75 for 2501-3000 and 0.76 for altitude ≥ 3001 masl (Table 8).

Table 7. Estimates of morphological diversity index (H') for 17 qualitative traits of barley accessions within the region of origins and altitude ranges, the value of H' ranges from 0 to 1, with greater values corresponding to greater diversity in the corresponding class. Mean bootstrap estimates are given with the corresponding standard error

Traits	Regions						Altitude ranges						Overall mean diversity
	Oromia	Amhara	Tigray	SNNP	BSE	BSs.e	≤2000	2001-2500	2501-3000	≥3001	BSE	BSs.e	
	H'	H'	H'	H'			H'	H'	H'	H'			
StP	0.46	0.69	0.96	0.58	0.83	0.02	0.88	0.73	0.79	0.64	0.80	0.02	0.72
LfC	0.87	0.99	0.95	0.87	0.77	0.03	0.84	0.82	0.75	0.96	0.77	0.03	0.89
StBr	0.00	0.00	0.00	0.00	-	-	0.00	0.00	0.00	0.00	-	-	-
GIH	0.65	0.76	0.89	0.91	0.8	0.05	0.00	0.86	0.79	0.99	0.81	0.05	0.88
GIC	0.94	0.99	0.99	0.91	0.9	0.01	0.93	0.99	0.92	0.94	0.90	0.02	0.94
LmC	0.89	0.77	0.96	0.79	0.68	0.02	0.91	0.99	0.75	0.83	0.68	0.02	0.86
AwC	0.41	0.64	0.90	0.83	0.67	0.04	0.52	0.74	0.81	0.68	0.66	0.03	0.68
KrRw	0.91	0.88	0.73	0.99	0.97	0.01	0.98	0.88	0.93	0.95	0.97	0.01	0.94
SpDs	0.91	0.83	0.81	0.95	0.89	0.02	0.86	0.89	0.88	0.92	0.90	0.02	0.90
GICM	0.79	0.95	0.68	0.83	0.82	0.02	0.95	0.94	0.78	0.93	0.83	0.02	0.91
LmCM	0.91	0.97	0.94	0.88	0.88	0.01	0.95	0.92	0.88	0.87	0.88	0.01	0.89
LmAB	0.76	0.76	0.79	0.99	0.85	0.04	0.89	0.89	0.89	0.65	0.84	0.05	0.76
LmA	0.00	0.00	0.00	0.00	-	-	0.00	0.00	0.00	0.00	-	-	-
BkSh	0.89	0.93	0.97	0.97	0.94	0.02	0.95	0.97	0.93	0.90	0.94	0.02	0.92
BkLn	0.87	0.83	0.91	0.86	0.81	0.01	0.95	0.92	0.84	0.89	0.81	0.02	0.90
KrSh	0.91	0.92	0.96	0.99	0.93	0.01	0.98	0.96	0.90	0.94	0.92	0.01	0.94
KrC	0.91	0.96	0.92	0.84	0.89	0.01	0.96	0.93	0.93	0.90	0.89	0.01	0.92
	0.71	0.76	0.79	0.77			0.74	0.79	0.75	0.76			
Overall mean in Ethiopia					0.76								

Abbreviations: H' =Shannon-Weiner index, BSE=Bootstrap estimate, BSs.e=Bootstrap standard error, StP=Stem pigmentation, LfC=Leaf color, StBr=Stem branching, GIH=Glume hairiness, GIC= Glume color at hard dough stage, LmC=Lemma color at hard dough stage, AwC=Awn color, KrRw=Kernel rows, SpDs=Spike density, GICM=Glume color at physiological maturity stage,

LmCM=Lemma color at physiological maturity stage, LmAB=Lemma awn barbs, LmA=Lemma awn, BkSh=Beak shape, BkLn=Beak length, KrSh=Kernel shape and KrC=Kernel color



Figure 7. Morphological traits variations were observed during the field trial

The majority of the qualitative traits such as spike, kernel and leaf related traits showed very high diversity index values over the four altitude ranges (Table 8), indicating that the barley accessions had high divergence for these traits. However, a very low H' value was estimated for stem branching ($H'=0.00$) and lemma awn ($H'=0.00$) in all altitude ranges. The barley accessions showed higher diversity at an altitude range of 2001-2500 masl (Table 8, Figure 7).

Table 8. Mean square values from the analysis of variance of diversity index (H') for the region of origins and altitude ranges based on 15 morphological traits

Traits	Region of origins(df=3)	Altitude ranges (df=3)
	Mean square	Mean square
StP	0.08 ^{**}	0.020 ^{***}
LfC	0.007 ^{**}	0.0070 ^{ns}
GlH	0.008 ^{ns}	0.363 ^{**}
GlC	0.020 ^{**}	0.432 ^{***}
LmC	0.003 ^{**}	0.002 ^{ns}
AwC	0.149 ^{ns}	0.002 ^{ns}
KrRw	0.022 ^{**}	0.004 [*]
SpDs	0.014 ^{ns}	0.017 ^{ns}
GlCM	0.027 [*]	0.387 ^{**}
LmCM	0.002 ^{**}	0.008 [*]
LmAB	0.025 ^{ns}	0.032 [*]
BkSh	0.001 ^{ns}	0.0002 ^{ns}
BkLn	0.003 ^{ns}	0.0002 ^{ns}
KrSh	0.003 [*]	0.0008 ^{ns}
KrC	0.001 ^{**}	0.003 [*]

Abbreviations: StP=Stem pigmentation, LfC=Leaf color, GlH=Glume hairiness, GlC= Glume color at hard dough stage, LmC= Lemma color at hard dough stage, AwC=Awn color, KrRw= Kernel rows, SpDs=Spike density, GlCM=Glume color at physiological maturity stage, LmCM=Lemma color at physiological maturity stage, LmAB= Lemma awn barbs, BkSh= Beak shape, BkLn=Beak length, KrSh=Kernel shape and KrC=Kernel color. Significant codes: 0 '****' 0.001, '***' 0.01, '**' 0.05, 'ns' 0.1, 'ns' 1, ns-non significant.

The analysis of variance of diversity index (H') based on the regions showed highly significant ($P<0.01$) variation for stem pigmentation, leaf color, glume color at hard dough stage, lemma color at hard dough and maturity stage, kernel rows and color. There were also significant

variations for glume color at physiological maturity stage and kernel shape ($P<0.05$) (Table 8) showing a variation for useful morphological traits of breeding importance. A very high significant difference ($P<0.001$) was observed at altitudinal ranges for stem pigmentation and glume color at hard dough stage (Table 8). The altitudes exhibited a highly significant difference ($P<0.01$) in traits of glume hairiness and glume color at physiological maturity stage. However, kernel rows and color, lemma color at physiological maturity stage and lemma awn barbs were significant variations ($P<0.05$) (Table 8).

4.1.4. Principal component analysis

A Principal component analysis (PCA) was also used to visualize variation among 85 accessions for 15 qualitative traits in the barley accessions (Figure 8A), which refine and reduce the number of selection criteria into a few meaningful and practically useful traits. The PCA on 15 qualitative traits described 21.91% and 16.37% of the total variation in PC1 and PC2 percent of the total variance (Figure 8A). Qualitative traits such as kernel color (0.37), glume color (0.37), lemma color (0.42), glume color at physiological maturity (0.43) and lemma color at physiological maturity stage (0.44) were the major loadings on the PC1 (Figures 8A and B).

Other traits, such as kernel rows (-0.46), leaf color (-0.42) and spike density (-0.36) were the major contributors to PC2 (Figures 8A and B) showing that yield traits including rowed types, spike density (kernel weight) and leaf color (light-harvesting to maximize productivity) had also a large contribution to the total morphological diversity in the barley accessions. Further, other qualitative traits which loaded highly on the other principal components should also be considered (Figures 8A and B). For example, traits such as beak shape (-0.45) and length (-0.41) and spike density (0.5) were the major contributors to the morphological variability in PC3 (Figures 8A and B). The first three principal components (PC1, PC2 and PC3) accounted for 49.3% of the total variation (Table 9).

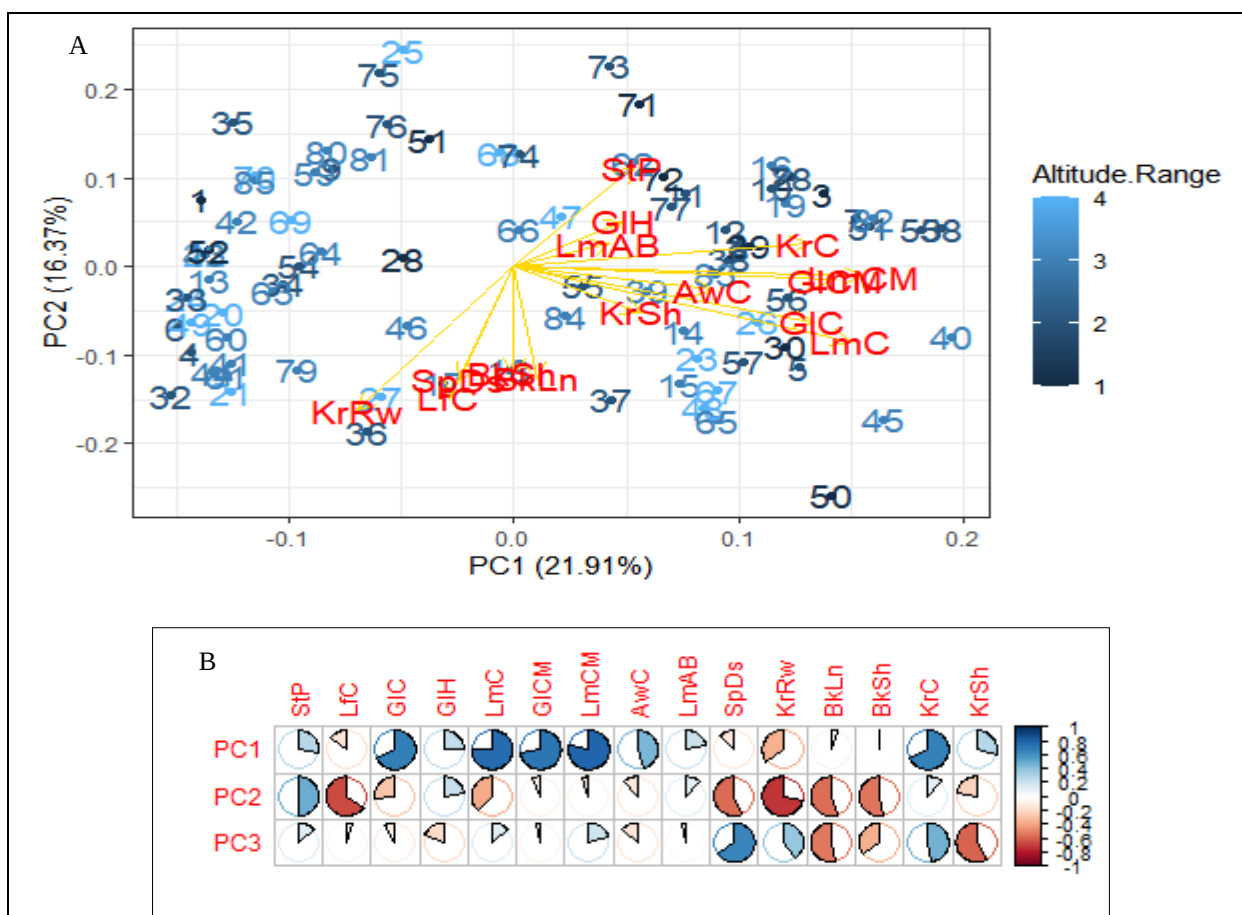


Figure 8. Variation and relationship of morphological traits of 85 barley accessions evaluated at Kulumsa during the 2020 and 2021 main cropping seasons. A. PC represents 15 morphological traits of barley accessions (for accession names refer to Table 2). Dots or numbers are barley accessions at various altitude ranges (1= ≤ 2000 , 2= 2001-2500, 3= 2501-3000 and 4 = ≥ 3001 , according to the legend). Golden vectors represent the traits in the PCA. PC1 and PC2 are shown on the X and Y axis, respectively, aside from their explained variance. B. Correlations between derived PC variables and original variables in the morphological traits. Correlation coefficients are reported in colors, according to the legend to the right. Abbreviations: StP=Stem pigmentation, LfC=Leaf color, GIH=Glume hairiness, GIC=Glume color at hard dough stage, LmC= Lemma color at hard dough stage, AwC=Awn color, KrRw= Kernel rows, SpDs=Spike density, GICM=Glume color at physiological maturity stage, LmCM=Lemma color at physiological maturity stage, LmAB=Lemma awn barbs, BkSh= Beak shape, BkLn= Beak length, KrSh= Kernel shape and KrC=Kernel color.

Table 9. Total variance explained by the three principal components extracted from a morphological data set of 15 traits in 85 barley accessions grown at Kulumsa in the 2020 and 2021 main cropping seasons

Principal Components	Standard deviation	Proportion of Variance	Cumulative Proportion
PC1	1.812	0.219	0.219
PC2	1.567	0.164	0.383
PC3	1.30	0.11	0.496

Abbreviation: PC=Principal component

4.1.5. Heat map analysis

The Heat map analysis based on 17 morphological traits of 85 barley accessions was grouped into three clusters (Figure 9, Table 10). Cluster I contained the largest number of barley accessions (40) followed by Cluster II (25) and Cluster III (20). Besides, the number of accessions per cluster varied from 20 accessions in cluster III to 40 accessions in cluster I (Figure 9, Table 10). Cluster I included accessions from four regions with the prevailing percentage in Amhara (12.94%) followed by Oromia (11.76%), Tigray (11.76%) and SNNP (10.59%) regions. The majority of the accessions were mainly found between 2001-3000 masl. Cluster II contributed 8.24% and 10.59% of accessions from the SNNP and Oromia regions, respectively. Most of the accessions included from an altitude of 2001-3000 masl. Cluster III consisted of less number of accessions from four regions with a high percentage in the Amhara (11.76%) and the accessions encompassed both in the medium and above 3001 masl (Figure 9, Table 10).

Qualitative traits did not cluster the 85 barley accessions along the collection region of origins and altitude ranges (Figure 9, Table 10). For example, Cluster-I was the largest cluster containing 40 accessions which were composed of 11 accessions from Amhara (Agew Awi, West and East Gojjam, North Gondar and Shewa zone), 10 accessions from Oromia (Arssi, Bale, East Hararghe and Welega, East and North Shewa zone), and 10 accessions from Tigray (Central, East and South zone) and 9 accessions from SNNP (Gamo-Gofa, Sidama, North Omo, and Gurage zone). This result indicated that barley accessions from different collection origins were grouped into varied clusters with different sizes (Figure 9, Table 10).

Table 10. Clustering of 85 barley accessions into three groups using morphological traits, the distribution of accessions across the region of origins and altitude ranges

Regions	Cluster I	Cluster II	Cluster III	Total
Oromia	10	9	3	22
Amhara	11	6	10	27
Tigray	10	3	2	15
SNNP	9	7	5	21
Total	40	25	20	85
Altitude Ranges				
≤2000	3	6	2	11
2001-2500	14	8	7	29
2501-3000	18	8	4	30
≥3001	5	3	7	15
Total	40	25	20	85

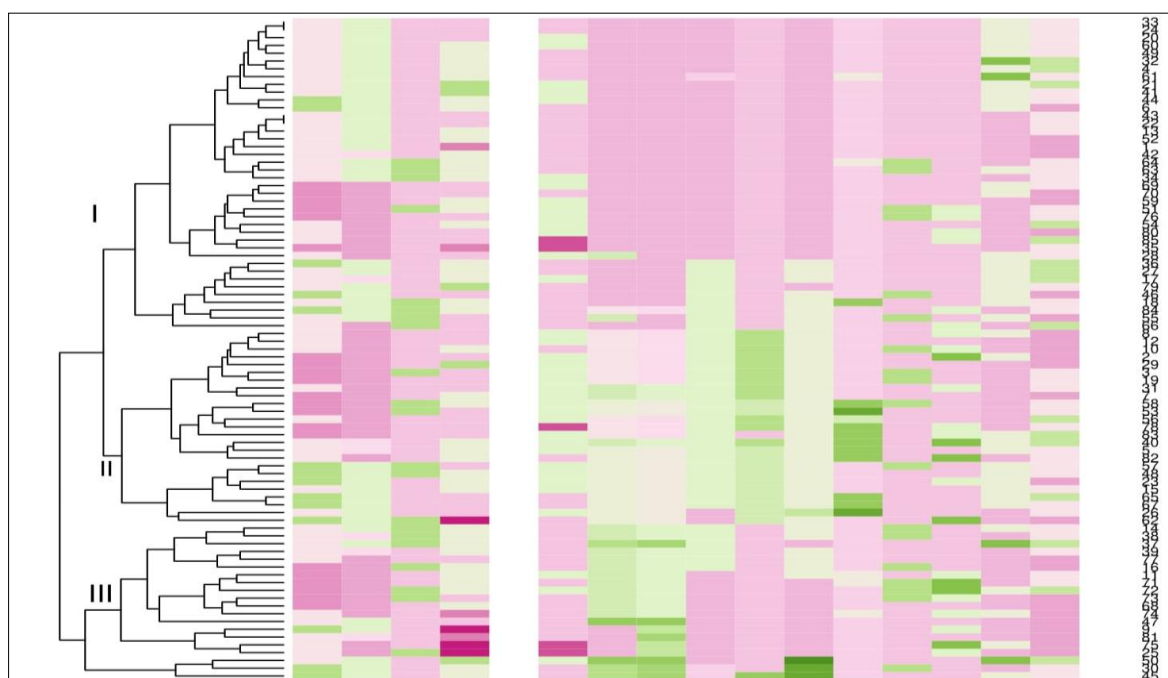


Figure 9. Heatmap analysis shows similarity distance based on the color distribution among 85 barley accessions of 17 qualitative traits evaluated at Kulumsa in the 2020 and 2021 main cropping seasons (for accession name and collection origins refer to Table 2). On the left of the matrix, barley accessions are grouped into 3 distinct clusters. Abbreviations: StP=Stem pigmentation, LfC=Leaf color, StBr=Stem branching, GlH=Glume hairiness, GlC=Glume

color at hard dough stage, LmC= Lemma color at hard dough stage, AwC=Awn color, KrRw= Kernel rows, SpDs=Spike density, GlCM=Glume color at physiological maturity stage, LmCM=Lemma color at physiological maturity stage, LmAB=Lemma awn barbs, LmA=Lemma awn, BkSh=Beak shape, BkLn=Beak length, KrSh=Kernel shape and KrC=Kernel color.

4.2. Quantitative traits variations in Ethiopian barley germplasm

4.2.1. Analysis of the variance (ANOVA)

Analysis of the variance (ANOVA) computed using combined from two seasons is presented in Table 11. Mean squares of all quantitative traits showed highly significant ($p < 0.001$) variations, except days to emergency and the number of tillers per plant. Similarly, the seasonal/environmental variance (σ^2_{sn}) was significant ($p < 0.001$) for most quantitative traits, but not for days to emergency, flag leaf length, spike length, awn length, number of tillers per plant, number of fertile tillers per plant, lodging and harvest index. In addition, the genotype by season interaction variance ($\sigma^2_{gen \times sn}$) was significant ($p < 0.001$) on the majority of quantitative traits except for the number of tillers per plant, number of fertile tillers per plant, grain filling period and harvest index (Table 11).

4.2.2. Broad sense heritability

Broad sense heritability (H^2) of over seasons for the assessed quantitative traits was presented in Table 12. The H^2 ranged from 16% for the number of tillers per plant to 94% for days to heading and flowering. Grain yield per hectare (GYHa), spike weight (SpW), grain yield per plant (GYPP), thousand kernels weight (TKW), number of kernels per plant (NKP), biomass yield per plant (BMYPP), days to physiological maturity (DPM), days to heading (DH) and flowering (DF) exhibited high heritability ($> 75\%$). Moderate ($> 50\%$) broad sense heritability was obtained for plant height, spike length, number of fertile tillers per plant, lodging, biomass yield, grain filling period and rate, harvest index per plant and hectare. On the other hand, days to emergency, flag leaf and awn length, the number of tillers per plant and biomass production rate showed a lower ($< 50\%$) heritability.

Table 11. Grand BLUP means (GM), the estimate of variance components including phenotypic (σ^2_p), genotypic (σ^2_g), season (σ^2_{sn}), genotype by season interaction variance ($\sigma^2_{gen \times sn}$), residual variance (σ^2_r) and coefficient of variations (CV) for each trait based on the combined analyses of variance over the two seasons

Traits	GM	σ^2_{gen}	Significance	σ^2_{sn}	Significance	$\sigma^2_{gen \times sn}$	Significance	σ^2_r	σ^2_{ph}	CV
DE	6.69	0.05	ns	0.11	ns	0.10	**	0.36	0.26	8.59
DH	63.01	30.75	***	6.22	**	1.35	**	4.63	38.32	3.42
DTF	66.00	31.82	***	8.48	**	1.42	**	5.09	41.72	3.43
DPM	98.41	33.74	***	30.78	**	2.22	**	7.34	66.74	2.75
PH	101.5	14.20	***	74.76	**	11.64	***	27.35	100.6	5.15
FLL	16.15	0.90	**	1.49	ns	1.17	***	3.09	3.56	10.89
SpL	7.14	0.40	***	0.001	ns	0.19	***	0.47	0.59	9.55
AwL	13.50	0.21	*	1.34	ns	0.33	**	1.08	1.88	7.70
NTPP	3.68	0.01	ns	0.05	ns	0.002	ns	0.14	0.062	10.16
NFTP	2.62	0.04	***	0.01	ns	1.92	ns	0.11	1.97	12.45
NKP	32.60	54.18	***	6.08	*	0.03	ns	21.85	60.29	14.34
SpW	1.71	0.12	***	0.11	**	0.04	***	0.06	0.27	14.49
TKW	40.45	17.84	***	8.89	**	3.64	**	11.61	30.37	8.42
Ldg	3.04	0.23	***	0.001	ns	0.10	**	0.32	0.33	18.53
GYPP	1.44	0.09	***	0.09	**	0.03	***	0.05	0.21	15.46
GYHa	3.77	0.23	***	0.27	**	0.08	***	0.15	0.58	10.27
BMYP	3.92	0.43	***	0.40	*	0.09	***	0.17	0.92	10.47
BMYPHa	12.28	1.29	***	6.02	*	0.79	***	1.60	8.1	10.30
GFP	32.43	5.61	***	6.86	**	0.37	ns	9.33	12.84	9.42
GFR	117.2	202.26	***	44.31	*	106.90	***	231.68	353.47	12.99
BMPR	124.2	46.87	***	317.77	*	90.48	***	171.86	455.12	10.56
HIP	36.3	6.88	***	3.16	ns	3.12	***	8.40	13.16	8.10
HI	31.0	6.62	***	3.62	ns	0.56	ns	7.05	10.8	8.57

Abbreviation: DE= days to emergency, DH=days to heading, DTF=days to flowering, DPM=days to physiological maturity, PH=plant height, FLL=flag leaf length, SpL= spike length, AwL=awn length, NTPP=number of tillers per plant, NFTP=number of fertile tillers per plant, NKP=number of kernels per plant, SpW=spike weight, Ldg=lodging, TKW=thousand kernels weight, GYPP=grain yield per plant, GYHa=grain yield per hectare (in t/ha), BMYP=biomass yield per plant (gram), BMY= biomass yield (t/ha), BMPR= biomass production rate (kg/ha/day), GFR=grain filling rate (kg/ha/day), GFP= grain filling period (day), HIP= harvest index per plant, HI=harvest index. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 'ns' 0.1 'ns' 1.

4.2.3. Expected genetic advance

Expected genetic advance (GA) showed wide variations, ranging from 0.08 in the number of tillers per plant to 24.79 in the grain filling rate (Table 12). Moderate genetic advance was recorded for thousand kernels weight (8.97%), plant height (10.95%), days to heading (11.99%), days to flowering (12.51%), number of kernels per plant (14.56%), biomass production rate (15.38%), days to physiological maturity (15.48%), and the highest in grain filling rate (24.79%) (Table 12). In addition, the highest genetic advance as a percent of the mean was recorded for biomass yield (29.60%), grain yield (31.21%), biomass yield per plant (41.84%), number of kernels per plant (44.65%), spike weight (48.20%), grain yield per plant (51.13%), and the number of fertile tillers per plant (64.01%) (Table 12). Other traits such as days to heading, days to flowering and physiological maturity, plant height, spike length, grain filling period, biomass production rate, harvest index per plant and hectare revealed a moderate (10-20%) genetic advance as a percent of the mean (Table 12).

4.2.4. Phenotypic and genotypic coefficients of variation

Phenotypic coefficient of variation (PCV) values ranged from 6.77% for the number of tillers per plant to 53.57% for the number of fertile tillers per plant. The highest PCV (>20%) was revealed for grain yield (20.20%) and biomass yield per hectare (23.18 %), number of kernels per plant (23.82%), biomass yield per plant (24.47%), spike weight (30.39%), grain yield per plant (31.82%) and number of fertile tillers per plant (53.57 %). However, the lowest PCV (<10%) values were recorded in the number of tillers per plant (6.77%), days to emergency (7.62%), days to physiological maturity (8.30%), days to heading (9.82%) and flowering (9.79%) (Table 12). Genotypic coefficient of variation (GCV) values also ranged from 2.72 to 22.58%. Among the morphological traits, moderate GCV (10-20%) was recorded for thousand kernels weight (10.44%), grain yield per hectare (12.72%), Lodging (15.78%) and biomass yield per plant (16.73%). Higher genotypic coefficients of variation (>20%) values were obtained in spike weight (20.26%), grain yield per plant (20.83) and number of kernels per plant (22.58%) (Table 12). However, the number of tillers per plant (2.72%), days to emergency (3.34%), awn length (3.39%) and plant height (3.71%) showed the very lowest GCV values (<5%).

Table 12. Estimate of variance components including broad sense heritability (H^2), phenotypic coefficient of variation (PCV) and genotypic (GCV), genetic advance (GA) and genetic advance as a percent of the mean (GAM) for each trait in pooled data over the two seasons

Traits	H^2 (%)	PCV (%)	GCV (%)	GA	GAM
DE	25	7.62	3.34	0.26	3.93
DH	94	9.82	8.80	11.99	19.02
DTF	94	9.79	8.55	12.51	18.95
DPM	92	8.30	5.90	15.48	15.73
PH	53	9.9	3.71	10.95	10.79
FLL	40	11.68	5.87	1.55	9.63
SpL	66	10.76	8.86	1.04	14.63
AwL	33	10.16	3.39	0.93	6.90
NTPP	16	6.77	2.72	0.08	2.23
NFTP	58	53.57	7.63	1.68	64.01
NKP	91	23.82	22.58	14.56	44.65
SpW	77	30.39	20.26	0.82	48.20
TKW	79	13.62	10.44	8.97	22.17
Ldg	64	18.90	15.78	0.76	24.91
GYPP	78	31.82	20.83	0.74	51.13
GYHa	75	20.20	12.72	1.18	31.21
BMYP	83	24.47	16.73	1.64	41.84
BMYPHa	62	23.18	9.25	3.63	29.60
GFP	69	11.05	7.30	5.09	15.71
GFR	64	16.04	12.13	24.79	21.15
BMPP	35	17.18	5.51	15.38	12.38
HIP	65	10.1	7.23	4.86	13.38
HI	56	10.60	8.30	3.79	12.23

Abbreviation: DE=days to emergency, DH=days to heading, DTF=days to flowering, DPM= days to physiological maturity, PH= plant height, FLL= flag leaf length, SpL=spike length, AwL=awn length, NTPP=number of tillers per plant, NFTP=number of fertile tillers per plant, NKP= number of kernels per plant, SpW=spike weight, Ldg=lodging, TKW=thousand kernels weight, GYPP=grain yield per plant, GYHa=grain yield(in t/ha), BMYP=biomass yield per plant (gram), BMY=biomass yield (t/ha), BPP= biomass production (kg/ha/day), GFR=grain filling rate (kg/ha/day), GFP=grain filling period (day), HIP=harvest index per plant, HI=harvest index.

4.2.5. Phenotypic and genotypic correlation

Phenotypic and genotypic association estimates among all possible pairs of morphological traits are presented in Tables 13 and 14. The phenotypic correlation coefficient of grain yield was highly significant ($p < 0.001$) showing a positive phenotypic correlation with number of fertile tillers ($r = 0.46^{***}$), number of kernels per plant ($r = 0.52^{***}$), spike weight ($r = 0.55^{***}$), grain ($r = 0.58^{***}$) and biomass yield ($r = 0.53^{***}$) per plant, biomass yield per hectare ($r = 0.88^{***}$), lodging ($r = 0.61^{***}$), grain filling rate ($r = 0.81^{***}$) and biomass production rate ($r = 0.78^{***}$), harvest index per plant ($r = 0.47^{***}$) and harvest index per hectare ($r = 0.57^{***}$). However, plant height ($r = 0.06$), flag leaf length ($r = 0.16$) and spike length ($r = 0.17$) revealed non-significant negative phenotypic correlation (Table 13).

Phenological traits such as days to heading ($r = 0.40^{***}$), days to flowering ($r = 0.40^{***}$), days to physiological maturity ($r = 0.52^{***}$) and grain filling period ($r = 0.30^{***}$) were highly significant ($p < 0.001$) a positively correlated with grain yield. Grain yield was highly significant ($p < 0.001$) a positive genotypic association with the majority of morphological traits except for flag leaf length ($r = 0.53^{***}$) and spike length ($r = 0.34^{***}$) a negatively highly significant, and plant height ($r = 0.11$) a negatively non-significant genotypic correlation (Table 14). Genotypic correlation coefficient values were high for most of the quantitative traits as compared to corresponding phenotypic correlation coefficient values.

4.2.6. Principal component and cluster analysis

4.2.6.1. Principal component analysis

A principal component analysis (PCA) was used to observe the general pattern for the difference in quantitative traits of barley genotypes (Figure 10). This enabled the categorization of the major traits accounting for the greater share in the visualized variations among barley genotypes. The PC1 and PC2 explained about 46.15 and 17.06% of the total variation, respectively (Figure 10).

Table 13. Phenotypic correlation coefficients for twenty two quantitative traits of 125 barley genotypes performed using the combined data over two seasons

Traits	DE	DH	DTF	DPM	PH	FLL	SPL	AwL	NTPP	NFTP	TKW
DH	0.42***										
DTF	0.41***	0.99***									
DPM	0.41***	0.88***	0.88***								
PH	-0.07 ^{ns}	0.06 ^{ns}	0.06 ^{ns}	0.11 ^{ns}							
FLL	-0.27**	0.05 ^{ns}	0.06 ^{ns}	0.03 ^{ns}	0.33***						
SPL	-0.10 ^{ns}	-0.14 ^{ns}	-0.15 ^{ns}	-0.19*	0.22*	0.18*					
AwL	0.19*	0.31***	0.30***	0.26**	0.20*	0.13 ^{ns}	0.33***				
NTPP	0.02 ^{ns}	0.28**	0.28**	0.28**	-0.03 ^{ns}	0.17 ^{ns}	0.03 ^{ns}	0.02 ^{ns}			
NFTP	0.04 ^{ns}	0.34***	0.32***	0.44***	0.08 ^{ns}	-0.02 ^{ns}	-0.09 ^{ns}	0.15 ^{ns}	0.47***		
TKW	-0.08 ^{ns}	-0.09 ^{ns}	-0.09 ^{ns}	-0.08 ^{ns}	0.22*	0.17 ^{ns}	0.38***	0.28**	0.12 ^{ns}	0.15 ^{ns}	
NKP	0.25**	0.44***	0.44***	0.55***	-0.04 ^{ns}	-0.07 ^{ns}	-0.54***	-0.01 ^{ns}	0.17 ^{ns}	0.32***	-0.51***
SpW	0.29***	0.50***	0.51***	0.61***	0.08 ^{ns}	0.00 ^{ns}	-0.46***	0.10 ^{ns}	0.20*	0.38***	-0.16 ^{ns}
Ldg	0.35***	0.44***	0.43***	0.51***	0.11 ^{ns}	-0.23*	-0.13 ^{ns}	0.19*	0.15 ^{ns}	0.46***	0.21*
GYPP	0.24**	0.45***	0.46***	0.58***	0.13 ^{ns}	0.02 ^{ns}	-0.44***	0.09 ^{ns}	0.20*	0.40***	-0.12 ^{ns}
GYHa	0.24**	0.40***	0.40***	0.52***	-0.06 ^{ns}	-0.16 ^{ns}	-0.17 ^{ns}	0.13 ^{ns}	0.28**	0.46***	0.12 ^{ns}
BMYP	0.22*	0.58***	0.59***	0.68***	0.25**	0.11 ^{ns}	-0.39***	0.15 ^{ns}	0.20*	0.41***	-0.14 ^{ns}
BMYPHa	0.23**	0.56***	0.56***	0.64***	0.08 ^{ns}	-0.06 ^{ns}	-0.26**	0.14 ^{ns}	0.27**	0.45***	-0.01 ^{ns}
GFP	0.06 ^{ns}	-0.14 ^{ns}	-0.15 ^{ns}	0.34***	0.11 ^{ns}	-0.06 ^{ns}	-0.12 ^{ns}	-0.06 ^{ns}	0.03 ^{ns}	0.29**	0.01 ^{ns}
GFR	0.20*	0.47***	0.48***	0.31***	-0.10 ^{ns}	-0.10 ^{ns}	-0.09 ^{ns}	0.16 ^{ns}	0.26**	0.28**	0.12 ^{ns}
BMPR	0.03 ^{ns}	0.13 ^{ns}	0.14 ^{ns}	0.17 ^{ns}	0.04 ^{ns}	-0.09 ^{ns}	-0.21*	0.01 ^{ns}	0.18*	0.29**	0.03 ^{ns}
HIP	0.18*	0.003 ^{ns}	0.003 ^{ns}	0.14 ^{ns}	-0.17 ^{ns}	-0.16 ^{ns}	-0.38***	-0.06 ^{ns}	0.14 ^{ns}	0.24**	-0.01 ^{ns}
HI	0.11 ^{ns}	-0.12 ^{ns}	-0.13 ^{ns}	-0.04 ^{ns}	-0.27**	-0.22*	0.07 ^{ns}	0.02 ^{ns}	0.11 ^{ns}	0.18*	0.27**

Abbreviations: DH=days to heading, DTF=days to flowering, DPM= days to physiological maturity, PH= plant height, FLL= flag leaf length, SpL=spike length, AwL=awn length, NTPP=number of tillers per plant, NFTP=number of fertile tillers per plant,

SpW=spike weight, Ldg=lodging, TKW=thousand kernels weight, GYPP=grain yield per plant, GYHa=grain yield per hectare(in t/ha), BMYPP= biomass yield per plant (gram), BMYHa=biomass yield per hectare (t/ha), BPR=biomass production (kg/ha/day), GFR=grain filling rate (kg/ha/day), GFP=grain filling period (day), HIP=harvest index per plant, HI=harvest index per hectare. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘ns’ 0.1 ‘ns’ 1.

Table 13. Phenotypic correlation *Continued*

Traits	NKP	SpW	Ldg	GYPP	GYHa	BMYPP	BMYHa	GFP	GFR	BMPR	HIP	HI
NKP												
SpW	0.84***											
Ldg	0.36**	0.43***										
GYPP	0.83***	0.98***	0.43***									
GYHa	0.52***	0.55***	0.61***	0.58***								
BMYPP	0.80***	0.94***	0.42***	0.94***	0.53***							
BMYHa	0.62***	0.63***	0.58***	0.64***	0.88***	0.67***						
GFP	0.27**	0.28**	0.22*	0.29**	0.30***	0.26**	0.23***					
GFR	0.35***	0.38***	0.46***	0.40***	0.81***	0.37***	0.73***	-0.31***				
BMPR	0.44***	0.41***	0.40***	0.46***	0.78***	0.42***	0.86***	0.08ns	0.73***			
HIP	0.56***	0.65***	0.29**	0.71***	0.47***	0.45***	0.37***	0.26**	0.30***	0.39***		
HI	0.01 ^{ns}	0.05 ^{ns}	0.30***	0.07 ^{ns}	0.57***	-0.06 ^{ns}	0.12 ^{ns}	0.18*	0.44***	0.16 ^{ns}	0.33***	

Abbreviations: NKP=number of kernels per plant, SpW=spike weight, Ldg=lodging, TKW= thousand kernels weight, GYPP=grain yield per plant, GYHa=grain yield per hectare(in t/ha), BMYPP=biomass yield per plant (gram), BMYHa=biomass yield per hectare (t/ha), BPR= biomass production (kg/ha/day), GFR=grain filling rate (kg/ha/day), GFP= grain filling period (day), HIP= harvest index per plant, HI=harvest index per hectare. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘ns’ 0.1 ‘ns’ 1.

Table 14. Genotypic correlation coefficients for twenty two quantitative traits of 125 barley genotypes based on the combined data analyses over two seasons

Traits	DE	DH	DTF	DPM	PH	FLL	SPL	AwL	NTPP	NFTP	TKW
DH	0.80***										
DTF	0.74***	1.00***									
DPM	0.81***	0.92***	0.91***								
PH	-0.63***	0.10 ^{ns}	0.10 ^{ns}	0.14 ^{ns}							
FLL	-0.46***	0.11 ^{ns}	0.11 ^{ns}	0.02 ^{ns}	0.67***						
SPL	-0.30***	-0.18*	-0.20*	-0.26**	0.40***	0.08 ^{ns}					
AwL	0.70***	0.48***	0.47***	0.41***	0.40***	0.49***	0.53***				
NTPP	1.00***	0.64***	0.62***	0.67***	0.29***	0.31***	-0.20*	0.39***			
NFTP	0.07 ^{ns}	0.49***	0.47***	0.64***	0.27**	-0.09 ^{ns}	-0.08 ^{ns}	0.50***	0.92***		
TKW	-0.13 ^{ns}	-0.13 ^{ns}	-0.14 ^{ns}	-0.13 ^{ns}	0.33***	0.30***	0.55***	0.44***	0.29***	0.27**	
NKP	0.53***	0.47***	0.48***	0.59***	-0.06 ^{ns}	-0.15 ^{ns}	-0.70***	-0.11 ^{ns}	0.48***	0.47***	-0.62***
SpW	0.67***	0.56***	0.57***	0.69***	0.11 ^{ns}	-0.05 ^{ns}	-0.66***	-0.06 ^{ns}	0.71***	0.58***	-0.38***
Ldg	0.67***	0.53***	0.52***	0.62***	0.02 ^{ns}	-0.33***	-0.22*	0.14 ^{ns}	0.54***	0.84***	0.19*
GYPP	0.52***	0.50***	0.52***	0.63***	0.16 ^{ns}	-0.06 ^{ns}	-0.64***	-0.07 ^{ns}	0.77***	0.61***	-0.32***
GYHa	0.55***	0.43***	0.44***	0.57***	-0.11 ^{ns}	-0.53***	-0.34***	0.26**	0.73***	0.72***	0.14 ^{ns}
BMYP	0.41***	0.62***	0.64***	0.74***	0.39***	0.18*	-0.55***	0.12 ^{ns}	0.64***	0.60***	-0.28**
BMYPHa	0.73***	0.72***	0.72***	0.84***	0.17 ^{ns}	-0.39***	-0.55***	0.39***	0.77***	0.81***	-0.03 ^{ns}
GFP	0.24***	-0.13 ^{ns}	-0.14 ^{ns}	0.27**	0.13 ^{ns}	-0.24**	-0.17 ^{ns}	-0.12 ^{ns}	0.02 ^{ns}	0.44***	-0.02 ^{ns}
GFR	0.48***	0.55***	0.56***	0.46***	-0.12 ^{ns}	-0.42***	-0.27**	0.34***	0.67***	0.50***	0.17 ^{ns}
BMPR	0.43***	0.28**	0.29**	0.38***	0.22*	-0.70***	-0.71***	0.29**	0.58***	0.73***	0.08 ^{ns}
HIP	0.55***	0.006 ^{ns}	-0.009 ^{ns}	0.11 ^{ns}	-0.37***	-0.55***	-0.60***	-0.38***	0.73***	0.42***	-0.22*
HI	-0.003 ^{ns}	-0.26**	-0.25**	-0.14 ^{ns}	-0.61***	-0.45***	0.20*	-0.15 ^{ns}	0.22*	0.27**	0.41***

Abbreviations: DH=days to heading, DTF=days to flowering, DPM=days to physiological maturity, PH=plant height, FLL=flag leaf length, SpL=spike length, AwL=awn length, NTPP=number of tillers per plant, NFTP=number of fertile tillers per plant, SpW=

spike weight, Ldg=lodging, TKW=thousand kernels weight, GYPP=grain yield per plant, GYHa=grain yield per hectare(in tons/ha), BMYPP=biomass yield per plant (gram), BMYHa=biomass yield per hectare (t/ha), BPR=biomass production (kg/ha/day), GFR=grain filling rate (kg/ha/day), GFP=grain filling period (day), HIP= harvest index per plant, HI=harvest index. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘ns’ 0.1 ‘ns’ 1.

Table 14. Genotypic correlations *continued*

Traits	NKP	SpW	Ldg	GYPP	GYHa	BMYPP	BMYHa	GFP	GFR	BMPR	HIP	HI
NKP												
SpW	0.97***											
Ldg	0.45***	0.52***										
GYPP	0.95***	0.98***	0.50***									
GYHa	0.59***	0.67***	0.87***	0.70***								
BMYPP	0.90***	0.97***	0.50***	0.96***	0.61***							
BMYHa	0.80***	0.90***	0.97***	0.89***	0.92***	0.89***						
GFP	0.32***	0.36***	0.30***	0.36***	0.38***	0.33***	0.34***					
GFR	0.45***	0.53***	0.77***	0.55***	0.83***	0.48***	0.78***	-0.21*				
BMPR	0.77***	0.86***	1.00***	0.91***	0.95***	0.79***	0.83***	0.27**	0.83***			
HIP	0.65***	0.65***	0.32***	0.71***	0.62***	0.50***	0.51***	0.31***	0.47***	0.77***		
HI	-0.03 ^{ns}	-0.04 ^{ns}	0.34***	0.03 ^{ns}	0.75***	-0.16 ^{ns}	0.44***	0.30***	0.60***	0.82***	0.52***	

Abbreviations: NKP=number of kernels per plant, SpW=spike weight, Ldg=lodging, GYPP=grain yield per plant(gram), GYHa=grain yield per hectare(in tons/ha), BMYPP=biomass yield per plant (gram), BMYHa=biomass yield per hectare (tons/ha), BPR= biomass production (kg/ha/day), GFR=grain filling rate (kg/ha/day), GFP= grain filling period (day), HIP= harvest index per plant, HI=harvest index per hectare. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘ns’ 0.1 ‘ns’ 1.

The observed variations in PC1 were dominantly contributed by days to heading, days to flowering and physiological maturity, biomass and grain yield per hectare, number of kernels per plant, and grain filling rate. The variations in PC2 were largely contributed by thousand kernels weight, biomass production rate, days to heading, days to flowering and physiological maturity (Figure 10).

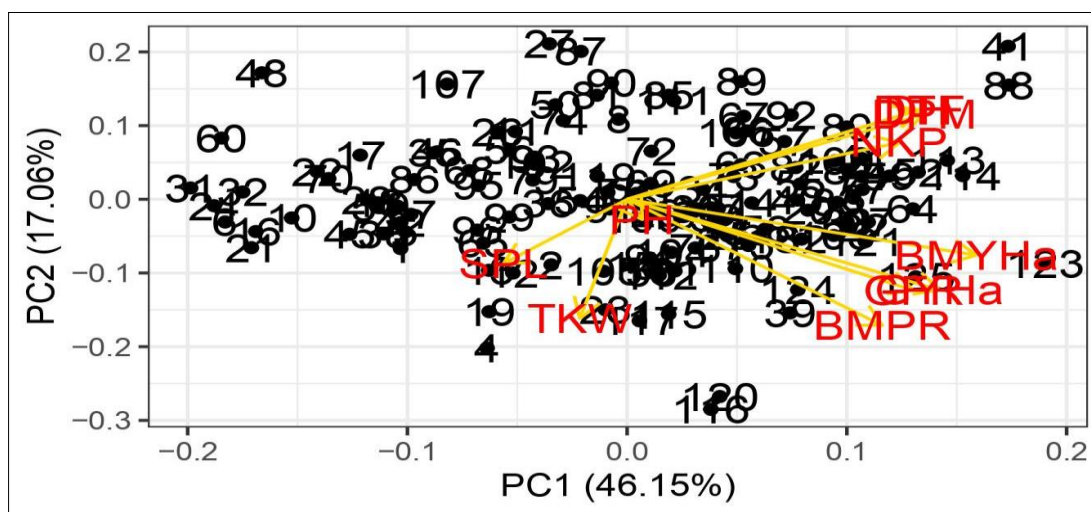


Figure 10. PC representing quantitative traits of barley genotypes evaluated at Kulumsa during the 2021 and 2022 main cropping seasons. Red vectors represent the abbreviated codes for the traits (variables) in the PCA. PC1 and PC2 are shown on the X and Y axis, respectively, aside from their explained variance.

4.2.6.2. Cluster analysis

Clustering of the entire 125 barley genotypes revealed three major clusters (Figure 11). The number of barley genotypes in each of the clusters ranged from twenty five in cluster II to sixty barley genotypes in cluster III (Figure 11). The numbers of genotypes per cluster various from 25 genotypes in cluster II to 60 genotypes in cluster III (Figure 11). In the cluster analysis 60 genotypes (48%) were grouped in cluster III, 40 genotypes (32%) in cluster I, and the result 25 genotypes (20%) into cluster II (Figure 11). Hence, the result revealed that genotypes from varied geographical origins that are even far apart were clustered together and genotypes collected from the same regions were placed under different groups. The result indicated that the genotypes were distributed in varied clusters showing existence of variations between the studied genotypes.

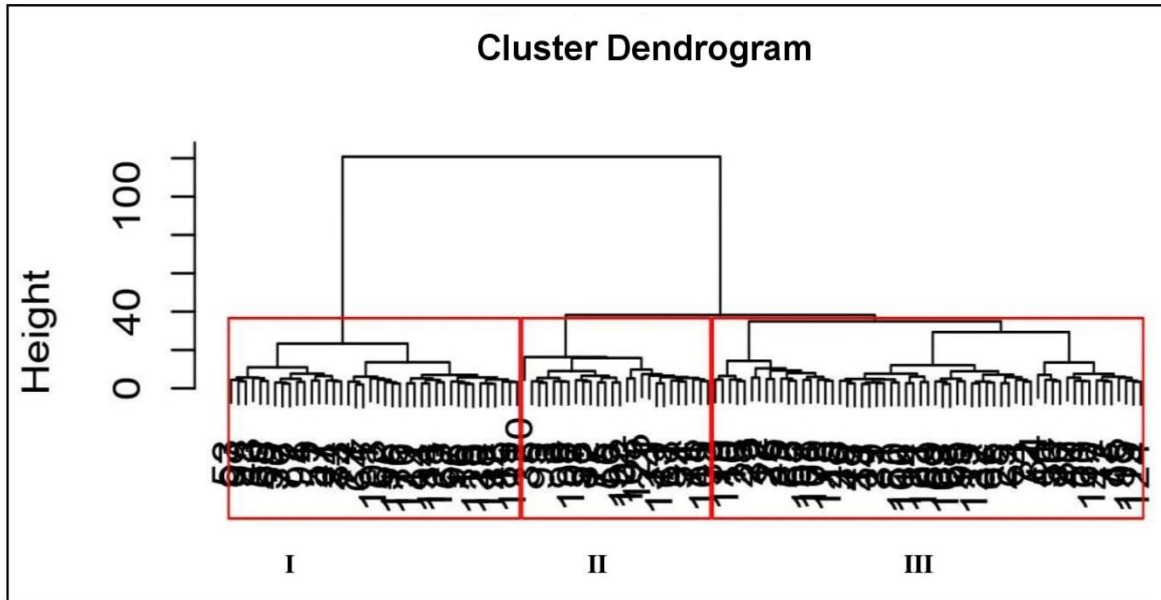


Figure 11. Cluster analysis of barley genotypes using pooled data (for genotypes code or numbers 1 to 125 refer to Table 2). Red color line represents the clusters for the genotypes in the cluster dendrogram. Each cluster had its specific feature for its cluster formation. Close the matrix shows the strong relationship among the barley genotypes.

4.3. Molecular study using SNP markers

4.3.1. SNP variation and markers distribution

The single nucleotide polymorphism (SNP) distribution of the 10,103 SNPs remaining after imposing a quality control threshold was plotted in 10Mbp (megabase pair) window size across the *H.vulgare* genome (Figure 12). Variant distribution was not completely uniform across the chromosomes. We detected an average of 97 SNPs per 10 Mb. The highest SNP density (>129 SNPs/10Mb) was observed on chromosomes Chr2, Chr3, Chr6 and Chr7. The medium SNP density (between 33 to 129 SNPs/10Mbp) was observed on chromosomes Chr4 and Chr5. However, the lowest average SNP density was found on Chr1 (<33 SNPs/10Mb).

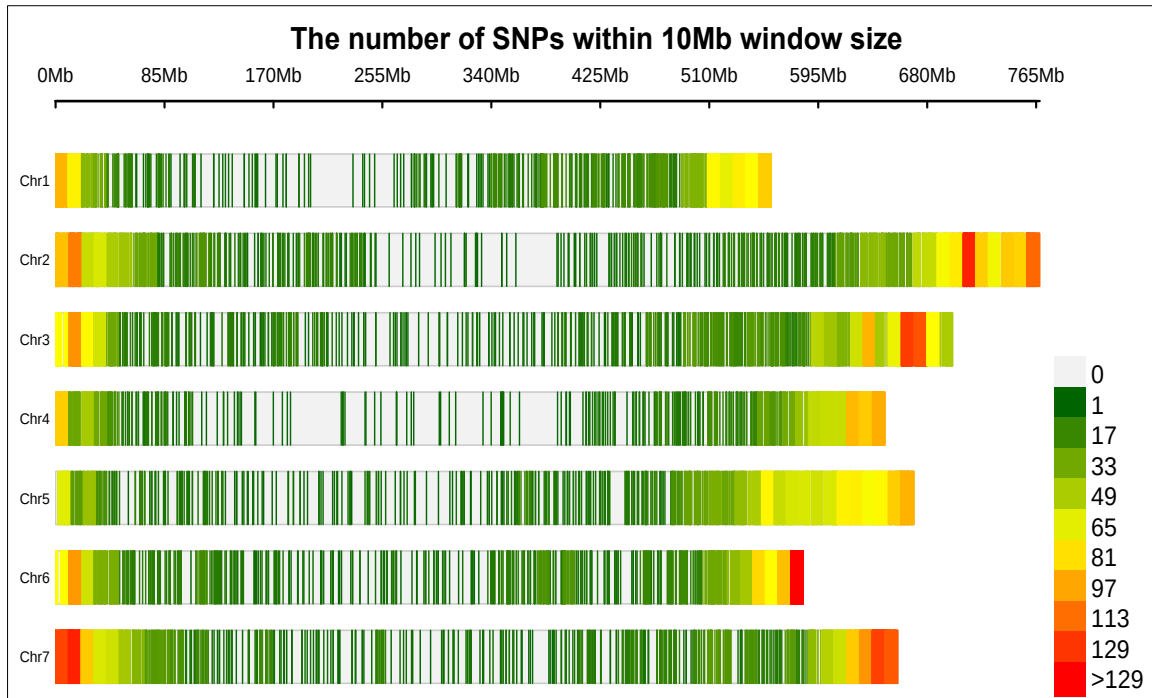


Figure 12. Distribution of SNP markers within 10Mb window size across seven chromosomes. Colored bars are SNP counts in 10Mb intervals

4.3.2. Genetic diversity and relationship

The genetic parameters included gene diversity, heterozygosity (the ratio of observed to expected heterozygosity), minor allelic frequency (MAF) and the polymorphic information content (PIC) of the 10,103 SNP markers of 105 barley genotypes were presented in Table 15. The result showed that the average genetic diversity was 0.253 and ranged from 0.177 to 0.311. The highest gene diversity (0.311) was observed for genotypes collected from the Amhara region followed by the Oromia (0.288), SNNP (0.266), Tigray (0.221) and ICARDA (0.177). The PIC values ranged from 0.151(ICARDA) to 0.271(SNNP) with an average polymorphism value of 0.216, which indicates high genetic diversity between the barley genotypes.

The MAF ranged from 0.092 to 0.146, with an average of 0.118. Genotypes from the Oromia region showed the highest MAF (0.146) followed by ICARDA (0.145) collection and Amhara (0.107) genotypes. However, the lowest MAF was exhibited in the SNNP (0.101) and Tigray (0.092) genotypes. In addition, the observed heterozygosity (H_o) ranged from 0.015 in the

Tigray to 0.088 in the Oromia genotypes with an average of 0.045. On the other hand, the expected heterozygosity (He) was higher than that of observed heterozygosity (Ho), ranging from 0.133 to 0.215 with an average of 0.163.

Table 15. Gene diversity, observed heterozygosity (Ho), expected heterozygosity (He), minor allele frequency (MAF) and the polymorphism information content (PIC) of the 105 barley genotypes.

Regions	Genotypes	GD	Ho	He	MAF	PIC
Amhara	31	0.311	0.071	0.158	0.107	0.195
Oromia	32	0.288	0.088	0.215	0.146	0.211
SNNP	21	0.266	0.027	0.145	0.101	0.271
Tigray	19	0.221	0.015	0.133	0.092	0.250
ICARDA/Other	2	0.177	0.022	0.164	0.145	0.151
Average values	105	0.253	0.045	0.163	0.118	0.216

Abbreviations: GD=Gene diversity, Ho=Observed heterozygosity, He=Expected heterozygosity, MAF=Minor allele frequency, PIC=Polymorphism information content, SNNP=Southern Nations, Nationalities and Peoples, ICARDA=International Centre for Agricultural Research in the Dry Areas.

4.3.3. Analysis of molecular variance

The analysis of molecular variance (AMOVA) showed that the proportion of variance within the barley populations was significantly higher (52.85%, $P < 0.0001$) than the variation within accessions (46.43%, $P < 0.0001$) (Table 16). In contrary, a significantly lower level of genetic variation (0.72%, $P < 0.0001$) was recorded among the barley populations (Table 15).

Table 16. Analysis of molecular variance (AMOVA) of 105 barley population based on SNP markers

Source of variation	Mean square	Estimated variance	Proportion of Variance (%)	Probability (p) value
Among populations	2349.75	10.8	0.72	***
Within populations	1742.11	820.77	52.85	***
Within accessions	475.33	582.41	46.43	***
Total	1588.53	1413.98	100	

Significant codes: 0 '***' 0.001, '**' 0.01, '*' 0.05, 'ns' 0.1, 'ns' 1, ns-non significant.

4.3.4. Genetic differentiation and gene flow

Pairwise genetic differentiation (F_{ST}) and gene flow (N_m) among barley populations was computed using SNP markers (Table 17). The lower values of 0.019, 0.022, and 0.038 genetic differentiations were recorded between the barley genotypes originating from Amhara and Oromia, Amhara and SNNP, and SNNP and Oromia regions, respectively (Table 17). Although moderate genetic differentiation was recorded between the Tigray and SNNP (0.117) followed by Tigray and Amhara (0.089) and Tigray and Oromia (0.088) (Table 17). This revealed that the barley genotypes obtained from those regions of origin the existence in the highest genetic variations and distant relationships. The values for gene flow (N_m) between barley populations ranged from 0.131 to 0.001 (Table 17). The result showed that relatively higher N_m occurred between Amhara and Tigray (0.131), and Amhara and Oromia (0.071) barley genotypes while the lowest (0.001) between SNNP and Tigray (Table 17). This relatively higher level of gene flow shows the existence of genetic material exchange among barley genotypes collected from various geographic regions.

Table 17. Pairwise genetic differentiation (F_{ST} , below diagonal) and gene flow (N_m , above diagonal) among barley populations using SNP markers

Regions	Amhara	SNNP	Oromia	Tigray
Amhara		0.012	0.071	0.131
SNNP	0.022		0.015	0.001
Oromia	0.019	0.038		0.021
Tigray	0.089	0.117	0.088	

Abbreviation: SNNP = Southern Nations, Nationalities and Peoples

4.3.5. Genetic distance and identity

A high genetic distance (0.08) was recorded among barley genotypes obtained from Tigray and ICARDA, which indicates the existence of high genetic differences among the genotypes (Table 18). The genetic distance ranged from 0.002 to 0.08. Besides, the genetic identity ranged from 0.971 to 0.998, and the highest genetic identity was found among Amhara and Tigray, Amhara and ICARDA, and Oromia and SNNP. However, a lower (0.971) genetic

identity value was observed between genotypes originating from SNNP and ICARDA (Table 18).

Table 18. Genetic distance (below diagonal) and genetic identity (above diagonal) between 105 barley genotypes based on SNP markers

Regions	Amhara	Oromia	SNNP	Tigray	ICARDA/Other
Amhara		0.991	0.993	0.998	0.998
Oromia	0.009		0.998	0.990	0.990
SNNP	0.007	0.012		0.991	0.971
Tigray	0.012	0.021	0.004		0.992
ICARDA/Other	0.002	0.011	0.031	0.08	

Abbreviations: SNNP=Southern Nations, Nationalities and Peoples, ICARDA=International Centre for Agricultural Research in the Dry Areas.

4.3.6. Principal coordinate analysis (PCoA)

Four clusters were observed which accounted for 47.7% and 13.5 % of the total variation in PC1 and PC2 of the total variance, respectively (Figure 13). Oromia and Amhara genotypes were loadings in PC1 whereas Tigray and SNNP regions were the major loadings to PC2 (Figure 13). The lowest total variance was obtained for PC3 (6.7%) and PC4 (5%) (Figure 14). As shown in Figure 14, the third and consecutive PCs resulted in a lower percentage of contribution to the total genetic variance. The PCoA groups showed slightly unclear-cut separation due to an explicit genetic relatedness between the Amhara and SNNP, and Oromia and Amhara genotypes. The first group assembled Amhara and SNNP genotypes. The second group consisted of Tigray genotypes, and the last group contained the remaining genotypes from Oromia found in all groups (Figure 13).

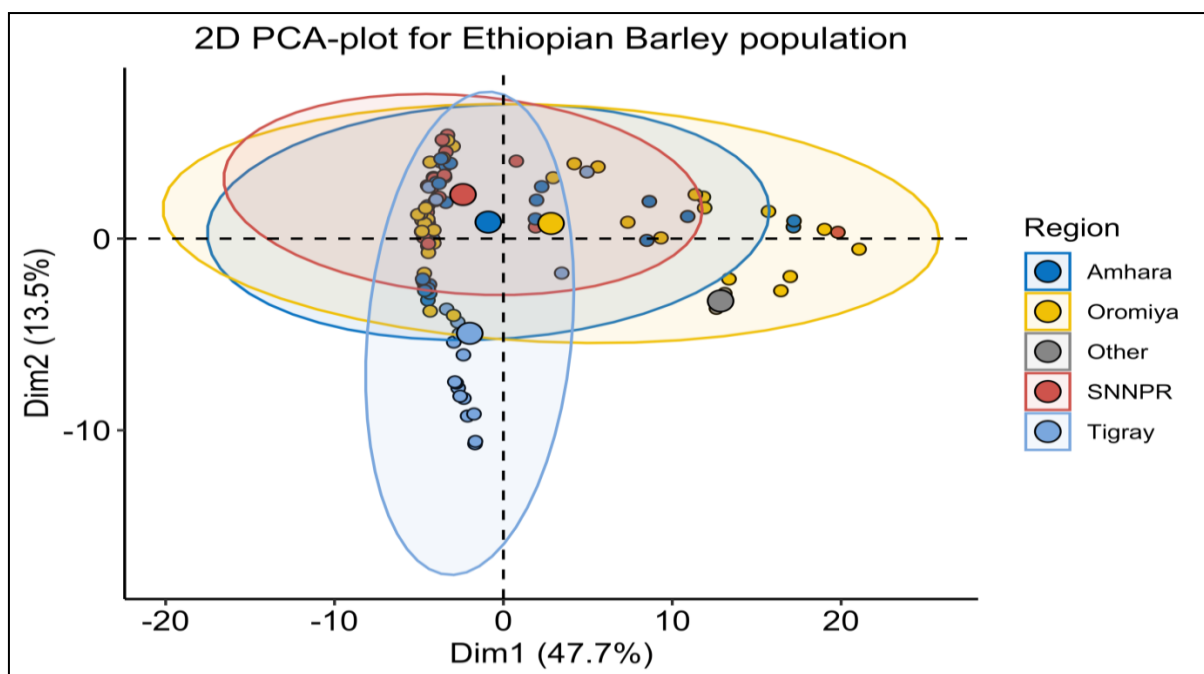


Figure 13. Principal coordinate analysis showing the clustering among the region of origins of 105 barley genotypes using 10,103 SNP markers. The genotypes are grouped into three main groups. Colored dots are genotypes from various region of origins and grouped by regions, according to the legend. PC1 and PC2 are shown on the X and Y-axis, respectively, aside from their explained variance.

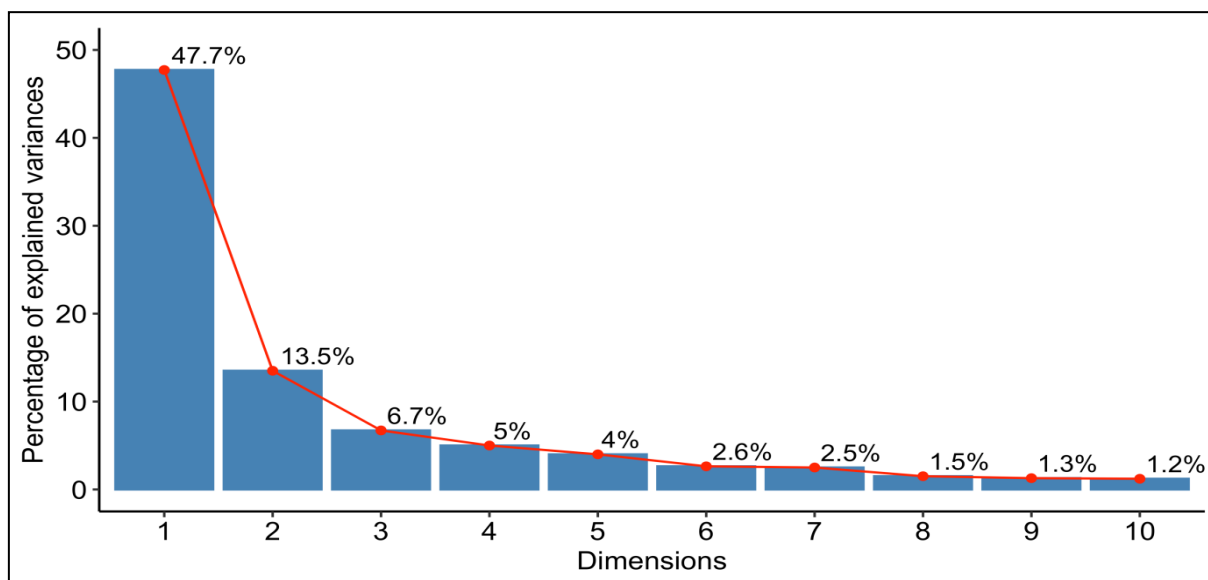


Figure 14. Percentages of explained variances for 105 barley genotypes using SNP markers

4.3.7. Genetic relatedness

We performed genetic relatedness analysis using the refined SNP dataset of 105 genotypes. The heat map was used to visualize the genetic relatedness across the population. The heat map plot depicted four clusters (Figure 15, Table 19). Cluster I contained the largest number of barley genotypes (n=60) followed by cluster III (n=16), cluster IV (n=15) and cluster II (n=14). The number of genotypes belonging to distinctive clusters varies from 14 in Clusters II to 60 in Cluster I (Figure 15, Table 19). Of all, cluster I was the largest cluster, consisting of 3(2.9%), 16(15.4%), 18(17.2%) and 23(21.9%) from Tigray, Oromia, SNNP and Amhara regions, respectively. In terms of altitude based clustering, the majority of the genotypes were included within an altitude of 2001-3000 masl. However, cluster II consists of a small number of barley genotypes, commonly from Amhara (4.8%) and Oromia (7.6%) regions originated at medium and high altitude ranges (Figure 15, Table 19). Cluster III encompassed genotypes only from Oromia (1.9%) and Tigray (13.3%) regions. The greatest number of genotypes included within an altitude of 2001-2500 masl. Cluster IV comprises genotypes from all regions with the highest percentage in the Oromia (5.7%) and the genotypes comprised at the medium altitude (Figure 15, Table 19).

Table 19. Clustering of 105 barley genotypes into four groups using SNP markers, the distribution of genotypes across the region of origins and altitudinal ranges

Regions	Cluster I	Cluster II	Cluster III	Cluster IV	Total
Oromia	16	8	2	6	32
Amhara	23	5	0	3	31
Tigray	3	0	14	2	19
SNNP	18	1	0	2	21
ICARDA/Other	0	0	0	2	2
Total	60	14	16	15	105
Altitude Ranges					
≤2000	8	1	2	3	14
2001-2500	22	5	9	8	44
2501-3000	21	3	5	3	32
≥3001	9	5	0	1	15
Total	60	14	16	15	105

Abbreviation: ICARDA= International Centre for Agricultural Research in the Dry Areas

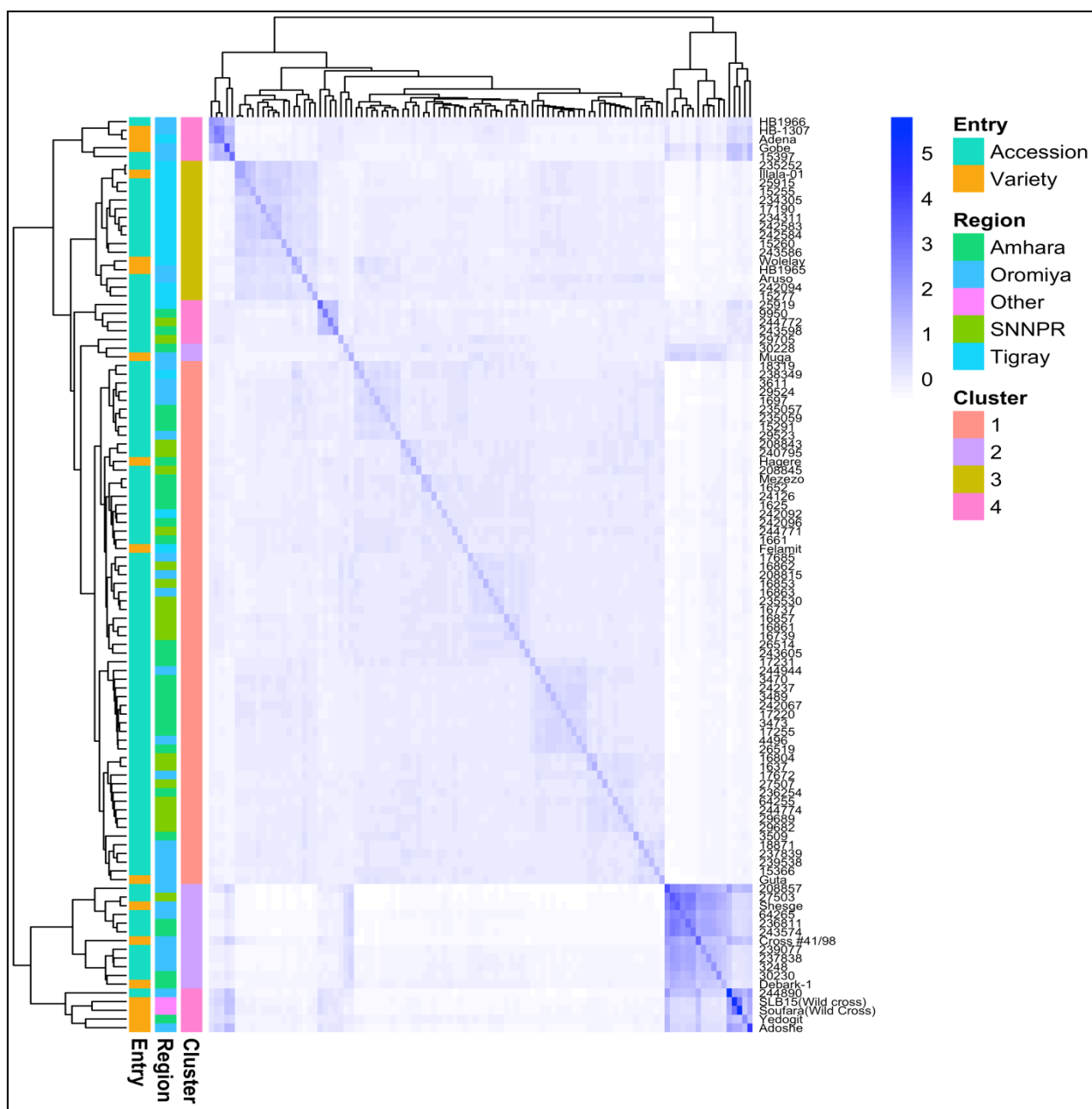


Figure 15. Heat map analysis shows relationship distance based on the color distribution among 105 barley populations genotyped using 10,103 SNP markers (for genotypes name and collection origins refer to Table 2). Colored plots on the left side are entry genotypes, regions, and clusters at the various stages, according to the legend to the right. On the left of the matrix, barley genotypes are grouped into four distinct clusters. Abbreviations: SNNP=Southern Nations, Nationalities and Peoples, Unknown= unidentified genotype names.

4.3.8. Population structure

A total of 10,103 SNP markers were used for the population structure analysis of the 105 barley genotypes. The best number of K, which clearly defined the number of populations as K= 4, revealed that four subpopulations should include all the 105 barley genotypes with a great probability (Figure 16). Each K is shown in diverse colors (Figure 16), the subpopulations described in blue-green and purple showed a high proportion of the variation with the barley genotypes confirming the results of AMOVA and heat map.

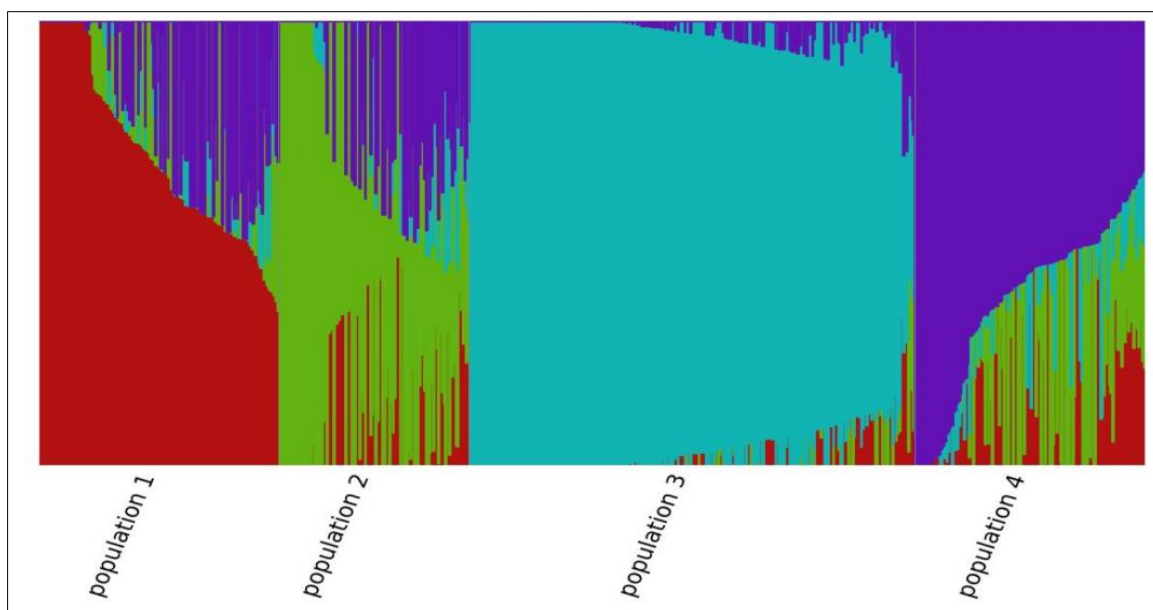


Figure 16. Population structure of 105 barley genotypes (K=4). Each vertical bar represents genotypes that are divided into K-colored segments.

4.4. Assessment of the genetic improvement of food barley varieties

4.4.1. Analysis of variance

The results of ANOVA for all traits of barley varieties studied in two consecutive main cropping seasons of 2020 and 2021 are presented in Table 20. The analysis of variance for the tested barley varieties indicated a significant difference for all traits except harvest index per plant (Table 20), showing the presence of variability among the tested varieties. Season effects were significant ($p < 0.05$) for spike weight, thousand kernels weight, grain yield per plant and hectare, biomass yield per plant and hectare, and grain filling period, and highly significant

($p < 0.001$) only for days to physiological maturity and plant height. Although the interaction (Var*Sn) effect was non-significant for most assessed morphological traits (Table 20).

Table 20. Analysis of variance for morphological traits of food barley varieties grown over two consecutive main cropping seasons in 2020 and 2021

Traits	Mean Squares						
	Variety	Var*Sn	Season(Sn)	Residual	Mean	LSD	CV
DH	33.43***	1.20 ^{ns}	4.43 ^{ns}	5.70	63.55	3.19	3.76
DTF	33.83***	1.85 ^{ns}	5.77 ^{ns}	5.19	66.48	3.34	3.43
DPM	36.24***	3.04*	23.31**	7.06	99.94	4.09	2.66
PH	30.09***	2.38 ^{ns}	96.06**	38.30	102.04	5.93	6.06
FLL	2.47**	1.08 ^{ns}	0.37	2.90	16.06	1.92	10.61
SpL	0.28**	0.07 ^{ns}	0.03 ^{ns}	0.46	6.90	0.64	9.82
NFTP	0.03**	0.00 ^{ns}	0.07 ^{ns}	0.00	2.74	0.23	9.73
NKP	33.74***	0.00 ^{ns}	2.72 ^{ns}	37.44	36.36	5.61	16.83
Ldg	0.19**	0.12*	0.00 ^{ns}	0.27	3.43	0.57	15.20
SpW	0.07**	0.04*	0.13*	0.09	1.90	0.32	15.46
TKW	18.51***	2.83 ^{ns}	8.29*	11.16	40.63	3.93	8.22
GYPP	0.05**	0.03*	0.12*	0.06	1.62	0.29	15.52
GYHa	0.36***	0.00 ^{ns}	0.12*	0.11	4.01	0.40	8.47
BMYP	0.35***	0.07 ^{ns}	0.52*	0.21	4.23	0.56	10.96
BMYPHa	0.82***	0.00 ^{ns}	4.55*	1.66	12.87	1.10	10.02
GFP	6.75**	2.78 ^{ns}	5.26*	6.43	33.49	3.13	7.57
GFR	312.64***	64.95*	0.87	105.47	120.75	14.97	8.50
BMPP	76.98***	0.00 ^{ns}	222.69 ^{ns}	161.00	128.85	11.04	9.85
HIP	2.83 ^{ns}	4.30**	2.01 ^{ns}	7.81	37.92	2.64	7.37
HI	6.74***	0.38 ^{ns}	5.89 ^{ns}	7.00	31.40	2.67	8.45

Abbreviations: DH= days to heading, DTF=days to flowering, DPM=days to physiological maturity, PH=plant height(cm), FLL= flag leaf length(cm), SpL=spike length(cm), AwL= awn length(cm), NTPP=number of tillers per plant, NFTP= number of fertile tillers per plant, NKP=number of kernels per plant, SpW=spike weight (gram), Ldg=lodging, TKW=thousand kernels weight (gram), GYPP=grain yield per plant (gram), GYHa=grain yield per hectare (in t/ha), BMYPP=biomass yield per plant (gram), BMYHa=biomass yield per hectare (t/ha), BPP= biomass production (kg/ha/day), GFR=grain filling rate (kg/ha/day), GFP=grain filling period (day), HIP= harvest index per plant, HI=harvest index. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 'ns' 0.1 'ns' 1, respectively.

4.4.2. Grain yield improvement

Grain yield over the breeding periods showed consistent improvement (Table 21). The result showed that the average grain yields of barley varieties released in the 1980s, 1990s, 2000s, 2010s, and after 2015s were 4.22, 3.52, 3.71, 4.18 and 4.61 t/ha, respectively (Table 21). The increase in grain yield of released varieties over the average of local checks (Aruso and Muga), were 38.65, 15.94, 22.11, 37.58 and 51.61% for the varieties released in the 1980s, the 1990s, 2000s, 2010s, and after 2015's, respectively (Table 21).

Table 21. Average grain yield of food barley varieties released over six breeding periods and increment over the local checks (Aruso and Muga)

Barley Genotypes	Release period	Average Grain yield (t/h)	Grain yield increment over local checks	
			t/ha	%
Local checks (Aruso and Muga)	Pre1970s	3.04	-	-
IAR-485 and FBHB-42	1980s	4.22	1.18	38.65
Shege, Abay, and Meserach	1990s	3.52	0.48	15.94
Dimtu, Mezezo, Basso, Harbu, Setegn, Estayish, Dinsho, Yedogit, Defo, Biftu, HB-1307, Bentu, Guta, Agegnehu, Tilla	2000s	3.71	0.67	22.11
Dribie, Felamit, Abdane, Hriti, Fetina, EH 1493, Cross #41/98 and Gobe	2010s	4.18	1.14	37.58
Illala-01, Illala-02, Robera, Adena, Wolelay, HB1965, HB1966, Debark-1 Hagere and Adoshe	After 2015s	4.61	1.57	51.61

The release period and the name of the improved food barley varieties refer to Table 2.

Across tested varieties, the average grain yield over two seasons has ranged between 3.02 t/ha in Tilla to 5.09 t/ha in Adoshe (Appendix 4). As a result of two years (2020 and 2021 main growing seasons) assessments, we revealed that the barley varieties HB1965 (4.76 t/ha), HB1966 (4.91 t/ha), Debark-1 (4.99 t/ha) and Adoshe (5.09 t/ha) had markedly higher grain yield performance compared to local checks and older varieties (Appendix 4). This indicated a significantly higher grain yield was obtained from the most recently released barley varieties.

The regression analysis revealed an increase in the estimated average annual rate in grain yield of $0.021 \text{ t ha}^{-1} \text{ yr}^{-1}$ or $21 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Figure 17, Table 22). This indicated that there was a highly significant improvement in the grain yield of barley through breeding over the past breeding periods. Besides, the relative annual genetic improvement was 0.69% per year in the grain yield potential of barley varieties (Figure 17, Table 22).

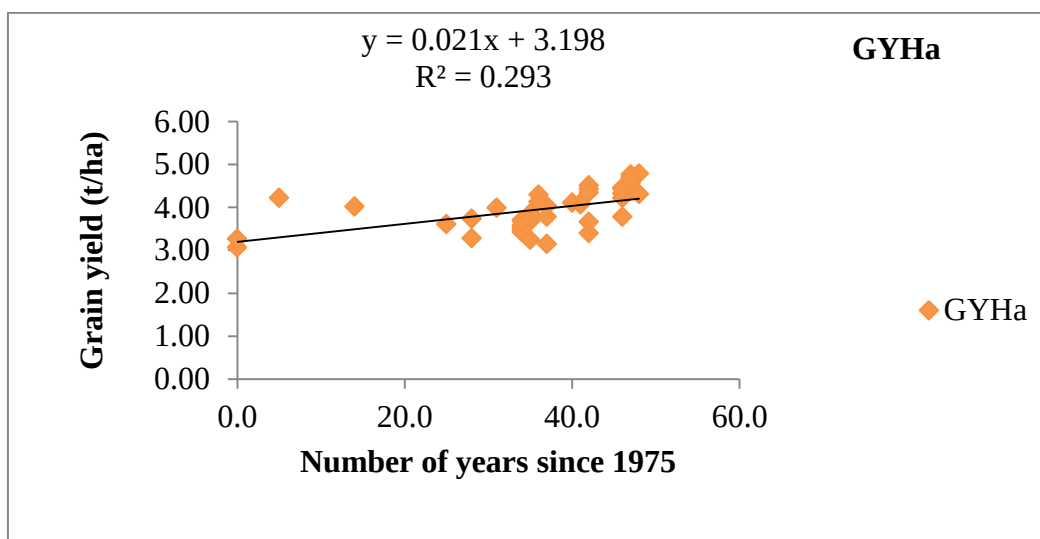


Figure 17. Relationship between the number of years since varieties were released and grain yield (in t/ha)

4.4.3 Yield components

Thousand kernels weight (TKW) have been the most critical trait in barley breeding. The highest TKW was recorded in the variety Wolelay (53.23g) followed by Fetina (50.62 g), Illala-01 (45.83g), IAR-485 (45.26g), Gobe (45.24g) and Setegn (44.98) (Appendix 4). The estimated annual rate of genetic improvement of TKW was 0.054g per thousand kernels weights per year (Figure 18, Table 22) and the relative genetic improvement of 0.15% per year (Figure 18). This indicated a non-significant positive increment over the released periods (Table 22). The HI has also increased with the year of variety release from 26.59 in Muga (local check) to 35.22 in the Gobe variety (Appendix 4). The varieties Wolelay (34.05), HB1965 (34.10), Adoshe (34.41), IllaIa-02 (34.52) and Gobe (35.22) had revealed the highest HI compared to others. In this study the HI of the developed varieties showed an increasing trend for the corresponding improvement of grain yield, indicating new varieties have high

harvest index than the local checks. The regression analysis per year was found to be 0.078 yr⁻¹ with a relative genetic improvement of 0.28%, showing a progressive and significant increase with the year of release (Table 22).

Table 22. Estimation of mean values, regression coefficients (b), the annual relative genetic improvement (RGI), coefficient of determination (R²) and intercept of yield and yield components of barley varieties evaluated at Kulumsa during the 2020 and 2021 main cropping seasons

Traits	Overall mean	Mean of local checks	Regression coefficients (b)	RGI (% yr ⁻¹)	Intercept	R ² (%)
GYHa	3.95	3.04	0.021***	0.69	3.20	29.27
GYPP	1.58	1.32	0.002	0.15	1.54	0.36
TKW	40.57	36.99	0.054	0.15	38.70	2.89
SpW	1.85	1.60	-0.002	-0.13	1.88	0.07
NKP	35.97	28.62	0.079	0.28	33.17	2.58
NFTP	2.69	2.64	0.002	0.08	2.62	2.97
HIP	36.39	35.38	-0.016	-0.05	36.95	0.12
HI	31.17	27.75	0.078***	0.28	28.40	35.24

Abbreviations: GYHa=grain yield per hectare (t/ha), GYPP=grain yield per plant (gram), TKW= thousand kernels weight (gram), SpW= spike weight (gram), NKP=number of kernels per plant, NFTP= number of fertile tillers per plant, HIP=harvest index per plant, HI=harvest index per hectare. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘ns’ 0.1 ‘ns’ 1, respectively.

Other yield-component traits such as spike weight, number of fertile tillers per plant, number of kernels per plant, grain yield per plant and harvest index per plant were also the most important traits in barley improvements (Table 22). For those traits, higher values were recorded in most of the improved varieties compared to local checks while there was no linear increase with the year of variety release (Appendix 4). Regression analysis indicates grain yield per plant (0.002g of yield yr⁻¹), number of kernels per plant (0.079 kernels per spike yr⁻¹), and number of fertile tillers per plant (0.002 tillers per plant yr⁻¹) in barley varieties have a non-significant positive increase with the year of variety release (Table 22). However, spike

weight (-0.002g yr^{-1}) and harvest index per plant (-0.016 yr^{-1}) showed a non-significant negative trend (Table 22), showing little or no genetic improvement in these two traits.

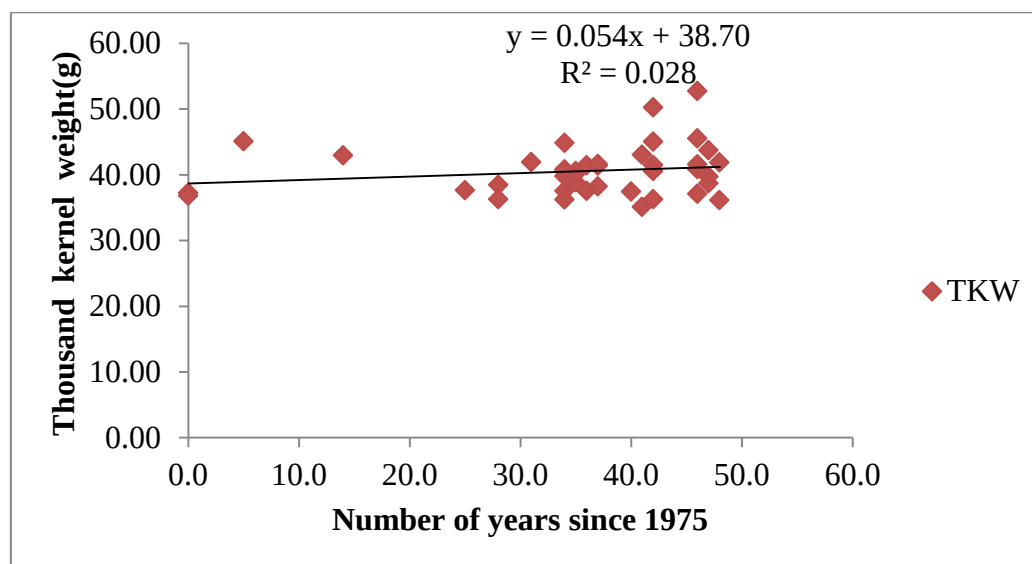


Figure 18. Relationship among the number of years since varieties were released and thousand kernels weight (g).

4.4.4. Growth and production traits

The plant height of the newly released varieties was markedly shorter than the oldest varieties and local checks. The PH ranged from 94.16 (Adoshe) to 110.94 cm (Aruso) (Appendix 4). This trait had a negatively significant ($P < 0.01$) trend in the year of release. The yearly genetic improvement was -0.12cm yr^{-1} and a relative percentage of -0.11% per year (Table 23). On the other hand, higher biomass yield was produced by the newly released varieties compared to the local checks and oldest varieties (Table 23). The biomass yield ranged from 11.08t/ha for Tilla to 14.49 t/ha for Debark-1. The improved varieties of HB-1307(13.87 t/ha), HB1966 (13.92 t/ha), Adoshe (14.09 t/ha) and Debark-1 (14.49 t/ha) produced the highest biomass yield (Appendix 4). The BMYHa has significantly increased at the rate of $0.019\text{ t ha}^{-1}\text{ yr}^{-1}$ (19 kg/ha yr^{-1}) and the relative annual genetic improvement was estimated to be 0.16% per year (Figure 19, Table 23).

Similar to biomass yield, the highest biomass production and grain filling rate were recorded for newly released varieties than the local checks and older varieties. Higher biomass

production rates of 135.8, 136.69, 138.53, 140.88 and 144.01 kg ha⁻¹ day⁻¹ were obtained for varieties Abdane, Wolelay, Debark-1, Adoshe and Illala-01, respectively (Appendix 4). Correspondingly, we recorded the highest value of grain filling rate in the variety Illala-01 (157.74 kg/ha/day) followed by Debark-1 (155.28 kg/ha/day), Hagere (145.94 kg/ha/day), Adoshe (142.34 kg/ha/day) and Wolelay (141.67 kg/ha/day) varieties (Appendix 4). Biomass production and grain filling rate were highly significant with the year of variety release. The genetic improvement of 0.16 and 0.57 kg ha⁻¹ day⁻¹ yr⁻¹ in BPR and GFR, respectively (Table 23). The relative annual improvement was also estimated to be 0.14% yr⁻¹ for biomass production and 0.60% yr⁻¹ for grain filling rate (Table 23).

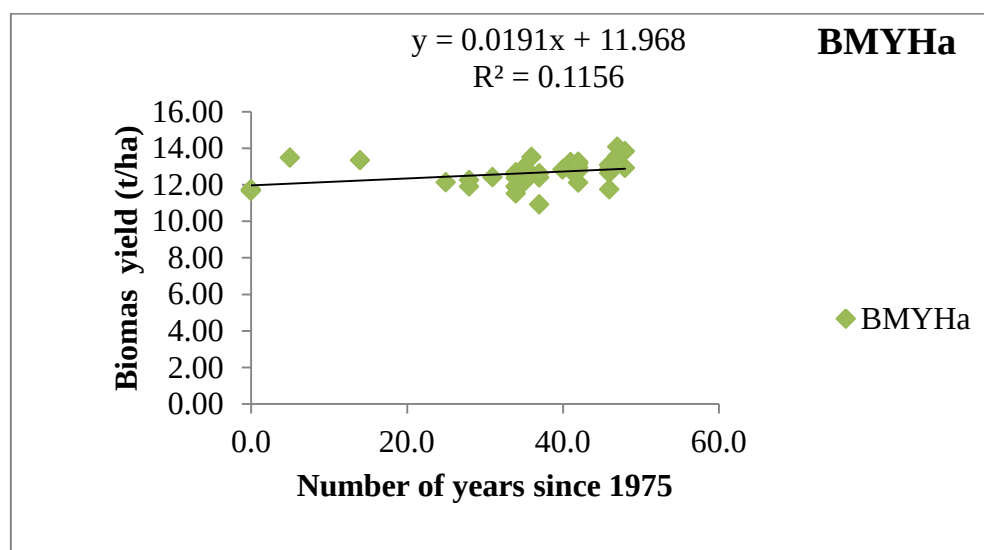


Figure 19. Relationship between the number of years since barley varieties were released and biomass yield (t/ ha)

4.4.5. Phenological traits

Phenological traits of the modern varieties found a shorter number of days to attain heading, flowering and physiological maturity stages. The oldest varieties of Dimtu, Cross #41/98, EH 1493 and FBHB-42 as well as the local check (Muga) have longer days to heading, flowering and maturity than recent varieties (Appendix 4). The phenological traits revealed a non-significant negative trend over the 48 years of barley breeding. The annual rate of genetic improvement in DH, DTF and DPM was estimated to be -0.14, -0.14 and -0.13 days yr⁻¹ with relative percentage improvement of 0.22, 0.21 and 0.13% yr⁻¹, respectively (Table 23).

However, the grain-filling period showed a non-significant increasing trend within a year of release. The estimated annual rate in GFP was 0.004 days yr⁻¹ with a relative genetic value of 0.02% yr⁻¹ (Table 23).

Table 23. Estimation of mean values, regression coefficients (b), the annual relative genetic improvement (RGI), coefficient of determination (R²) and intercept of phenological, growth and production traits of barley varieties grown at Kulumsa during the 2020 and 2021 main cropping seasons

Traits	Overall mean	Mean of local checks	Regression coefficients (b)	RGI (% yr ⁻¹)	Intercept	R ² (%)
DH	63.47	65.74	-0.14	-0.22	68.08	7.86
DTF	66.42	68.62	-0.14	-0.21	71.03	7.75
DPM	99.74	101.30	-0.13	-0.13	104.18	6.64
PH	101.78	108.70	-0.12**	-0.11	105.93	20.07
FLL	16.11	16.10	-0.004	-0.03	16.23	0.28
SpL	7.01	7.83	-0.02	-0.26	7.35	8.96
AwL	13.46	13.69	-0.004	-0.03	13.61	3.45
Ldg	3.31	2.94	0.006	0.20	3.10	3.60
BMYP	4.17	3.75	-0.007	-0.19	4.42	2.30
BMYPHa	12.65	11.84	0.019*	0.16	11.97	11.54
BPR	125.80	117.93	0.16***	0.14	120.20	26.05
GFR	119.52	95.53	0.57***	0.60	99.42	29.25
GFP	33.12	32.97	0.004	0.02	33.01	0.04

Abbreviation: DH= days to heading, DTF=days to flowering, DPM= days to physiological maturity, PH= plant height(cm), FLL=flag leaf length(cm), SpL=spike length(cm), AwL=awn length(cm), Ldg=lodging, BMYP=biomass yield per plant (gram), BMYPHa=biomass yield (t/ha), BPR=biomass production (kg/ha/day), GFR=grain filling rate (kg/ha/day), GFP=grain filling period (day). Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 'ns' 0.1 'ns' 1, respectively.

4.4.6. Correlation of grain yield with morphological traits

In this study, the grain yield revealed a positively highly significant ($p < 0.001$) correlation with biomass yield per hectare ($r = 0.86^{***}$), Lodging ($r = 0.59^{***}$), biomass production rate ($r = 0.81^{***}$) and grain filling rate ($r = 0.70^{***}$) (Figure 20, Table 24). These highly significant

traits highly contributed to the current grain yield improvement in released barley varieties. Significant ($p < 0.01$) positive correlation of grain yield with harvest index ($r = 0.87^{**}$), thousand kernels weight ($r = 0.34^*$) and number of fertile tillers per plant ($r = 0.37^*$) (Figure 20, Table 24). Other traits such as spike weight ($r = 0.11$), number of tillers per plant ($r = 0.14$), awn length ($r = 0.05$), number of kernels per plant ($r = 0.18$), grain ($r = 0.16$) and biomass yield ($r = 0.03$) per plant showed positively non-significant ($p > 0.05$) association with grain yield (Figure 20, Table 24). However, plant height ($r = -0.18$), flag leaf length ($r = -0.15$), spike length ($r = -0.26$) and harvest index per plant ($r = -0.20$) had a negatively non-significant ($p > 0.05$) association with grain yield (Figure 20, Table 24). On the other hand, phenological traits (DH, DTF, DPM and GFP) found a non-significant positive correlation with grain yield (Figure 20, Table 24).

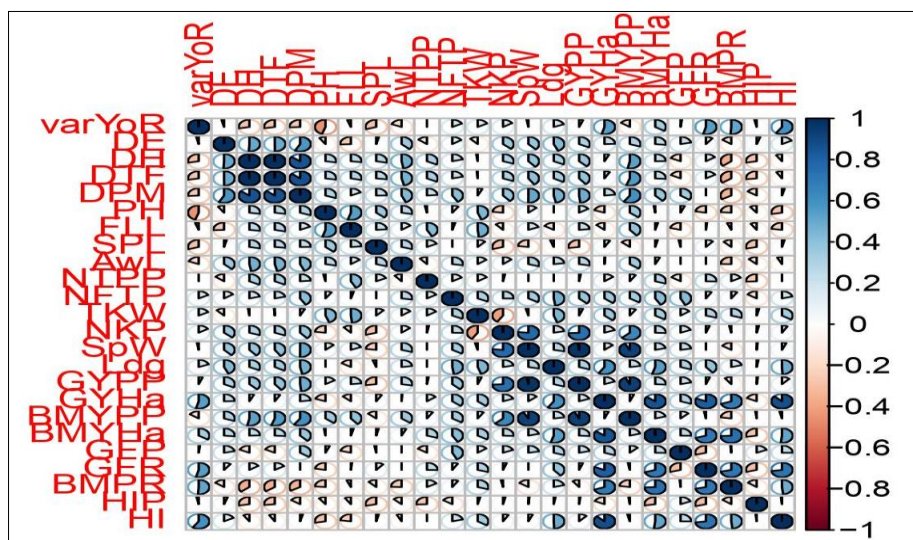


Figure 20. The correlation coefficient among morphological traits of improved barley varieties was evaluated at Kulumsa during the 2020 and 2021 main cropping seasons. Correlation coefficients are reported in colors, according to the legend to the right. Abbreviations: DH= days to heading, DTF=days to flowering, DPM=days to physiological maturity, PH=plant height(cm), FLL= flag leaf length(cm), SpL=spike length(cm), AwL= awn length(cm), NTPP=number of tillers per plant, NFTP= number of fertile tillers per plant, NKP=number of kernels per plant, SpW=spike weight (gram), Ldg=lodging, TKW=thousand kernels weight (gram), GYPP=grain yield per plant (gram), GYHa=grain yield per hectare (in t/ha), BMYPP=biomass yield per plant (gram), BMYHa=biomass yield per hectare (t/ha), BPR= biomass production (kg/ha/day), GFR=grain filling rate (kg/ha/day), GFP=grain filling period (day), HIP= harvest index per plant, HI=harvest index.

Table 24. The association between phenotypic traits of released barley varieties evaluated at Kulumsa during the 2020 and 2021 main cropping seasons

Traits	YoR	DH	DTF	DPM	PH	FLL	SpL	AwL	NTPP	NFTP	NKP
YoR											
DH	-0.26										
DTF	-0.25	0.99***									
DPM	-0.24	0.85***	0.86***								
PH	-0.42**	0.28	0.28	0.30							
FLL	-0.06	0.27	0.28	0.16	0.56***						
SpL	-0.26	0.29	0.28	0.25	0.35*	0.22					
AwL	-0.15	0.45**	0.44**	0.42**	0.31	0.15	0.29				
NTPP	-0.01	0.32*	0.32*	0.22	-0.03	0.43**	0.18	-0.15			
NFTP	0.16	0.24	0.24	0.45**	0.14	0.07	0.002	0.27	0.16		
NKP	0.14	0.32*	0.32*	0.41**	-0.20	-0.12	-0.28	0.14	0.01	0.24	
SpW	-0.05	0.40*	0.41**	0.50	0.16	0.18	-0.22	0.31*	-0.03	0.33*	0.76***
TKW	0.16	-0.03	-0.03	0.05	0.47**	0.50**	0.15	0.16	0.15	0.34*	-0.46**
Ldg	0.20	0.32*	0.32*	0.47**	0.05	-0.16	-0.22	0.24	-0.02	0.47**	0.18
GYPP	0.04	0.39*	0.40**	0.49**	0.19	0.23	-0.004	0.30	-0.01	0.38*	0.73***
GYHa	0.55***	0.08	0.10	0.24	-0.18	-0.15	-0.26	0.05	0.14	0.37*	0.18
BMYP	-0.17	0.53***	0.55***	0.58***	0.38*	0.39*	-0.14	0.36*	0.40	0.36*	0.63***
BMYPHa	0.34	0.26	0.27	0.39*	-0.02	0.03	0.11	0.11	0.30	0.44**	0.24
GFP	0.01	-0.17	-0.17	0.37*	0.05	-0.22	-0.40	0.02	-0.16	0.43**	0.20
GFR	0.55***	-0.34*	0.17	-0.01	-0.20	0.01	0.17	0.03	0.25	0.08	0.02
BMPR	0.51***	-0.34*	-0.33*	-0.32*	-0.22	-0.06	-0.06	-0.20	0.19	0.08	-0.06
HIP	-0.06	-0.30	-0.28	-0.16	-0.09	-0.09	-0.29	-0.14	-0.27	-0.19	0.02
HI	0.60***	-0.09	-0.09	0.04	-0.27	-0.25	0.09	-0.05	-0.03	0.19	0.02

Abbreviations: YoR=year of variety release, DH=days to heading, DTF=days to flowering, DPM=days to physiological maturity, PH= plant height(cm), FLL= flag leaf length(cm), SpL=spike length(cm), AwL=awn length(cm), NTPP=number of tillers per plant, NFTP= number of fertile tillers per plant, NKP= number of kernels per plant, SpW= spike weight(g), Ldg=lodging, TKW=thousand kernels weight (gram), GYPP=grain yield per plant (gram), GYHa=grain yield per hectare(in t/ha), BMYP= biomass yield per

plant (gram), BMYHa= biomass yield per hectare(t/ha), BPR= biomass production (kg/ha/day), GFR=grain filling rate (kg/ha/day), GFP= grain filling period (day), HIP= harvest index per plant, HI=harvest index. Significance codes: 0 ‘****’ 0.001 ‘***’ 0.01 ‘**’ 0.05 ‘ns’ 0.1 ‘ns’ 1, respectively.

Table 24. Phenotypic correlation *Continued*

Traits	TKW	SpW	Ldg	GYPP	GYHa	BMYP	BMYHa	GFP	GFR	BMPR	HIP	HI
SpW	-0.08											
Ldg	0.44**	0.25										
GYPP	0.02	0.98***	0.27									
GYHa	0.34*	0.11	0.59***	0.16								
BMYP	0.06	0.92***	0.20	0.94***	0.03							
BMYHa	0.43**	0.19	0.54***	0.23	0.86***	0.18						
GFP	0.13	0.21	0.31	0.20	0.30	0.12	0.26					
GFR	0.28	-0.03	0.38*	0.03	0.81***	-0.06	0.69***	-0.31				
BMPR	0.29	-0.18	0.21	-0.13	0.70***	-0.25	0.75***	-0.02	0.72***			
HIP	0.03	-0.01	0.02	-0.004	-0.20	-0.03	-0.30	0.18	-0.32*	-0.17		
HI	0.32*	0.03	0.49**	0.08	0.87**	-0.07	0.52***	0.24	0.73***	0.49**	-0.03	0.00

Abbreviations: NKP=number of kernels per plant, SpW=spike weight(g), Ldg=lodging, TKW=thousand kernels weight (g), GYPP=grain yield per plant (g), GYHa=grain yield(in t/ha), BMYP=biomass yield per plant (g), BMYHa= biomass yield (t/ha), BPR= biomass production (kg/ha/day), GFR=grain filling rate (kg/ha/day), GFP=grain filling period (day), HIP=harvest index per plant, HI=harvest index. Significance codes: 0 ‘****’ 0.001 ‘***’ 0.01 ‘**’ 0.05 ‘ns’ 0.1 ‘ns’ 1, respectively.

4.4.7. Basis of barley yield variation

The stepwise regression analysis using grain yield as a dependent variable was carried out in this study to identify the major critical traits that mainly contributed to the yield variation. Therefore, our work revealed biomass yield and harvest index were the major critical traits that mainly contributed to most of the yield variation (99%) among the food barley varieties (Table 25). This distinctly contributed to the difference in grain yield between the barley varieties.

Table 25. Stepwise regression analysis of grain yield of barley as a dependent variable on the other traits as independent variables

Location	Conducted Year	Variable entered	Parameter estimated	Constant (Intercept)	Partial R ²
Kulumsa	2021 and 2022	Harvest index	0.38***		0.77
		Biomass yield	0.18**	-6.30	0.22
					99%

CHAPTER FIVE

5. DISCUSSIONS

5.1. Qualitative traits variations

5.1.1. Distribution of qualitative traits of barley across regions

The yield of barley can be enhanced by combining both qualitative and quantitative traits with other morphological traits (Tshikunde *et al.*, 2019). In this study, high frequency distribution was obtained in the green stem in Oromia followed by the purple stem (basal only) in Tigray regions. A comparable result reported by Gadissa *et al.* (2021a) showed that most collections of barley revealed green stem pigmentation. The highest percentage frequency distribution of light green and medium green leaves was recorded in the Tigray and Amhara regions, respectively. The high frequency of green stem and light green leaf traits in barley accessions can be valorized in drought resistance breeding. Indeed, the stay-green trait, retaining green leaf area, which is related to the retention of chlorophyll content has been identified as a key target trait for improving light interception and utilization and can contribute to enhancing cereal yield potential under moisture deficit growing conditions (Tshikunde *et al.*, 2019). Among the region of origins, awn colors of barley accessions revealed diverse distributions. Amber or white was the most common color while reddish, yellow, brown and black colors were with low frequencies. Similarly, in study of Derbew *et al.* (2013), most of the barley landraces were amber or white in awn color followed by red and yellow. On the contrary, Bouhaouel *et al.* (2019) reported yellow awn color (99%) was dominant in Tunisian barley accessions.

The highest percentage frequency distributions of white and purple GLC colors were recorded in the current study. In contrast, Hadado *et al.* (2009) found that the most frequent glumes color was white followed by brown with black and yellow colors. Moreover, white and black colors were frequently obtained glumes colors at the physiological maturity stage. The amber or normal and purple were the most prevalent LmCs at the hard dough stage. Amber or normal, grey and black colors were largely obtained lemma colors at maturity stage. This study explained by the dominance of the traits may be due to farmers' selections for desirable

traits being key forces in determining the variations of the barley accessions (Kebebew *et al.*, 2001).

Awned LmA was monomorphic while awnless, awnleted, sessile and elevated traits were absent. This result is comparatively consistent with the awned spikes that were predominant (96% and 89%) of barley materials from India, Syria, Canada, the USA and Mexico (Kaur *et al.*, 2022) and from the world collection (Tolbert *et al.*, 1979), respectively. The presence of awn is an advantageous trait in cereals (Ahmadi *et al.*, 2018; Ntakirutimana and Xie, 2020) which may be associated with tolerance to drought stress climate as well as an adaptive structure against leaf diseases (Paperson and Ohm, 1975; Negassa, 1986), yet further evaluations are required to understand the relationship between awn length, and grain yield potential in moisture deficit area as well in leaf disease affected environments. Research report showed that the presence of awns helps the spikes to maintain higher rates of photosynthesis and WUE throughout the grain filling period as well as significantly enhance kernel dry weight in barley (Bort *et al.*, 1994).

Spike related traits of barley accessions revealed varied distribution among region of origins, which are useful traits of smallholder farmers in Ethiopia (Mancini *et al.*, 2017) as well as a selection tool that provides key traits for yield enhancement in breeding (Gao *et al.*, 2015; Allel *et al.*, 2017). Intermediate spike density was frequently found in the Amhara region followed by lax type in the Tigray region and dense type in the Oromia region. Two-rowed is the prevalent type of KrRw in the Tigray region whereas six-rowed was commonly found both in the Oromia and Amhara regions, respectively. The predominance of these traits may be related to the presence of better precipitations in the highlands of Oromia and Amhara than in the lowlands of the Tigray regions. These results are in agreement with Bouhaouel *et al.* (2019) and Tolbert *et al.* (1979) who reported the dominance of the six-rowed number in the Tunisian barley collection and the world barley collection, respectively. Six-rowed barley is associated with a higher number of seeds per spike and enhanced productivity at higher elevations and high rainfall areas (Pourkheirandish and Komatsuda, 2007), while the two-rowed barley is frequently produced in marginal areas (García del Moral *et al.*, 2003).

Slightly curved and straight types of beak shape, long and medium types of beak length, elongated and elongated-flattened kernel shapes showed the highest frequency distribution in barley accessions. This material can be valorized in barley quality improvement in Ethiopia and beyond. Indeed, the landraces of barley from Ethiopia are a source of genes that control useful nutrient traits, such as high lysine and protein quality and content (Munck *et al.*, 1970). The kernel size and shape related traits showed different distribution among regions. This may be associated with local requirements to cultural preferences such as cooking and taste qualities, and local adaptation. Earlier research reports showed that kernel shape is one of the most determinant traits for the preparation of food and taste qualities (Sun *et al.*, 2013), and malting quality (Nielsen, 2003).

In our study, kernel colors (KrC) of barley accessions revealed also diverse distribution among the region of origins. The presence of diverse KrC could be linked to the local requirement of farmers' traits for making cultural purposes (Asfaw, 1988), which could ensure the *in-situ* conservation of indigenous knowledge by the farming community in Ethiopia (Abebe *et al.*, 2010). White kernel color was predominant in barley accessions, which may be associated with Ethiopian smallholder farmer selection pressures operating for the white phenotypic class (Demissie and Bjørnstad, 1996). A similar finding was reported (Assefa and Labuschagne, 2004; Hadado *et al.*, 2009) indicating that the majority of Ethiopian barley landraces have white kernel color. In contrary, the barley accessions were largely characterized by the grey kernel color in Tunisia (Bouhaouel *et al.*, 2019).

5.1.2. Distribution of qualitative traits of barley across altitude ranges

The frequency distribution of the qualitative traits of barley accessions was also recorded in relation with four altitude ranges. Research report showed that variations of barley traits at different altitudes may have resulted from the random or selective accumulation of certain genes at high or low altitudes and partly from farmers' selection of morphological types (Asfaw, 1989). The highest percentage frequency distribution was obtained in the green stem and medium green leaf at higher altitudes. On the other hand, high frequency distribution was the purple stem (basal only) at medium and light green leaf at lower altitudes. Leaf traits are important for the breeding of enhanced grain yield in cereals (Mathan *et al.*, 2016; Shaaf *et al.*,

2019), traits related to photosynthetic efficiency as well as water balance are less exploited in barley breeding programs in Ethiopia. Leaf traits could be useful for enhancing the performance of Triticale under drought stress growing conditions (Lonbani and Arzani, 2011). Therefore, it is good to consider strategic breeding that combines leaf traits with measured traits to enhance the grain yield of barley under both favourable and marginal production conditions.

The distribution of the two classes of glume hairiness (GLH), glabrous and hairy, indicated a strong trend in the four altitude ranges. Glabrous increased its frequency of distribution from the lower (≤ 2000) to higher (≥ 3001) altitudinal ranges. However, the hairy type of GLH decreased its distribution in lowlands (≤ 2000) and was absent in highlands (≥ 3001). Higher altitude in Ethiopia is characterized by high rainfall, low temperature and frost (Etana *et al.*, 2020). The abundance of glabrous in the highland and hairy in the lowland may be associated with adaptive significance in drought and frost-affected areas in Ethiopia. A similar finding was reported in this study by Belay *et al.* (1997) indicating that the greater parts of Ethiopian wheat landraces have glabrous glume with preset at the highest altitudes. Glume hairiness is usually common in Ethiopian wheat (Negassa, 1986), and it can be used by breeders engaged in resistance breeding to insects. In general, the adaptive implication of glume hairiness has not been clearly well-known (Sourour and Hajer, 2008), a previous finding by Warham (1988) showed that the function of glume hairiness is to protect the glume from insects and to prevent diseases.

The predominance of glume color at the hard dough stage was white color at higher altitudes followed by purple color at medium altitudes. At maturity, white and purple colors also commonly occurred at altitudes 2501-3000 and ≤ 2000 masl, respectively. The variations of glume colors from the medium altitudes towards the lower and higher altitudes were decreased; this is possible due to the direct influence of both human and natural selection (Shumet and Tesema, 2014). Amber or normal LmC was predominately found at higher altitudes followed by purple color recorded at lower to medium altitudes during the hard dough and maturity stages. This predominance of LmC in this study is in line with Demissie and Bjørnstad (1996), the amber or white or normal color was the prevalent lemma color in Ethiopian barley collections.

Intermediate spike density (SpDs) was the most frequent from medium to higher altitude ranges. Lax SpDs commonly occurred at lower altitudes while the dense type was a relatively higher frequency at higher altitudes. This result is in agreement with the finding of Kebebew *et al.* (2001), who reported that intermediate SpDs was largely found at higher altitudes whereas the lax type concentrated below 2650 masl. Research report showed a significant association between altitude and spike related traits in Ethiopian barley (Asfaw, 1989). Indeed, variation in altitude ranges affects barley cultivation in Ethiopia by influencing the amount of rainfall and temperature (Engels, 1994). Further, moisture deficit is decreased from lower altitudes towards higher altitude ranges in central Ethiopia (Etana *et al.*, 2020). Besides, the distribution of kernel rows types was different in the four altitude ranges. The six-rowed type in barley accession was mainly found at higher altitude ranges whereas two-rowed barley was more common in lower altitudes. This could be related to local adaptation/related to the amount and distribution of rainfall and temperature (Demissie and Bjørnstad, 1996; Kebebew *et al.*, 2001). Two-rowed barley over six-rowed barley is earlier to mature, which is a useful trait for breeding to escape high temperature and low precipitations during grain filling periods (Kandic *et al.*, 2018), while six-rowed barley is a high yielder type at higher altitudes and high rainfall areas (Pourkheirandish and Komatsuda, 2007).

Beak length (BkLns), beak shape (BkSh) and kernel shape (KrSh) showed a varied distribution in the four altitude ranges. The highest percentage frequency distribution of long BkLn and slightly curved BkSh were common at higher altitudes while the high frequency of elongated-flattened KrSh was mainly found at lower altitude ranges. This showed that most of them showed high variations may be due to their being particularly influenced by altitude, climate and soil conditions and human selections. Previously described by the human being dependent on the yields for its existence or a variation of desirable traits (Fuller, 2007). The high frequency of long BkLn and slightly curved BkSh at higher altitudes and elongated-flattened KrSh at lower altitudes in this study indicates that these barley accessions may be valorized in quality and yield enhancement breeding programs. Yield has been highly related to enhanced kernel size in cereals (Tshikunde *et al.*, 2019). Kernel size is characterized by the grain width, grain length and thousand kernel weights (Li *et al.*, 2019). Variation of kernel size related traits in Ethiopian barley germplasm has received little research attention. Therefore, future barley research should focus on understanding the variation and relationship

among kernel related traits and grain yield potential, which will enhance the efficiency of genetic selection in barley breeding and improvement programs.

This study also showed a wide range of kernel color (KrC) distribution in the four altitude ranges. High percentage frequency distribution was obtained in the white kernel color at higher altitudes followed by the purple color at the lower with black color at higher altitude ranges. The white color was the most abundant for all altitude ranges. Furthermore, the white color showed an increasing trend from low to high altitude while the brown color showed decreasing trend from low to high altitude ranges. The possible factor for the highest frequency of the white kernel colors could be the human selection for barley against the pigmented strains (Demissie and Bjørnstad, 1996). The white color showed an increasing trend from low to high altitude, while the brown color showed decreasing trend from low to high altitude ranges. This may be related to the local requirement of smallholder farmers for making various traditional foods and local adaptive evolution.

5.1.3. Diversity index of qualitative traits

The result showed that the overall mean values for H' ranged from 0.71 in the Oromia to 0.79 in the Tigray regions, indicating high H' in the barley accessions for current and future barley breeding purposes. The highest H' values were observed in the Amhara, Tigray and SNNP regions while the lowest H' values were obtained in the Oromia region. Relatively overall lower estimates of H' in the Oromia region are probably associated with the high adoption of improved varieties of barley by smallholder farmers in the highlands of the region (Dawit and Zewdie, 2019). Therefore, future collection campaigns should focus on remote areas of the Oromia region to capture the barley diversity for the breeding program.

The H' values for each qualitative trait, over regions and altitude ranges, varied from 0.68 for awn length to 0.94 for kernel rows, glume color and kernel shape with an overall mean of 0.76, indicating the accessions harnesses many useful traits and merit further evaluation. The overall mean H' obtained in this study was higher than that reported in earlier Ethiopian barley germplasm collection (Demissie and Bjørnstad, 1996, H' =0.71; Addisu *et al.*, 2018, H' =0.55), Nepalese naked barley landraces (Yadav *et al.*, 2018, H' = 0.73), Algerian barley accessions (Taibi *et al.*, 2019, H' =0.53), Tunisian barley landraces (Bouhaouel *et al.*, 2019, H' =0.28) and

the world barley collection (Tolbert *et al.*, 1979, $H'=0.50$). This is likely due to variations in the source and type of barley materials, and the variation of studied qualitative traits. In general, our results confirm the huge variation of Ethiopian barley genetic resources and need further evaluation for breeding purposes.

Across the altitude ranges, the overall mean value for H' was 0.74 for altitude ≤ 2000 masl to 0.79 for altitude ranges 2001-2500 masl, suggesting high genetic diversity and this calls for barley breeding to valorize these resources to improve new barley varieties for different growing areas. The barley accessions indicated higher diversity at medium altitude ranges (2001-2500 masl), this may be associated with climatic variability and agro-ecological heterogeneity in these altitude ranges (Demissie and Bjørnstad, 1997). Our result is in agreement with the highest variation of traits at medium altitude ranges in the Ethiopian barley collection (Shumet and Tesema, 2014). The analysis of the variance of the diversity index (H') based on the regions and altitudes showed a significant variation for various qualitative traits, showing a variation for useful morphological traits of breeding importance. Previous research reports showed high variability for agro morphological traits in Ethiopian barley collection (Demissie and Bjørnstad, 1997; Abebe *et al.*, 2010), North African barley germplasm (Allel *et al.*, 2017) and Algerian barley accessions (Taibi *et al.*, 2019), which could be a source of a new gene for valorizing in future barley improvement program for world agriculture.

5.1.4. Principal component and clustering analysis

The PCA on 15 qualitative traits described 38.3% of the total variation in the first two PC percent of the total variance and could be considered as the major factors that determine morphological variation in barley accessions. These percentages of variance captured in PC axes were previously reported by different researchers using morphological traits for Ethiopia barley collections (Demissie and Bjørnstad, 1996), Nepalese naked barley landraces (Yadav *et al.*, 2018) and Algerian barley accessions (Taibi *et al.*, 2019). Qualitative traits such as kernel color, glume and lemma colors at hard dough stage, glume and lemma colors at physiological maturity were the major loadings on the PC1 whereas kernel rows, leaf color and spike density were the major contributors to PC2. The majority of the morphological diversity among barley

accessions was brought because of these major qualitative traits and should be emphasized in barley breeding.

The yield of barley can be enhanced by combining spike related traits both qualitative and quantitative traits with other morphological traits (Tshikunde *et al.*, 2019). Hence, breeding combines spike related traits with useful agronomic traits to boost grain yield. Further, other qualitative traits such as spike density, beak shape and length were the major contributors to the morphological variability in PC3. The first three principal components (PC1, PC2 and PC3) accounted for 49.3% of the total variation and could be considered the major factors that determine morphological variation in barley. Our result comparatively agrees with Enyew *et al.* (2019) who showed the first three PCs contributed 74.20% of the total variations among the 48 Ethiopian barley accessions.

The Heat map analysis based on seventeen qualitative traits of 85 barley accessions was grouped into three clusters. Similar to current findings, previous studies grouped North African barley germplasm into three clusters (Allel *et al.*, 2017). In the present study, the number of accessions per cluster varied. Clustering accessions into the phenotypically similar cluster of diverse collections is valuable for barley breeding such as selecting parents for crossing (Abebe *et al.*, 2010; Allel *et al.*, 2017). Morphological traits did not cluster the barley accessions along the collection region of origins and altitude ranges. This showed that barley accessions from different collection origins (Amhara, Oromia, Tigray and SNNP) were grouped into varied clusters with different sizes. This admixture between barley accessions regardless of their collection region of origins could be associated with the continuous exchange of seeds of landraces among smallholder farmers throughout the country (Abebe *et al.*, 2010; Yadava *et al.*, 2018; Alemu *et al.*, 2020; Woldeyohannes *et al.*, 2020).

5.2. Quantitative traits variations

5.2.1. Estimation of variation components

Studies under field-based research approaches are used to improve and understand the diversity of barley. The present study showed a wide range of genetic variations among the quantitative traits. A significant difference was obtained among barley genotypes in most

assessed traits. This showed the existence of significant genetic variation among the tested genotypes, and this provides a great range for selecting superior barley genotypes. The seasonal/environmental variance (σ^2_{sn}) was also significant for most evaluated quantitative traits, showed the presence of wide variations. The distinctive agro-ecological conditions and differences in natural and artificial selection pressure may have resulted in significant variations (Kebebew *et al.*, 2001). Abebe *et al.* (2010) indicated that traits that are significant from the farmers' point of view and undergoing selection pressure revealed high genotype variance compared to traits that were not considered important by farmers.

The estimated H^2 provides good insight into the quality of a breeding program for traits (Gregorio *et al.*, 2016). Higher H^2 (>75) was observed for grain yield per plant and hectare, spike weight, thousand kernels weight, number of kernels per plant, biomass yield per plant, and phenological traits (DPM, DH and DF). This high heritability may be due to mainly genetic rather than environmental factors. The higher H^2 values for these quantitative traits suggested a higher selection response. Similarly, Addisu and Shumet (2015) found high H^2 for the traits biomass yield per plant, days to maturity, thousand kernels weight, number of grains per spike and grain yield. Genetic advance as a percent of the mean can be classified as high ($>20\%$), moderate (10-20%) and low (0-10%) (Johnson *et al.*, 1955). Based on this cutting point, the highest expected genetic advance as a percent of the mean was found for the traits of grain yield per plant and hectare, biomass yield per plant and hectare, spike weight, number of kernels and fertile tillers per plant, indicating that these traits may be under the greater influence of genetic factors. High H^2 coupled with high GAM, is an indication that significant improvement in barley yield can easily be achieved. Shegaw *et al.* (2013) revealed high GAM for a trait such as a susceptibility to lodging, flag leaf width, spikelet per spike and grain yield per plant.

5.2.2. Coefficients of variation and correlation

High PCV ($>20\%$) values were obtained for grain and biomass yield per hectare, spike weight, number of kernels and fertile tillers per plant, biomass and grain yield per plant. This indicated highly affected by the environment and less useful for the direct selection of superior genotypes. According to Sivasubramaniah and Menon (1973), PCV and GCV values were

classified as low (0-10%), moderate (10-20%) and high ($\geq 20\%$). The highest GCV values ($>20\%$) for spike weight, grain yield and number of kernel per plant, showing the existence of adequate scope for selection and improvement in these traits. However, number of fertile tillers per plant, days to emergency, awn length and plant height had the lowest rate of difference and is not greatly important traits for selection. In contrast, low ($<10\%$) genotypic and phenotypic coefficient of variation in all the quantitative traits of Ethiopian barley was reported by Gadissa *et al.* (2021a). In the present study, the PCV estimates were higher than the related GCV estimates for the quantitative traits. The higher PCV estimates suggest a strong environmental influence on the expression of the phenotypic traits. These lower values of GCV signify that selection of desirable traits could be challenging due to the high role of environmental factors in genetic variations.

In the present study, the phenotypic and genotypic correlation coefficient of grain yield was highly significant and had a positive correlation with most of the quantitative traits. This could be estimated among the traits that were vital for barley breeders to perform the strategic selection. The strongest phenotypic correlation was found between grain yield with biomass yield per hectare, biomass production and grain filling rate. The highest genotypic association was also found among grain yield with these three traits. In contrary, Gadissa *et al.* (2021a) revealed that the phenotypic and genotypic correlations of grain yield were strongly correlated with the number of grains per plant, thousand kernels weight, and effective fertile tillers. In addition, Geleta *et al.* (2019) showed significant positive genotypic and phenotypic associations of thousand kernels weight, biomass yield per hectare, awn length and plant height with grain yield. In our study, genotypic correlation coefficient values were higher for most of the quantitative traits compared to corresponding phenotypic correlation coefficient values. This indicates the greater inherent association of the traits with grain yield.

5.2.3. Principal component and cluster analysis

PCA was vital to estimate the contribution of each trait for the total observed variations. The first two principal components (PCs) described a variation of 63.21% and might be measured as the most important factors that determine quantitative trait variation in barley genotypes. Similar to this percentages of variance were earlier reported by Abebe *et al.* (2010), the first

two PCs explained about 60.23% of the total variation amongst accessions for the nine quantitative traits. Quantitative traits with the most contribution in PC1 were grain filling rate, grain and biomass yield per hectare, biomass production and grain filling rate whereas, in PC2 significant the difference was predominantly attributed to thousand kernels weight and biomass production rate. The variation of this quantitative trait implied that there may be a margin to further boost valuable traits as well through a crossing of advanced breeding to support barley yield potential. The cluster analysis also delineated the barley genotypes into three groups based on the region of origins and altitude ranges. Clustering based on regions and altitudes has also been reported in Ethiopian barely (Mekonnen *et al.*, 2015; Addisu *et al.*, 2018).

5.3. Molecular study using SNP markers

5.3.1. Genetic diversity and relationship

Genetic diversity study of germplasm is the best way to understand ancestry relations and to achieve proficient management of crop genetic resources to improve breeding programs (Al-Abdallat *et al.*, 2017; Ketema *et al.*, 2020). Such genetic diversity analysis was essential for plant breeders to perform strategic integration and target selection while preserving important economic traits of individual crops. In this study, the highest gene diversity (GD= 0.311) was revealed in barley genotypes while the average gene diversity was 0.253, which is lower than that indicated in worldwide barley genotypes (Sun *et al.*, 2011, GD =0.388), in ICARDA spring barley collections (Amezrou *et al.*, 2018, GD=0.366), in Nordic spring barley collections (Bengtsson *et al.*, 2017, GD= 0.359) and Egyptian barley collections (Elakhdar *et al.*, 2018, GD= 0.550).

The PIC range was 0.151 to 0.271 with an average polymorphism value of 0.216. A previous study indicated that PIC values >0.5 mean highly informative markers, 0.5> PIC >0.25 is an informative marker, and PIC <0.25 is a fairly informative marker (Botstein *et al.*, 1980). Therefore, our results showed that the SNP markers were fairly informative and polymorphic. The average and range values of polymorphism observed in the present study were lower than the reported values of 0.474 to 0.652 with an average value of 0.552 in barley landraces (Dido *et al.*, 2021), 0.34 to 0.83 with an average of 0.57 in barley collections (Nyiraguhirwa *et al.*,

2021). The lower PIC obtained in this study may be due to the sample size or less number of genotypes studied and the samples containing improved varieties. Higher MAF (MAF= 0.146) with an average value of 0.118 was found in barley genotypes, indicating further valuable genes can be exploited from those genotypes. The lower MAF obtained may be due to the lower number of genotypes studied from those regions. Our result is lower than that reported for the MAF that ranged from 0.517 to 0.520 in Ethiopian sorghum with a mean value of 0.518 (Girma *et al.*, 2020).

The observed heterozygosity was higher ($H_o=0.088$) in the Oromia region, indicating the existence of high genetic variability between the genotypes. This may be due to the high rate of outcrossing in barley (Parzies *et al.*, 2000). In this work, the average observed heterozygosity was smaller ($H_o=0.045$) than the average expected heterozygosity ($H_e= 0.163$), showing highly related among the barley genotypes. This could be related to the existence of gene flow along with the growing season. Our result is lower than the reported ranges observed from 0.594 to 0.662, and the expected 0.688 to 0.773 heterozygosity for Ethiopian barley landraces (Dido *et al.*, 2021). This is probably due to the source of genotypes and the samples included improved varieties, which are obtained in the lower heterozygosity ranges in our study.

5.3.2. Analysis of molecular variance

A higher genetic variation of 52.85% was observed within the populations, showing the variations within the barley genotypes in the regions. This confirmed that Ethiopia has been recognized as a centre of diversity for barley (Harlan, 1969). However, in our study, the genetic diversity of barley genotypes was 0.253, which was lower than for the North African germplasm (Allel *et al.*, 2017, 60.31%). The existence of variations in our work may be because of the outcrossing that leads to introducing new genetic components into the population. The large genetic diversity within a population could be a result of natural adaptation or extensive exchange of seeds among farmers between environments (Uba *et al.*, 2021). Therefore, the presence of genetic variation within the populations obtained in this study can be utilized in breeding programs to enhance barley productivity, the parental line selection from within the populations could be more valuable compared to among the

populations. The AMOVA also showed that genetic variation (46.43%) within accessions, this could be associated with climatic variability and agro-ecological heterogeneity among the region of origins. The presence of diversity within accessions confirmed the potential of a genetic variation, which is the source material for barley breeding (Hadado *et al.*, 2010; Dido *et al.*, 2021). The utilization of diversity within accession using pure-bred selection has been confirmed to provide germplasm for desirable traits (Lakew *et al.*, 1997). The presence of genetic variation within the population and accessions suggests that there might be a margin for barley improvement in genomic selection for cultivar development.

5.3.3. Genetic differentiation and gene flow

The genetic variance fixation index (F_{ST}) was an evaluation of population variations because of genetic structure (Dido *et al.*, 2021; Tehseen *et al.*, 2021). According to Botstein *et al.* (1980), the F_{ST} values in a range of 0.0 to 0.05 are commonly considered as lower differentiation, in the range of 0.05 to 0.15 indicates moderate genetic differentiation, 0.15 to 0.25 large differentiations, and more than 0.25 shows strong differentiation among the population. In the present study, lower values of (F_{ST} =0.019, 0.022, 0.038) genetic differentiations were observed. This showed that the barley genotypes originating from those regions of origins had very close ancestry, and the close origins may be due to seed exchange that can help barley breeding to exploit hybrids. In this study, the closest pairwise value (F_{ST} =0.019) was observed between the Amhara and Oromia genotypes while distant dissimilarity (F_{ST} =0.117) was observed between the SNNP and Tigray genotypes.

Tigray genotypes were most distantly (F_{ST} =0.089-0.117) related to populations of other regions, which showed geographical origins could be the basis of a genetic variation. The genotypes obtained from the Tigray region may be exploited as a source of breeding materials to improve genetic diversity in barley breeding through hybridization programs. Our study result revealed moderate genetic differentiation (F_{ST} =0.117) for the pairwise comparison among the barley populations implying a smaller amount of genetic relatedness and this indicated a lesser genetic relationship. This result was confirmed by genetic differentiation largely higher in marginal populations than in the favorable environment. Marginal stands are grouped by geographic and genetic separation because of spatial segregation and restricted

gene flow (Tóth *et al.*, 2019). A similar finding was reported by Dido *et al.* (2021) indicating moderate genetic differentiation ($F_{ST} = 0.082$) in the Ethiopian barley population. On the contrary, the largest genetic differentiation ($F_{ST} = 0.257$) was revealed between the barley population by Allel *et al.* (2017).

Gene flow is considered one of the most important determinants of the genetic structure of crop populations (Robledo-Arnuncio *et al.*, 2014). According to Wright (1978), the gene flow was classified into high (≥ 1), medium (0.25-0.99), and low (0.0-0.249). Based on this cutting point, the result showed that gene flow in the overall population was very low. This indicates there is a higher genetic divergence between barley populations. Hence, the barley genotypes originating from the regions could be utilized as a source of materials to enhance genetic variation in barley breeding. Besides, relatively certain gene flow was observed between Amhara and Oromia ($N_m = 0.131$) and Amhara and Tigray ($N_m = 0.07$) populations, which indicates that barley genotypes are closely related. This may be due to the consequence of the high exchange of materials among farmers from those regions. In contrary to the present study, the highest level of gene flow was reported in Ethiopian barley landraces (Teklemariam *et al.*, 2022, $N_m = 2.02$) and North African barley germplasm (Allel *et al.*, 2017, $N_m = 1.44$).

5.3.4. Genetic distance and identity

Genetic distance is the measure of genetic differences that exist among individuals or populations and can be measured by allelic differences (Nei, 1987). The present study showed the highest genetic distance (0.08) between genotypes derived from Tigray and ICARDA, which indicated that the barley genotypes had a high genetic difference. This could be due to the influence of eco-geographical differences on the existence of high genetic distance. On the other hand, the least genetic distance (0.002) was recorded between Amhara and ICARDA, showing that the barley genotypes have a great genetic relationship. Research report explained that the lower genetic distance among collection regions probably has high levels of the farmer to farmer seed exchange and gene flow across regions (Girma *et al.*, 2020). The genetic identity ranged from 0.971 to 0.998. The least genetic identity was observed between genotypes collected from SNNP and ICARDA. However, the highest genetic identity (0.998) was from Amhara and Tigray, Oromia and SNNP, and Amhara and ICARDA. This is possibly

due to natural selection for shared genetic components being the key force in determining the high genetic identity between the geographical origins. Genetic divergence as the lower cause is a result of geographical isolation and distinctive agro-ecological conditions shared by genetic materials (Guillot and Rousset, 2013). Our result is relatively similar to the genetic identity of North African barley germplasm (0.956) reported in an earlier study by Allel *et al.* (2017).

5.3.5. Principal coordinate and genetic relatedness analysis

The first two PCoA accounted for 61.2% of the total genetic variation. This highlights the potential of highly informative and selective SNP markers for genetic studies in barley, which might underpin conservation and future breeding efforts. Besides, this result showed genetic differences exist among genotypes confirming the result indicated by AMOVA, which revealed a significant genetic variation within populations and accessions. There was a comparison to the present study that has been reported in earlier studies on barley germplasm (Elakhdar *et al.*, 2018; Nyiraguhirwa *et al.*, 2021). The heat map analysis also grouped the genotypes into four major clusters, reflecting the origin of the genotypes and their genetic relationships. Likewise, the North African region of barley collection from 14 countries was grouped into four clusters (Nyiraguhirwa *et al.*, 2021). Our result is also parallel with clustering the Ethiopian barley landraces into three clusters (Dido *et al.*, 2021; Teklemariam *et al.*, 2022) and the Egyptian barley genotypes into three main groups using SNP markers (Elakhdar *et al.*, 2018).

The present result has shown that the distribution pattern of the genotypes into different groups indicated the existence of significant genetic variations among the barley genotypes. For example, among the clusters, cluster I was the largest cluster containing 60 genotypes (57.2%), and most of the genotypes included an altitude of 2001-3000 masl. These stated that structure analysis grouped the barley genotypes with greater genotypic similarity and this may be used as a source of breeding material to enhance genetic variants in barley breeding. Clustering genotypes into genotypically similar clusters of diverse collections are significant for barley improvements such as selecting parents for hybrid (Elakhdar *et al.*, 2018; Kumar *et al.*, 2021).

In the genotypes, groups were admixed into the varied clusters irrespective of their collection origins. For instance, genotypes collected from the Oromia region (32) were grouped into four clusters (sixteen genotypes in cluster I, eight genotypes in cluster II, two genotypes in cluster III and six genotypes in cluster IV). Thirty one genotypes that were collected from the Amhara region also clustered into three groups; 23 genotypes in cluster I, 5 genotypes in cluster II and 3 genotypes in cluster IV. This finding agrees with the admixture could be associated with gene flow facilitated by the continuous exchange of seeds among smallholder farmers in various agro-ecologies in the shared market and contentious introduction of new seeds into the respective growing regions (Alemu *et al.*, 2020; Woldeyohannes *et al.*, 2020; Dido *et al.*, 2021). The analysis of population structure also confirms the barley genotypes clustered into four subgroups, while they showed no clear clustering pattern of grouping. These unstructured grouping and the mixed genetic background suggested that the genotype shared a similar lineage possibly due to the high exchange of planting material between the region of origins. A similar result was reported by the absence of consistent population structure among the Ethiopian barley landraces (Abebe and Léon, 2013; Teklemariam *et al.*, 2022) and Jordan barley germplasm (Thormann *et al.*, 2018), which was related to high seed mediated gene flow.

5.4. Genetic improvement Study

5.4. 1. Grain yield improvement

Grain yield is the most useful trait for evaluating the breeding progress of barley crops (Laidig *et al.*, 2017). In this work, the yield of barley over the breeding periods showed consistent improvement. The grain yield of developed barley varieties was increased over the average of local checks (Aruso and Muga). The highest grain yield increment (37.57 and 51.61%) was observed during the two breeding periods, in the 2010's and after 2015's over the average local checks (Aruso and Muga). An earlier study on food and malt barley by Wondimu *et al.* (2011) indicated that an increment in grain yield of 51% and 38% of modern varieties over the farmer's variety (Balami) and the oldest improved variety (IAR/H/485), respectively. Likewise, Amsal *et al.* (1995) and Yadav *et al.* (2021) showed an increment in grain yield potential of recently released varieties of wheat compared with old varieties.

The highest grain yield of 4.76, 4.91, 4.99 and 5.09 t/ha was obtained from the newly released varieties of HB1965, HB1966, Debark-1, and Adoshe. This shows markedly higher grain yield in recently released varieties than the local checks and older varieties. This highest grain yield obtained from the newly released barley varieties may be due to the exploitation of breeding materials. Laidig *et al.* (2017) revealed a significant increase in barley yield due to the effective utilization of breeding materials. The regression analysis of the present study revealed an increase in the estimated average annual rate in grain yield of 0.021 t/ha yr⁻¹ or 21kg ha⁻¹ yr⁻¹. This showed that there was a highly significant improvement in the grain yield of barley through breeding over the past breeding periods. Our result is similar to Ortiz *et al.* (2002) who reported an improvement in yield at the annual rate of 22 kg ha⁻¹ for six-rowed barley varieties in Nordic spring barley breeding. On the other hand, this average rate of increase in grain yield potential of barley was lower than 44.24 kg ha⁻¹ yr⁻¹ (Wondimu *et al.*, 2011), 66.8 kg ha⁻¹ yr⁻¹ yield of wheat (Miri, 2009) and 24.27 kg ha⁻¹ yr⁻¹ of wheat cultivars (Yadav *et al.* 2021). The relative annual genetic improvement was 0.69% per year in the grain yield potential of barley varieties, which is relatively comparable to a previous study of wheat cultivars (0.54% yr⁻¹) (Yadav *et al.*, 2021).

5.4.2. Yield related and other morphological traits

Thousand kernels weights (TKW) have been the most critical trait in barley breeding (Li *et al.*, 2019). The highest TKW was obtained from the recently developed varieties. This may be due to the direct selection effect on grain size during the breeding of barley, which is maintained by the positive increment. The estimated annual rate and relative genetic improvement were 0.054g per TKW and 0.15% per year over the past breeding periods, respectively. Our work showed a linear increment in grain yield over the years with a consistent increase in thousand kernels weight. While the improvement made over the years on the TKW of barley was a positive non-significant increase. This requires further research that will allow for increasing the TKW of barley crops. Psota *et al.* (2009) found an increase in TKW of 0.18g yr⁻¹ in spring barley varieties, which is higher than the current study.

The harvest index (HI) which is a valuable trait of productivity is the ratio of the dry weight of desirable cereal material to the total above-ground dry matter produced by the cereals (Simith

et al., 1999). In this study, the HI of the varieties showed an increasing trend for the corresponding improvement of grain yield of the modern varieties, indicating new varieties have high HI compared to the local checks and other varieties. According to Cossani *et al.* (2022), the harvest index was the main driver of yield improvement in barley. The regression analysis per year for HI was found to be 0.078 yr^{-1} with a relative genetic improvement of $0.28\% \text{ yr}^{-1}$, indicating a significant progressive increase with the year of variety release. Our result is relatively higher than Ortiz *et al.* (2002) who revealed that the improvement in harvest index was 0.0008 yr^{-1} in two-rowed and 0.0018 yr^{-1} in six-rowed barley genotypes.

Regression analysis of grain yield per plant, numbers of kernel and fertile tillers per plant in barley varieties have also positively increased trends but non-significant change with the year of variety release. Additionally, spike weight and harvest index per plant showed a non-significant negative trend with the year of variety release. This shows the genetic improvement of these traits less improvement with the years, with new varieties having less genetic progress in these traits. Improvement has received little research attention for these traits. Hence, barley breeders will give special attention to those useful traits. The range of plant height (PH) varied. The PH of the newly released varieties was markedly shorter than the oldest varieties and local checks. This trait had a negatively significant trend in the year of release period. The grain improvement was -0.12 cm yr^{-1} with a relative percentage of -0.11% per year, showing that PH reduced within a year. Correspondingly, Ortiz *et al.* (2002) reported an annual rate of change of -0.2 cm yr^{-1} for two-rowed and -0.16 cm yr^{-1} for six-rowed barley varieties. Similarly, the released varieties showed a significant PH decrease in wheat varieties reported by Donmez *et al.* (2001) and Miri (2009).

As a result of two seasons of assessment, we revealed that the modern barley varieties HB1966, Adoshe and Debark-1 produced higher biomass yields than local checks and oldest varieties. Varieties with higher grain yield also had higher biomass yield. This is possibly due to relatively better rainfall distributions and lower temperature at Kulumsa that resulted in extended vegetative growth resulting in higher biomass yield with grain yield. Biomass yield has significantly increased at the rate of $0.019 \text{ t ha}^{-1} \text{ yr}^{-1}$ and the relative annual genetic improvement was estimated to be 0.16% per year. In addition, the highest biomass production and grain filling rate were recorded in the recently released varieties (Illala-01, Adoshe,

Debark-1, Hagere and Wolelay) compared to other varieties and local checks. This may be due to the new varieties having gained more photosynthetic efficiency than the local checks and oldest varieties. Biomass production and grain filling rate were highly significant with the year of variety release, indicating effectively progressed for the last years of barley breeding.

Phenological traits (DH, DTF and DPM) of the newly developed varieties found a shorter number of days to attain heading, flowering and physiological maturity stages than the local check (Muga) and other old varieties (Dimtu, Cross #41/98, EH 1493 and FBHB-42. Besides, these phenological traits revealed a non-significant negative trend over the years, showing against the year of variety release which is the early days to attain heading, flowering and maturity stages. However, the grain-filling period showed a non-significant positive increasing trend. The previous study by Abeledo *et al.* (2003) reported a non-significant reduced trend in days to heading and maturity in barley.

5.4.3. Association and basis of grain yield variations

An association study assisted to determine the extent of the relationship among the morphological traits (Fellahi *et al.*, 2013). In our study, we obtained a highly significant positive association between grain yield with biomass yield, biomass production and grain filling rate, and a significant positive correlation with harvest index, thousand kernels weight and number of fertile tillers. These significant associations showed highly contributed to the current grain yield improvement in released barley varieties. On the other hand, plant height, flag leave length, spike length and harvest index per plant had a negatively non-significant relation with grain yield. Phenological traits such as grain filling period, days to heading, days to flowering and physiological maturity found a non-significant positive correlation with grain yield. Wondimu *et al.* (2011) reported that grain yield showed a non-significant negative relation with days to flowering and physiological maturity while positively associated with grain filling period. The stepwise regression analysis using grain yield as a dependent variable was also carried out in the present study to identify the major critical traits that mainly contributed to the yield variation. Therefore, the result revealed biomass yield and harvest index were the major critical traits that mainly contributed to most of the yield variation (99%) among the food barley varieties.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

The results obtained in our study showed a wide morphological diversity in region of origins and different altitudinal ranges. The majority of the qualitative traits indicated enormous variation in Amhara, SNNP and Tigray regions with medium altitude ranges. These are a sign of the potential of the regions and altitudes in contributing toward primary barley diversity areas. The first three principal components accounted for 49.3% of the total variation and could be considered the major factors that determine qualitative variation in barley and should be emphasized in the barley breeding program. This study mainly provides useful information for further barley breeding as well as critical indication for *in-situ* conservation. The result shows that a wide collection from various region of origins and altitude ranges is very useful than barley collection from specific agro-ecology, suggesting capturing important alleles diversity from different growing environments. Thus, this material could be manipulated for improvement either through the usual conventional breeding (direct selection) or advanced tools to study the genetic basis of phenotypic differences of these traits.

Moreover, the ANOVA showed a high range of variations in the majority of the quantitative traits. The genotypes and season variance were highly significant for most quantitative traits. Besides, the highest H^2 and GAM were obtained for agronomic traits such as days to heading, days to flowering, days to physiological maturity, number of kernel per plant, spike weight, thousand kernels weight, grain yield per hectare, grain and biomass yield per plant in the present study, indicating that the expression of these traits is mainly controlled by additive gene effects. Thus, direct and simple selection can be effective for the breeding of barley for those traits. Low H^2 and GAM were found for measured traits such as days to emergency, awn length, number of tillers per plant and biomass production rate, indicating a stronger contribution from non-additive gene effects on the expression of these traits. As a result, heterosis breeding may be more effective than selection for improving these traits.

Grain yield was highly significant a positive and strongest genotypic and phenotypic association with biomass yield per hectare, biomass production and grain filling rate. Genotypic correlation coefficient values were the highest for the majority of the quantitative

traits as compared to corresponding phenotypic correlation coefficient values, indicating the greater genetically linked correlation of the traits with grain yield. To quantify the morphological variation of quantitative traits between genotypes was also perform a principal component analysis (PCA). The result showed the first two principal components (PCs) described a total variation of 63.21% and might be measured as the most important factors that determine quantitative trait variation in barley genotypes. The SNP data generated using advanced molecular tools provide useful information that can be utilized in breeding and genetic research in barley. Based on SNP data, the barley genotypes were genetically divergent. The AMOVA indicated high genetic variations within the populations and accessions. This high diversity could be the foundation for developing and generating desirable new barley varieties with superior grain yield potential and wide adaptability, enhanced with abiotic and biotic resistant traits.

The genetic improvement over the years due to association changes in the morphological traits is also key for a critical understanding of the breeding challenges. In the present study, grain yield has increased linearly at the rate of $0.021\text{t ha}^{-1} \text{yr}^{-1}$ or $21 \text{ kg ha}^{-1} \text{yr}^{-1}$ with the relative annual yield improvement estimated to be 0.69% per year. The main improvement comes through consequent rounds of breeding involving the introductions and indigenous local materials in the barley breeding programs in Ethiopia. The introduction of exotic germplasm and local collections of barley leads to an improvement in barley yield. The results obtained in the genetic improvement study, grain yield has increased linearly. This showed barley grain yield could still be increased while the current result revealed a medium yield gain from the past improvement program. Hence, a wide study, of multiple locations and seasons is suggested to understand the yield potential of barley genotypes and their interaction consequences for crop improvement. Overall, the results revealed that Ethiopian barley germplasm exhibited greater genetic diversity for useful traits. This provides numerous opportunities for barley breeding and improvement via conventional programs and advanced tools.

6.2. Recommendations

The overall findings of this study allowed for the assessment of barley genotypes with adequate morphological and molecular variations, better yield, and biotic and abiotic stress tolerance traits having implications for collecting conservation, and appropriate use of germplasm. We also suggest that it be carefully taken into account as a source of genetic material for the crossing of specific and targeted traits to enhance productivity, tolerance to abiotic and abiotic stresses, and quality of barley products. The major recommendations and directions for future research are to

1. Extend the testing of this genetic material in different environments to assess the phenotypic performance of yield and yield-related traits, physiological traits, and farmer-preferred traits and disease tolerant traits and then to develop new, highly productive barley varieties that addressing the local requirement of barley growing areas in Ethiopia.
2. The presence of phenotyping data and the SNP data will be applied for current and future research using genome-wide association and genomic selection for economically useful traits in barley. Therefore, the genotype and phenotype traits association (GWAS) will be carried out using the SNP dataset to take advantage of the unique gene variation for agronomic, physiological and smallholder farmer-preferred traits and disease-resistant traits. This information will be applied for current and future research using genome-wide association and genomic selection for economically useful traits in barley.
3. Characterizing the variation in the material's nutritional properties to find genotypes with significant attributes for marketability and diet improvement in Ethiopia.
4. To determine the segregation of quantitative trait loci of breeding relevance, conduct crosses with selected parents to produce recombinant populations.
5. These results demonstrated genetic diversity among the Ethiopian barley genotypes can be also applied to suggest approaches, such as spatial analysis to optimize *in-situ* conservation of barley genetic resources, mining SNPs and linkage analysis, high-density SNP mapping of the barley genome.

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8. APPENDICES

Appendix 1. Mean monthly rainfall (mm) and temperature (°C) of Kulumsa Agricultural Research Center (2011-2021)

Month	2011			2012			2013			2014			2015			2016		
	Rain fall	T.M AX	T.M IN	Rain fall	T.M AX	T.M IN	Rain fall	T.M AX	T.M IN	Rain fall	T.M AX	T.M IN	Rain fall	T.M AX	T.M IN	Rain fall	T.M AX	T.M IN
January	0.0	23.3	5.7	0.0	23.7	5.6	12.4	23.8	4.1	0.0	23.2	5.9	1.7	23.6	4.7	3.6	22.8	7.8
Febru ary	0.2	24.4	6.7	0.0	24.5	6.0	0.0	24.8	6.1	7.0	23.8	8.1	0.0	25.0	6.6	12.5	23.3	7.3
March	20.	24.4	8.1	8.80	25.1	7.6	115	23.4	7.8	33.4	23.7	8.4	39.7	23.8	8.1	59.1	23.4	9.6
April	70.9	24.5	9.0	158	22.4	9.9	81.7	22.4	9.5	73.7	22.6	9.2	52.0	23.3	9.6	107	22.0	10.9
May	127	22.3	10.4	59.4	22.3	9.53	104	22.7	9.17	145	23.3	9.48	196	22.6	10.9	88.6	21.3	9.70
June	37.9	23.1	10.1	34.1	23.3	8.75	79.5	22.4	9.5	37.5	23.3	9.9	64.4	22.9	10.2	59.3	22.7	10.3
July	134	22.5	9.9	151	22.4	9.1	154	21.7	9.4	68.0	22.1	10.1	60.5	23.6	10.2	149	21.6	10.4
August	133.0	21.1	9.64	223	21.6	9.31	183	20.6	10.7	87.8	21.7	9.69	154	22.7	9.18	136	21.3	10.9
Septem ber	152.9	20.6	9.62	137	20.8	9.28	60.7	21.1	8.86	97.2	20.6	9.37	96.3	22.1	9.65	134	21.7	10.8
October	36.9	19.9	7.9	68.7	20.2	7.4	103	20.1	8.9	105	19.8	9.6	55.6	20.8	9.8	71.1	19.3	7.96
Novem ber	57.20	20.5	8.37	3.20	21.5	6.73	116	20.8	8.07	57.8	20.1	7.62	48.2	20.9	8.22	34.5	19.8	6.29
Decem ber	0.00	21.7	5.19	12.3	22.4	5.73	0.0	21.9	4.42	16.5	22.1	7.28	2.60	22.7	7.22	20.7	21.5	6.30
Annual	769.2	22.4	8.38	855	22.5	7.90	1010	22.1	8.1	729	22.2	8.7	771	22.8	8.61	876	21.7	9.02

Abbreviations: T= Temperature, MAX=Maximum, MIN=Minimum Source: Kulumsa Agricultural Research Center/ unpublished data.

Appendix 1. *Continued*

Month	2017			2018			2019			2020			2021		
	Rainfall	T. MAX	T. MIN	Rainfall	T. MAX	T. MIN	Rainfall	T. MAX	T. MIN	Rainfall	T. MAX	T. MIN	Rainfall	T. MAX	T. MIN
January	45.07	22.63	6.86	0.00	23.55	9.67	0.00	24.87	9.14	8.80	23.65	11.2	0.00	23.94	9.31
February	15.20	23.80	7.47	43.90	25.31	10.37	15.40	26.48	11.26	3.90	25.51	11.3	5.60	24.61	11.29
March	197.6	22.80	9.87	30.20	25.22	12.51	16.50	27.55	12.22	68.40	26.44	13.4	0.40	26.82	12.27
April	124.0	21.72	10.94	165.1	24.14	13.22	147.0	26.40	13.24	104.6	26.21	12.9	70.30	26.39	13.35
May	82.99	22.10	10.31	57.20	24.70	13.19	131.3	26.19	12.79	94.70	24.90	12.9	0.00	24.85	12.49
June	36.64	22.92	10.24	127.3	22.66	12.77	152.4	24.20	13.12	123.7	23.76	12.9	59.90	24.82	11.95
July	115.7	22.05	10.93	121.6	21.37	11.72	195.1	23.41	12.3	251.4	21.23	13.0	170.6	20.62	13.01
August	197.6	21.98	10.15	199.3	21.35	12.33	66.10	21.74	12.55	143.5	21.2	12.9	131.9	21.34	12.62
September	269.7	21.32	10.19	70.20	22.82	12.01	119.2	21.84	12.13	130.8	28.14	12.4	191.2	20.90	11.91
October	76.60	20.90	10.27	15.80	24.22	12.03	65.20	22.58	12.55	64.00	24.05	12.8	64.30	22.78	12.62
November	2.50	20.96	8.61	31.50	23.43	11.50	28.30	23.83	11.94	1.00	23.52	12.2	6.00	23.65	11.41
December	0.00	21.70	6.72	0.60	24.01	10.44	0.00	22.36	11.48	3.20	22.98	10.7	0.00	23.34	9.29
Annual	1164	22.06	9.39	862.7	23.55	11.82	936.5	24.27	12.06	998.0	24.28	12.8	8	700.2	23.66
															11.79

Abbreviations: T= Temperature, MAX=Maximum, MIN=Minimum, Source: Kulumsa Agricultural Research Center/ unpublished data.

Appendix 2. Predominant glumes and lemma colors of the barley accessions at the hard dough stage (A) white/normal and B) purple colors. The traits are measured with a scale following the barley descriptors (for measuring scales for the traits refer to Table 3).



Appendix 3. Qualitative traits of diverse barley accessions (A) various glumes and lemma color at physiological maturity stage A1) purple color changed to black glumes and lemma colors, A2) purple changed to white glumes and grey lemma colors, A3) purple changed to white glumes and lemma color during the maturity stage



Appendix 4. Mean performance of phenological, growth, yield and yield related traits of barley varieties grown at Kulumsa during the main cropping seasons of 2021 and 2022

Genotype	NFTP	TKW	NKP	SpW	GYHa	BMYPa	GFR	BMPR
Aruso	2.57	36.78	27	1.62	3.13	11.78	95	120.72
Muga	2.7	37.21	30	1.59	2.95	11.9	96.07	115.15
IAR-485	2.78	45.26	40	2.32	4.32	13.79	122.77	131.81
FBHB-42	2.62	42.98	38	2.09	4.11	13.62	125.11	123.69
Shege	2.95	37.6	42	2.05	3.61	12.26	114.54	119
Abay	2.64	36.17	35	1.85	3.21	12.06	103.99	119.42
Meserach	2.66	38.37	36	1.85	3.75	12.46	119.71	129.33
Dimtu	2.63	41.92	37	2.14	4.05	12.63	116.68	118.5
Mezezo	2.71	39.82	37	1.87	3.36	12.06	101.67	122.74
Basso	2.46	41.03	38	1.86	3.69	12.66	127.95	133.58
Harbu	2.72	40.61	38	1.95	3.55	11.7	103.59	120.63
Setegn	2.85	44.98	29	1.81	3.74	12.15	102.95	122.75
Estayish	2.72	37.54	39	1.93	3.47	12.92	111.19	133.37
Dinsho	2.65	36.17	31	1.57	3.44	12.57	104.14	131.28
Yedogit	2.85	40.48	37	1.93	3.93	13.17	106.54	133.23
Defo	2.88	40.05	35	1.9	3.7	12.83	107.9	129.93
Biftu	2.64	38.93	38	1.84	3.16	12.27	101.8	127.09
HB-1307	2.95	41.44	40	1.97	4.46	13.87	123.25	134.57
Bentu	2.67	37.47	40	1.92	4.21	12.88	126.9	134.66
Guta	2.76	41.57	36	1.97	4.11	12.67	121.52	132.22
Agegnehu	2.79	41.58	40	2.05	3.79	12.89	106.02	123.23
Tilla	2.61	38.29	35	2	3.02	11.08	100.6	113.78
Dribie	2.75	37.46	44	2.01	4.22	13.07	112.19	131.03
Felamit	2.82	35.07	40	1.89	4.22	13.09	138.44	129.72
Abdane	2.8	43.15	37	2.03	4.15	13.48	122.25	135.8
Hriti	2.71	36.16	38	1.86	3.4	12.34	117.64	123.76
Fetina	2.76	50.62	29	1.68	3.66	12.97	122.69	131.5
EH 1493	2.8	40.64	42	2.05	4.5	13.4	135.02	125.69
Cross	2.93	41.46	41	1.97	4.58	13.53	135.79	127.42
Gobe	2.77	45.24	27	1.53	4.73	13.26	128.05	132.63
Illala-01	2.61	45.83	24	1.51	4.61	13.45	157.74	144.01
IllaIa-02	2.65	40.82	26	1.38	4.5	12.8	138.02	133.89
Robera	2.86	41.69	40	2.16	4.33	13.1	129.45	132.33
Adena	2.64	37.01	42	1.97	3.8	11.89	118.54	123.11
Wolelay	2.86	53.23	28	1.69	4.65	13.29	141.67	136.69
HB1965	2.68	38.64	40	1.97	4.76	13.44	136.04	132.43

HB1966	2.9	43.88	39	2.03	4.91	13.92	113.16	129.73
Debark-1	2.93	39.82	40	2.08	4.99	14.49	155.28	138.53
Hagere	2.65	42.1	37	2.2	4.45	13.13	145.94	134
Adoshe	2.69	36.09	43	1.72	5.09	14.09	142.34	140.88

Abbreviations: NFTP= number of fertile tillers per plant, NKP=number of kernels per plant, SpW=spike weight (gram), TKW=thousand kernels weight (gram), GYHa=grain yield per hectare (in t/ha), BMYHa=biomass yield per hectare (t/ha), BPR= biomass production (kg/ha/day), GFR=grain filling rate (kg/ha/day).

Appendix 4. *Continued*

Genotype	HI	PH	SPL	GFP	DH	DTF	DPM
Aruso	28.92	110.94	8	33.8	59.5	62.72	96.61
Muga	26.59	106.37	7.66	32.1	71.99	74.51	105.96
IAR-485	30.97	108.97	7.2	34.6	67.75	71.62	106.43
FBHB-42	30.61	109.88	6.36	32.9	80	82.68	114.92
Shege	30.26	98.62	7.34	32.3	71.75	73.54	105.12
Abay	27.86	103.17	7.2	32.2	68.45	70.91	102.33
Meserach	31.23	97.52	6.37	32	60.2	63.16	94.19
Dimtu	32.04	102.85	7.53	34.8	71.75	75.18	110.21
Mezezo	29.14	102.91	6.88	33.8	61.15	63.97	98.16
Basso	29.67	104.28	7.21	30.1	60.91	63.68	92.52
Harbu	31.98	106.1	6.72	34.8	57.85	60.37	95.62
Setegn	31.46	107.28	7.18	36.3	58.55	61.3	98.47
Estayish	28.05	103.5	6.32	32.1	59.73	63.23	94.96
Dinsho	28.46	103.83	6.93	33.6	57.61	59.74	93.81
Yedogit	30.14	98.58	6.21	36.4	57.61	60.91	98.62
Defo	29.12	101.7	6.41	34.5	60.67	64.36	99.01
Biftu	27.2	102.77	6.36	32.7	59.02	62.08	95.01
HB-1307	31.52	104.56	7.05	35.5	64.92	67.68	103.79
Bentu	32.71	94.18	6.54	33.1	57.37	59.98	93.62
Guta	32.6	102.02	6.7	33.7	56.67	59.24	93.59
Agegehu	29.88	102.27	7.1	35.4	67.27	70.73	106.88
Tilla	29.87	97.82	6.34	31.6	63.03	65.74	96.48
Dribie	32.19	97.32	6.74	36.9	58.08	60.64	99.1
Felamit	32.3	94.85	6.74	31	67.98	71.41	101.31
Abdane	30.67	101.82	6.87	34	61.38	64.39	98.64
Hriti	28.68	97.68	6.46	30.3	67.98	70.94	99.87
Fetina	28.86	110.75	7.1	31	65.15	67.9	97.77

EH 1493	32.91	103.79	7.62	33.2	72.7	76.36	109.27
Cross	33.49	105.03	6.83	33.6	72.23	75.17	108.57
Gobe	35.22	96.47	7	36	59.02	62.26	99.26
Illala-01	33.85	95.96	6.97	30.1	58.32	61.55	90.22
Illala-02	34.52	95.1	7.23	32.9	58.55	61.34	94.21
Robera	32.77	98.04	6.31	33.3	61.62	64.85	98.05
Adena	33.24	98.77	6.55	32.5	59.26	62.52	94.83
Wolelay	34.05	108.71	7.26	32.8	61.38	63.85	95.97
HB1965	34.1	104.17	6.8	34.7	64.45	67.63	102.26
HB1966	33.95	103.63	6.51	40.9	62.8	65.57	109.36
Debark-1	32.96	101.39	7.41	32.2	71.28	73.78	105.28
Hagere	33.59	105.06	7.16	31.2	63.74	66.74	96.99
Adoshe	34.41	94.16	7.03	35	62.32	65.06	100.3

Abbreviations: DH=days to heading, DTF=days to flowering, DPM=days to physiological maturity, PH=plant height (cm), SpL=spike length (cm), HI=harvest index.