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Characterization of Genetic Variation among Ethiopian Barley (*Hordeum vulgare* L.) Genotypes

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Stuttgart-Hohenheim, 2019

Wosene Gebreselassie Abtew

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List of abbreviations

AMMI	Additive main effect and multiplicative interaction
ANOVA	Analysis of variance
ASV	AMMI stability value
CSA	Central Statistical Agency of Ethiopia
DAPC	Discriminant analysis of principal components
EARI	Ethiopian Agricultural Research Institute
G x E	Genotype by environment interaction
GBS	Genotyping by sequencing
HARC	Holeta Agricultural Research Center
IBC	Institute of Biodiversity Conservation
ICARDA	International Center for Agricultural Research in the Dry Areas
IPCA	Interaction principal component axis
IPK	The Leibniz Institute of Plant Genetics and Crop Plant Research
JUCAVM	Jimma University College of Agriculture and Veterinary Medicine
NGS	Next generation sequencing
NJ	Neighbor joining tree
SNNP	Southern Nations Nationalities and Peoples' region of Ethiopia
SSC	Static stability coefficient
SUP	Superiority index

Executive summary

Barley (*Hordeum vulgare* L.) is a major cereal crop in Ethiopia and accounts for 8% of the total cereal production based on cultivation area. Farmers may face unpredictable rainfall and drought stress patterns such as terminal drought where rainfall ends before crops have completed their physiological maturity, which then poses a challenge to crop production. The absence of efficient weather forecasts and a lack of efficient communication channels for resource-poor farmers ask for the development of varieties that are robust to such irregularities. A goal of plant breeding for areas with variable climate and limited resources for agricultural inputs is to produce stable varieties with higher average yield across diverse environments and growing conditions. Genotype by environment (G x E) interactions, however, frequently interfere with the selection of widely adapted genotypes. Landraces represent over 90% of the cultivated barley diversity of Ethiopia, and reflect a deeply rooted and ancient relationship between barley and Ethiopian farmers. Knowledge about the yield stability of existing Ethiopian barley varieties and landraces under changing environmental variables is important for the future development of barley varieties with high and stable yields. Therefore, it is useful to evaluate the robustness of barley varieties against late onset and early termination of rainfall.

In addition, yield components are quantitative with substantial influence of environment. Yield components also compensate each other in trait correlation dynamics. Since grain yield is a more complex trait than its components, environmental effects and genotype-by-environment (G x E) interactions for grain yield are stronger than for its components. Therefore, indirect selection of yield components may be more efficient than selection on grain yield per se to obtain higher yielding and stable cultivars. A study, therefore, was

initiated to 1) characterize the response of a diverse set of barley genotypes to different locations and variable planting dates and identify genotypes with wide adaptation and stable performance and/or genotypes with specific altitude and planting date 2) determine traits that contribute to high and stable yields across a range of different environments and planting dates 3) determine the pattern of population structure and genetic parameters among genotypes conserved in Ethiopian and German gene banks in for different period of time as well as currently growing in farmers' field. In order to meet the objectives 18 genotypes were tested at four different sowing dates with 15 days interval in different locations (Ambo and Jimma) and years (2012 and 2013). In addition, we investigated relationships among traits in these different situations, with the ultimate aim of identifying traits with reduced sensitivity to environmental effects that may contribute to higher yield stability.

Considering the genotypes and environments, both genotypes (G) and G x E interaction variance components were highly significant for grain yield, with a ratio of approximately 1:1. Of the 16 environments, 12 grouped into two clusters which largely corresponded to test locations. The tested genotypes revealed a wide variation for both static and dynamic yield stability measures. Compared to improved cultivars, farmers' landraces displayed higher average static stability and similar superiority indices (dynamic stability). These landraces are therefore a source of germplasm for breeding resilient barley cultivars. Staggered planting proved to be a useful method for evaluating genotype stability across environmental factors beyond location and season. In addition, we also noticed that compensatory relationship between kernels per spike and thousand kernel weight in landraces. Kernels per spike and number of fertile tillers can be proposed as robust traits in barley breeding for a wider adaptation as they had significant and consistent positive total

effects on grain yield.

In order to determine the pattern of population structure and genetic parameters among genotypes of different origin and gene banks, DNA samples were subject to double-digest by ApeK1 and Hind III enzymes. After sequencing, raw read was checked for major quality parameters. Sequence reads were then filtered for sequencing artifacts and low quality reads (preprocessing). The pre-processed reads were aligned to genome of barley cultivar Morex to call SNPs. Values of observed heterozygosity (H_o) ranged from 0.250 to 0.337 and were higher than the expected heterozygosity (H_e) that varied from 0.180 to 0.242 in genotypes of all origins. The inbreeding coefficient (F_{IS}) values that ranged between -0.240 and -0.639 across the regions were also higher and negative suggesting existence of excess outcrossing than expected. Based on the inferred clusters by the ADMIXTURE, high F_{st} values were observed between clusters suggesting high genetic differentiation among the genotypes tested though differentiation was not based on location. In addition, genetic differentiation computed based on the predetermined location, altitude and source of genotypes suggested weak differentiation among the groups.

These results indicate that, in Ethiopia, barley genetic variation between regions and altitudes were less pronounced than within region and altitude variations. This calls for the germplasm collection strategies to be cautious in considering location and altitude as a main factor of variation thus strategies should focus on exploiting the within region variation also for better germplasm conservation and utilization. The static yield stability of landrace has to be utilized by breeders for their wider recommendations for those farmers who cannot afford use of farm inputs and specific cultivars. In addition, the relative robustness as well as plasticity of traits sorted by the current study can be

incorporated in the breeding strategy of barley in Ethiopia.

Zusammenfassung

Gerste (*Hordeumvulgare* L.) ist eine bedeutende Getreideart in Äthiopien und macht 8% der gesamten Getreideerzeugung, in Bezug auf die Anbaufläche, aus. Unvorhersehbaren Regen- und Dürreereignisse, wie z.B. Dürren, bei denen der Regen endet, bevor die Gerste ihre physiologische Reife erreicht hat, stellen die Pflanzenproduktion in Äthiopien vor große Herausforderungen. Der mangelnde Zugang für Landwirte zu effizienten Wettervorhersagesystemen und Kommunikationskanäle erfordern die Entwicklung von Sorten, die extremen Wetterereignissengegenüber tolerant sind. Ein Ziel der Pflanzenzüchtung, für Gebiete mit Extremwetterereignissen und begrenzten Ressourcen für landwirtschaftliche Betriebsmittel, ist die Erzeugung umweltstabiler Sorten mit höherem Durchschnittsertrag in unterschiedlichen Umwelten und unter verschiedenen Wachstumsbedingungen. Genotyp - Umwelt Interaktionen (G x E) erschweren jedoch häufig die Auswahl von Genotypen die sich an unterschiedliche Umweltbedingungen anpassen können. Landrassen machen über 90% der kultivierten Gerstenvielfalt Äthiopiens aus und spiegeln eine tief verwurzelte und anhaltend, gewachsene Beziehung zwischen der Gerste und äthiopischen Landwirten wider. Das Wissen über die Ertragsstabilität bekannter äthiopischer Gerstensorten und Landsorten, unter sich ändernden Umweltbedingungen, ist für die zukünftige Entwicklung von Gerstensorten mit hohen und stabilen Erträgen wichtig. Daher ist es unabdingbar, die Robustheit von Gerstensorten im Hinblick auf Schwankungen in der Niederschlagsmenge und den Niederschlagszeitpunkten zu beurteilen.

Darüber hinaus sind Ertragskomponenten, quantitative Merkmale,die stark von der Umwelt beeinflusst werden. Da der Kornertrag einkomplexeres Merkmal ist als die ertragsbestimmenden Komponenten, sind die Umwelteinflüsse und die Wechselwirkungen zwischen Genotyp und Umwelt (G x E) für denKornertrag stärker als für Komponenten

die den Kornertrag bestimmen. Daher kann eine indirekte Selektionanhand vonErtragskomponenten effizienter sein als die per se Selektion auf Basis des Kornertrags, um Ertragreichere und stabilere Sorten zu erhalten. Daher wurde diese Studie initiiert um 1) die Reaktion verschiedener Gerstengenotypen auf verschiedene Standorte und Aussaattermine zu beurteilen und Genotypen mit einerweiten Anpassung und stabilen Leistung zu identifizieren, 2) Merkmale zu bestimmen, die zu hohen und stabilen Erträgen in einer Reihe unterschiedlicher Umwelten beitragen, 3) die Populationsstruktur und genetische Parameter von äthiopischen und deutschen Genbankakzessionen, sowie aktuellen Gerstensorten, zu erfassen.

Um diese Ziele zu erreichen, wurden 18 Genotypen an vier verschiedenen Aussaatterminen im Abstand von 15 Tagen an verschiedenen Orten (Ambo und Jimma) und Jahren (2012 und 2013) getestet. Darüber hinaus untersuchten wir die Beziehungen zwischen Merkmalen in diesen verschiedenen Situationen mit dem Ziel, Merkmale mit verminderter Empfindlichkeit gegenüber Umwelteinflüssen zu identifizieren, die zu einer höheren Ertragsstabilität beitragen können.

Sowohl die Genotyp- als auch die GxE Varianzkomponenten sind mit einem Verhältnis von etwa 1:1, für das Merkmal Kornertrag, von großer Bedeutung. Von den 16 Umweltenwurden 12 in zwei Cluster eingruppiert, die weitestgehend den Versuchsstandortenentsprechen. Die getesteten Genotypen zeigen eine große Variation, sowohl für die statische als auch für die dynamische Messung der Ertragsstabilität. Im Vergleich zu aktuellen Sorten zeigen die Landrassen der Landwirte eine höhere durchschnittliche statische Stabilität und eine ähnliche dynamische Stabilität. Diese Landrassen sind daher ein wertvoller Genpool für die Züchtung von widerstandsfähigen Gerstensorten. Die zeitversetzteAussaat erwies sich als nützliche Methode zur Beurteilung

der Stabilität von Genotypen über Umweltfaktoren hinweg. Darüber hinaus haben wir auch eine kompensatorische Beziehung zwischen Kornanzahl pro Ähre und Tausendkorngewicht in Landrassen festgestellt. Die Kornanzahl pro Ähre und die Anzahl der fruchtbaren Bestockungstriebeerwiesen sich als robuste Merkmale für eine breitere Adaption an unterschiedliche Umweltbedingungen, da sie einen signifikanten und beständig, positiven Effekt auf den Kornertrag haben.

Um die Populationsstruktur und genetische Parameter zwischen Genotypen verschiedener Herkunft und Genbanken zu bestimmen, wurden DNA-Proben mit ApeK1- und HindIII-Enzymenbearbeitet. Nach der Sequenzierung wurde die Rohdaten auf wichtige Qualitätsparameter überprüft. Sequenzen wurden gefiltert, um Artefakte zu eliminieren und Sequenzen mit geringer Qualität zu entfernen (Vorverarbeitung). Die vorverarbeiteten Sequenzen wurden an dem Genom der Gerstensorte Morexausgerichtet, um SNPs zu identifizieren. Die Werte der beobachteten Heterozygosität (Ho) lagen im Bereich von 0,250 bis 0,337 und waren höher als die erwartete Heterozygosität (He), die in der gesamten Population von 0,180 bis 0,242 variierte. Die Werte für den Inzuchtkoeffizienten (F_{IS}), liegen zwischen -0,240 und -0,639, sind ebenfalls höher und negativ, was auf eine übermäßige Auskreuzung als erwartet hindeutet. Basierend auf den von ADMIXTURE abgeleiteten Clustern wurden hohe F_{st}-Werte zwischen den Clustern beobachtet. Dies lässt auf eine hohe genetische Differenzierung zwischen den getesteten Genotypen schließen, jedoch ließ sich keine ortsabhängige Differenzierung feststellen. Darüber hinaus deutet die genetische Differenzierung, die basierend auf Ort, Höhe und Sortentyp berechnet wurde, auf eine schwache Differenzierung zwischen den Gruppen hin.

Diese Ergebnisse zeigen, dass in Äthiopien die genetische Variation der Gerste zwischen

Regionen und Höhenlagen weniger ausgeprägt war als innerhalb von Region und Höhenlagen. Dies erfordert, dass Strategien zur Sammlung von genetischen Ressourcen sich nicht nur an Standort und Höhenlage als Hauptvariationsfaktor ausrichten. Vielmehr sollten sich Strategien zum Erhalt des Gersten-Genpools darauf konzentrieren, die Variationen innerhalb der Regionenund Höhenlagen zu nutzen um die genetische Diversität aufrecht zu erhalten. Die statische Ertragsstabilität der Landsortensollte von Züchtern für ihre Empfehlungen für Landwirte berücksichtigt werden. Darüber hinaus kann empfohlen werden die relative Robustheit sowie die Plastizität von Merkmalen, die durch die aktuelle Studie identifiziert wurden, in die Züchtungsstrategie von Gerste in Äthiopien einzubeziehen.

1. Chapter 1: General introduction

1.1 Origin and domestication of barley

Cultivated barley (*Hordeum vulgare* L.) is believed to be domesticated from its closest wild relative *Hordeum vulgare* subsp. *Spontaneum* (C. Koch) Thell. in the Fertile Crescent (the comparatively moist and fertile land of western Asia, the Nile Valley and the Nile Delta of northeast Africa), about 10,000 years ago (Schmid et al., 2018). It is considered as one of the founder crops of agriculture in western Asia, first appearing in the archaeological record in the 8^{th} and 7^{th} millennia BC (Badr et al., 2000, Jones et al., 2011). Since domestication, its cultivation area has expanded spectacularly, especially within temperate regions of the Northern and Southern hemispheres which subsequently has resulted in a large diversity of both landraces and modern elite varieties (Schmid et al., 2018). The origin of barley is probably one of the controversial aspects of the crop (von Bothmer et al., 2003). Even if, the most widely accepted hypothesis was origin was a Fertile Crescent based on the presence of *H. spontaneum* the closest ancestor of cultivated barley in the region, reports emerged claiming a wider area of origin from Morocco to Tibet due to the existence of wild ancestor of barley in Morocco, Ethiopia, Cyprus, Crete, Libya, Iraq, Iran, Turkey, Afghanistan and Israel that is outside the proposed area of origin (Molina-Cano et al., 1999). This has further expanded the thought of possible multiple origins of the crop (Molina-Cano et al., 2005). Furthermore, Orabi et al. (2007) suggested that independent domestication might have been taken place at the horn of Africa (Ethiopia and Eritrea) as an outcome of studying barley using 38 nuclear SSR and five chloroplast SSR markers.

1.2 Global distribution of barley

Based on the average data of 20 years, Russian Federation leads the production with nearly 18 million tons a year followed by Germany and France each producing about 10.7 and 10.3 million tonnes, respectively. Europe is the major producer with 62.6% of the world's barley production with Asia's share to be 14.1%. Americans, Oceania and Africa contribute 13.3, 6.6 and 3.2% of global barley production (FAOSTAT, 2018). Several grains of barley have been recovered from archeological sites at Wadi Kubbaniya, near Aswan in Egypt. The sites are typical Late Paleolithic and are firmly dated between 18,300 and 17,000 years ago. They seem to represent a very early use of ground grain in the Nile Valley, and evidence is presented for its continued use over the subsequent 6000 years. The Egyptian findings possibly record an initial stage of food production, and if they indeed do, then they suggest that food production may not have been brought about by environmental stress and may not have led inevitably to radical social changes (Wendorf et al., 1979). The results of research done by Badr and co-workers are in favour of the hypothesis that the Israel-Jordan area is the region in which barley was brought into culture. In the same work, it was reported that the Himalayas can be considered a region of domesticated barley diversification (Badr et al., 2000).

1.3 Barley genetic diversity

In the global portal to information about Plant Genetic Resources for Food and Agriculture, the current number of global barley accessions conserved is estimated to be 193,023 (Genesys, 2014). This number seems underestimated because the Global Crop Diversity Trust estimated 290,820 barley accessions with Canada, USA and Brazil being countries holdig largest numbers of barley accessions in 2008, an estimate which is

expected to have increased by this time (Harold and Valkoun, 2011). The European barley Database (EBDB) alone contains approximately 156,000 barley accessions (IPK, 2014) and ICARDA comprises about 27,000 accessions of which nearly 2,000 (7%) are wild relatives (Global Diversity Trust, 2014). The USDA National Small Grains Collection also possesses 33,176 accessions (Muñoz-Amatriaín et al., 2014). More than 15,000 barley accessions have been maintained in the gene bank of Ethiopian Institute of Biodiversity (IBC, 2015) until mid of 2014. This number is expected to increase, as there have been accessions from regular collections coming to the database almost every year (personal communication). The country is also blessed with huge genetic and morphological diversity of barley. For instance, more genetic diversity was reported in Ethiopia than other countries of north Southwest Asia, the Middle East, North and Northeast Africa, and South Arabia (Pomortsev et al., 2013). This was evidenced by the fact that Ethiopian accessions were grouped to many clusters in relation to accessions from the above mentioned countries.

1.4 Importance of barley in Ethiopia

Barley is a major cereal grain grown for food, animal feed and alcohol. It is the fourth most important cereal crop in the world after wheat, maize, and rice. Russian Federation, Germany, and France are the top producers worldwide more than 141 million tonnes of barley was produced globally in 2016 (FAOSTAT, 2018). Barley is mainly used as animal feed in developed countries followed by malt. However, in many countries like Ethiopia and Tibet, it is a major food crop. Farmers also use barley straw as animal feed in West Asia, North Africa, Ethiopia, Eritrea, Yemen, the Andes region and East Asia (Akar et al., 2004).

Barley is among the major cereal crops in Ethiopia and accounts for about 8% of the total national cereal production. In the 2017/18 growth season, 951,993.15 hectares of land was covered by barley in peasants' farms in Ethiopia with a total harvest of 2,052,996.4 tons (CSA, 2018). Ethiopia is considered as a centre of diversity of barley with the widest morphological diversity (Lakew et al., 1997). Barley is believed to be the oldest domesticated crop long before other cereals were known in recorded history (Bekele et al., 2005). The existence of extremely variable climatic and edaphic conditions in Ethiopia allowed barley to be cultivated from 1400 to over 4000 meters above sea level (m.a.s.l) (Asfaw, 2000). Barley is used as a food and beverage in more than 20 different ways in the country. Bread and Injera (pancake-like bread) are among the major types of barley products to consume. The grain is roasted and consumed as kolo (snack bread). Roasted or cooked grain is also consumed alone or mixed with beans and peas. The powder is made to porridge either boiled or raw. Kinche (a type of bulgur) is another type of food prepared from semi-milled grains of barley (Shewayrga and Sopade, 2011). The existence of genetic diversity has special significance for improving productivity and maintenance of diversity in a country like Ethiopia (Worede et al., 2000). Even though barley is cultivated in almost all parts of the country, Arsi, Bale, Shewa, Gojam, Gonder, Welo, and Tigray are the most important barley producing regions accounting for more than 85% of the country’s total production (Abebe et al., 2010).

1.5 Barley genome and next generation sequencing (NGS)

The cultivated barley (*H. vulgare* L.) is a self fertilized diploid (2n=2x=14) plant with a large haploid genome of 5.1 Gb. The International Barley Genome Sequencing Consortium constructed a genome-wide physical map of barley cultivar (cv.) Morex by high-information-content fingerprinting and contig assembly of 571,000 BAC clones

(~14-fold haploid genome coverage) originating from six independent BAC libraries. It is an integrated and ordered physical, genetic and functional sequence resource that describes the barley gene-space in a structured whole-genome context (The International Barley Genome Sequencing Consortium, 2012). A characteristic of the barley genome is the abundance of repetitive DNA and it was observed that approximately 84% of the genome is comprised of mobile elements or other repeat structures (Mayer et al., 2012). In addition, the interspersed copia-like retrotransposon BARE-1 comprises about 7 % of the barley genome (Manninen and Schulman 1993). The genome of barley has seven pairs of chromosomes among which chromosome 2H is believed to be the longest, followed in length by 5H, 3H, 7H, 4H, 6H and 1H (Pedersen et al. 1995). A physical map of 4.98 Gb, with more than 3.90 Gb anchored to a high-resolution genetic map was developed recently revealing 79,379 transcript clusters, including 26,159 'high-confidence' genes with homology support from other plant genomes (Mayer et al., 2012).

Advances in DNA sequencing technology and the development of high-throughput sequencing plant forms (NGS) have enabled the scientific community unraveling several hundreds of single nucleotide polymorphisms (SNPs) for different application. In barley, the NGS has been used in diversity study by whole genome sequencing, exome sequencing and gene expression studies. Recently Mascher et al. (2014) reported exome sequencing of phenotypic bulks of a mapping population of barley segregating for a mutant phenotype that increases the rate of leaf initiation. In addition exome capture-based (re)-sequencing was used to reveal large numbers of SNPs enabling the precise allocation of *H. bulbosum* introgressions in barley (Wendler et al., 2014). Nowadays, detailed laboratory protocols required for enrichment and sequencing as well as detailed step-by-step instructions for the bioinformatics analysis of the resulting data is available (Bayer et

al., 2019).

Next Generation Sequence technology has recently advanced to the extent that Genotype-by- sequencing (GBS) has become affordable and boosted the efficiency of determining the molecular genetic diversity of crop plants of large genome size like barley recently (Elshire et al. 2011, Peterson et al., 2014). The simplicity in terms of approach, high specificity coupled with reproducibility makes it preferable by many molecular population genetics researchers. Moreover, it enables to address important regions of the genome that are inaccessible to sequence capture approaches. Currently, more than 15,000 barley accessions have been maintained in the gene bank of Ethiopian Institute of Biodiversity (IBC, 2015). In addition, over 3,350 Ethiopian barley accessions have been conserved in IPK's Genebank information system, GBIS (http://gbis.ipk-gatersleben.de/GBIS_I/) in Germany since 1970's.

1.6 Production constraints of barley in Ethiopia

Ethiopia is known for having very large amount of barley genetic diversity. The huge genetic diversity is attributed to the diverse agroecological conditions, long history of barley cultivation and immense cultural practices (Bekele et al., 2005). The existence of genetic diversity has special significance for improving productivity and maintenance of diversity in a country like Ethiopia, which is characterized by highly varied agroclimates and diverse growing conditions (Worede et al., 2000). According to Harlan (1969), barley is one of the oldest cultivated crops and has been grown in Ethiopia for at least 5000 years. Barley is cultivated in almost all parts of the country but Arsi, Bale, Shoa, Gojam, Gondar, Wollo, and Tigray are the major barley producing regions accounting for more than 85% of the country’s total production (Abebe et al., 2010).

Barley is predominantly cultivated in high altitudes (2000 m.a.s.l.) and in some regions in two distinct seasons: Belg (February–May) that relies on the short rainfall period from March to April and Meher which is the main season cropping (June–December) that relies on the long rainfall period from June to September (Lakew et al., 1997; Bekele et al., 2005). The Meher barley contributes to more than 85% of the total Ethiopian barley production (Bekele et al., 2005). Despite barley can tolerate more adverse growing environments such as drought or lower soil fertility than wheat, it has some production constraints among which unpredictable rainfall pattern is the main along with poor supply of improved seed, untimely supply of inputs like fertilizers (Begna et al., 2014). Farmers faces unpredictable rainfall and drought stress patterns such as terminal drought where rainfall ends before crops have completed their physiological maturity (Cheung et al., 2008), which then poses a challenge to productivity. The absence of efficient weather forecasts and lack of efficient communication channels for resource-poor farmers ask for the development of varieties that are robust to such irregularities. Therefore, it is useful to evaluate the robustness of barley varieties against late onset and early termination of rainfall.

According to a research based on 30 years (1977-2007) meteorological data conducted in Central Rift Valley of Ethiopia, which represents major cereal based farming systems, there was a high special and temporal variation in moisture and temperature. In a short rainy season that runs from March to May the total average rainfall varied spatially from 178 to 358 mm with a coefficient of variation of 32-50%. In the main rainy season (June – September) total average rainfall was recorded from 420 to 680 mm with a coefficient of variation of 15-40%. During the same 30 years period number of rainy days was seen

decreased but intensity per rainfall event was increased for the main rainy season, which could attribute to soil and nutrient losses through erosion and run-off. The reduced number of rainy days increased the length of intermediate dry spells by 0.8 days per decade leading to crop moisture stress during the cropping season. As to temperature, the same study showed that there was an increasing trend of temperature of 0.12-0.54oC per decade (Kassie et al., 2014). In general, meteorological projections suggested that by 2080, annual rainfall will change by -40 mm. The rain fall will increase during non-growing seasons (November-December) but decrease during crop season thus the length of growing season would be expected to reduce by 12-35%. Moreover, annual mean temperature will increase by 1.4-4.1 ^{o}C by 2080 (Kassie et al., 2014). This alarms that future climate trends i.e., moisture and temperature variability pose major risks to agriculture that solely depend on rainfall. Therefore, adaptation strategies are needed to cope with the risks, make farming sustainable towards food security.

In crops like barley grain yield is determined by agronomic traits like, number of spikes per plant, number of kernels per spike and 1000-kernel weight that each contribute directly and indirectly to final grain yield (Fischer and Edmeades 2010). Most yield components are complex traits that are highly influenced by environment where they grow. Since grain yield is a more quantitative trait than yield contributing agronomic traits, environmental effects and genotype-by-environment (GxE) interactions for grain yield are more stronger than for yield contributors (Baenziger et al. 2011).Therefore, indirect selection of yield components may be more efficient than direct selection on grain yield per se to obtain higher yielding cultivars (Puri et al. 1982). Therefore, it is imperative to characterize the existing barley genotypes for the prevailing contrasting environments and look for robust and reliable traits that can be used as a selection index. In addition, in order to make wise

decision on how to utilize and conserve the available barley genetic resource, studies have to be done on genetic diversity of the crop. This study, thus, was initiated to address the above research questions.

1.7 Objectives

1. Characterize the response of a diverse set of barley genotypes to different locations and variable planting dates and identify genotypes with wide adaptation and stable performance and/or genotypes with specific adaptation to defined environmental conditions (specific altitude or planting date).
2. Determine traits that contribute to high and stable yields across a range of different environments and planting dates.
3. Investigate the pattern of population structure and determine genetic parameters among genotypes conserved in Ethiopian and German gene banks as well as currently growing in farmers' field to forward information as an input for plant breeders and conservationists

Chapter 2: Ethiopian barley landraces show better yield stability and comparable yield to improved varieties in multi-environment field trials

2.1 Abstract

Barley (*Hordeum vulgare* L.) is a major food crop in Ethiopia. A high inter-annual rainfall variability, concomitant variable planting dates and unpredictable drought stress at any time during the rainy season are severe constraints to barley production in Ethiopia. To study genotype by environment (G x E) interactions and grain yield stability, we evaluated 18 barley genotypes (three landraces and 15 improved cultivars) for yield and flowering time in two locations (Ambo and Jimma) and four staggered sowing dates over two years (2012-2013) giving a total of 16 environments. We observed wide phenotypic variation over environments for both grain yield (677-2,944 kg ha^{-1}) and days to 50% flowering (63-82 days). Considering the 18 genotypes and 16 environments, both genotype (G) and G x E interaction variance components were highly significant for grain yield, with a ratio of approximately 1:1. The G x E analysis revealed that the first two interaction principal component axes (IPCA1 and IPC2) in an additive main effect and multiplicative interaction (AMMI) model explained 66.1% of the total G x E interaction for grain yield ($P < 0.001$). Of the 16 environments, 12 grouped into two clusters, which largely corresponded to test locations. The tested genotypes revealed a wide variation for both static and dynamic yield stability measures. Compared to improved cultivars, farmers' landraces displayed higher average static stability (e.g. IPCA1; $P = 0.017$) and similar superiority indices (dynamic stability). These landraces are therefore a source of germplasm for breeding resilient barley cultivars. Staggered planting proved to be a useful method for evaluating genotype stability across environmental factors beyond location and season.

Key words: G x E interaction, AMMI, stability, landrace, barley, Ethiopia

2.2 Introduction

Barley (*Hordeum vulgare* L.) is a major cereal crop in Ethiopia and accounts for 8% of the total cereal production based on a cultivation area of 1,018,753 hectares in 2013 (CSA, 2013). Ethiopia is a center of barley diversity (Lakew et al., 1997) with a high level of morphological variation between landraces that resulted from adaptation to diverse climatic conditions and soil types. Long- term geographic isolation likely contributed to this diversity (Mekonnon et al., 2014) because barley is a founder crop of Old World agriculture and may have been cultivated in Ethiopia for the last 5,000 years (Bekele et al., 2005). In the present time, farmers cultivate barley in Ethiopia from 1,400 to over 4,000 meters above sea level (m.a.s.l) under highly variable climatic and edaphic conditions (Asfaw, 2000). Barley is used as food, fodder and beverage in more than 20 different ways, which reflects its cultural and nutritional importance (Shewayrga and Sopade, 2011; Abraha et al., 2013). One key challenge in barley breeding is to develop varieties that are able to face the challenges of changing climatic conditions and agricultural systems.

A frequent goal of plant breeding for areas with limited resources for agricultural inputs is to produce varieties with higher average yield across diverse environments. Genotype by environment (G x E) interactions, however, frequently interfere with the selection of widely adapted genotypes (Ceccarelli and Grando, 1997). Although the breeding of varieties adapted to specific environments and cultivation practices is an alternative strategy to address the problem of low yield, changing weather patterns during periods of crop cultivation require the development of varieties with high yield stability in fluctuating

environments. This notion is supported by 40 years of meteorological data, which indicate a decrease in rainfall from June to September (the main cropping season in most parts of Ethiopia) in the south western and central parts of Ethiopia (Cheung et al., 2008). Consequently, temperature and rainfall extremes may differ substantially between locations (Mekasha et al., 2014).

Landraces represent over 90% of the cultivated barley diversity of Ethiopia (Hadado et al., 2010), and reflect a deeply rooted and ancient relationship between barley and Ethiopian farmers. So far, the national agricultural system did not deliver significantly better performing cultivars that are suitable for the cropping system of resource-poor smallholder farmers and may replace landraces (Mulatu and Lakew, 2011). Therefore, knowledge about the yield stability of existing Ethiopian barley varieties and landraces under changing environmental variables is important for the future development of barley varieties. Moreover, although barley landraces are widely cultivated in Ethiopia and considered to be an important source of genes for stability traits, information about their yield stability across variable environments is currently very limited in the scientific literature.

In an eco-geographically diverse environment like Ethiopia, crop production is highly dependent on the timing of local growth seasons, and on the distribution and total amount of rainfall. Farmers may face unpredictable rainfall and drought stress patterns such as terminal drought where rainfall ends before crops have completed their physiological maturity (Cheung et al., 2008), which then poses a challenge to crop production. The absence of efficient weather forecasts and a lack of efficient communication channels for resource-poor farmers ask for the development of varieties that are robust to such

irregularities. Therefore, it is useful to evaluate the robustness of barley varieties against late onset and early termination of rainfall.

In this study, our main goal was to test whether a staggered planting date in different locations and years allows identifying genotypes with low G x E and stable yields. We used this approach to compare the yield performance of a diverse set of Ethiopian barley landraces and improved cultivars and to test for differences in the environmental stability between the two groups.

2.3 Materials and methods

2.3.1 Genetic material

Eighteen Ethiopian barley genotypes consisting of 15 improved cultivars and three landraces were included in the experiment. The cultivars and one widely used landrace were obtained from Holetta Agricultural Research Center (HARC) of Ethiopia and two local landraces were obtained from barley growers at Jimma and Ambo, respectively. The landraces represent the dominant landraces of the region. The improved cultivars were chosen based on their diversity in adaptation and genetic background. They are grown in different parts of the country and differ in traits like stress tolerance and grain yield (Table 2.1).

Table 2. 1Summary of Ethiopian barley genotypes used in the study

Code	Name	Selection history	Desirable traits of the variety other than yield
G1	Dribie	Selection from ICARDA germplasm	Tolerant to drought
G2	Agegnehu	Released cultivar derived from a landrace accession # 218950 obtained from the Ethiopian Institute of Biodiversity (EIB) through pure line selection	Tolerant to major barley leaf diseases (*Pyrenophora teres* and *Rhynchosporium secalis*) and adapted to low moisture areas
G3	Biftu	Released cultivar derived from a farmers variety 'Shasho' through pure line selection	Early vigor and tolerant to shoot fly (*Deliaflavibasis* Stein) and suitable for both main and short seasons
G4	Estayish	Released cultivar derived from a landrace accession # 218963 obtained from EIB through pure line selection	High quality grain (white seeded), high market value
G5	Meserach	Released cultivar derived from a farmers' variety 'Kulumsa' through pure line selection	Early maturing and tolerant to major leaf diseases (*Pyrenophora teres* and *Rhynchosporium secalis*)
G6	Shedeho	Released cultivar derived from a landrace accession # 3381 obtained from EIB through pure line selection	High quality grain (white seeded), high market value
G7	Misccal 21	Selection from ICARDA germplasm and released as dual purpose barley (food and malt)	High yielding with good malting quality; resistance to lodging with multiple disease resistance
G8	HB42	Released cultivar, a cross made at Holetta from IAR/H/81/ Composite 29 //Compound14/20 / Coast	Resistant to scald (*Rhynchosporium secalis*) and good biomass yield
G9	EH1493	Released cultivar, a cross made at Holetta from white sasa/ Composite 29//white sasa	High yielding, late maturing
G10	HB1307	Released cultivar, a cross made at Holetta from Awura gebs-1/IBON 93/91	High yielding, lodging resistant, resistant to leaf diseases *(Pyrenophora teres* and *Rhynchosporium secalis)* with good biomass yield and white seeded
G11	Jimma Local (local check)	Farmers' variety (landrace) at Jimma, Ethiopia	Early maturing
G12	Dimtu	Released cultivar derived from a landrace accession # 3369 obtained from EIB through pure line selection	Good yield under low input conditions with good biomass yield
G13	Basso	Released cultivar derived from a landrace accession # 4731 obtained from EIB through pure line selection	Suitable for main and short seasons
G14	Cross 41/98	Released cultivar, cross made at Holetta from 50-16/3316-03// HB42/Alexis	High yielding, late maturing
G15	Abay	Released cultivar derived from a landrace accession # 3357 obtained from EIB through pure line selection	High quality grain (white seeded) with long spike and medium to early maturity
G16	Ambo Local (local check)	Farmers' variety (landrace) at Ambo, Ethiopia	Suitable for main season with big grain size
G17	Balame	Dominant farmers' variety (landrace) at West Shoa, Ethiopia	Tolerant to low soil fertility and drought, good flour quality
G18	Shege	Released cultivar derived from a landrace accession # 3336 obtained from EIB through pure line selection	Good yield under low input conditions and tolerant to major leaf diseases (*Pyrenophora teres* and *Rhynchosporium secalis*)

2.3.2 Description of the study area

The experiment was conducted at two locations in Ethiopia, Ambo and Jimma that differ in altitude, soil type and land coverage mean annual rainfall and other characteristics (Table 2.2).

Table 2. 2 Characteristics of the two test locations in Ethiopia

Characteristic	Location	
	Ambo	Jimma
Position relative to Addis Ababa	135 km West	365 km Southwest
Latitude	8°57'N	7°42'N
Longitude	37°45'E	36°48'E
Altitude (m.a.s.l.)	2,005	1,790
Mean annual rainfall (mm, average over 20 years)	1,041	1,625
Min., Mean and Max Temperature (°C) over 20 years	10.2, 18.0 and 26.3	11.3, 18.5 and 26.5
Soil type	Clay	Clay loam
Soil organic matter (%)	5.14 - 5.54	5.93 - 6.33
Soil Cation exchange capacity (meq/100 gm soil)	36.0 - 37.2	31.6 - 33.8
Soil pH (Gerba et al., 2013)	6.63 - 6.85	6.11 - 6.19
Land coverage	Crops like wheat, barley and maize	Denser in forest coverage as part of tropical rainforest
Total rainfall (mm) in the 2012 growing season (June-December)	894	880
Total rainfall (mm) in the 2013 growing season (June-December)	887	1,036

2.3.3 Definition of environments

We defined the different environments as combinations of two locations (Jimma and Ambo), two seasons (2012, 2013) and four sowing dates (done in approximately 15 day intervals between mid-June and end of July in each year), resulting in a total of 16 environments (Table S2.1). No serious moisture stress was experienced after all four sowing dates in the two seasons and locations except at the fourth sowing date at Jimma in 2012. In both years, the rainy season finished earlier at Ambo than Jimma (Figure S2.1).

2.3.4 Experimental design

A randomized complete block design (RCBD) was used for each combination of location, season and sowing date. The dimension of a single plot was 2.4 m width and 2.5 m length (6 m^2) and it was planted with 12 rows at a distance of 0.2 m between, which corresponded to HARC recommendations.

2.3.5 Trial management

Fifty-one grams of barley seeds were manually drilled per plot as recommended by HARC. Fertilizer was applied to each trial field as 100 kg diammonium phosphate (DAP) and 50 kg urea per hectare split into two time points. 15 g of Urea and 30 g of DAP were added to a plot at time of sowing and the same amount at the tillering stage. The trial plots were weeded by hand.

2.3.6 Data collection

The traits measured were grain yield and days to 50% flowering. To measure grain yield,

matured spikes were harvested from ten inner rows of each plot when the seeds were mature. The spikes were then further dried and threshed. The clean seeds were dried in the oven until the moisture content was zero to avoid a bias in moisture content between different harvests. The yield was adjusted to 12.5% moisture content in kg ha^{-1}. To determine days to 50% flowering, the date was counted from sowing to 50% of the spikes were completely emerged from the leaf sheaths in a plot based on visual assessment.

2.3.7 Statistical analysis

The grain yield data were analysed with GenStat for Windows 17th Edition (VSN International, 2014). A two-way ANOVA determined the effect of environment on grain yield, and a four-way interaction ANOVA was carried out to examine the main and interaction effects of factors on grain yield with the following model:

$$X_{ijklm} = \mu + Y_i + G_j + L_k + S_l + (YG)_{ij} + (YL)_{ik} + (GL)_{jk} + (YS)_{il} + (GS)_{jl} + (LS)_{kl} + (YGL)_{ijk} + (YGS)_{ijl} + (YLS)_{ikl} + (GLS)_{jkl} + (YGLS)_{ijkl} + \varepsilon_{ijklm} \quad (1)$$

where X_{ijklm} = the value of treatment in the i^{th} Year, j^{th}Genotype, k^{th} Location, l^{th} Sowing date and m^{th} replication; μ = grand mean; $Y_i = i^{th}$ Year; $G_j = j^{th}$ Genotype; $L_k = k^{th}$ Location; $S_l = l^{th}$ Sowing date; $(YG)_{ij.}$.. = interactions between Year, Genotype, Location and Sowing date etc.; and ε_{ijklm} = error of $X_{ijklm.}$

An additive main effect and multiplicative interaction (AMMI) model was used to dissect the G x E interaction (Gauch, 1992) using the Meta Analysis function in GenStat. Each

combination of location, season and sowing date was considered as an environment giving a total of 16 environments. The AMMI model for 18 genotypes and three replications was defined as (Gauch 2013):

$$Y_{ijr} = \mu + \alpha_i + \beta_j + \sum \lambda_k \gamma_{ik} \delta_{jk} + \rho_{ij} + \tau_{r(e)} + \varepsilon_{ijr} \quad (2)$$

where Y_{ijr} = yield of the i^{th} genotype in the j^{th} environment for replicate r, μ = the grand mean, α_i = the genotype deviation from the grand mean, β_j = the environment deviation, λ_k = the singular value for the interaction principal component (IPC) k, γ_{ik} = the eigenvector value for genotype i and component k, δ_{jk} = the eigenvector value for environment j and component k, ρ_{ij} = the residual, $\tau_{r(e)}$ = the block effect for replication r within environment j, and ε_{ijr} = the error.

2.3.8 Stability analysis

The static and dynamic yield stability concepts describe the differential response of genotypes to variable environments (Becker and Leon, 1988). Under the static stability concept, the yield performance of genotypes remains constant in different environments, whereas under the dynamic stability concept the response of a stable genotype to the environment is parallel to the average response of all genotypes in the trial (Becker and Leon, 1988). We estimated the following stability indices with GenStat:

(1) *Superiority index (SUP)*: This index, proposed by Lin and Binns (1988), measures the distance in grain yield of a given genotype to the genotype with the maximum

performance in each environment. It consists of a non-parametric analysis, which is simpler and addresses the limitations of a linear regression analysis (Oliveira et al., 2013). A small SUP value indicates a better fit of a genotype to the dynamic stability concept.

(2) *Static stability coefficient (SSC)*: This index measures the consistency of genotype performance for grain yield. It is based on environmental variances i.e. the variance of yields of each genotype over test environments (Lin et al., 1986; Becker and Leon, 1988). A low value (closer to zero) of this coefficient indicates a better fit of a genotype to the static stability concept.

(3) *The first interaction principal component axis (IPCA1)*: IPCA1 values obtained from the AMMI model indicate the position of genotypes on an AMMI biplot. Genotypes with an absolute value close to zero have a higher static stability.

(4) *AMMI stability value (ASV):* This value is calculated from the IPCA1 and IPCA2 scores of each genotype in the AMMI model and the two main principal component axes (PC1 and PC2; Zali et al., 2012). This parameter also follows the static stability concept and ranks genotypes with low values as more stable (Purchase et al., 2000).

To test for differences in the stability parameters between landraces and cultivars, we used the Mann-Whitney U (Wilcoxon rank-sum) test.

2.4 Results

2.4.1 Environmental means, repeatability and differentiation among entries

The field trials revealed a strong effect of the environment on grain yield (Figure 2.1).

Environmental means for grain yield differed between the 16 environments and ranged from 677 to 2,944 kg ha^{-1}, with an overall mean of 1,447 kg ha^{-1} (2-way ANOVA, $P < 0.001$; Table 2.3 and Table S2.2). Pairwise comparisons of factors revealed that (i) the later (fourth) sowing date produced lower yields (1,191 kg ha^{-1}) than the earlier (first) sowing date (1,364 kg ha^{-1};t-test, $P < 0.05$); (ii) genotypes performed better at the Ambo site (1,873 kg ha^{-1}) than at the Jimma site (1,182 kg ha^{-1}; t-test, $P < 0.001$) and (iii) genotypes performed better in 2013 (1,593 kg ha^{-1}) than 2012 (1,463 kg ha^{-1}; t-test, $P < 0.05$). The environment also affected the number of days to 50% flowering with means ranging from 63 to 82 days (2-way ANOVA, $P < 0.001$; Tables 2.3 and S2.2). Late sowing caused a longer time span to 50% flowering (73 days) than early sowing (66 days; t-test, $P < 0.01$). Genotypes differed for grain yield and days to 50% flowering with ranges from 525 to 2,119 kg ha^{-1} forgrain yieldand 58 to 88 days to 50% flowering (2-way ANOVA, $P < 0.001$; Tables 2.3 and S2.2). The three top yielding genotypes were improved varieties whereas the lowest yielding genotype, Balame (G17) was the landrace most widely used by Ethiopian farmers. Grain yield was negatively correlated with days to 50% flowering across the 16 environments, but with variable significance levels. The correlation coefficients between grain yield and days to 50% flowering ranged from -0.33 to -0.88 (Table S2.2). Estimated repeatability ranged from 0.46 to 0.92 for grain yield, and from 0.53 to 0.98 among environments (Table S2.2). Repeatability did not differ between locations or sowing dates.

Table 2. 3ANOVA showing the effects of genotypes, environments and G x E interaction on grain yield and days to 50% flowering of 18 barley varieties grown in 16 environments (location-season-sowing date combinations in Ethiopia)

Source of variation	D.F	Grain yield		Days to 50% flowering	
		MS	Variance	MS	Variance
Genotype	17	8,479,572***	165,140	3,584.1***	73.0
Environment	15	17,750,843***	318,481	1.515.5***	26.6
G x E	255	552,873***	151,831	81.7***	22.0
Error	574	97,383	97,383	15.8	15.8
Total	863				

DF, degree of freedom; MS, means squares.

***
significant at P < 0.001 probability level.

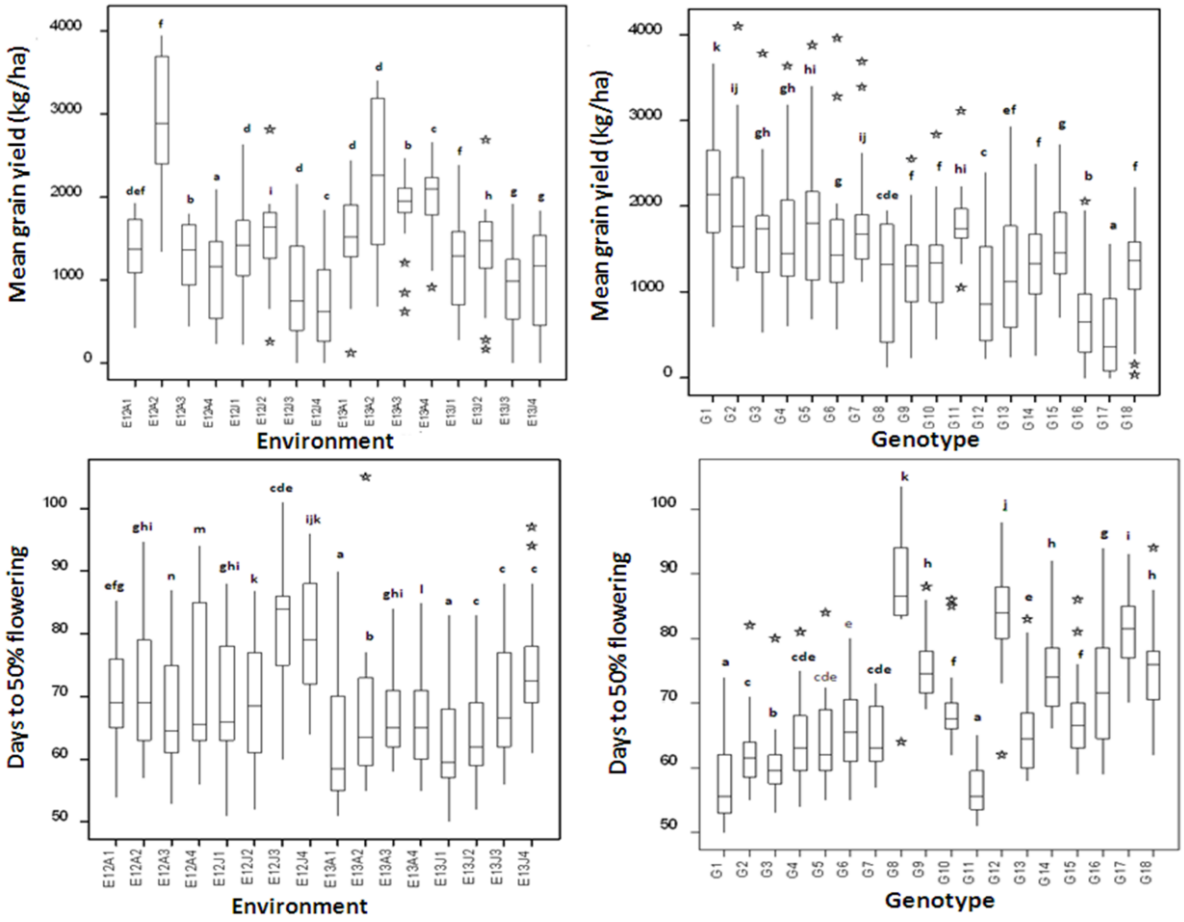

Figure 2. 1 Boxplots for mean grain yield (top left and right) and days to 50% flowering (bottom left and right) as affected by the 16 environments and 18 genotypes, respectively. Individual letters (a-n) above each box plot shows significant differences and box plots with different letters are significantly different ($P < 0.05$) from each other. Stars above box plots indicate outliers.

2.4.2 Variance components

Genotype (G), environment (E) and genotype-by-environment (G x E) interaction affected both grain yield and days to 50% flowering (2-way ANOVA, $P < 0.001$). For grain yield, the ratio of G to G x E variance was nearly one (Table 2.3). A combined ANOVA of genetic and environmental factors revealed significant effects of G ($P < 0.001$), genotype-by-location (G x L; $P < 0.001$), genotype-by-sowing date (G x SD; $P < 0.001$) and genotype-by-year (G x Y; $P < 0.01$) for grain yield. Ratios of G variance to G x SD, G x L and G x Y interactions were about two, three and nine times, respectively (Table 2.4).

Table 2. 4 Combined ANOVA showing mean square and variance components of grain yield and days to 50% flowering of 18 barley genotypes in 2012 and 2013

Source of variation	D.F	Grain yield		Days to 50% flowering	
		MS	Variance	MS	Variance
Genotype (G)	17	8,479,572***	152,320	3,584.0***	74.0
Location (L)	1	92,588,723***	209,549	3,586.0***	6.1
Year (Y)	1	3,291,308**	6,248	7,995.0***	15.5
Sowing date (SD)	3	26,326,767***	144,169	2,480.0***	8.9
G x Y	17	592,165**	16,611	100.2***	0.1
G x L	17	2,063,589***	46,886	141.1***	1.8
G x SD	51	505,003***	87,994	60.8***	8.4
G x Y x L	17	304,863***	5,285	96.8***	1.3
G x Y x SD	51	463,031***	93,197	85.9***	1.2
G x L x SD	51	487,641***	84,449	81.6***	12.6
G x Y x L x SD	51	321,820**	132,557	67.5***	25.9
Residual	574	97,383	97,383	15.8	15.8
Replication	2	153,190		26.4	
Total	863				

DF, degree of freedom; MS, means squares.

*, **, *** significant at $P < 0.05$, $P < 0.01$, $P < 0.001$ probability level, respectively.

2.4.3 Level of genotype x environment interactions

The AMMI analysis of variance for grain yield and days to 50% flowering of the 18 barley genotypes evaluated in 16 environments showed that G x E had a significant effect on trait values ($P < 0.001$). The environment explained 48.3% of the total sum of squares implying that the environments were sufficiently diverse to differentiate between genotypes. The remaining 26.1% and 25.6% of the variation resulted from genotype and G x E effects, respectively. The partitioning of the G x E interaction revealed that IPCA1 captured 44.4% and IPCA2 21.7% of variation in grain yield. Similarly, 43.9% and 20.2% of the interaction was explained by IPCA1 and IPCA2, respectively, for days to 50% flowering. The mean squares of the two components (IPCA1 and IPCA2) differed significantly (AMMI ANOVA, $P < 0.001$) and explained a total of 66.1% and 64.1% of the variance of the G x E interaction in grain yield and days to 50% flowering, respectively (Table 2.5, Figure 2.2A, B).

Table 2. 5ANOVA of the AMMI model with 18 barley genotypes based on grain yield and days to 50% flowering in 16 environments

Source of variation	Grain yield			Days to 50% flowering		
	DF	MS	% explained by IPCAs	DF	MS	% explained by IPCAs
Treatments	287	1,921,248***		287	364.1***	
Genotype (G)	17	8,479,572***		17	3584.1***	
Environment (E)	15	17,750,843***		15	1515.5***	
Block	32	140,171*		32	12.7	
G x E	255	552,873***		255	81.7***	
IPCA 1	31	2,018,458***	44.4	31	295.1***	43.9
IPCA 2	29	1,056,456***	21.7	29	145.3***	20.2
IPCA 3	27	407,506***	7.8	27	137.7***	17.8
IPCA 4	25	409,232***	7.3	25	50.9***	6.1
IPCA 5	23	300,351***	4.9	23	44.5***	4.9
IPCA 6	21	304,051***	4.5	21	25.4*	2.6
IPCA 7	19	231,757***	3.1	-	-	-
Residual	80	110,541		99	7.2	
Error	544	95,072		544	16.0	
Total	863	704,058		863	131.7	

DF, degree of freedom; MS, mean Squares; IPCA, interaction principal component axis.

*, **, *** significant at $P < 0.05$, $P < 0.01$, $P < 0.001$ probability level, respectively.

Environments and genotypes showed much variation for both traits in terms of main effects and their interaction. For example, genotype G11 located close to the origin in the biplot and showed low IPCA1 and IPCA2 values suggesting little interaction with the environment and a good performance for grain yield compared to other genotypes. In contrast, G5, G8 and G18 were the most unstable genotypes because they were more distant to the origin of the biplot. With respect to the contribution of environments to G x E interactions, environments 13A3, 13A2 and 12A2 contributed most as indicated by their

distance to the origin in the biplot (Figure 2.2A) and allowed a better discrimination of genotypes. Environments 12J3, 12J4, 12A3 and 13A4 had the least effect on G x E interaction.

Among the 16 environments, 12 grouped into two clusters of seven and five environments, respectively, with a clear separation to the remaining four environments (Figure 2.2 left). All environments except one of the first cluster were located in Jimma and showed high repeatability values ranging from 0.64 to 0.91. The environments of the second cluster were all located in Ambo (with one exception) and also showed a high repeatability ranging from 0.63 to 0.92.

2.4.4 Estimation of stability parameters and difference between cultivars and farmers' landraces

Superiority index (SUP) values ranged from 149 to 1,969, and static stability coefficient (SSC) values from 211 to 791. They indicate large differences among tested genotypes for both dynamic and static yield stability (Table 2.6). Based on three static stability parameters, the three landraces had a higher static stability because the overall average rank was 4 for the landraces and 11 for the modern cultivars. Significant differences between the landraces and the modern cultivars were observed for the three static stability parameters SSC, IPCA1 and ASV, but not for the dynamic stability parameter SUP (Table 2.7).

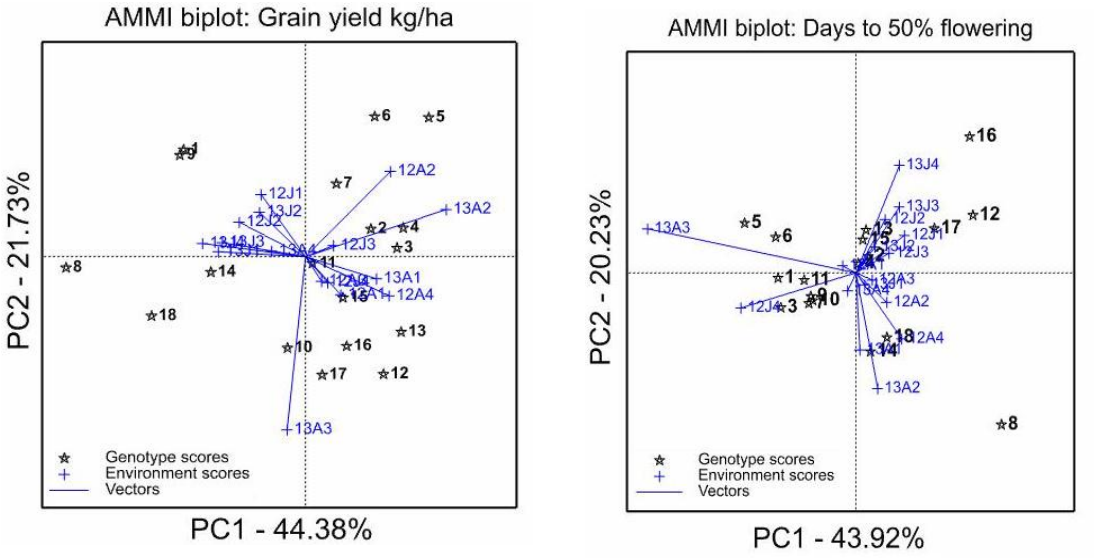

Figure 2. 2 AMMI biplots showing relationships among 18 barley genotypes and 16 environments (location-season-sowing date combinations in Ethiopia) for grain yield (left) and days to 50% flowering (right).

Table 2. 6 Mean grain yield (kg ha-1) and estimated yield stability parameters of 18 barley genotypes evaluated across 16 environments (location-season-sowing date combinations in Ethiopia)

Genotype	Grain yield		SUP		SSC		IPCA1		ASV	
	Value	Rank	Value	Rank	Value	Rank	Value	Rank	Value	Rank
G1	2119	1	149*	1	522*	10	-19.3	14	39.6	14
G2	1922	2	204	2	657	15	10.3	7	21.2	7
G3	1749	6	395	7	566	12	14.5	10	29.6	8
G4	1695	7	368	6	664	16	15.5	13	31.0	10
G5	1819	5	333	5	791	18	19.5	15	40.0	16
G6	1516	8	398	8	780	17	11.0	8	22.9	12
G7	1848	3	224	3	559	11	4.9	4	10.6	3
G8	1186	15	1133	16	473	9	-37.9	18	77.3	18
G9	1317	13	840	12	395	6	-19.9	16	40.8	15
G10	1347	9	728	10	385	5	-2.9	3	7.0	4
G11(LR)	1847	4	270	4	211	1	1.1	1	2.4	1
G12	1034	16	1115	15	580	13	12.3	9	25.6	11
G13	1262	14	905	14	618	14	15.1	12	31.1	13
G14	1330	12	742	11	321	3	-15.0	11	30.6	9
G15	1461	10	489	9	361	4	5.9	5	12.3	2
G16(LR)	734	17	1507	17	458	8	6.5	6	13.8	6
G17(LR)	528	18	1969	18	274	2	2.6	2	6.9	5
G18	1337	11	882	13	437	7	-24.3	17	49.8	17

LR=landraces, SUP= superiority index, SSC= static stability coefficient, IPCA1= the first interaction principal component axis, ASV= AMMI stability value, * = numbers are divided by 1000

Table 2. 7 Summary of Mann-Whitney U (Wilcoxon rank-sum) test showing significant difference in static yield stability between landraces and improved cultivars

Stability parameter	Mean rank of landraces	Mean rank of cultivars	P-value
SSC	4	11	0.039*
IPCA1	3	11	0.017*
ASV	4	11	0.027*
SUP	13	9	0.25NS

SSC, static stability coefficient; IPCA1, the first interaction principal component axis; ASV, AMMI stability value; SUP, superiority index. * significant at $P < 0.05$, NS non-significant

2.5 Discussion

2.5.1 Relative effects of location, year and sowing dates on grain yield

The two locations for the field trials were selected on the basis of their differences in agro-ecological features. Ambo represents a temperate, intermediate highland region with intensified barley production. The area is mainly known for the production of cereals like barley and wheat. Jimma is located in the hot and humid zone of tropical rain forest of the southwestern part of Ethiopia. Since its elevation is in the mid-altitude range, it is characterized by denser tree coverage. The main crops of this region are maize and sorghum, although wheat and barley are also produced. As shown in Figure S2.1, the two locations differ in the pattern of rainfall distribution, and in the minimum and maximum temperature that likely contribute to the effect of the two locations on the grain yield performance of barley.

The staggered sowing dates were chosen to include the regular date of sowing according to the local sowing calendar, but included earlier and later dates to produce a larger environmental variation, in particular drought stress at different stages of plant development, in order to evaluate diverse local conditions on yield and G x E interactions.

We examined the overall grain yield performance of genotypes in relation to the growth conditions of the different environments. The low grain yield at Jimma ranged between 896 to 1,284 kg ha^{-1} and may result from the moisture stress experienced at the flowering stage of environments 12J3, 12J4 and 13J4 in combination with the extended rainfall during the maturity stage. Drought stress during flowering can strongly affect yield in barley (Vaezi et al. 2010). In contrast, the late sowing dates of 2013 at Ambo (13A3 and

13A4) did not result in drought stress during flowering and did not affect grain yield much, possibly because the higher amount of rain prior to the end of the rainy season was stored in the soil. Residual soil moisture contributes to the completion of developmental stages in barley and other crops (Asfaw, 2000). In general, location and sowing dates displayed highly significant effects on grain yield of barley in our set of genotypes (Table 2.4) and the staggered planting was seen as additional means to allow genotypes respond differently to the array of environments apart from location and year difference. The combination of year, location and staggered planting date efficiently creates a diversity of environments to test the environmental stability of barley genotypes. However, the effect of location was the strongest because it divided the genotypes in to two groups based on grain yield performance (Figure 2.2A).

2.5.2 Patterns of G x E interaction

The multi-environment testing of genotypes to assess G x E interactions and genotype yield stability plays an important role in either selecting widely adapted genotypes to be used across different environments, or in selecting genotypes specifically adapted to a particular sub-set of environments. In this regard, different trials assessed the differential response of barley across environments and mainly accounted for location and seasonal variation (Abdipur and Vaezi, 2014; Sarkar et al., 2014; Mehari et al., 2014). To fully exploit the differential responses of genotypes under a wider range of environments apart from location and year differences, testing genotypes at different sowing dates enables to include more environmental variables like moisture levels or atmospheric and soil temperature regimes which also appear in farmers' fields. As expected, our trial revealed a substantial genotype-by-sowing date (G x SD) interaction ($P < 0.001$; Table 2.4)

suggesting that genotypes differed in their ability to cope with early versus late planting dates. Understanding such patterns may allow specific variety recommendations and optimized selection of varieties by farmers, depending on the actual sowing date and given that an appropriate seed system is in place.

The dissection of G x E interactions in the current trial suggested that 12 out of the 16 environments grouped into two clusters or mega-environments. These clusters largely corresponded to the two locations, Jimma and Ambo, suggesting that genotypes that produce high grain yields in both highly distinct environments (locations) can be considered as adapted genotypes for these locations.

2.5.3 Specific advantages of landraces over improved cultivars

Among the genotypes investigated, 11 were pure line selections from local landraces, four resulted from crosses followed by successive selfing, and three were farmer landraces. The four stability parameters analyzed in the study were based on either the static stability concept i.e., genotypes with stable and high yield (SSC, IPCA1 and ASV) or the dynamic stability concept i.e., genotypes that respond with a higher yield if the environment improves (SUP). The G11 landrace (Jimma Local) was the most stable of all genotypes by all three static stability parameters. Another landrace (G17, Balame) was classified as the second most stable genotype by two of the three measures although the mean grain yield was not high. The landraces showed a higher static stability than improved cultivars (Table 2.7), which was also observed in previous studies in maize (Salazar et al., 2007), wheat (Jaradat, 2013) and field crops in general (Oliveira et al., 2013). The higher genetic diversity of landraces highly contributes to their increased stability (Ceccarelli, 1994).

Since barley is mainly a self-pollinated crop, barley landraces are mixtures of mostly homozygous genotypes (Brown, 1978; Rodriguez et al., 2012) and landraces with a better mean grain yield can readily be utilized or be used as a basis for further improvement provided that static stability is considered important by the farmers and breeders. Improved cultivars like G1, G2 and G7 performed better than farmers' landraces in terms of dynamic stability (SUP), but the differences were not statistically significant. Improved cultivars usually tend to respond better to optimal environmental conditions than landraces (Pswarayi et al., 2008), and hybrids of winter barley showed a higher dynamic yield stability than lines (Mühleisen et al. 2014). The wide range of SUP values in our trial for both landraces and improved cultivars suggest that both types of varieties can be improved significantly for dynamic stability. The current study included three landraces: the dominant farmers' variety in West Shoa region of Ethiopia (Balame) and two other landraces from the location where the field experiment was conducted (Ambo Local and Jimma Local). An inclusion of more landraces from other barley growing regions might be helpful to fully investigate the relative performance in terms of grain yield and stability of improved cultivars and barley landraces in Ethiopia. However, the present results suggest that the G11 landrace (Jimma Local) is the best candidate for risk-averse farmers who prefer static stability combined with high mean yield. In contrast, genotypes G1 (Dribie), G2 (Agegnehu) and G7 (Misccal 21) are improved cultivars with a high dynamic stability and are suitable varieties for farmers favouring dynamic response to better growing conditions and providing higher inputs.

2.5.4 Scope for exploiting specific adaptation to factors that are known before planting

The AMMI biplot grouped the testing environments into two groups characterized by the two locations (Figure 2.2A), which indicates that selection needs to be done separately for the two regions if the breeding objective is specific adapting cultivars for the locations. Although the grouping was based on location, the highly significant G x L and G x Y x L, G x Y x SD interaction effects (Table 2.4) suggest that the selection of new barley varieties requires field trials in different and multiple years, but also at different sowing dates to asses yield stability by accounting for variation in the beginning and end of the rainfall season. This notion is supported by a study in sweet sorghum, which reported a high G x E interaction with sowing date as the largest contributor to the interaction (Reddy et al. 2014). Some genotypes performed very well in specific environments, and their specific adaptation can be attributed to *a priori* known factors like location.

The differential performance of genotypes over test environments raises the question, which traits are mainly responsible for the differences. For example, genotype G1 was identified as best overall genotype for the Jimma location because of its high yield, whereas genotype G11 exhibited the best static stability. Both were the two most early flowering genotypes among the 18 tested (Figure 2.1 and Table S2.2). They reached the stage of 50 % flowering plants on overall average at 58 (G1) and 57 days (G11) after sowing, respectively, which was 12 and 13 days earlier than the average over all genotypes (70 days). This result and the negative correlation of grain yield and flowering time in 13 of the 16 environments indicates the importance of early flowering for yield performance and stability. Similarly, early flowering genotypes of wheat showed less yield

reduction after stress than late flowering genotypes (Talukder et al., 2014), and early maturing Ethiopian barley landraces performed better than late maturing ones in a year of high season-end drought (Sinebo et al., 2010). Therefore, breeders can consider days to 50% flowering as a target trait in breeding programs aimed at yield stability.

For a breeder to choose which stability concept to apply, the inclination of farmers to take a risk is relevant. In case of a high preference of farmers to avoid risk by preferring lower but stable yield over a high yield under optimal environmental conditions and inputs, static stability parameters should be applied to selection. A dynamic stability concept can be considered as selection criterion, if farmers are willing to accept a higher risk. The barley varieties and landraces used in our study showed a wide range of both static and dynamic stability measures, which indicates the presence of genetic variation to improve both types of stability. Yield stability can be achieved by two different mechanisms, namely individual buffering and population buffering (Ceccarelli et al., 1991). Individual buffering is influenced by traits like responsive tillering, photoperiod-sensitive flowering and resistance to biotic and abiotic stress factors as was shown in pearl millet (Haussmann et al., 2012). At the population level, intra-population variation in flowering time may buffer unpredictable and unfavorable growth conditions. Such a buffering was observed in oat, where grain yield differed significantly between a mix of genotypes and the individual pure lines in response to stress (Helland and Holland 2001). Individual buffering is frequently believed to be a property of heterozygous crops and difficult to exploit in self-pollinated diploid crops like barley (Ceccarelli et al., 1991). Since modern line cultivars are highly uniform, population buffering is not possible. Therefore, a possible strategy for barley breeding in a diverse and changing environment as in Ethiopia is to combine

different selected genotypes in a mixture, providing different trait combinations to achieve sustainable population buffering. Traditional landraces are mixture of genotypes which might explain the higher static stability observed for them in the present study.

2.5.6 Need to further develop the Ethiopian seed system

The current barley seed system of Ethiopia is mainly informal because of the highly diverse structure of agriculture (Abay et al., 2011). Farmers usually get seed for next season from their previous harvest, neighbors or local open markets. Commercial plant breeding or seed companies actively involved in the seed system are almost non-existent. Seeds are seldom provided by public research institutes or local agricultural extension services to barley growers though efforts have been made to create formal seed system. As location and sowing date factors are predictable ahead of planting, seeds of the appropriate cultivars must be made available to the growers on a very short-term basis, to enable exploitation of specific adaptations. This requires decentralized seed production of required cultivars, and a strengthening of the local, informal and semi-formal seed sector in Ethiopia, in order to make the seed available on time. As long as the seed sector is unable to provide on time the seed of specifically adapted cultivars, promotion of widely adapted cultivars identified by the approach used in this study are possibly the better short-term strategy to follow.

2.6 Conclusions

The analysis of 18 barley genotypes grown in 16 environments (location-season-sowing date combinations in Ethiopia) with the AMMI statistical model revealed that a staggered sowing date enabled to exploit G x E patterns beyond location and season. The major

proportion of the total variation in grain yield was explained by location followed by sowing date. The year of cultivation had a smaller effect than location and sowing date as shown by the variance components. Adaptation to a specific location was detected for the G15 (Abay) cultivar, while others showed a wider adaptation. The observed G x E patterns can be exploited by barley breeders and farmers by a tactical choice of varieties to be cultivated depending on the actual location and sowing date. Landraces showed, on average, higher static yield stability than improved cultivars with a comparative grain yield. Our study showed that by including staggered planting dates in combination with different years and locations, a diversity of environments can be created to test the environmental stability of barley genotypes if resources for field trials are limited as in developing countries like Ethiopia. For further breeding efforts, the number of environmentally diverse environments has the strongest effect on the analysis of G x E interaction and the number and type of location used to select for improved varieties likely have the strongest effect in producing future-proof barley cultivars for Ethiopian agriculture.

2.7 Acknowledgement

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2.9 Supplementary table and figure

Table S2. 1 Sixteen environments used for evaluation of barley genotypes

Code	Location	Sowing date	Code	Location	Sowing date
12A1	Ambo	June 9, 2012	13A1	Ambo	June 11, 2013
12A2	Ambo	June 26, 2012	13A2	Ambo	June 26, 2013
12A3	Ambo	July 13, 2012	13A3	Ambo	July 12, 2013
12A4	Ambo	July 28, 2012	13A4	Ambo	July 27, 2013
12J1	Jimma	June 13, 2012	13J1	Jimma	June 13, 2013
12J2	Jimma	June 28, 2012	13J2	Jimma	June 28, 2013
12J3	Jimma	July 14, 2012	13J3	Jimma	July 14, 2013
12J4	Jimma	July 30, 2012	13J4	Jimma	July 30, 2013

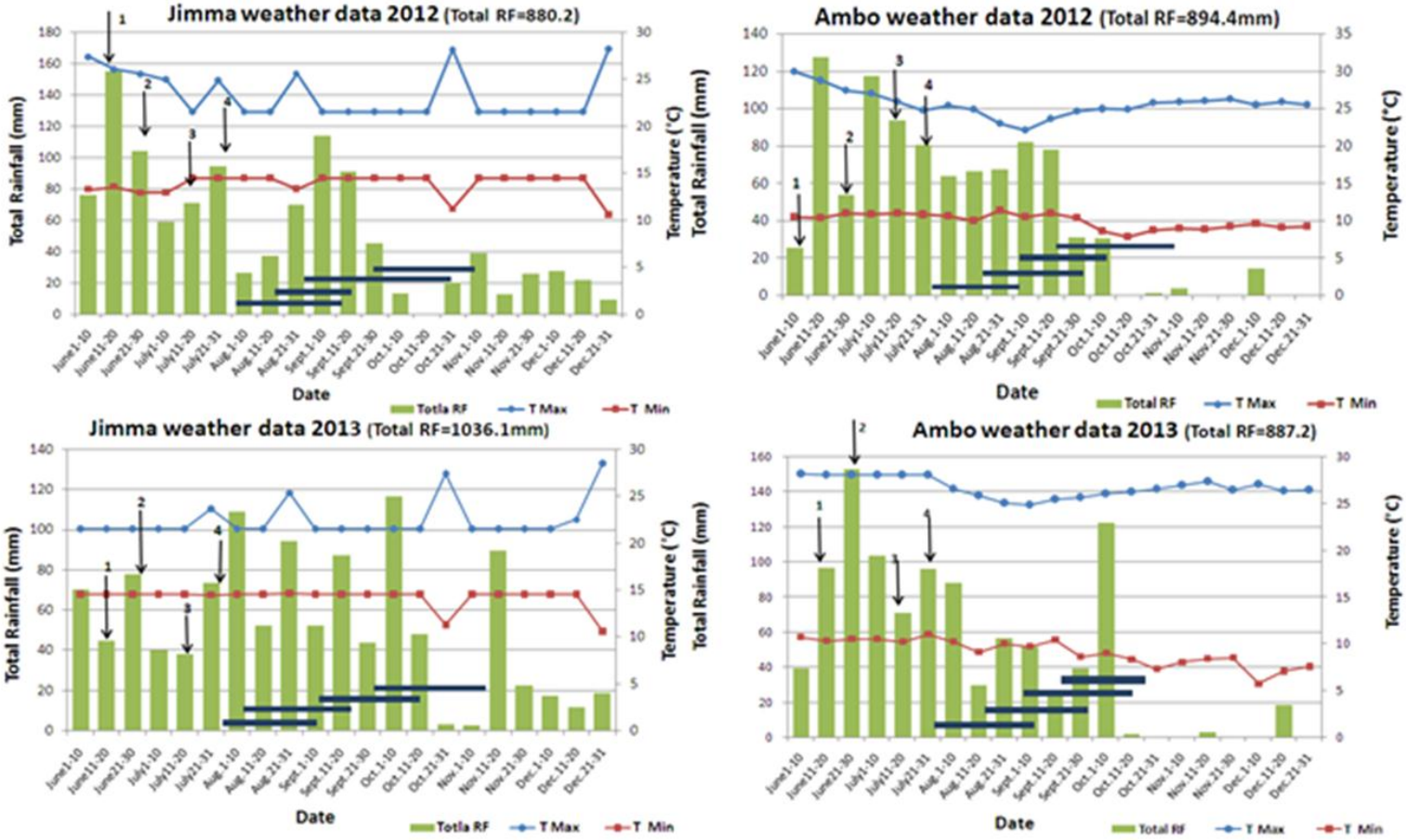

Figure S2. 1 Total rainfall, minimum and maximum temperature of the study areas in 2012 and 2013 crop season. The four sowing dates are indicated with arrows and the black horizontal line represents the time taken to 50% flowering from bottom to top in order of 1st, 2nd, 3rd and 4th sowing dates

Table S2. 2 Mean grain yield (kg ha^{-1}) of the18 genotypes across 16 environments

	Environments																
Genotypes	12A1	12A2	12A3	12A4	12J1	12J2	12J3	12J4	13A1	13A2	13A3	13A4	13J1	13J2	13J3	13J4	Mean
1†	1731	3665	1665	591	2639	2812	2160	1227	1725	2318	2107	2662	2389	2687	1925	1595	2119
2	1777	4099	1254	1299	1888	1809	1407	1128	2381	3183	2466	2288	1214	1745	1539	1266	1922
3	1930	3781	1723	1086	1193	1260	1830	1847	1763	2669	1810	2145	1368	1488	532	950	1749
4	1465	3637	1432	1394	1715	1379	1303	689	2148	3185	2073	2067	1056	1705	600	1065	1695
5	1801	3879	1804	2093	2183	1892	1209	681	2440	3400	846	2160	1243	1456	943	1070	1819
6	1224	3962	1394	1463	1760	1839	799	564	1531	3279	1207	2041	1248	1850	1023	936	1516
7	1360	3689	1485	1738	1703	1916	1445	1122	1279	3390	1829	2626	1888	1652	1248	1408	1848
8	426	1881	698	395	1610	1738	323	230	121	681	1863	1951	1581	1192	1444	1839	1186
9	1218	2544	1164	232	1446	1732	392	353	1298	1421	618	2130	1480	1309	1264	1615	1317
10	1386	2837	1296	1504	1053	1513	570	704	1515	1234	2239	1828	1585	1139	696	453	1347
11	1696	3113	1747	1836	1662	1737	1431	1596	2105	2182	1832	2232	1329	1832	1049	1696	1847
12	1082	2396	715	998	685	1080	593	265	1668	2244	2387	1394	616	280	222	255	1034
13	1756	2935	1699	1233	915	1012	734	293	1302	2280	2027	1781	633	541	367	244	1262
14	1016	2501	939	537	1267	1707	590	258	1361	1685	1984	1667	1371	1295	1116	1483	1330
15	1725	2690	1474	1434	1055	1362	765	1184	1902	2724	1946	2295	697	1485	1235	1440	1461
16	736	2056	841	613	352	649	248	-	651	1916	1956	1114	463	774	-	-	734
17	553	1340	440	452	220	258	-	-	941	1284	1562	912	280	165	-	-	528
18	1252	1980	1336	275	1394	1563	157	37	1125	1331	2225	2221	1613	1503	934	1540	1337
Mean	1341	2944	1284	1065	1374	1514	887	677	1514	2245	1832	1973	1225	1339	896	1048	1447
S.E±	130	134	104	234	105	108	116	93	255	269	262	186	168	182	186	159	
LSD (5%)	374	385	300	671	302	310	332	267	734	773	752	534	483	524	535	458	
Repeatability	0.91	0.89	0.89	0.92	0.78	0.92	0.82	0.63	0.75	0.77	0.71	0.82	0.81	0.75	0.46	0.64	0.78

† See Table 2.1 for genotype codes

Table S2. 3 Days to 50% flowering of the18 genotypes across 16 environments

Genotypes	Environments																Mean
	12A1	12A2	12A3	12A4	12J1	12J2	12J3	12J4	13A1	13A2	13A3	13A4	13J1	13J2	13J3	13J4	
1[†]	54	57	53	56	51	55	67	74	53	55	65	60	50	52	57	64	58
2	65	63	61	63	63	61	82	71	55	59	58	59	57	57	62	71	63
3	62	62	59	60	56	58	66	80	57	58	65	58	53	54	60	62	61
4	65	69	62	64	64	67	81	75	54	59	60	61	59	59	60	69	64
5	68	62	63	60	62	62	75	72	55	59	84	60	57	59	62	70	64
6	71	67	63	65	66	67	80	78	55	59	78	62	60	59	63	70	66
7	65	68	62	63	63	60	70	73	57	71	71	62	59	60	63	69	65
8	86	95	87	94	86	84	101	94	90	105	64	85	83	83	83	88	88
9	74	79	75	75	72	77	86	88	70	73	79	71	69	69	72	77	75
10	69	74	69	67	66	68	86	85	66	65	70	67	62	64	66	70	70
11	54	57	53	59	58	52	60	64	51	55	65	55	52	55	56	61	57
12	81	86	81	87	88	85	98	96	73	73	62	81	79	83	88	97	84
13	68	63	61	64	65	69	83	81	58	58	58	65	59	62	67	76	66
14	76	79	74	88	78	69	89	92	70	73	66	72	68	68	74	74	76
15	69	69	66	66	68	71	86	81	59	63	63	65	59	62	67	78	68
16	72	78	73	71	79	78	85	-	61	64	59	65	61	69	87	94	73
17	81	83	82	85	88	87	93	-	72	77	76	77	80	81	83	85	82
18	76	79	76	88	76	76	86	94	71	70	62	71	68	63	77	77	76
Mean	70	72	68	71	69	69	82	81	63	66	67	66	63	64	69	75	70
S.E±	1.3	1.2	1.4	2.4	2.1	2.2	3.6	2.8	1.5	3.0	3.7	0.7	0.9	1.1	1.6	2.7	
LSD (5%)	3.7	3.3	3.9	6.8	6.0	6.3	10.4	8.2	4.2	8.6	10.7	2.0	2.6	3.1	4.7	7.8	
Repeatability	0.90	0.87	0.73	0.79	0.93	0.97	0.95	0.89	0.97	0.96	0.93	0.81	0.94	0.83	0.52	0.98	0.87
Correlation (GY x DtF[††])	-0.67[*]	-0.62[*]	-0.81[**]	-0.44	-0.80[**]	-0.80[**]	-0.88[**]	-0.69[*]	-0.33	-0.77[**]	-0.49[*]	-0.46	-0.74[**]	-0.65[*]	0.85[**]	-0.54[*]	

See Table 2.1 for genotype codes, [††] Days to flowering
[*,**] significant at P < 0.05, P < 0.01probability level, respectively.

Chapter 3: Utilizing relative robustness of agronomic traits in diverse environments as selection criterion for grain yield stability in Ethiopian barley

3.1 Abstract

Barley (*Hordeum vulgare* L.) is an important food crop of smallholder farmers in the Ethiopian highlands. Yield stability across years and variable planting dates is a major variety adoption criterion for these farmers. The present experiment aimed to study relationships among barley agro-morphological traits in 16 environments (location-year-sowing-date combinations) and to identify traits contributing to greater grain yield stability. Eighteen Ethiopian barley genotypes were grown in two locations and four staggered sowing dates over two years (2012-2013) and 14 traits were evaluated. Effects of genotype (G), location (L), sowing date (SD) and year (Y) with all interactions were highly significant ($P < 0.01$) for six out of 15 traits measured. Relatively high genotypic coefficients of variation were observed for grain yield, kernels per spike and days to first flowering (25.9%, 18.6% and 13.0%, respectively) while the lowest genotypic coefficients of variation were seen in harvest index, total number of tillers and thousand-kernel weight. A compensatory relationship was observed between kernels per spike and thousand-kernel weight in landraces. Total number of tillers, number of fertile tillers and kernels per spike showed stability across different site-season-sowing-date combinations. Kernels per spike and number of fertile tillers can be proposed as robust traits in barley breeding for a wider adaptation as they had significant and consistent positive total effects on grain yield.

Key words: barley, Ethiopia, genotypic correlation coefficient, yield components, yield stability

3.2 Introduction

A central goal of many plant breeding programs is to develop cultivars that perform well in diverse environmental conditions within the target production environment (TPE). The selection of environmentally stable genotypes among progeny of crosses is a major challenge because genotypes respond differentially to environments and show genotype by environment interaction (GxE). This interaction usually reduces the association between phenotypic and genetic variation if the best genotypes in one environment to perform poorly in another (Baye et al., 2011). Determining the type and extent of GxE of genotypes is therefore an important task of breeding programs aimed at producing high yielding and environmentally stable cultivars (Keneni et al., 2016). The breeding of new varieties that fulfill both criteria is particularly challenging in regions that are characterized by a high diversity of environments within short geographic distances although the shuttle breeding approach by Norman Borlaug demonstrated that selection of varieties in different environments is a suitable approach for developing highly productive varieties with stable yields that can be cultivated over large TPEs (Borlaug, 2007).

The extent of genetic gain in a breeding program strongly depends on the heritability and the extent of GxE (Xu et al., 2017). The selection of traits with low heritabilities such as grain yields or of traits with high levels of GxE requires large field trials at multiple locations to reduce the error and increase the precision of variance estimate (Mühleisen et al., 2014). When breeding for smallholder farmers in highly variable environments, breeders need to make a decision regarding the appropriate adaptation strategy, i.e., whether to select for wide or for specific adaptation, and whether yield stability needs to be specifically selected for. The optimal strategy depends on the extent of GxE interaction with predictable and unpredictable environmental factors (Ceccarelli, 1989). If GxE

interactions with predictable factors such as location and sowing date are of the cross-over type (different genotypes best at different locations or sowing dates) and repeatable across years, then selection should be targeted for specific adaptation to these locations or sowing dates. However, if GxE interactions with predictable factors are not repeatable across years because of strong genotype-by-year interactions, selection for wide adaptation and yield stability becomes important. Since grain yield frequently shows high levels GxE interaction, especially in highly variable target environments, it is difficult to find cultivars with both a higher grain yield and wide adaptation (Graybosch and Peterson, 2012). Therefore, breeding programs need to consider whether to breed for higher grain yield and specific adaptation or for moderate yield with wider adaptation. In developing countries like Ethiopia where budget constraints prevail and very diverse climatic conditions occur within short distances in combination with high inter-annual rainfall variability, breeding for specific adaptation is impractical as this would require multiple breeding programs. Instead, breeding for wider adaptation and yield stability in combination with a high grain yield seems more advisable.

In cereal crops like barley, grain yield is a function of yield components like plant density, number of spikes per plant, number of kernels per spike and 1000-kernel weight that each contribute directly and indirectly to final grain yield (Shi et al., 2009; Peltonen-Sainio et al., 2007; Fischer and Edmeades, 2010). Most yield components are quantitative traits that are affected by the environment. Since grain yield is a more complex trait than its components, environmental effects and genotype-by-environment (GxE) interactions for grain yield are stronger than for yield components (Baenziger et al., 2011). Therefore, indirect selection of yield components may be more efficient than selection on grain yield per se to obtain higher yielding cultivars (Puri et al., 1982). Since multiple yield

components are available for selection with different GxE interactions and heritability values, a key question is which combination of yield components improves genetic gain compared to direct selection of yield. In addition, yield components compensate each other in trait correlation dynamics if the relationship between traits is not static between environments or developmental stages. For instance, at low plant population density per unit area, more tillers are produced per plant (Herrera et al., 1994; de Rouw and Winkel, 1998). Lower number of spikes (fertile tillers) can also be compensated by larger numbers of kernels per spike (Lafond, 1994). A partial loss of flowers resulting from pest or mechanical damage has the effect that remaining flowers tend to develop larger grains with a higher thousand-kernel weight. These compensation effects reflect phenotypic plasticity, which can contribute to yield stability (Herrera et al., 1994; Berenguer and Faci, 2001).

If a given yield component is considered as suitable trait for selection, it needs to have a high heritability and a strong genetic correlation with overall grain yield. In barley, several studies investigated trait correlations. For example the number of fertile tillers and number of kernels per spike had a strong effect on yield in 86 barley genotypes that were cultivated under rainfed condition for two consecutive years in Jordan (Al-Tabbal and Al-Fraihat, 2011). Another study at two locations and two consecutive years in Ethiopia with 100 Ethiopian landraces and breeding material from the International Center for Agricultural Research in Dry Areas (ICARDA) showed that number of spikes per square meter, kernel number per spike and thousand-kernel weight were principal yield components with positive and significant genotypic correlation coefficients to grain yield (Setotaw et al., 2014). However, these studies were limited to just a few locations or test environments. Given the high inter-annual rainfall variability in Ethiopian barley growing

regions, which is further increased by the ongoing climate change (Cheung et al., 2008), it is necessary to consider also the effect of sowing date on the relationship between agronomic traits, yield components and yield to identify robust trait relationships that are suitable for selection. Simple correlations between traits cannot be considered as sole source of information for indirect selection because they are strongly dependent on environmental conditions. Instead, the genetic correlation has to be extracted from the overall phenotypic correlation. Genetic correlation is the proportion of variance that two traits share because of genetic causes and it provides information to which extent measurements of one trait contain information about other traits (Thompson and Meyer, 1986).

Correlation coefficients have long been used by breeders to determine the relationship between traits. However, they cannot explain the direct and indirect effects of each trait on the ultimate target trait (yield) (Bhatt, 1973). This problem is addressed by path analysis, which is used to estimate the direct and indirect effects of the independent variables on the dependent variable (yield) to develop selection criteria in different crops (Li, 1976). In multivariate analyses of causal effects on complex parameters like grain yield different approaches like structural equation models (SEM) (Tarka, 2018) and path analysis (Setotaw et al., 2014)can be used to identify causal relationships between factors and dependent variables. Path analysis is frequently preferred because it utilizes all observed factors, unlike SEM that considers latent (hidden) variables if the number of recorded casual variables is insufficient. In addition, path analysis differentiates between direct and indirect correlations, which facilitate the interpretation of results (Jeon, 2015).

In this study, we use path analysis to investigate the relationship between yield and its

components in a genetically diverse set of Ethiopian barley varieties and landraces tested at two locations, two seasons and four staggered sowing dates, which together represent 16 environments. In a previous analysis of grain yield (Chapter 2) these environments cluster into two groups that mainly differentiate between the two locations, and we identified genotypes with low GxE interactions and stable yields. In the present study, we include 14 additional traits like spike length, fertile tiller and thousand-kernel weight to investigate the effect of yield components on total yield. By adding planting dates as factor in addition to the location and season effects, we accommodated different on-farm situations like early, medium and late planting to investigate trait relationships in different situations. Our main objective was to identify traits with reduced sensitivity to environmental effects that contribute to yield stability. In addition, we compared yield stability between landraces and cultivars and investigated, which traits contributed to different levels of grain yield in both groups of genotypes.

3.3 Materials and methods

3.3.1 Genetic material

Eighteen barley genotypes consisting of 15 released cultivars, one widely used landrace and two local landraces were compared in the experiment (Table 3.1). With the exception of the two local landraces, seeds for all genotypes were provided by the Holetta Agricultural Research Center (HARC) of Ethiopia. The two local landraces were obtained from barley growers from the Jimma and Ambo areas of Ethiopia. The genotypes were selected to represent the genetic diversity of currently used cultivars for traits like stress tolerance and grain yield. The selection history of the released cultivars is described in Chapter 2.

Table 3. 1 Barley genotypes used in the study

Name	Status	Peculiar characteristics
Dribie	Released cultivar	Tolerant to drought
Agegnehu	Released cultivar	Tolerant to major barley leaf diseases (*Pyrenophorateres* and *Rhynchosporiumsecalis*) and adapted to low moisture areas
Biftu	Released cultivar	Early vigor and tolerant to shoot fly (*Deliaflavibasis* Stein) and suitable for both main and short seasons
Estayish	Released cultivar	High quality grain (white seeded), high market value
Meserach	Released cultivar	Early maturing and tolerant to major leaf diseases (*Pyrenophorateres* and *Rhynchosporiumsecalis*)
Shedeho	Released cultivar	High quality grain (white seeded), high market value
Misccal 21	Released cultivar	High yielding with good malting quality; resistance to lodging with multiple disease resistance,
HB42	Released cultivar	Resistant to scald (*Rhynchosporiumsecalis*) and good biomass yield
EH1493	Released cultivar	High yielding, late maturing
HB1307	Released cultivar	High yielding, lodging resistant, resistant to *(Pyrenophorateres* and *Rhynchosporiumsecalis)* with good biomass yield and white seeded
Dimtu	Released cultivar	Good yield under low input conditions with good biomass yield
Basso	Released cultivar	Suitable for main and short seasons
Cross 41/98	Released cultivar	High yielding, late maturing
Abay	Released cultivar	High quality grain (white seeds) with long spike and medium to early maturity
Shege	Released cultivar	Good yield under low input conditions and tolerant to major leaf diseases (*Pyrenophorateres* and *Rhynchosporiumsecalis*)
Balame	Dominant landrace	Tolerant to low soil fertility and drought, good flour quality
Ambo Local	Local landrace	Suitable for main season with big grain size
Jimma Local	Local landrace	Early maturing

3.3.2 Experimental field sites

Field trials were conducted at two locations in Ethiopia, namely Ambo and Jimma. The Ambo site is located in Western Ethiopia with geographic coordinates $8^0$57'N and $37^0$45'E at an altitude of 2005 m.a.s.l. It represents a temperate (intermediate highland) climate in the agro-ecological zonal classification. The Jimma site is located in the southwest part of the country with latitude $7^0$42'N and longitude $36^0$48'E. Its elevation is 1790 m.a.s.l. and represents a hot and humid agro-ecological zone. The field trial site at Jimma received a relatively higher rainfall during the growing months in the two seasons (2012/13 and 2013/14). It also showed a relative higher minimum temperature compared to Ambo in the two growing seasons. Rainfall and temperature readings of the experimental sites were obtained from portable weather stations installed at each experimental site (Table 3.2)

Table 3. 2 Rainfall and temperature data of the field trial sites in Ethiopia.

Growing season (June-December)	Ambo			Jimma		
	Total rainfall (mm)	Mean minimum temp. ^{o}C	Mean maximum temp. ^{o}C	Total rainfall (mm)	Mean minimum temp. ^{o}C	Mean maximum temp. ^{o}C
2012/13	134.14	9.93	25.64	158.71	12.93	26.53
2013/14	141.43	9.11	25.69	159.29	14.10	26.39

We conducted the field trials in two consecutive growing seasons (2012/13 and 2013/14) and used four sowing dates per year in approximately 15-day intervals between mid-June and end of July in each year (Table S3.1). The first sowing date was intended to represent early moisture stress, which happens when farmers sow to anticipate the beginning of the rainfall season. The second sowing date represents the normal sowing date for which sufficient rainfall throughout the growing season is expected. The third sowing date was chosen to test for the effect of drought stress just before physiological maturity, and the fourth date to evaluate the effects of terminal drought stress during grain filling.

3.3.3 Experimental design

A randomized complete block design (RCBD) was used for each location-year-sowing date combination. One experiment (environment) had three blocks in which three replications of the genotypes were randomly assigned to each block. Plots were 2.4 m wide and 2.5 m long with a total area of 6 m^2 to accommodate 12 rows with 0.2 m distance according to the recommendation of the HARC. In each plot, 51 grams of barley seed were drilled manually as recommended by HARC. Fertilizer was applied at 100 kg diammonium phosphate (DAP) and 50 kg urea per hectare in two batches, i.e. 15 g of urea and 30 g of DAP were added to each plot at sowing time and the same amounts at the tillering stage. This amount of fertilizer is recommended by HARC. The trial plots were kept weed free by hand weeding.

3.3.4 Data collection

Fourteen agronomic traits including grain yield were recorded (Table 3.3).

Table 3. 3 Description and units of agronomic and morphological traits measured or calculated.

Description	Description/Calculation	Unit
Height at 45 days	Plant height was measured from soil surface to the tip of the longest young leaf 45 days after sowing	cm
Dry weight at 45 days	Ten plants were randomly cut at collar 45 days after sowing and oven-dried till moisture become 0%.	g
Days to first flowering	The date from sowing to the spike of first plant is completely emerged	days
Days to 50% flowering	The date from sowing to 50% of the spikes completely emerged from the leaf sheaths in a plot	days
Days to maturity	The date when majority of the plants in each plot have reached maturity. Data was collected when the seeds were hard (difficult to divide by thumbnail)	cm
Height at maturity	Measured in cm from the soil surface up to the tip of the spike (excluding the awns) at maturity from ten randomly taken plants	cm
Spike length	Measured from the base of the spike to the tip of the apical spikelet, excluding awns at maturity from ten randomly taken plants	cm
Total tiller	Number of tillers (productive and non-productive) were counted per plant of ten randomly taken plants at maturity	number
Fertile tiller	Number of tillers producing spikes were counted from ten randomly taken plants at maturity	number
Kernels per spike	Number of kernels (seeds) per spike was counted from 10 spikes collected randomly at maturity	number
Thousand-kernel weight	Weight of 1000 random kernels was measured after harvest and adjusted to 12.5% moisture content	g
Biological yield	Dry weight of spikes plus dry weight of stover	kg/ha
Harvest index	Dry weight of grain divided by dry weight of the total above-ground biomass	
Grain yield	Weight of clean kernels adjusted to 12.5% moisture content	kg/ha

In ANOVA and variance component analyses, all fourteen traits were considered. However, biological yield was excluded in correlation and path analyses in order to avoid autocorrelations.

3.3.5 Statistical analysis

We used two types of models for the analysis of the data. In the first model, all variables were fixed with the exception of the block within the environment, which was considered as random effect. This model was used to evaluate the contribution of effects to the total variance and their significance. The second model contained only random effects and was used to estimate variance components. We used these two types of models because genotype and environment can be modeled as fixed effects (Gauch, 2006) but also as random effects, since both factors can be viewed a random samples of a large population of genotypes or possible target environments (Atlin et al., 2000).

The linear fixed-effect models were fitted with Restricted Maximum Likelihood (REML) with the *lme* function of *nlme* R package. To account for variance heterogeneity, variance estimates were weighted for each location, year and planting date to accommodate with the *varIdent* and *varComb* functions. The following model was used for the analysis:

$$X_{ijklm} = \mu + G_i + L_j + Y_k + S_l + (GL)_{ij} + (GY)_{ik} + (GS)_{il} + (LY)_{jk} + (LS)_{jl} + (YS)_{kl} + (GLY)_{ijk} + (GLS)_{ijl} + (GYS)_{ikl} + (LYS)_{jkl} + (GYLS)_{ijkl} + \varepsilon_{ijklm}$$

where X_{ijklm} = the phenotypic value of the i^{th} genotype, j^{th} location, k^{th} year, l^{th} sowing date and m^{th} replication; μ = grand mean; G_i= i^{th}genotype;L_j= j^{th} location; Y_j= k^{th} year; S_l= l^{th} sowing date; $(GL)_{ij}$... = interactions between genotype, location, year and sowing date etc.; and ε_{ijklm}= error of X_{ijklm}. The only factor considered random was block in location and planting date. The residuals ε_{ijklm} were modeled with a different variance for each location, year and planting date. Significance of factors were tested with a Wald-*F* Test on the full model.

Variance components of genotype, location, year and sowing date for each trait were

computed with GenStat using a REML model. The genotypic (V_g), phenotypic (V_p) and error (V_e) variances were estimated as follows:

$$V_g = [\mathrm{MSG} - \mathrm{MSE}/r]$$

$$V_p = [\mathrm{MSG}/r]$$

$$V_e = [\mathrm{MSE}/r]$$

Where MSG = mean squares of genotypes, MSE = mean squares of error and r = number of replications.

Broad-sense heritability was estimated as

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{GxE}^2/k + \sigma_E^2/rk},$$

where $\sigma^2_{G,}$ σ^2_{GXE} and $\sigma^2_{\varepsilon}$are genotypic, genotype-by-environment and residual variance components respectively, k the number of environments (location-season-sowing date combinations) and r the number of replicates. Repeatability was also estimated for all traits in each environment according to Wolak et al. (2012)

Correlation coefficients between all independent traits and grain yield were calculated with the *chart correlation* function in the R package *Performance Analytics* version 1.4.3541 (Peterson et al., 2014). We then analyzed possible causal relationships between two or more phenotypic variables with path analysis to examine the relative strength of direct and indirect relationships among variables (Dewey and Lu, 1959; Shipley, 2000). We conducted the path analysis separately for Ambo and Jimma locations and in combination to examine the direct and indirect effects of all other traits on grain yield using the GENRES software (Pascal International, 1994). A standardized partial

regression coefficient known as path coefficient (Dewey and Lu, 1959) was estimated to determine the direct and indirect effects of yield components on grain yield as:

$r_{ij} = P_{ij} + \sum_{rik} P_{kj}$

where r_{ij} is the mutual association between the independent trait (*i*) and dependent trait (*j*) as measured by the correlation coefficient, P_{ij}, is the component of direct effects of the independent trait (*i*) on the dependent variable (*j*), $\sum_{rik} P_{kj}$ is the summation of indirect effects of a given independent traits via all other independent traits. In the analysis, grain yield was treated as the dependent variable and all remaining traits as grain yield determining variables that affect grain yield. Means and ranges of yield and selected yield-related traits were computed over location and season for landraces and cultivars with *P*-values to test for trait differences between the two groups.

3.4 Results

3.4.1 Environmental means, repeatability and variance components

Mean values for grain yield of the 18 genotypes in the 16 environments ranged from 677 to 2,944 kg ha^{-1}, with an overall mean of 1,447 kg ha^{-1}. Estimated repeatability for grain yield ranged from 0.46 to 0.92 across environments with mean value of 0.78 (Table S3.2). Linear mixed effects model for the 15 phenotypic traits showed that effects of genotype (*G*), location (*L*), sowing date (*SD*) and year (*Y*) contributed much more to the explained variation of each trait than the interaction terms (Figure 3.1A), but the relative contribution of these four factors to trait variation differs among traits. For example, location had the strongest effect on days to maturity, thousand seed weight and grain yield, whereas genotype had the strongest effect on days to first flowering. Although F values for some trait-factor or trait-interaction term can be small, the majority of them were highly significant indicating significant effects of all four main factors and their interaction on trait variation ($P < 0.01$ and $P < 0.001$; Figure 4.1B). Traits differ with respect to the number of significant factors or interaction terms. For example, the two early traits height and dry weight at 45 days show the lowest proportion of significant terms, as well as seeds per spike. Among interaction terms, location x year, location x sowing date and year x sowing date showed the largest effect on explained variation which indicates the environmental interactions have a larger effect on trait variation and G x E interactions (Table 3.4).

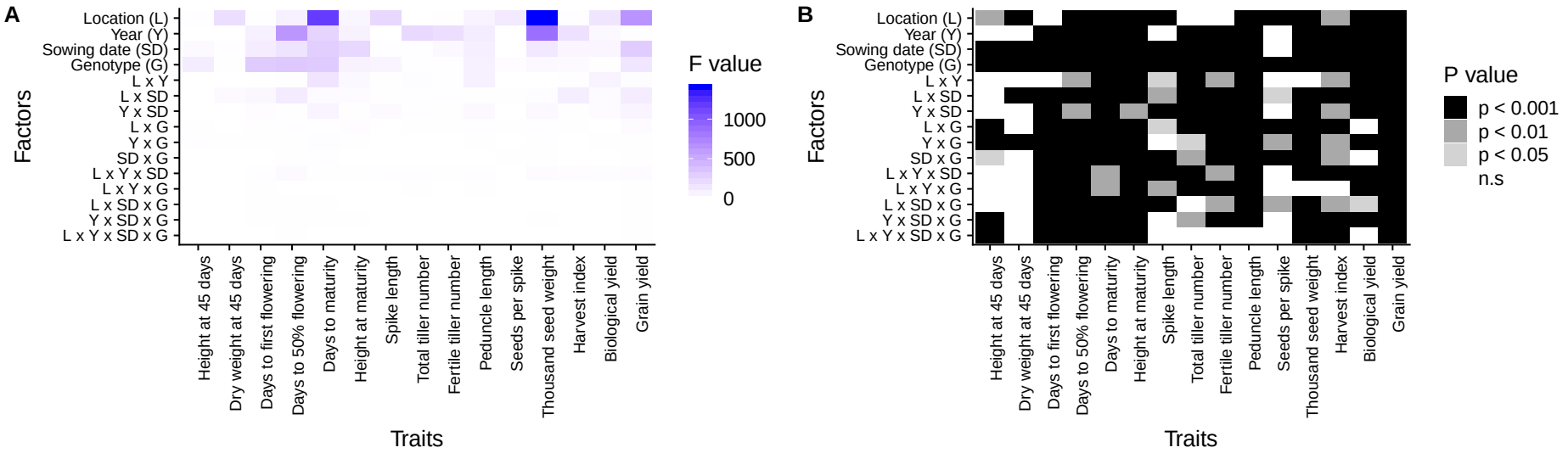

Figure 3. 1 Graphical representation of linear mixed model analysis of the factors location, year, sowing date, genotype and their interaction terms for15 phenotypic traits evaluated in 16 environments. A) F-Values of the linear mixed models. B) P values for each factor and interaction terms. P values are not corrected for multiple testing.

Table 3. 4 Estimated mean, variance components and heritability (H^2) for agronomic traits of 18 barley genotypes evaluated over two locations, two years and four sowing dates representing 16 environments in Ethiopia.

Trait	Mean value	Variance component								H^2
		G	G x Y	G x L	G x SD	G x L x Y	G x SD x Y	G x L x SD	G x L x SD x Y	
Height at 45 days	51.68	42.2***	7.31***	2.22***	0.98*	0.43	2.66***	0.72	2.59	0.95
Dry weight at 45 days	1.17	0.01***	0.013***	0.018	0.038	0.001	0.001	0.077***	0.004	0.51
Days to first flowering	59.69	60.0***	0.16***	1.19***	4.88***	1.57***	1.33***	8.06***	8.99***	0.99
Days to 50% flowering	69.63	76.6***	0.1***	1.8***	8.4***	1.3***	1.2***	12.6***	25.9***	0.98
Days to maturity	104	64.9***	8.28***	8.49***	10.35***	23.48***	24.42***	1.07***	15.16***	0.96
Height at maturity	79.51	39.6***	5.14***	6.44***	13.07***	13.46***	13.00***	12.31***	21.91***	0.95
Spike length	6.95	0.44***	0.024***	0.007***	0.062**	0.049***	0.057***	0.020***	0.179***	0.61
Total tillers	3.29	0.09***	0.085*	0.053***	0.073**	0.140***	0.126***	0.067***	0.098***	0.60
Fertile tillers	2.75	0.01***	0.030*	0.011***	0.047***	0.040***	0.075***	0.046***	0.087***	0.82
Kernels per spike	26.02	3.61***	2.33**	17.80***	38.74**	0.11	0.02	1.76	1.03	0.81
Thousand-kernel weight	31.99	3.12***	0.38***	3.78***	0.13***	1.13	1.00***	0.90***	6.55***	0.88
Harvest index	0.34	0.01***	0.0008**	0.0003***	0.0004***	0.0012***	0.0009***	0.0001***	0.0009***	0.34
Biological yield	4441	309,478***	349,315**	197,828***	786,555**	109,413***	587,086***	337,533***	1,561,588***	0.92
Grain yield	1447	152,320***	16,611**	46,886**	87,994***	5,285***	93,197***	84,449***	132,557***	0.78

G = genotype, *L*= location, *SD* = sowing date, *Y*= year. *, ** and ***Significant at P<0.05, P<0.01 and P<0.001 level, respectively

Variance components were estimated with an ANOVA model (Table 3.4). The variance component for interaction terms genotype by location (*G* x *L)* and genotype by sowing date (*G* x *SD)* were higher than the genetic variance component for the trait kernel per spike, but the overall the values of interaction terms, i.e. *G* x *L* x *SD* x *Y* variance was low, which suggest a relative stability of the trait. Estimates of heritability ranged from 0.34 for harvest index to 0.99 for days to first flowering. For grain yield, the heritability estimate was 0.78 (Table 3.4 and S3.2). Overall, heritability estimates were high which may be explained by the large number of 16 environments with three replications each.

3.4.2 Relationships between traits

To investigate the relationships between traits, we calculated the correlation coefficient between each pair wise combination of traits. When the overall effects of the four factors genotype, location, sowing date and year (*G*, *L*, *SD* and *Y*) are taken into account, phenological traits (days to first flowering, days to 50% flowering and days to maturity) showed the strongest negative correlation with yield ($P < 0.001$), indicating an advantage of early flowering and maturity in the target environments. A positive correlation exists between grain yield and kernels per spike ($P < 0.001$), fertile tiller ($P < 0.001$) and height at 45 days ($P < 0.01$) (Figure 3.2). A strong negative correlation between phenological traits and grain yield was also seen when the two locations were analysed separately (Figures S3.1 and S3.2). Among yield component traits, kernels per spike and thousand-grain weight were negatively correlated based on total mean values (-0.37, $P < 0.05$) and in Ambo (-0.39, $P < 0.001$) (Figures3.2and S3.1). Also, days to 50% flowering and fertile tiller were negatively correlated in the complete set (-0.56, $P < 0.001$) as well as in Ambo (-0.53, $P < 0.001$) and Jimma (-0.38, $P < 0.01$) indicating that later flowering was associated with lower numbers of fertile tillers. Correspondingly, late maturity was

associated with lower numbers of fertile tillers (Figure 3.2, S3.1 and S3.2).

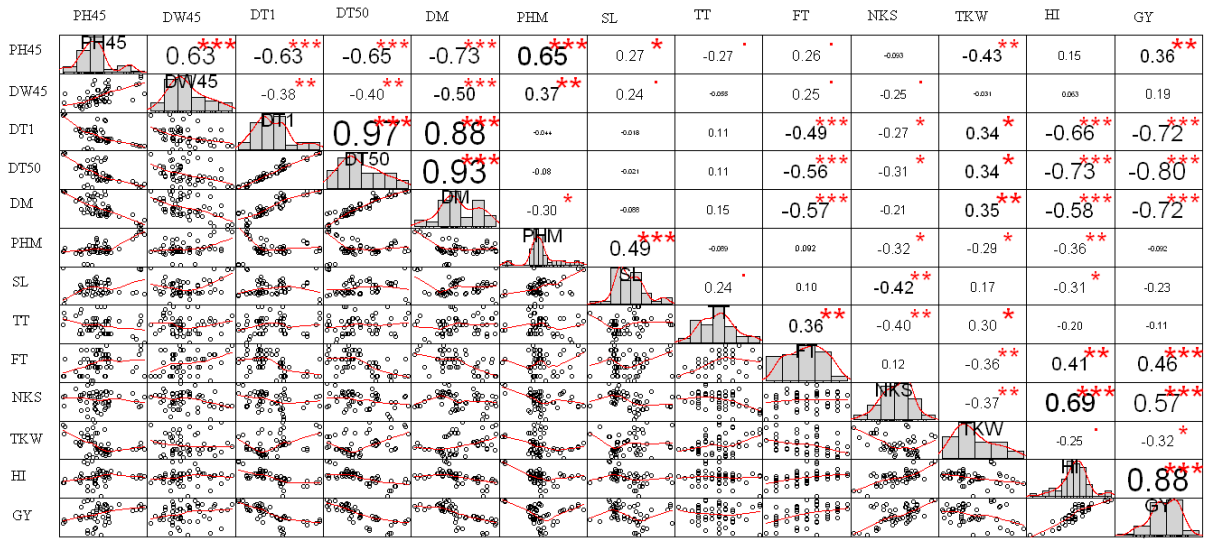

Figure 3. 2 Correlation among the characters derived from combined analysis over two locations, two years and four sowing dates in 18 barley genotypes. ·, *, **, ***, significant at P < 0.1, P < 0.05, P < 0.01 and P < 0.001, respectively. PH45 = Height at 45 days after sowing, DW45 = Dry weight at 45 days after sowing, DT1 = Days to first flowering, DT50 = Days to 50% flowering, DM = Days to maturity, PHM = Height at maturity, TT = Total number of tillers, FT = Number of fertile tillers, SL = Spike length, NKS = Number of kernels per spike, TKW = Thousand kernel weight, HI = harvest index and GY = Grain yield

3.4.3 Differences between cultivars and landraces

Our sample of 18 genotypes contains 15 cultivars and 3 landraces. Although the number of cultivars was much larger than of landraces in our set, we tested for differences in trait values between both groups to investigate whether a separation into cultivars and landraces differ in their response to environmental variation. For this analysis, we compared mean trait values over replicates and sowing dates in four environments (two locations x two years) in a t-test (Table S3.3). The traits were considered because they are grain yield components. Grain yield was not considered in this analysis because our aim was to see which grain yield component contributed to the grain yield stability in

landraces. Of four yield-related traits, three were significantly different between both groups. Landraces had higher values for spike length and thousand kernel weights, but lower numbers of kernels per spike. (Table S3.3).

3.4.4 Genetic correlations between grain yield and other traits

The genotypic correlation coefficient (r_g) calculated over all environments and separately for the two locations showed that several phenological traits (days to first flowering, days to 50% flowering and days to maturity) exhibited a strong negative genotypic correlation with grain yield if the average over all planting times is used (Table 3.5). In contrast, grain yield was consistently positively correlated with number of fertile tillers and kernels per spike. Other traits like height at 45 days and thousand-kernel weight were not correlated with yield in all three analyses (Table 3.5).

Days to first flowering, days to 50% flowering and days to maturity had a negative direct effect and positive indirect effect on grain yield at Ambo, Jimma and a combined analysis. We observed a significant ($P < 0.01$) total effect of the same traits on grain yield if both locations are analysed separately or together. In addition, kernels per spike consistently showed a positive effect on grain yield at Ambo ($P < 0.05$), Jimma ($P < 0.01$) and the combined analysis ($P < 0.01$). Thousand kernel weight showed a significant ($P < 0.05$) negative total effect on grain yield only at Ambo. Fertile tillers also had positive total effect on grain yield at Ambo ($P< 0.01$), Jimma ($P < 0.05$) and in the combined analysis ($P < 0.05$) (Table 3.5). Moreover, kernels per spike showed a relative consistency to have significant ($P < 0.05$, $P < 0.01$) total effect on grain yield over four sowing dates at both locations in the two growing season (Figure3.3; Table 3.5).

Table 3. 5 Direct and indirect effect of agronomic characters on grain yield of 18 barley genotypes and respective genotypic correlation coefficient (total effect) at two locations (combined across four sowing dates and two (2012 and 2013) growing seasons and combined across the two locations, four sowing dates and two years) in Ethiopia.

Trait	Ambo			Jimma			Combined (location, sowing date and year)		
	Direct effect	Indirect effect†	Total effect (r_g†)	Direct effect	Indirect effect	Total effect (r_g)	Direct effect	Indirect effect	Total effect (r_g)
Height at 45 days	-1.086	1.699	0.613**	-0.885	1.050	0.165	0.563	-0.188	0.375
Dry weight at 45 days	0.091	0.365	0.456	0.521	-0.480	0.041	-0.083	0.327	0.244
Days to first flowering	-1.189	0.385	-0.804**	-1.700	1.089	-0.611**	-1.305	0.573	-0.732**
Days to 50% flowering	-1.895	1.033	-0.862**	0.011	-0.711	-0.700**	2.114	-2.928	-0.814**
Days to maturity	-1.563	0.772	-0.791**	0.272	-0.796	-0.524*	0.005	-0.742	-0.737**
Height at maturity	-0.666	0.876	0.210	1.099	-1.182	-0.083	0.340	-0.454	-0.114
Spike length	-0.222	0.244	0.022	-0.097	-0.184	-0.281	0.060	-0.340	-0.280
Total tillers	-0.557	-0.030	-0.587*	-0.403	0.536	0.133	-0.336	0.168	-0.168
Fertile tillers	0.329	0.333	0.662**	1.026	-0.479	0.547*	0.764	-0.229	0.535*
Kernels per spike	0.602	-0.025	0.577*	0.466	0.200	0.666**	0.259	0.376	0.635**
Thousand-kernel weight	-0.431	-0.131	-0.562*	0.069	0.003	0.072	0.339	0.700	-0.361
Harvest index	-1.404	2.151	0.747**	-0.578	1.534	0.956**	1.248	-0.319	0.929**
Residual		0.17			0.06			0.10	

†Genotypic correlation coefficient. Figures in bold show significant (*) and highly significant (**) with t=0.468 (P<0.05) and t=0.590 (P<0.01), respectively, for df = n-2, where n is the number of genotypes.

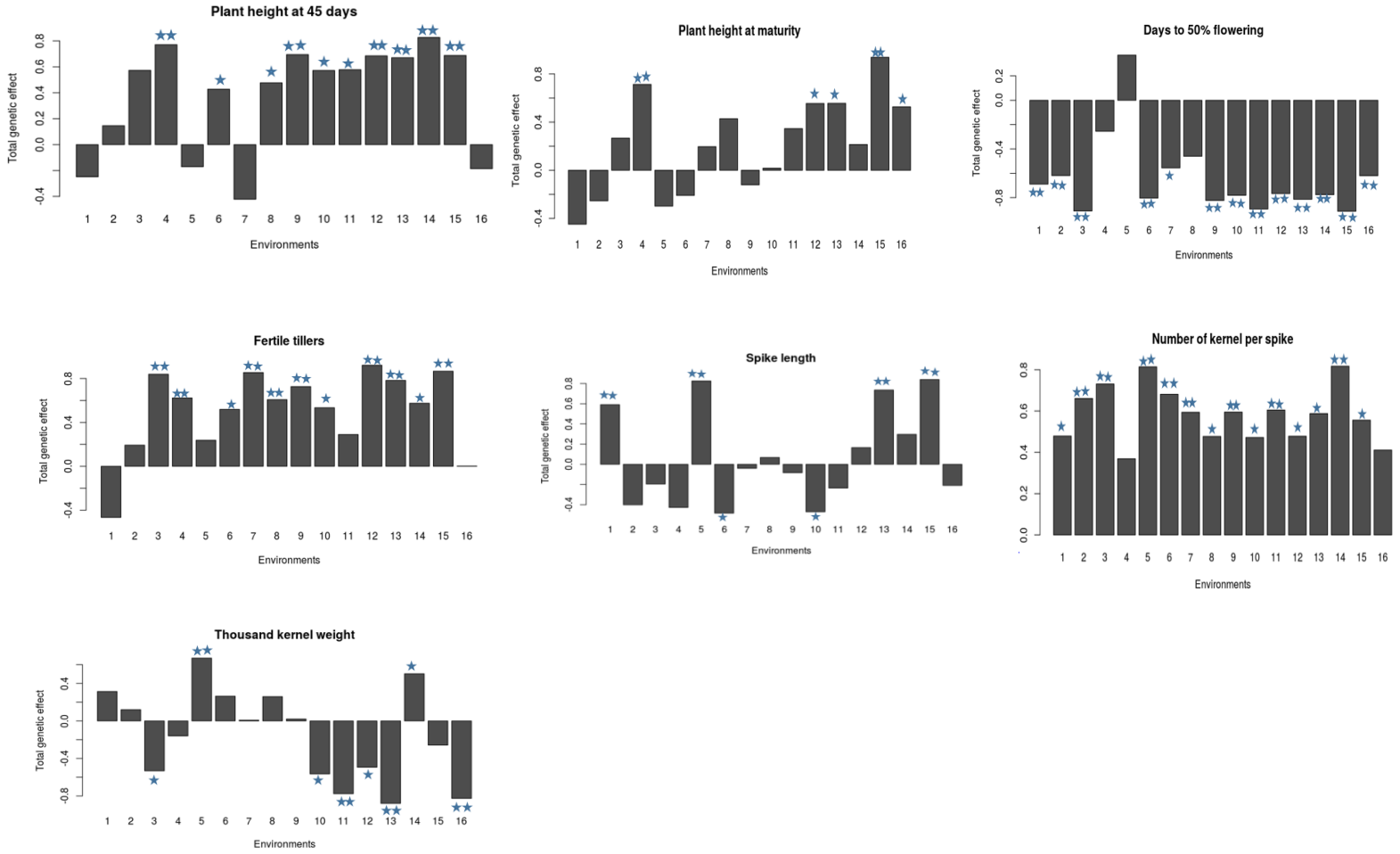

Figure 3. 3 Total effect of some agronomic traits on grain yield of 18 barley genotypes in 16 environments. *= (P <0.05, *t* = 0.468), **= (P <0.01, t = 0.590).

3.5 Discussion

3.5.1 Environments and genotypes for assessing genetic correlations

For most traits investigated including grain yield, highly significant ($P < 0.01$) effects were observed for all four factors (*G*, *L*, *SD* and *Y*), justifying the relevance of the factors included in the experiment. Location and season differences are apparent in affecting yield and yield components as addressed by some earlier works in barley using path coefficient analysis (Carpici and Celik 2012; Pržulj et al. 2013; Setotaw et al. 2014). In barley, grain yield is determined by three yield components: spike number per m^2, kernel number per spike and thousand-kernel weight (Grafius 1964). Correspondingly, we found in our study significant positive effects on grain yield from fertile tillers and kernel number per spike (Table 6). Another study reported that spike number per m^2 was the primary determinant of grain yield (Dofing and Knight 1994), which could be due to unequal stand establishment. On the other hand, Singh et al. (1987) found that grain yield in barley was significantly correlated with plant height and spike length and these two components had high positive direct effects on grain yield. In contrast, plant height was not consistently correlated with grain yield across our three analyses, rather a positive genotypic correlation (r_g=0.61**, Table 6) was seen only at Ambo but neither at Jimma nor in the combined analysis.

In this experiment, traits that did not show consistent genotypic correlation with grain yield across the three analyses were height at 45 days, total tiller, thousand-kernel weight and biological yield. Total tiller showed a negative genotypic correlation with grain yield at Ambo (r_g= -0.587, Table 6) suggesting that at the drier, temperate location, genotypes with tendency of bearing many tillers (parts of them not fertile) might end up with sterile

tillers that take assimilates without bearing seeds. Those genotypes with lower numbers of total tiller (but higher numbers of fertile tillers) might have used assimilates effectively for the grain yield. In fact the correlation between the number of total tillers and number of fertile tillers was only moderate with r=0.36, and the scatter plot (Figure 3.2) indicated that such genotypes with lower number of total tillers and higher numbers of fertile tillers do actually exist.

In our previous chapter, AMMI analysis was done for grain yield with the same data. Accordingly, the 16 environments were grouped into two clusters. With regard to the location of experiments, Ambo and Jimma represent a near-ideal and a marginal area for barley production, respectively. Estimating genetic correlations between traits in different environments is useful in determining the predictive power of the analysis for indirect selection. The use of diverse environments enabled to unravel better the relationships of traits in changing environments considering the uncertainty of weather variables the barley production in Ethiopia may face in future. In countries like Ethiopia, where farmers usually face unpredictable rainfall patterns when rainfall ends before crops have completed their physiological maturity (Cheung et al., 2008) or delays in start of rainfall in the main cropping season, the inclusion of the sowing date factor would reveal the relationships of other traits with grain yield in those diverse conditions. Regarding the efficiency of our path analysis, the residual effects indicated the strength of the explanatory variables in the path model to determine the grain yield. The residual effects were 0.17, 0.06 and 0.10 for Ambo, Jimma and combined analysis (Table 3.5), which were fairly small justifying most of the variance in correlation was explained by the path analysis.

3.5.2 Trait compensations and yield stability in landraces

Yield compensation processes have been reported in cereals under different growth conditions. For instance Berenguer and Faci (2001) reported that lower plant density was compensated by greater tiller production, greater number of grains per panicle and a higher weight of grains in sorghum. A higher plant density in sorghum resulted in higher grain yield and grain number per panicle by 7.5 and 18.9 %, respectively than lower density; while thousand grain weight and plant height showed higher values in lower density than higher density (Herrera et al., 1994).

In the combined analysis of our cultivars and landraces, we observed a significant compensatory relationship between number of kernels per spike and thousand grain weight (r = -0.37 to -0.39) at Ambo and in the joint analysis of both locations (mean data of Ambo and Jimma) indicating competition among grains for assimilates during the grain filling period. The relationship was, however, not significant at Jimma indicating these compensatory relationships were environment-dependent, which may reflect little or no competition for assimilates during the grain filling period at Jimma, which is the more humid location.

The three traits kernels per spike, spike length and thousand grain weight showed a statistical significant mean difference between landraces and cultivars. In two cases (spike length and thousand kernel weight), landraces had larger trait values than cultivars across the four analyses and the reverse was true for kernels per spike where cultivars showed larger trait values. The longer spike coupled with lower number of kernels per spike justifies that landraces had larger seeds (thousand grain weight) than cultivars in the same trial. This seems in line with Yahiaoui et al. (2014) who showed that barley landraces had

larger seeds (higher thousand kernel weight) compared to released cultivars in nine out of ten trials conducted in Spain.

A separate analysis of landraces (Table S3.3) revealed a compensatory relationship between kernels per spike and thousand kernel weight. Kernels per spike was more stable whereas thousand kernel weight was variable (plastic), and therefore the most frequent compensation by landraces was done via grain size by making grain size smaller or larger depending on available growth factors. For example, significant differences (t = 2.2460; P = 0.0279) were observed for thousand kernel weight between sowing dates, depending on the moisture status in the field. In general, grain yield stability in landraces compared to cultivars could possibly be due to the stable traits i.e. total tiller, fertile tiller and kernel per spike (Table S3.4).

3.5.3 Relative robustness of traits as selection indices for grain yield

Among agronomic traits, fertile tiller ($P < 0.05$) and kernels per spike ($P < 0.01$) showed a consistent and significant genotypic correlation with grain yield with heritability estimates of 0.82 and 0.88, respectively (Table 3.4, Figure3.3, Table S3.4).The relative higher heritability of the above mentioned traits in relation to traits like spike length might have resulted from an increase in the genetic components, particularly the additive genetic variance in relation to environmental variance components (Bowman 1972). Therefore, kernels per spike and number of fertile tillers can be proposed as robust traits in barley breeding for a wider adaptation because of their positive total effects on grain yield at Ambo, Jimma and in the combined analysis. These traits also had considerably high heritability estimates to make them reliable for selection for grain yield. Similar results were reported on maize where number of grains per ear showed the largest direct effect (r

= 0.66) on yield, with high heritability (0.73) and therefore good reliability for indirect selection (Olivoto et al. 2017). Although previous studies on barley and wheat reported a positive genotypic correlation between thousand kernel weight and grain yield (Setotaw et al. 2014; Mądry et al. 2015), we observed a negative correlation because most high yielding genotypes had smaller seeds. As it was observed from the field data, genotypes like Ambo local had larger seed size but were not top grain yielding. The mean thousand kernel weight of these least yielding genotypes was larger (36.24 gm; t-test, $P < 0.01$) while the average of the other genotypes was 31.46 gm. As farmers and markets prefer larger size of seeds especially for traditional food (Kolo), further work is needed to improve seed size in those high yielding cultivars.

3.6 Conclusions

Kernels per spike and fertile tiller can be proposed as robust traits in barley breeding for a wider adaptation as they had significant ($P < 0.05$) and highly significant ($P < 0.01$) positive total effect at the two locations tested and in the combined analysis. These traits also had considerably high heritability estimates to make them more reliable for selection. With regard to yield stability of landraces in a comparison to cultivars, thousand kernel weight and phenological traits like days to 50% flowering and days to maturity were highly plastic, whereas of fertile tillers and kernel per spike were stable in landraces , which contributed to the higher stability for grain yield compared to cultivars.

3.7 Acknowledgement

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3.8 Supplementary tables and figures

Table S3.1 Sixteen environments used for evaluation of barley genotypes

Location	Date	Sowing	Location	Date	Sowing
Ambo	June 9, 2012	1	Ambo	June 11, 2013	1
Ambo	June 26, 2012	2	Ambo	June 26, 2013	2
Ambo	July 13, 2012	3	Ambo	July 12, 2013	3
Ambo	July 28, 2012	4	Ambo	July 27, 2013	4
Jimma	June 13, 2012	1	Jimma	June 13, 2013	1
Jimma	June 28, 2012	2	Jimma	June 28, 2013	2
Jimma	July 14, 2012	3	Jimma	July 14, 2013	3
Jimma	July 30, 2012	4	Jimma	July 30, 2013	4

Table S3. 2 Mean grain yield (kg ha^{-1}) of the 18 genotypes across 16 environments

	Environments																
Genotypes	12A1	12A2	12A3	12A4	12J1	12J2	12J3	12J4	13A1	13A2	13A3	13A4	13J1	13J2	13J3	13J4	Mean
1[†]	1731	3665	1665	591	2639	2812	2160	1227	1725	2318	2107	2662	2389	2687	1925	1595	2119
2	1777	4099	1254	1299	1888	1809	1407	1128	2381	3183	2466	2288	1214	1745	1539	1266	1922
3	1930	3781	1723	1086	1193	1260	1830	1847	1763	2669	1810	2145	1368	1488	532	950	1749
4	1465	3637	1432	1394	1715	1379	1303	689	2148	3185	2073	2067	1056	1705	600	1065	1695
5	1801	3879	1804	2093	2183	1892	1209	681	2440	3400	846	2160	1243	1456	943	1070	1819
6	1224	3962	1394	1463	1760	1839	799	564	1531	3279	1207	2041	1248	1850	1023	936	1516
7	1360	3689	1485	1738	1703	1916	1445	1122	1279	3390	1829	2626	1888	1652	1248	1408	1848
8	426	1881	698	395	1610	1738	323	230	121	681	1863	1951	1581	1192	1444	1839	1186
9	1218	2544	1164	232	1446	1732	392	353	1298	1421	618	2130	1480	1309	1264	1615	1317
10	1386	2837	1296	1504	1053	1513	570	704	1515	1234	2239	1828	1585	1139	696	453	1347
11	1696	3113	1747	1836	1662	1737	1431	1596	2105	2182	1832	2232	1329	1832	1049	1696	1847
12	1082	2396	715	998	685	1080	593	265	1668	2244	2387	1394	616	280	222	255	1034
13	1756	2935	1699	1233	915	1012	734	293	1302	2280	2027	1781	633	541	367	244	1262
14	1016	2501	939	537	1267	1707	590	258	1361	1685	1984	1667	1371	1295	1116	1483	1330
15	1725	2690	1474	1434	1055	1362	765	1184	1902	2724	1946	2295	697	1485	1235	1440	1461
16	736	2056	841	613	352	649	248	-	651	1916	1956	1114	463	774	-	-	734
17	553	1340	440	452	220	258	-	-	941	1284	1562	912	280	165	-	-	528
18	1252	1980	1336	275	1394	1563	157	37	1125	1331	2225	2221	1613	1503	934	1540	1337
Mean	1341	2944	1284	1065	1374	1514	887	677	1514	2245	1832	1973	1225	1339	896	1048	1447
S.E±	130	134	104	234	105	108	116	93	255	269	262	186	168	182	186	159	
LSD (5%)	374	385	300	671	302	310	332	267	734	773	752	534	483	524	535	458	
Repeatability	0.91	0.89	0.89	0.92	0.78	0.92	0.82	0.63	0.75	0.77	0.71	0.82	0.81	0.75	0.46	0.64	0.78

[†] See Table 2.1 for genotype codes

Table S3. 3 Means and ranges of barley landraces and cultivars for selected, yield-related traits evaluated in Jimma and Ambo for two years (combined across sowing dates), and P-values indicating the significance of difference.

Location	Year	Type	Mean value	Range across sowing dates	P-value
			Fertile tillers		
Jimma	2012	Landrace	2.3	1.8-2.7	0.7087
		Cultivar	2.2	1.8-2.4	
	2013	Landrace	2.7	2.2-3.1	0.0826
		Cultivar	3.3	2.5-4.6	
Ambo	2012	Landrace	2.4	1.7-3.6	0.6590
		Cultivar	2.3	1.3-3.2	
	2013	Landrace	3.1	3.0-3.1	0.7067
		Cultivar	3.1	2.2-3.6	
			Kernels per spike		
Jimma	2012	Landrace	15	11-20	0.0091**
		Cultivar	24	15-31	
	2013	Landrace	13	13-21	0.0010**
		Cultivar	20	20-29	
Ambo	2012	Landrace	16	16-26	0.0163*
		Cultivar	22	22-36	
	2013	Landrace	22	22-28	0.0272*
		Cultivar	24	24-34	
			Spike length		
Jimma	2012	Landrace	8.3	7.5-9.3	0.0042**
		Cultivar	7.1	6.1-8.1	
	2013	Landrace	8.1	7.6-9.1	0.0435*
		Cultivar	7.1	6.1-8.3	
Ambo	2012	Landrace	7.6	7.3-8.0	0.0016**
		Cultivar	6.1	5.0-7.1	
	2013	Landrace	7.1	6.7-7.7	0.0682
		Cultivar	6.5	5.0-7.2	
			Thousand kernel weight		
Jimma	2012	Landrace	33	31-36	0.0042**
		Cultivar	31	27-36	
	2013	Landrace	29	24-32	0.0435*
		Cultivar	28	19-37	
Ambo	2012	Landrace	42	37-44	0.0016**
		Cultivar	38	34-41	
	2013	Landrace	35	32-36	0.0482*
		Cultivar	32	31-36	

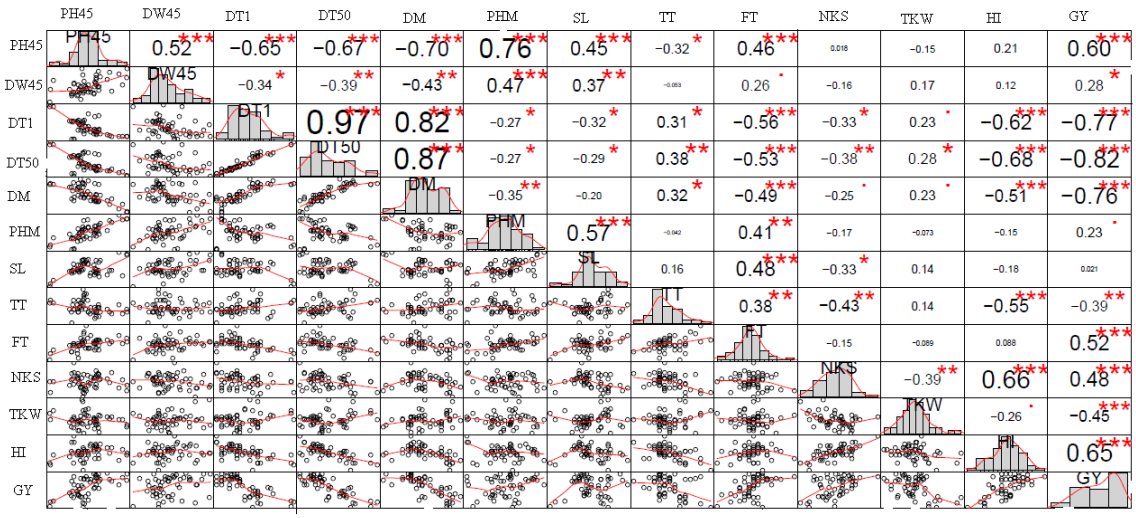

Figure S3. 1Correlation among the characters studied at Ambo for two years and four sowing dates in 18 barley genotypes.· , * , ** , *** , significant at P < 0.1, P < 0.05, P < 0.01 and P < 0.001, respectively. PH45 = Height at 45 days after sowing, DW45 = Dry weight at 45 days after sowing, DT1 = Days to first flowering, DT50 = Days to 50% flowering, DM = Days to maturity, PHM = Height at maturity, TT = Total tiller, FT = Fertile tiller, SL = Spike length, NKS = Kernel per spike, TKW = Thousand kernel weight, HI = harvest index and GY = Grain yield

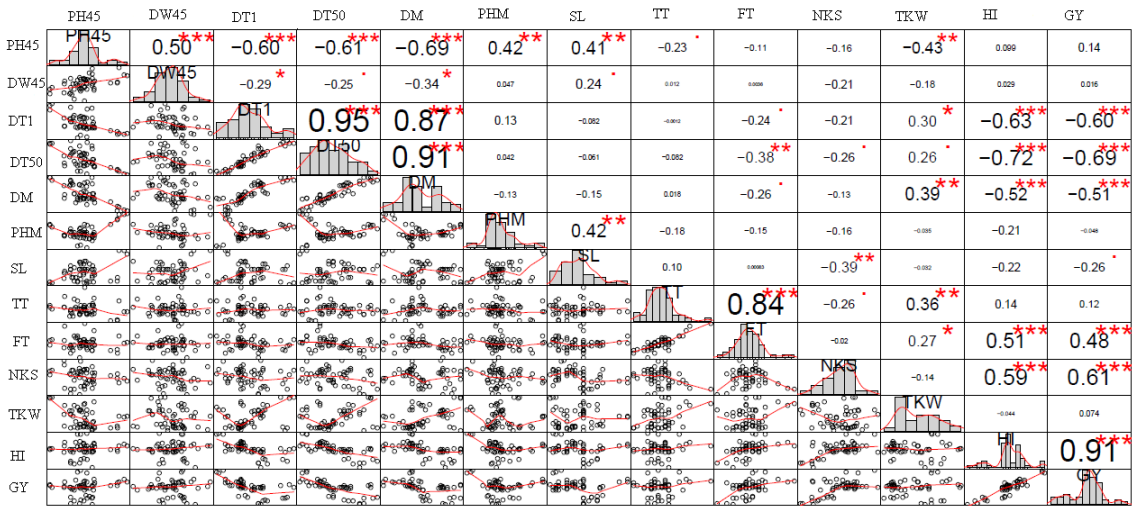

Figure S3. 2Correlation among the characters studied at Jimma for two years and four sowing dates in 18 barley genotypes. · , * , ** , *** , significant at P < 0.1, P < 0.05, P < 0.01 and P < 0.001, respectively. PH45 = Height at 45 days after sowing, DW45 = Dry weight at 45 days after sowing, DT1 = Days to first flowering, DT50 = Days to 50% flowering, DM = Days to maturity, PHM = Height at maturity, TT = Total tiller, FT = Fertile tiller, SL = Spike length, NKS = Kernel per spike, TKW = Thousand kernel weight, HI = harvest index and GY = Grain yield.

Table S3. 4Total effects of agronomic characters on grain yield of 18 barley genotypes evaluated in 16 contrasting environments differing in location and year and sowing date.

Location	Year	Sowing date	Total effect on grain yield of						
			PH45	PHM	DT50	FT	SL	NKS	TKW
Jimma	2012	1	-0.249	-0.448	-0.689**	-0.466	0.591**	0.479*	0.314
		2	0.146	-0.254	-0.619**	0.193	-0.401	0.661**	0.120
		3	0.573*	0.267	-0.910**	0.839**	-0.196	0.731**	-0.532*
		4	0.771*	0.713**	-0.255	0.623**	-0.428	0.369	-0.160
	2013	1	-0.171	-0.298**	0.371	0.238	0.826**	0.814**	0.670**
		2	0.428	-0.209	-0.804**	0.519*	-0.484	0.681**	0.264
		3	-0.422	0.196	-0.556*	0.855**	-0.040	0.594**	0.008
		4	0.477*	0.428	-0.460	0.608**	-0.068	0.477*	0.259
Ambo	2012	1	0.695**	-0.121	-0.824**	0.727**	-0.084	0.595**	0.020
		2	0.572*	0.017	-0.781**	0.534*	-0.471*	0.472*	-0.565*
		3	0.578*	0.346	-0.894**	0.290	-0.236	0.605**	-0.776**
		4	0.685**	0.555*	-0.767**	0.921**	0.166	0.478*	-0.494*
	2013	1	0.671**b	0.556*	-0.814**	0.783**	0.736**	0.588*	-0.879**
		2	0.827**	0.214	-0.776**	0.575*	0.297**	0.817**	0.503*
		3	0.688**	0.938**	-0.912**	0.867**	0.840**	0.556*	-0.258
		4	-0.186ns	0.527*	-0.620**	0.002	-0.209	0.411	-0.826**

PH45 = Height at 45 days after sowing, PHM = Height at maturity, DT50 = Days to 50% flowering, FT = Number of fertile tillers, SL = Spike length, NKS = Number of kernels per spike, TKW = Thousand kernel weight. *= (P <0.05, *t* = 0.468), **= (P <0.01, t = 0.590)

Chapter 4: Assessment of genetic diversity among Ethiopian barley (*Hordeum vulgare L.*) of different regional and temporal background using SNP markers

4.1 Abstract

Barley is among the oldest crops cultivated in Ethiopia in wide coverage. We present a report on Genotyping-by-Sequencing (GBS) of Ethiopian barley landraces in order to make population genetics analysis of currently growing landraces and those which were conserved for over four decades in gene bank. In total 222 barley genotypes were considered for this study. Barley seedlings were raised in greenhouse until two weeks old. Young leaf samples from a single plant were collected from each genotype and kept in silica gel for drying in vacuum plastic bags. DNA samples were subject to double-digest by ApeK1 and Hind III enzymes. After sequencing, raw read was checked for major quality parameters by FastQC and barcode splitting, sequence reads were filtered for sequencing artifacts and low quality reads (preprocessing) with custom Phyton scripts, bwa and FastQC. The pre-processed reads were aligned to genome of barley cultivar Morex using bwa. SNP calling was performed with SAMtools, bcfutils, vcfutils and custom Phyton scripts. The .vcf file was parsed to filter out SNP positions with a coverage of at least 30, where by at least ten reads had to confirm the variant nucleotide. Positions not fulfilling these criteria were marked and considered as missing data. Discriminant Analysis of Principal Components (DAPC) and Neighbour Joining tree (NJ) were inferred. The genetic structure of the genotypes (n=180) in the study was also analysed using ADMIXTURE. In addition, The DAPC showed genotypes differentiated between four clusters according to the Bayesian information criterion. In the analysis, seven principal components and three discriminate functions were retained that explained 40.8% of the variance. Values of observed heterozygosity (H_o) ranged from 0.250 to 0.337 and were

higher than the expected heterozygosity (H_e) that varied from 0.180 to 0.242 in genotypes of all origins. The inbreeding coefficient (F_{IS}) values ranged from -0.240 to -0.639 across the regions and were also higher and negative suggesting excess out crossing than the expected ones. Based on the inferred clusters by the ADMIXTURE, high F_{st} values were observed between clusters suggesting high genetic differentiation among the genotypes tested though differentiation was not based on location. In addition, genetic differentiation computed based on the predetermined location and type of genotype suggested that there were weak differentiation among the groups.

Key words: barley, diversity, Ethiopia, GBS, SNP

4.2 Introduction

Genetic diversity is key to progress in plant breeding and plant genetic resources are important source of genetic variation. Over past 100 years plant genetic resources were conserved. Two main strategies were developed: *in situ* and *ex situ*. The *in situ* strategy conserves species in their natural habitats while the *ex situ* involves conserving biological species out of their natural habitat. Key disadvantage of *ex situ* is lack of coevolution, whereas *in situ* allows species to coevolve and change over time. On the other hand, *ex situ* seeds need to be regenerated and therefore it is amenable to changes (or loss) of genetic variation by random drift. An additional effect is different levels of genetic diversity captured in different gene banks reflects collection strategy, storage, and regeneration procedures differential loss of seeds.

It is important to identify differences in genetic diversity between genebanks, but also to identify changes over time and between *ex situ* collections and area of *in situ* collection. This is particularly of interest because passport data are not complete and other means have to be found to identify genetically similar or distant accessions in the construction of core collections.

Rapid evolution of genotyping and sequencing allows large-scale analysis of genebanks and to test these hypotheses for both major and minor crops. Started with SSR, SNP arrays, reduced rep sequencing and now whole genome sequencing is in use. Among major crops barley is interesting to study because of wide environmental adaptation of wild and cultivated barley, evidence of local adaptation of different genes, having

substantial genetic diversity and multiple uses coupled with multiple selections by humans.

Among barley growing regions, Ethiopia is very important country because of its history of early use of barley after domestication (Engels, 1994) and named as one of center of diversity (Negassa 1985; Lakew et al., 1997, Hadado et al 2010). In addition, wide environmental variation in the country has led to local adaptation. Complex seed exchange networks and multiple uses has brought high rate in gain of genetic diversity (Abay et al., 2011). Furthermore, breeding has been closely linked to old landraces as they are the parents of improved varieties.

Today, genetic diversity of barley landraces is threatened (Abebe et al, 2010; Abdi, 2011) although the full diversity is not known yet. The recent expansion of road infrastructure and communication facilities enhanced the seed exchange practice of farmers and contributes further to local diversity (Abay et al., 2011), while crop improvement programs cause overall genetic erosion by replacing local landraces with a few selected cultivars. It therefore is important to determine native genetic diversity for an efficient conservation and utilization for future breeding.

Since the beginning of systematic collection by N. Vavilov, a very large number of accessions of crop species have been accumulated *ex situ*. For example, more than 15,000 barley accessions are stored in the gene bank of the Ethiopian Institute of Biodiversity (EBI, 2016). In addition, over 3,350 Ethiopian barley accessions have been conserved in IPK's Genebank information system, GBIS in Germany since 1970's (http://gbis.ipk-

gatersleben.de/GBIS_I/). Recently, Genotyping-by-sequencing (GBS) was performed for a total of 22,626 samples from the IPK, including Ethiopian collection (Milner et al., 2018). The disadvantage of *ex situ* conservation is the risk of creating population bottlenecks causing loss of genetic diversity and changes in gene frequencies over time because of seed mortality, genetic drift and inadvertent selection during seed regeneration cycles (Parzies et al., 2000). Despite the large size of *ex situ* collections, only a small proportion of the available diversity has been used for the improvement of barley cultivars. A main reason is that only a small proportion of exotic genetic diversity is useful for modern breeding approaches.

Despite the huge genetic resource available in *ex situ*, small portion of total barley germplasm collection has been utilized so far in breeding programmes and the research has yet to satisfy the needs of producers for improved cultivars for different farming systems (Mulatu and Lakew, 2011). Therefore in order to utilize the available materials, the existing population genetic diversity between the cultivars and conserved *ex situ* as well as those landraces at the hand of farmers should be studied.

The recent development of genotyping methods greatly facilitates the analysis of crop genetic diversity. For barley, multiple studies employed various marker-based and reduced representation sequencing such as exome capture sequencing to characterize genetic diversity in wild and cultivated barley. On a more regional level, barley diversity in Ethiopia was analysed with respect to spatial and temporal distribution using simple sequence repeat (SSR) markers (Hadado et al., 2010; Abebe and Leon, 2013). Among current methods for characterizing genetic diversity, RADseq has been used frequently

because it is cost-efficient, amenable to high-throuput analysis and provides sufficient data for the analysis of genetic diversity and related parameters. GBS used in crops (Elshiler et al. 2011, Peterson et al., 2014).

The aim of the present study was to compare the extent of diversity in barley gene bank accessions between two *ex situ* gene banks and between *ex situ* conserved and *in situ* conserved accessions. An important aspect is to investigate whether long term storage (over 40 years) in ex situ genebanks affects levels and patterns of genetic diversity in relation to cultivars under continous cultivation. We compare barley accessions from the Institute of Biodiversity Conservation (IBC) genebank recently collected from farmer's field, the German IPK genebank and improved cultivars from public breeding programs in Ethiopia. Since both collections in each genebank range in the thousands and the available passport information of accessions does not allow direct comparison of ex situ and in situ conserved landraces, we investigate randomly selected subsets from each collection that are large enough to obtain robust estimates of diversity within collections and allow establishing genetic relationships between collections.

4.3 Materials and methods

4.3.1 Plant material

In total 222 Ethiopian barley accessions of different genetic background were included in the study (Table S4.1). Among these, 146 represent landraces collected between 2004 and 2006 from major barley growing regions in Ethiopia with altitude ranges of 1642-3904 m.a.s.l. and geographical position ranging from 6^{o}51'N to 14^{o}13'N and from 36^{o}50'E to 39^{o}49'E collected by IBC (Figure 4.1). In addition, 48 genotypes were randomly selected

from the total of over 3000 Ethiopian barley accessions from the German genebank at IPK Gatersleben where they have been maintained since early 1970's. The remaining 28 genotypes were obtained from the Ethiopian Institute of Agricultural Research (EIAR) and are improved cultivars that were released from the early 1970's until 2012. After processing raw data, 180 genotypes were maintained i.e., 127, 28 and 25 genotypes from IBC, EIAR and IPK, respectively.

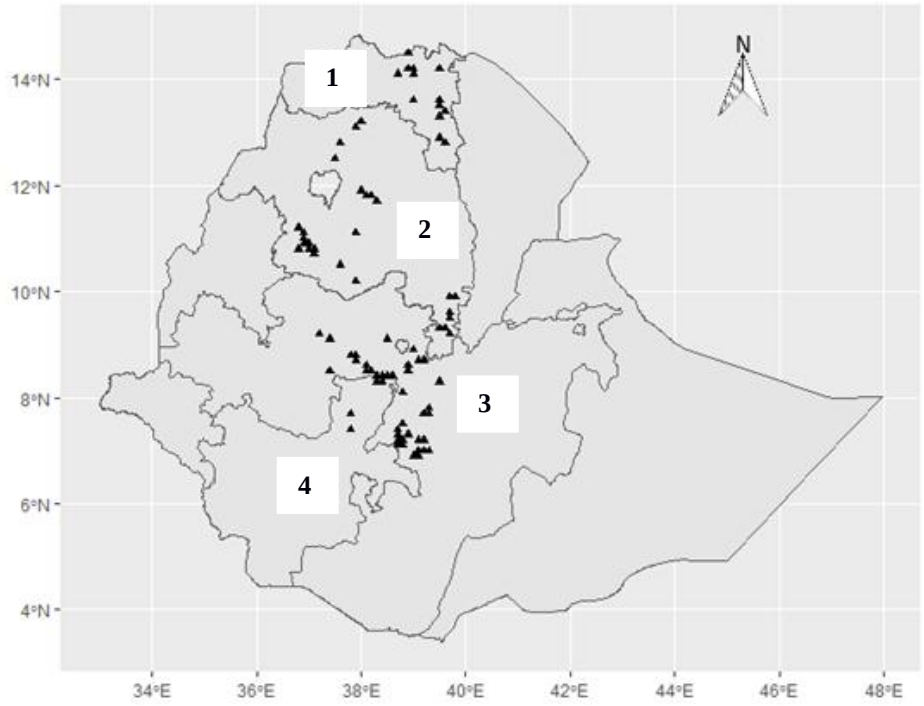

Figure 4. 1 Map of Ethiopia with the black dots showing the locations where accessions were collected from (1= Tigray, 2= Amhara, 3= Oromiya, 4= SNNP)

4.3.2 DNA extraction and Genotyping-by-Sequencing (GBS)

Barley seedlings were raised in greenhouse until two weeks old. Young leaf samples from a single plant were collected from each genotype and kept in silica gel for drying in vacuum plastic bags. The dried leaf samples were then ground in the lab using homogenizer. Genomic DNA was extracted with the CTAB mini preparation method

(Saghai-Maroof et al., 1984). DNA quality was checked by gel electrophoresis. The final DNA concentration of each DNA sample was maintained at 100 ng/µl.

GBS was carried out following the protocol by Elshire et al., (2011) with some modification as described in the following. DNA samples were double-digested with ApeK1 and Hind III restriction enzymes. A total of 222 genotypes i.e., 30 and 192 genotypes were sequenced separately after barcoding. Barcodes were obtained from Metabion International AG (http://www.metabion.com/home/index.php#) and Biomers.net Gmbh (http://www.biomers.net/?gclid=CJ2zzPOx6MkCFQQcwwodG60Eag).

Before sequencing the size distribution of PCR enriched GBS libraries was analyzed with an Agilent Technologies 2100 Bioanalyzer using a DNA 1000 chip and the average DNA fragment was found to be 260 bp. To achieve the best sequencing data quality, cluster densities needs to be optimized, which we achieved by accurate library quantification using qPCR followint the Illumina qPCR Quantification Protocol Guide.

4.3.3 Sequence read mapping and SNP calling

After the raw read was checked for major quality parameters by FastQC and barcode splitting, sequence reads were filtered for sequencing artifacts and low quality reads (preprocessing) with custom Phyton scripts, bwa (Li and Durbin, 2009) and FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). All reads with ambiguous 'N' nucleotides and reads with low quality values were discarded. The remaining sequence reads were demultiplexed into separate files according to their barcodes. After removal of the barcode sequence and end trimming, the reads had length of 90 bp.

The pre-processed reads were aligned to the online available genome of barley, cultivar Morex (ftp://ftpmips.helmholtz-muenchen.de/plants/barley/public_data/sequences/) using bwa. SNP calling was performed with SAMtools (Li et al., 2009), bcfutils, vcfutils and custom Python scripts. The .vcf file was parsed to filter out SNP positions with a coverage of at least 30, where by at least ten reads had to confirm the variant nucleotide. Positions not fulfilling these criteria were marked and considered as missing data. A distance matrix was calculated using SNP data as input.

4.3.4 Analysis of population structure and genetic diversity

For analyses of genetic diversity, the vcf file was converted to different file formats depending on the type of analysis requiring specific format using PGDSpider (Lischer and Excoffier, 2012). Different approaches were used to investigate the population structure of the genotypes. Discriminant analysis of principal components (DAPC) was computed using the R *adegenet* package (Jombart et al., 2010) to infer the number of clusters of genetically related individuals. This method is a multivariate statistical approach in which variance in the genotypes is partitioned into between and within group components to maximize discrimination between groups. Furthermore the genetic relationship among the genotypes was assessed using a neighbor-joining tree (NJ tree) based on pairwise distance matrix with ape package in R (Paradis et al., 2004).

Population structure was further studied with ADMIXTURE (Alexander et al., 2011). The number of subpopulations inferred ranged between 4 and 13 and cross validation was used to estimate the optimum number of cluster K (Alexander and Lange, 2011). Expected and observed heterozygosity along with other related parameters were computed using vcftools v0.1.13 (Danecek et al., 2011). Number of private alleles (NPA) and pairwise Gst

was computed with PopGenReport package in R version 3.1.3 (Adamack and Gruber, 2014). Expected and observed heterozygosity along with related parameters for each population were computed using vcftools v0.1.13 (Danecek et al., 2011).

4.4 Results

4.4.1 Analysis of the sequencing data

In the experiment, two separate sequencings were made with all the procedures being the same. The first set of sequences had 190,527,831 reads with 101 bp length that comprised 192 genotypes in total. After filtering and removing low quality reads, 166,617,846 reads were left with the number of reads per individual genotype varying from 282 to 4,545,905. The second set of sequence had a total of 31,054,310 reads with 110 bp length from 16 genotypes. After filtering and removing low quality reads, 25,214,600 reads were obtained with the numbers reads ranging from 298 to 3,899,082. Genotypes with less than 200,000 reads were excluded from further analysis and 180 genotypes from the two sets of sequences were mapped to barley reference genome of *Hordeum vulgare* cultivar Morex. The reference genome was constructed with 2,670,738 contigs with L50 of 1425 bp. Contigs less than 5000 bp were filtered out before mapping and the rest were maintained. The percentage of mapped reads per genotypes against the reference genome ranged from 15.87% to 53.15% with overall average of 26.13% (Table S4.2, Figure4.2). It was observed that there was a significant difference (t-test, P=0.0189) in the number of raw reads between two batches of reads from similar barcode adapters. However, we observed no significant difference between sources of genotypes neither in the number of raw reads (P=0.5529) nor number of mapped reads (P=0.5398) (Table S4.3 and S4.4).

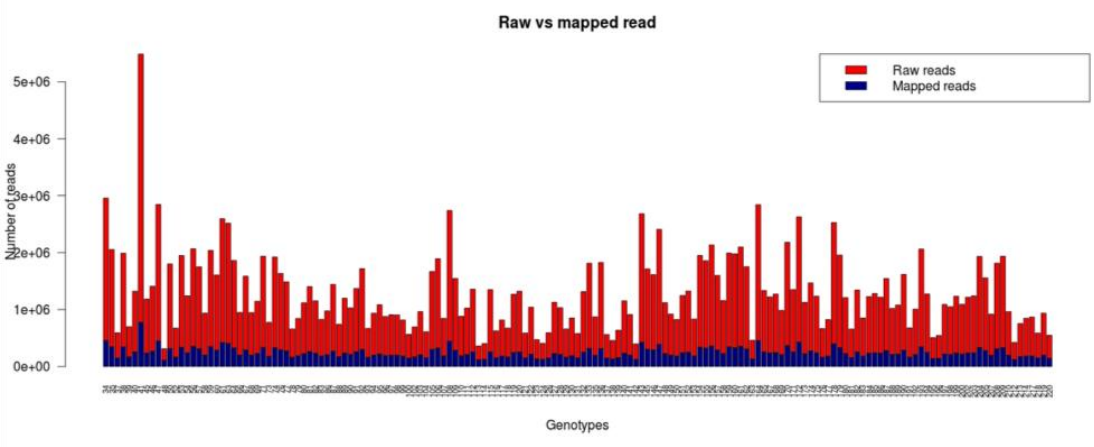

Figure 4. 2 Barplot showing proportion of raw and mapped reads across barcoded genotypes

4.4.2 Genetic structure of accessions

DAPC and NJ tree were inferred based on 180 x 3158 matrixes of allele counts. In addition, the genetic structure of the genotypes (= *180*) in the study was analyzed using ADMIXTURE.

The DAPC showed genotypes differentiated between four clusters according to the Bayesian information criterion (BIC; Figure4.3). In the analysis, seven principal components and three discriminate functions were retained that explained 40.8% of the variance. The largest cluster and smallest cluster contained 81 and 8 genotypes each. The rest two clusters had 40 and 31 genotypes (Figure4.4). However, the DAPC analysis did not show a differentiation of genotypes based on their region of origin

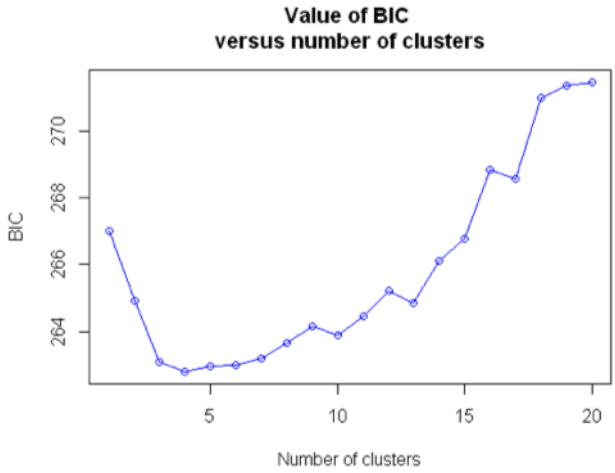

Figure 4. 3 Plot of BIC estimates used to infer the number of clusters for DAPC

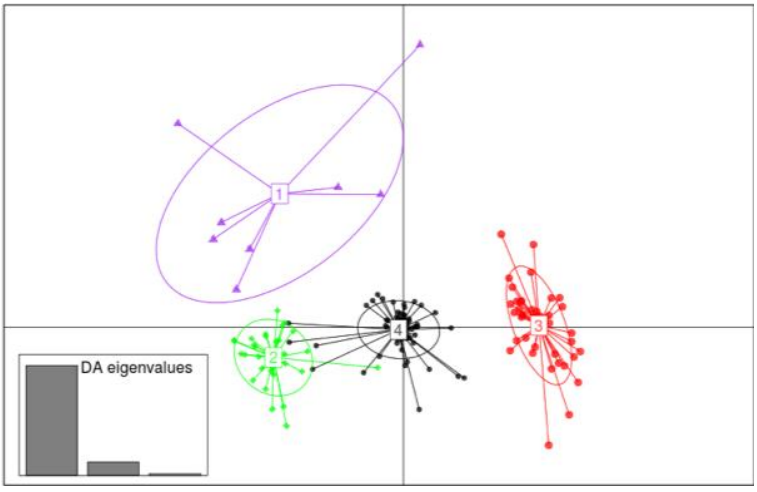

Figure 4. 4 Scatter plot of DAPC analysis showing the first two principal components of the analysis using data with missing values.

A neighbour joining (NJ) tree that was based on pair wise distance matrix separated the 180 genotypes in to several small groups (Figures 4.5 and 4.6). Like to the DAPC the NJ tree also did not show the groups clustered based on neither their origin of location nor altitudinal group. In the model based ADMIXTURE approach to infer population structure

of our materials, K=11 was the most likely number of cluster based on cross validation error (Figure S4.1). Accordingly, the admixture ancestry bar plot sorted by location did not clearly structure genotypes based on location except for Tigray region (Figure 4.7). We also sorted the population based on altitudinal gradient and no clear population structuring was observed either (Figure 4.8). In addition, same bar plot was made by sorting genotype by their type source (EIAR, IPK and landraces) based on K = 11. It was also noted that there was no clear population structuring based on the grouping (Figure 4.9).

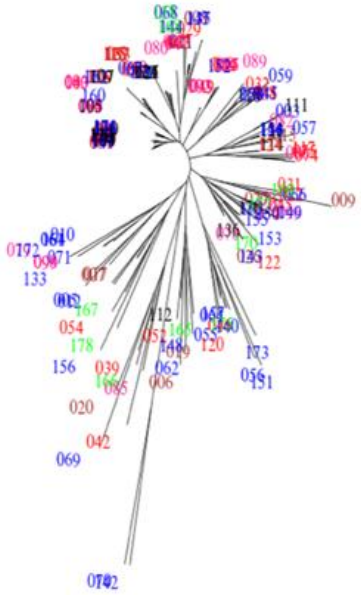

Figure 4. 5 NJ tree showing the relationships between accessions of different origin. Color depicts different regions where the genotypes were collected from (Oromiya = blue, Tigray = brown, Amhara = Red, SNNP = Green, EIAR = deep pink, IPK = black)

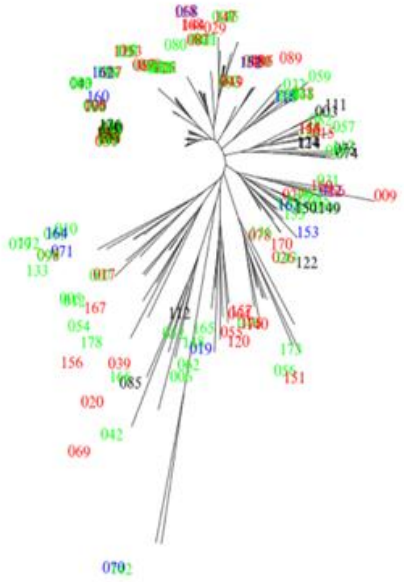

Figure 4. 6 NJ tree showing the relationships between accessions of different altitudinal gradient.Blue = <= 2000, red=2001-2500, green= 2501-3000, black = >= 3001 masl

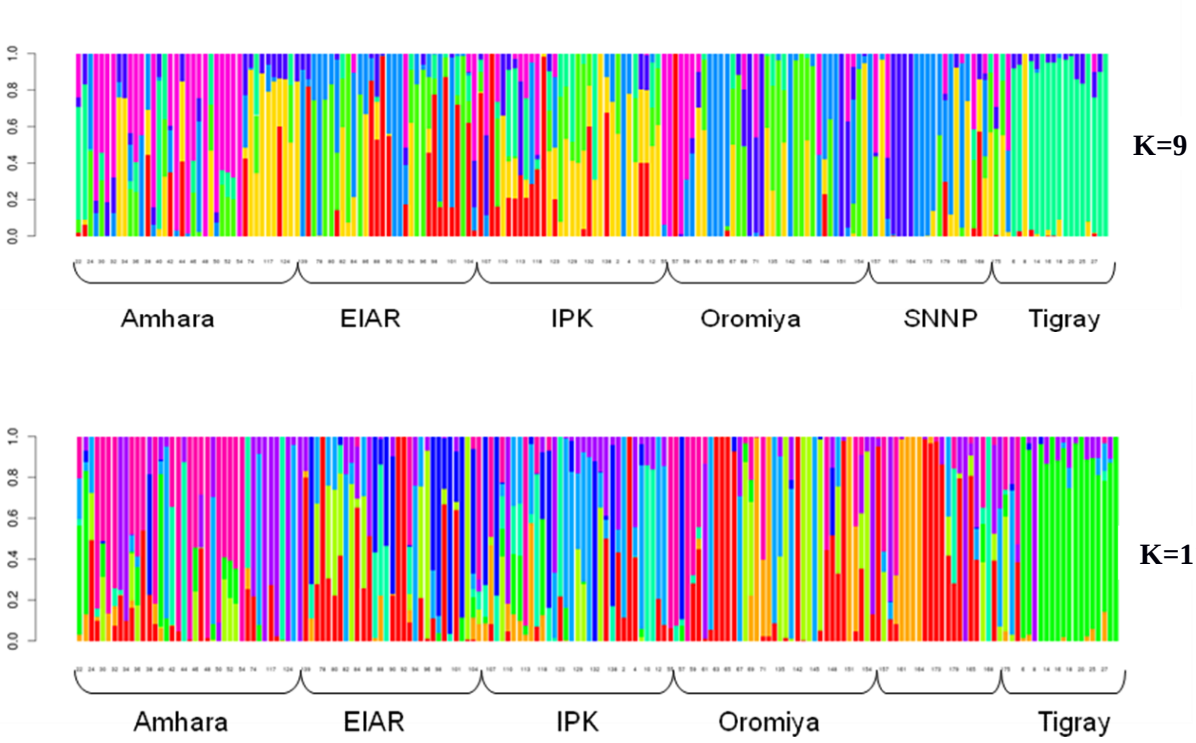

Figure 4. 7 Population structure generated by ADMIXTURE Version 1.23 among 180 barley genotypes (K=9 top and 11 bottom). Each vertical bar represents one genotype that is partitioned in to up to K colored segments

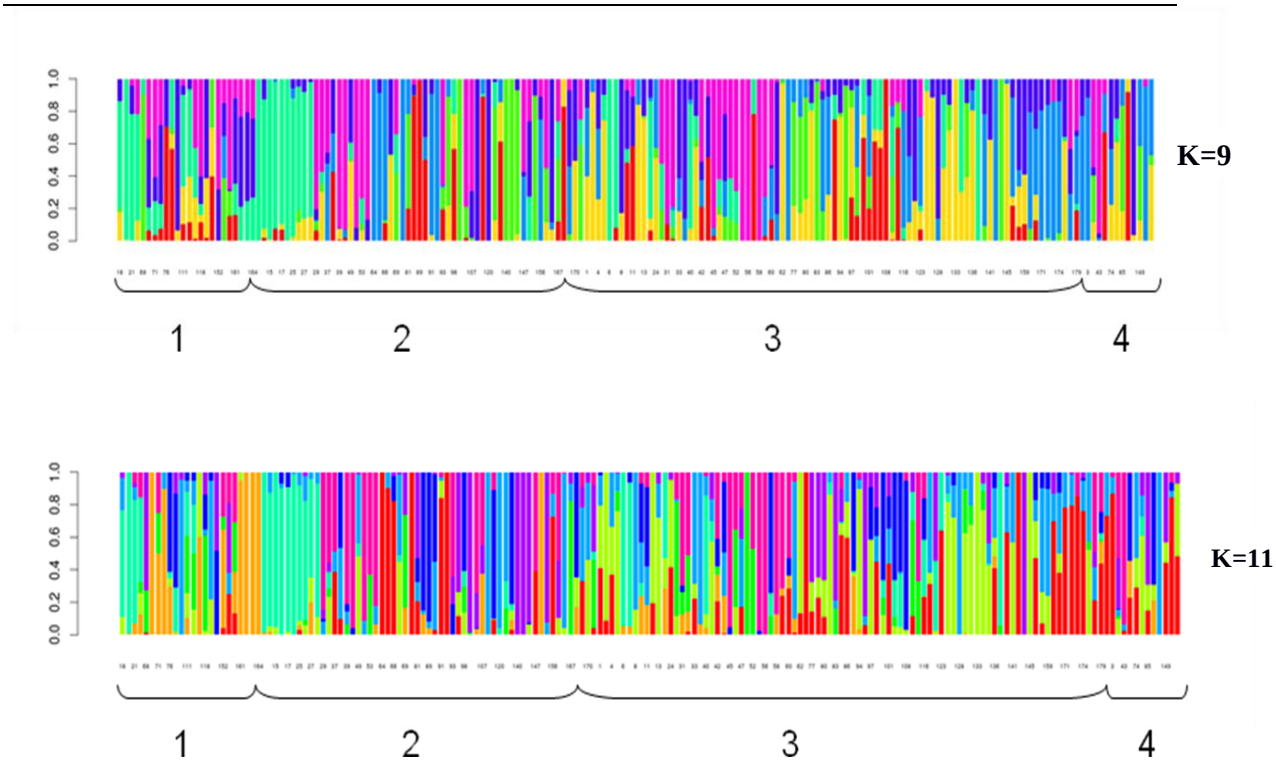

Figure 4. 8 Population structure generated by ADMIXTURE Version 1.23 among 180 barley genotypes (K=9 top and 11 bottom). Each vertical bar represents one genotype that is partitioned in to up to K colored segments. 1 = ≤ 2000, 2 = 2001-2500, 3 = 2501-3000, 4 = ≥ 3001

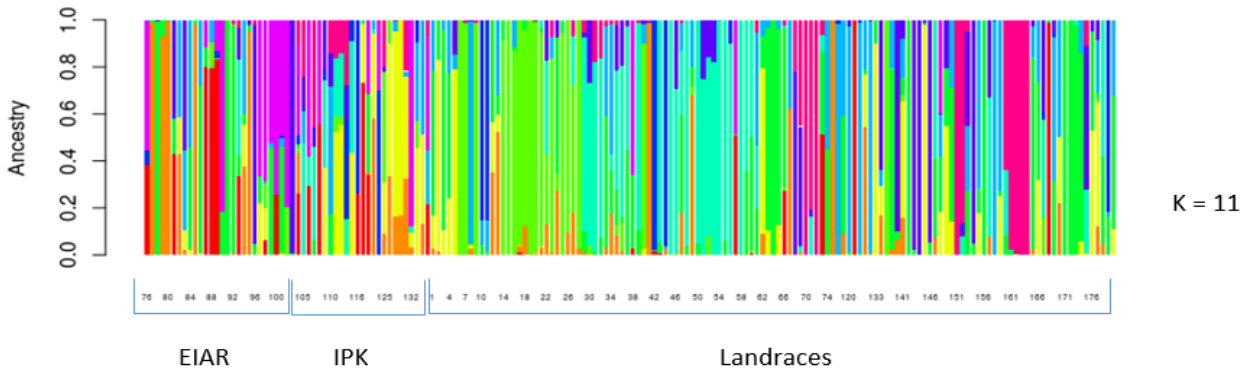

Figure 4. 9 Population structure generated by ADMIXTURE Version 1.23 among 180 barley genotypes (K= 11). Each vertical bar represents one genotype that is partitioned in to up to K colored segments.

4.4.3 Parameters of genetic diversity

In order to make comparison among genotypes of the different geographical origin, heterozygosity parameters were computed. Values of observed heterozygosity (H_o) ranged from 0.25 to 0.337 and were higher than the expected heterozygosity (H_e) that varied from 0.180 to 0.242 in genotypes of all origins. The inbreeding coefficient (F_{IS}) values which ranged between -0.240 and -0.639 across the regions were also higher and negative suggesting excess out-crossing than the expected ones (Table 4.1). Based on the inferred clusters by the ADMIXTURE, high F_{ST} values were observed between clusters suggesting high genetic differentiation among the genotypes tested though differentiation was not based on location (Table 4.2). In addition, genetic differentiation computed based on the predetermined location and type of genotype suggested that there were weak differentiation among the groups (Table 4.3).

Table 4. 1 Sample size and heterozygosity parameters of barley populations from different regions of Ethiopia and Germany

Parameter	Amhara	EIAR	IPK	Oromiya	SNNP	Tigray
N	40	28	25	60	10	17
H_o	0.284	0.251	0.337	0.267	0.301	0.260
H_e	0.221	0.193	0.242	0.190	0.182	0.180
F_{IS}	-0.240	-0.298	-0.309	-0.405	-0.639	-0.492
NPA*	953	77	489	122	2	31

* Number of private alleles

Table 4. 2Genetic (Fst) divergences between estimated populations (K=11)

Population	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9	Pop10
Pop2	0.485									
Pop3	0.466	0.384								
Pop4	0.456	0.380	0.400							
Pop5	0.422	0.420	0.429	0.433						
Pop6	0.457	0.397	0.431	0.391	0.416					
Pop7	0.472	0.376	0.387	0.392	0.417	0.388				
Pop8	0.446	0.438	0.436	0.418	0.433	0.416	0.415			
Pop9	0.471	0.385	0.420	0.374	0.398	0.396	0.409	0.420		
Pop10	0.436	0.458	0.457	0.449	0.451	0.473	0.453	0.443	0.459	
Pop11	0.478	0.420	0.451	0.385	0.452	0.395	0.399	0.437	0.402	0.470

Table 4. 3 Genetic differentiation among genotypes of different regions and types

	Oromiya	Tigray	Amhara	EIAR	IPK
Tigray	0.00371				
Amhara	0.00089	0.00181			
EIAR	0.00867	0.00176	0.00111		
IPK	0.01086	0.01449	0.02657	0.01691	
SNNP	0.00302	0.01547	0.00055	0.03469	0.03122

4.5 Discussion

4.5.1 GBS as a tool to assess genetic diversity

To assess the genetic diversity of barley different markers were used at different times. In Ethiopian barley a work by Abebe and Leon (2013) tried to study Ethiopian barley diversity using 15 SSR markers. In addition, other than Ethiopian barley, elsewhere also several works reported similar studies using different molecular markers among which Bernardo et al. (1997) who used isozyme markers and Nandha and Singh (2014) reported on gSSR and IST-SSR markers are some to mention. However, using SNPs generated by the Genotype-by-Sequencing (GBS) has some more advantages over the previous types of markers (Elshire et al., 2011). In our study although we generated large number of reads,

substantial number of reads could not be aligned to the *Hordeum vulgare* Morex reference genome. This could be attributed to the fact that the reference genome was not fully assembled rather it was composed of over 2.6 million contigs most of which were very small in size. The longest contig was only about 36,000 bp and the shortest was down to 700 bp. As a result, we filtered out very large number of short contigs out of the reference genome and the reads could not sufficiently align to the remaining contigs. In addition, as per suggested by Romay et al. (2013), the low number of mapped read could also be attributed to the limited sensitivity of the BWA software or a large number of presence/absence variation.

4.5.2 Diversity and population structure of Ethiopian barley

In our study, as revealed mainly by Admixture, we could not see clear pattern of population differentiation of Ethiopian barley under the study. This could be an evidence for the absence of geographical structure. We could not find genotypes clustered neither based on their origin of collection nor altitudinal classification. This lack of population explanation could be explained by the extensive seed exchange existing between barley farmers of different regions, contentious introduction of new seeds in to the respective barley growing regions and gene flow between regions (Asfaw 2000). Similar result was reported for genotypes of another crop, tef (*Eragrostis tef*), the most staple and indigenous crop of Ethiopia (Assefa et al., 2003; Abebe et al., 2010). In an attempt to study the genetic diversity Engels (1994) also pointed out that there was a very small fraction variation was explained between regions rather with in regions variation was higher among 3,700 Ethiopian barley germplasms collected from different regions of Ethiopia and maintained in gene bank.

In admixture, we detected a slight population differentiation between Tigray and the rest of population. This might be due to the geographical barrier as Tigray region is located at the peripheral part of the country. Abebe et al. (2013) also noticed similar pattern of differentiation in barley of this region against the rest of the regions based on barrier analysis. In addition, germplasm mobility from and to Tigray region seems less than other regions which are relatively central and business areas.In our materials, the observed heterozygosity was higher than the expected heterozygosity in all genotypes coming from all regions and IPK as well as improved (EIAR) materials (Table 4.1). This suggests the presence of high gene flow among the regions every growing season through seed exchange and outcrossing. In addition, the inbreeding coefficient (F_{IS}) was negative and relatively higher suggesting considerably higher outcrossing than the thought. In some studies the outcrossing rate in barley was estimated lower. For example, Chaudhary et al. (1980) and Abdel-Ghani et al. (2004) reported 0.35% and 0.34 %, respectively. However, that seems to be underestimated as opposed to other reports that report higher rates. Parzies et al. (2008) reported that they saw up to 6.2 an outcrossing rate in barley and Doll (1998) even reported more (10%) outcrossing rate in Canadian barley genotypes.

4.5.3 Temporal effect

Our materials were composed of genotypes with different time history. Among the 180 genotypes in the final analysis, 25 were obtained from the IPK which were conserved *ex situ* in gene bank at least for 50 years. The rest were either under current cultivation or conserved in gene bank for only the last five years. Therefore we expected some loss of diversity in IPK materials as a result of successive rejuvenation of the accessions in small plots in the gene bank to maintain the viability of seeds. However, we did not see population differentiation between the IPK materials and the rest. This seems due to the

IPK materials per se were highly diverse which were collected from different barley growing regions of the country and were picked randomly from the gene bank. In addition, number of samples might not be sufficient for detection. However compared to their number of samples, there were a high number of private alleles observed in IPK genotypes (Table 4.1). Private alleles are alleles that are found only in a single population among a broader collection of populations (Szpiech and Rosenberg, 2011). The lesser number of private alleles in other populations compared to the IPK materials might be due to the fact that they were in a contentious cultivation and gene flow process in comparison with the IPK materials which were conserved in a gene bank at least for half century.

4.6 Conclusion

Landrace highly contribute for the diversity of barley germplasm in Ethiopia as they are constituted by highly variable local populations. In our study the existence of high genetic diversity was revealed among barley genotypes of different regions that would play a key role in improvement of Ethiopian barley for different useful traits. In addition, it was shown that variation between regions and altitudes were less pronounced than within regions variations possibly attributed to the gene flow through barley seed exchange among farmers. This reality calls for the germplasm collection strategies to be cautious in considering location and altitude as a main factor of variation and strategies should focus on exploiting the within region variation for better germplasm conservation and utilization. As to the temporal effect between the IPK materials and other genotypes, we could not find sufficient genetic differentiation and hence it was not possible draw a conclusion whether population bottleneck existed in the IPK materials because of the long term*ex situ* conservation.

4.7 Acknowledgement

Keygene N. V. owns patents and patent applications protecting its Sequence Based Genotyping Technologies. This work was supported by the German Academic Exchange Service, DAAD through a scholarship to Wosene G. Abtew in Food Security Center, University of Hohenheim, with funds of the Federal Ministry for Economic Cooperation and Development (BMZ) of Germany. We thank the McKnight Foundation Collaborative Crop Research Program via discretionary research funds to B.I.G. Haussmann for financial support to first author in order to finish writing this article. We express our thanks to Elisabeth Kokai-Kota for laboratory assistance and Markus Stetter, Max Haupt and Patrick Thorwarth for their help with the sequence and population genetic analyses. Ethiopian Biodiversity Institute, Ethiopian Agricultural Research Institute and IPK Gatersleben are highly acknowledged for providing barley genetic materials.

4.8. Supplemantary tables and figures

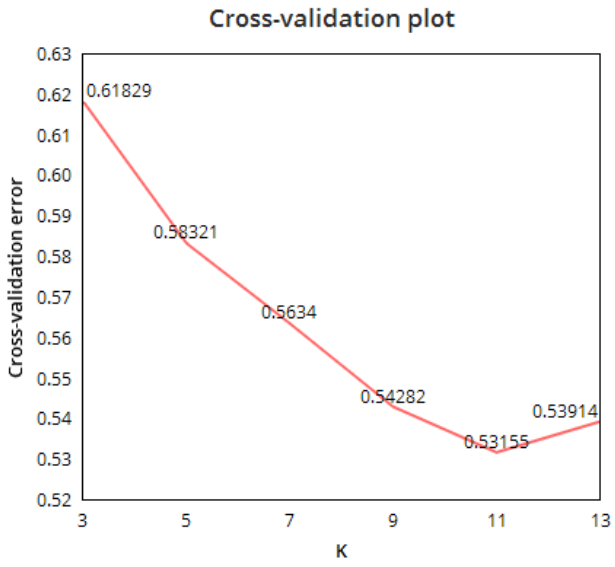

Figure S4. 1 Cross-validation error for Admixture

Table S4. 1 Barcodes used in the study

SN	Barcode Sequence	SN	Barcode Sequence	SN	Barcode Sequence	SN	Barcode Sequence	SN	Barcode Sequence	SN	Barcode Sequence
1	TTGTCA	31	CCACGCT	61	CGCCA	91	TGCGGA	121	CTCCGAC	151	GCTGGTTA
2	AATGCT	32	CTTGAAT	62	TTGTT	92	TAAGCA	122	GCACCTT	152	GATCTATA
3	GGCTAT	33	AACCACGT	63	GTGGC	93	GGTTCA	123	AGTGCTA	153	TGCGTAGC
4	AATCGA	34	CTTGTTGA	64	AAGGT	94	CAGTGT	124	CTAAGGC	154	GGAATCAA
5	CCGTAT	35	AGGTCGGT	65	GGCTC	95	TGGCAT	125	ATTGGTC	155	GCGTAGGA
6	TTAGCC	36	TAACGAGA	66	CCGAT	96	GATCAA	126	GTAACTA	156	GAATGGCT
7	GGCATA	37	GCCAACGT	67	GCCGT	97	ACTGTC	127	TAGAACA	157	TTCTCGAT
8	AGTATC	38	CTGTTGGA	68	CACGC	98	CGTCAC	128	CGTATAT	158	CGGAGGAT
9	GAACCT	39	CGTTAGGT	69	CTTCT	99	TTGTAC	129	GTTATTC	159	CGTGCCTA
10	CCTTGA	40	ACCATAGA	70	CCACC	100	CATAGA	130	CATGGTT	160	CCTGCACA
11	TGGACC	41	TGTTCTGA	71	TTCAC	101	ACCGAT	131	AATACGC	161	CGGCCTCA
12	ACTGAA	42	CTGGAGGT	72	AACAA	102	GGATTC	132	GTTCATT	162	GGCGACAT
13	CTGAGA	43	ACCACGTT	73	ACATT	103	AACTGA	133	CGTAACA	163	AATTCTGC
14	GATACC	44	GAACAATA	74	TTAGA	104	GACAGT	134	GCGATAT	164	ACTGCAAC
15	CGACAT	45	CTTATGAA	75	AGAAC	105	CCAGTA	135	GACAAGT	165	TTGTCA
16	AGTCGGT	46	GCCACAAT	76	AATCC	106	CACGTT	136	TAAGACT	166	AATGCT
17	CCTAAGA	47	CTGTGTTA	77	CGATAT	107	ATGCTC	137	ATCGTTA	167	TTACGA
18	TTCGTGA	48	TATAACGA	78	ATCCGT	108	TCGTTA	138	GCGTGAA	168	GGCTAT
19	ACGTGGT	49	GCACCATT	79	GAACTAT	109	GCATAA	139	TGCCACA	169	AATCGA
20	GGACAGT	50	CTTGGTAT	80	CACATGC	110	TGTCTA	140	TGCCTAT	170	CCGTAT
21	CACATGA	51	TGCCTCCA	81	TGAAGC	111	ACACGTA	141	AACCTGA	171	TTAGCC
22	CTGAGGT	52	AATAGTCA	82	GTACAC	112	CAAGCAT	142	GGCTGTAT	172	GGCATA
23	GAAGTCA	53	ACTGATCT	83	AGGTGC	113	ATCTCGA	143	CTGAGTGA	173	AAGCAT
24	CATTGGT	54	TATAT	84	GCTGGA	114	GACCTTC	144	ATGAAGGC	174	CTATGC
25	ACCTAGA	55	GGACT	85	TTGCGA	115	ATTGTCT	145	CTAGTGGT	175	TCCGCA
26	TGTCTCA	56	CCTTA	86	CGCATA	116	GCTGAGT	146	TTATTGCT	176	AGTATC
27	ATCGGTT	57	GGTGA	87	GAATCT	117	CACTCGT	147	GTCTTCAT	177	GAACCT
28	GTTCCTA	58	ATGCA	88	ACTTGT	118	TTCCGTA	148	TTGATTGC	178	CCTTGA
29	ACTGATT	59	GAATA	89	ACGATT	119	ACTAGAA	149	CCGTACCA	179	TGGACC
30	TTCCGAA	60	TGGAA	90	CTATGA	120	GCGGTTA	150	AAGCCGGT	180	GAATTC

Table S4. 2 No. of reads mapped to the reference genome

S.N	Barcode Adapter	Origin of accessions	Accession number	No. of reads after quality checks	No. of reads mapped to reference	Percentage of mapped reads
1	34	Amhara	8590	2501621	455821	18.22
2	35	Amhara	8577	1709458	345331	20.20
3	37	Amhara	8566	442066	149863	33.90
4	38	Amhara	8563	1646538	343387	20.86
5	39	Amhara	8561	531339	166680	31.37
6	40	Amhara	8560	1068634	252257	23.61
7	41	Amhara	8515	4714562	771582	16.37
8	45	Oromiya	16704	951024	233302	24.53
9	46	Oromiya	16703	1142369	266804	23.36
10	47	Tigray	15287	2397548	448684	18.71
11	48	Amhara	8592	203192	107997	53.15
12	50	Oromiya	16729	1485683	314815	21.19
13	52	Oromiya	16727	509054	164285	32.27
14	53	Oromiya	16726	1613723	334591	20.73
15	54	Oromiya	16725	1000520	240393	24.03
16	56	Oromiya	16723	1715539	351201	20.47
17	57	Oromiya	16710	1440472	309597	21.49
18	58	Oromiya	16751	735310	199946	27.19
19	59	Oromiya	16750	1690544	348196	20.60
20	60	Oromiya	16748	1323600	283901	21.45
21	61	Oromiya	16747	2173948	420791	19.36
22	63	Oromiya	16744	2109793	406390	19.26
23	64	Oromiya	16731	1536432	323687	21.07
24	66	Oromiya	16787	747255	200693	26.86
25	67	Oromiya	16786	1294999	289077	22.32
26	68	Oromiya	16782	748729	197556	26.39
27	69	Oromiya	16780	918640	226112	24.61
28	71	Oromiya	16759	1602294	334784	20.89
29	73	Oromiya	16752	594897	178179	29.95
30	74	SNNP	16800	1594008	330386	20.73
31	76	Oromiya	16798	1342325	291453	21.71
32	77	Oromiya	16796	1211882	274683	22.67
33	78	Oromiya	16791	496297	160995	32.44
34	79	Oromiya	16790	656209	188227	28.68
35	80	Oromiya	16789	894391	224609	25.11
36	81	Oromiya	16788	1140533	262293	23.00
37	82	SNNP	16817	920850	231651	25.16
38	83	SNNP	16816	640503	186586	29.13
39	86	SNNP	16804	769509	205725	26.73
40	87	SNNP	16803	1172740	267560	22.81
41	88	SNNP	16802	570163	171309	30.05

42	89	SNNP	16801	962802	234469	24.35
43	90	Oromiya	16885	817601	211429	25.86
44	91	Oromiya	16878	1114612	254872	22.87
45	92	Oromiya	16873	1418359	300312	21.17
46	93	Oromiya	16870	507058	161424	31.84
47	94	SNNP	16867	737151	196899	26.71
48	95	Oromiya	16863	866644	218438	25.21
49	96	SNNP	16857	686833	190198	27.69
50	97	SNNP	16856	712679	195870	27.48
51	98	Oromiya	17013	712392	194795	27.34
52	99	Oromiya	17012	632672	183059	28.93
53	100	Oromiya	16909	416459	146098	35.08
54	102	Oromiya	16907	528834	165817	31.36
55	103	Oromiya	16891	760982	202334	26.59
56	104	Oromiya	16889	457279	153642	33.60
57	105	Oromiya	16887	1370657	297449	21.70
58	106	Tigray	17148	1569149	323576	20.62
59	107	Tigray	17147	657698	187131	28.45
60	108	Tigray	17146	2297970	441465	19.21
61	109	Tigray	17145	1263822	282858	22.38
62	110	Oromiya	17020	692763	188555	27.22
63	111	Oromiya	17016	816030	209580	25.68
64	112	Oromiya	17015	1101600	256398	23.28
65	113	Oromiya	17014	243504	116379	47.79
66	114	Tigray	17180	281385	122029	43.37
67	115	Tigray	17175	1093970	254784	23.29
68	116	Tigray	17171	473266	153545	32.44
69	117	Tigray	17169	633106	182633	28.85
70	118	Tigray	17162	510897	163608	32.02
71	119	Tigray	17161	1023616	245577	23.99
72	120	Tigray	17160	1068609	252409	23.62
73	121	Tigray	17159	436794	150075	34.36
74	122	Amhara	17216	826097	216055	26.15
75	123	Amhara	17215	338171	132794	39.27
76	124	Amhara	17204	285171	122664	43.01
77	126	Tigray	17200	442070	150704	34.09
78	127	Tigray	17195	901306	226488	25.13
79	128	Tigray	17183	819581	212364	25.91
80	129	Tigray	17181	496962	160805	32.36
81	130	Amhara	17219	661787	189233	28.59
82	131	Amhara	17224	428785	150143	35.02
83	132	Amhara	17226	1063095	250614	23.57
84	133	Amhara	17227	1494253	318033	21.28
85	135	Amhara	17229	677925	193026	28.47
86	136	Amhara	17230	1513352	313864	20.74

87	137	Amhara	17232	412511	147449	35.74
88	138	Amhara	17233	325813	130763	40.13
89	139	Amhara	17234	479539	157557	32.86
90	140	Amhara	17235	919431	232349	25.27
91	141	Amhara	17236	714990	197894	27.68
92	142	Amhara	17237	273961	121791	44.46
93	143	Amhara	17238	2259143	423407	18.74
94	145	Amhara	17240	1409936	303625	21.53
95	146	Amhara	17242	1323535	289740	21.89
96	147	Amhara	17244	2021272	388106	19.20
97	148	Amhara	17245	894224	227002	25.39
98	149	Amhara	17247	724078	196474	27.13
99	150	Amhara	17248	641423	183895	28.67
100	151	Amhara	17249	1005635	241629	24.03
101	152	Amhara	17250	1076791	249517	23.17
102	153	Amhara	17251	649526	185117	28.50
103	154	Amhara	17253	1612880	337820	20.95
104	155	Amhara	17255	1534728	321108	20.92
105	156	Amhara	17257	1774846	360622	20.32
106	157	Amhara	17259	1312409	288101	21.95
107	158	Oromiya	18298	929281	228112	24.55
108	159	Oromiya	18299	1653699	340457	20.59
109	160	Oromiya	18301	1647684	329447	19.99
110	161	Oromiya	18302	1745797	353407	20.24
111	162	Oromiya	18305	1446957	305714	21.13
112	163	Oromiya	18306	328589	131482	40.01
113	164	Oromiya	18307	2390113	452632	18.94
114	165	Oromiya	18308	1080635	254486	23.55
115	167	Oromiya	18315	984290	237325	24.11
116	168	Oromiya	18316	1028152	244650	23.80
117	169	Oromiya	18317	778184	206340	26.52
118	170	Oromiya	18318	1813796	366026	20.18
119	171	Oromiya	18319	1094379	255875	23.38
120	172	Oromiya	18321	2198096	429778	19.55
121	173	Oromiya	18322	903030	223181	24.71
122	174	Oromiya	18324	1195126	271892	22.75
123	175	Oromiya	18327	992583	242135	24.39
124	176	Oromiya	18328	504638	161467	32.00
125	177	Amhara	18329	638306	182519	28.59
126	178	Amhara	18330	2130810	397057	18.63
127	179	Amhara	18331	1624742	331447	20.40
128	180	EIAR	Derbe	980801	226800	23.12
129	181	EIAR	Shedeho	498928	157566	31.58
130	182	EIAR	HB1307	1088747	253545	23.29
131	183	EIAR	Agegnehu	667046	184266	27.62

132	184	EIAR	Estayesh	994937	232272	23.35
133	185	EIAR	Abbay	1043943	239100	22.90
134	186	EIAR	Basso	982365	233959	23.82
135	187	EIAR	Biftu	1266564	277314	21.89
136	188	EIAR	Dimtu	810736	208863	25.76
137	189	EIAR	Meserach	861366	217024	25.20
138	190	EIAR	Shege	1332982	284981	21.38
139	191	EIAR	HB42	516080	162513	31.49
140	192	EIAR	EH1493	799421	207482	25.95
141	193	EIAR	41/98	1717998	342869	19.96
142	194	EIAR	M21	1027097	247590	24.11
143	195	EIAR	Balame	368716	137173	37.20
144	196	EIAR	Ardu12-60B	401847	142827	35.54
145	197	EIAR	Abdane	871688	217005	24.89
146	198	EIAR	Tiret	840857	209630	24.93
147	199	EIAR	Tilla	997219	236053	23.67
148	200	EIAR	Dinsho	874508	216557	24.76
149	202	EIAR	HB52	980132	234948	23.97
150	203	EIAR	HB1533	1001170	238694	23.84
151	204	EIAR	EH1847	1599562	332305	20.77
152	205	EIAR	IBON174/03	1278273	277928	21.74
153	207	EIAR	Bahati-1	723719	194343	26.85
154	208	EIAR	Holkr	1505188	308795	20.52
155	209	EIAR	Daffo	1606566	329402	20.50
156	210	IPK	2897	218240	54145	24.81
157	212	IPK	5680	765686	198198	25.89
158	213	IPK	6581	302507	119582	39.53
159	214	IPK	6590	582637	170584	29.28
160	217	IPK	6598	661829	184291	27.85
161	218	IPK	6599	682871	185437	27.16
162	219	IPK	7172	438261	148065	33.78
163	220	IPK	7410	735677	197963	26.91
164	222	IPK	13993	405536	142661	35.18
165	330	IPK	21656	2227566	642036	28.82
166	340	IPK	21654	1571037	323505	20.59
167	350	IPK	20454	1490408	308171	20.68
168	360	IPK	14346	2418392	444828	18.39
169	370	IPK	14299	560864	161654	28.82
170	380	IPK	14294	2137338	416801	19.50
171	390	IPK	14280	2413165	418112	17.33
172	400	IPK	14267	3614404	573534	15.87
173	410	IPK	21779	291648	120610	41.35
174	420	IPK	21772	831933	212824	25.58
175	430	IPK	21754	908585	226231	24.90
176	440	IPK	21752	205209	105603	51.46

177	450	IPK	21747	833545	223082	26.76
178	460	IPK	21739	1900927	379369	19.96
179	470	IPK	21666	1461917	312013	21.34
180	480	IPK	21657	445755	147677	33.13

Table S4. 3 ANOVA summary of total reads by region

Source	df	MS	F	P
Region	4	26511952373	0.76	0.552889
Error	150	350299175686		
Total	154	376811128059		

Table S4. 4 ANOVA summary of mapped reads by region

Source	df	MS	F	P
Region	4	25047987981	0.78	0.539839
Error	150	1203172913176		
Total	154	1228220901157		

5. General Discussion

5.1. Yielding potential of Ethiopian barley landraces

Barley landraces from Ethiopia have not been thoroughly exploited by modern breeding. This is mainly because landraces are predominantly grown by local farmers in a low or no agricultural inputs conditions that are highly adapted to specific soil, climatic and traditional management systems. These genetic resource landraces have been and are in a continuous process of evolution as a result of natural and artificial selection. Many breeding researches focused on introducing exotic materials from places like ICARDA and testing under optimum conditions for grain yield. These exotic cultivars outperform for grain yield local landraces under good management practices in selected testing sites; however, landraces often out yield the introduced material under the low input conditions (Lakew and Assefa, 2011). For such conditions, genetic variation from landraces should be exploited to improve productivity. As self-pollinated crop, barley landraces in Ethiopia contain large amount of readily available genetic variation for immediate use without the complexities of works in making crosses and selecting for a number of cycles with the masking effect of heterozygosity. However, the use of landraces has been unenthusiastic in most research programmes in developing countries because of their low yield. Reports including our study showed the presence of individual genotypes within landraces, which have a yield potential comparable to improved line cultivars (Lakew et al., 1997). So far, the Ethiopian Agricultural Research Institute has released 36 food barley cultivars by selection from landraces and exotic materials (EIAR, 2017) (http://www.eiar.gov.et/index.php/crop-research) (Accessed on 26/11/17). Among the released cultivars, most of them are pure lines. In fact, selection for pure lines from locally adapted landraces is only the first and simplest step in utilizing landraces in a plant

breeding program. The best option to maintain the population buffering of the landraces because of genetic heterogeneity is to use many superior pure lines to constitute mixtures. The inclusion of gene bank reserved genetic resources of barley is also advisable to fully exploit the genetic diversity in their respective collection areas. The generated pure line cultivars can be released as cultivars to achieve short term yield increases. In addition, they can be tested in mixtures to achieve better yields combined with yield stability. Furthermore, they can also be used in crossing program as recipients of useful genes which may not be present in these adapted populations.

5.2 Yield Stability

Grain yield stability is one of the most important needs of agriculture, especially in the tropical environment like Ethiopia. The ideal genotype of a given crop needs to be high yielding under any environmental conditions, but as genetic effects are prone to environment, most genotypes do not perform adequately across environments (Anwar et al., 2011). When there is interaction between genotype and environment, the relative ranking of cultivars for grain yield usually differs when genotypes are compared over a series of locations and/or seasons. This brings challenge in selecting genotypes for grain yield superiority over others (Monteverde et al. 2018).

In tropical countries like Ethiopia where climatic and soil properties are highly variant within a short distances, it is difficult to develop and recommend cultivars that perform best across regions or zones. Hence it calls for breeding/selecting cultivars that fit to each and specific crop growing areas. This is almost impossible on the basis that about 80% of the population (World Bank, 2017) occupying over 90% of the area in the country have

extremely variable climatic and soil conditions. In addition, the current versatile nature of climatic conditions like rainfall and temperature makes the environment unpredictable so that crop improvement for certain location is very challenging. Moreover, the agricultural research structure in Ethiopia is entirely public that gets funding from government treasury, which is unable to conduct breeding activities in every climatic and edaphic variable pocket areas. Therefore, the breeding strategy should focus on looking for genotypes that show reasonable and consistent yielding performance over diverse location and season.

5.3. Plasticity and stability traits as indices for stable grain yielding

To improve yield stability it is important to know more about the genetic basis of plant responses to fluctuating environments. Phenotypic plasticity is the capacity of a given genotype to give different phenotypic values for a given trait under different environmental conditions (Bradshaw, 2006). Trait robustness or stability in contrary happens when genotypes show relatively consistent trait value for certain traits under diverse conditions. In plants, even short-term environmental stimulus like climate warming can highly alter vegetation functional structure and its relation to productivity (Debouk et al., 2015). The mechanism by which plants maintain perpetuation of the species is by making some fitness traits to be stable (Fisher et al., 2017). Stability and plasticity are trait specific.While some traits exhibit plasticity with environmental changes some do not show such character making them stable. For example, seed weight is considered to be one of the most stable traits in tomatoes (Fisher et al., 2017) and carob tree (Turnbull et al. 2006). However, these trait plasticity and stability are specific to genotypes within a given species (Valladares et al., 2006; Grenier et al., 2016).

Adaptive phenotypic plasticity is not free of cost (Van Kleunen and Fischer, 2005) and the cost increases with the level of plasticity in the population (Lind and Johansson, 2009). This cost varies based on environment, species and genotype and can have large number of sources (DeWitt et al., 1998). The cost can include (a) maintenance costs like sensory structure and metabolic regulation mechanism of plasticity (Edelaar et al., 2005) (b) production costs explained by structure associated with plasticity could be more costly than a genetically governed one (Ernande and Dieckmann, 2004) (c) genetic costs i.e., genetic linkage with deleterious alleles at other loci, pleiotropy, and epistasis (Dechaine et al., 2007), and (d) ecological costs i.e., the interaction between the new phenotype and other species for example plastic response to herbivory reduces attractiveness to pollinators (Valladares et al., 2007)

In the last decades, many research papers reported trait plasticity and stability from the point of view of fitness, ecology and evolution aspects (Hodgins and Rieseberg, 2011; Debouk et al., 2015). Little or no information is available from the point of view of utilizing traits plasticity and stability in crop improvement to be able to gain crop yield consistently over varied environments and which traits contribute for the stability of yield. The current study found out which traits were stable in barley landraces and suggested as a cause for grain yield stability.

5.4. Ethiopian barley genetic diversity

Genetic diversity can easily be understood as individual species possessing genes or allelesthat encodes fordifferent features from other individuals of same species. It is crucial for improvement of any cultivated crop. Barley, as an early domesticated crop, possesses huge genetic diversity in it. Some diversity studies have been done in barley

both within certain localities and across the globe from simple morphological analysis to more detailed molecular analysis (Abebe et al., 2010; Muñoz-Amatriaín et al., 2014).

Ethiopia, with its diverse agro-ecological and climatic features, is named as one of the eight Vavilovian centers of origin (Ladizinsky, 1998). The altitudinal variation ranging from 110 m below sea level in areas of Kobar Sink to 4,620 m. a. s. l. at Ras Dashen, temperature and rainfall differences coupled with edaphic factors creates a wide range of ecological conditions in the country (Abebe et al, 2010). Landraces of major crops like barley, teff, wheat, sorghum, chickpea, field pea, faba bean, cowpea, linseed, castor bean and wild relatives of some of the world's important crops are abundant in Ethiopia. In Ethiopia, the main cereal staples include maize, wheat, barley; sorghum and finger millet are grown in different places. The continued interaction of cultivated crop plants with their wild relatives under diverse ecological, social, and economic conditions has made Ethiopia one of the hot spots of genetic diversity of landraces (Harlan 1969). Particularly in barley, landraces have closer ties with its wild relatives. For instance, in a study by Morrell et al. (2014) estimates of nucleotide sequence diversity indicated landraces retaining >80% of the diversity in wild barley.

5.5. General conclusion

With the objective of characterizing the response of different barley genotypes to contrasting environments, determining traits that contribute to the yield stability in barley landraces and investigating population structure of barley genotypes with different temporal and spatial background, 18 barley accessions and cultivars were investigated in two locations (Ambo and Jimma), two seasons (2012 and 2013) sown at four staggered

sowing dates. In addition 222 genotypes (some were also field studied) were genotyped using GBS to investigate population genetics parameters. The following conclusions can be inferred which can have significance contribution to barley breeding and conservation programs in Ethiopia:

1. Landraces showed, on average, higher static yield stability than improved cultivars with a comparative grain yield.
2. Environments under investigation were grouped in to two based on location i.e., location had higher influence than season and different sowing dates
3. Kernels per spike and fertile tiller can be proposed as robust traits in barley breeding for a wider adaptation as they had highly significant positive total effect on grain yield
4. Days to 50% flowering and Days to maturity were seen highly plastic along with thousand kernel weight while, fertile tiller and kernel per spike were seen stable in landraces leading the landraces relatively givingstable grain yield compared to cultivars.
5. In Ethiopian barley, genetic variation between regions and altitudes were less pronounced than within region and altitude variations. This calls for the germplasm collection strategies to be cautious in considering location and altitude as a main factor of variation and strategies should focus on exploiting the within region variation for better germplasm conservation and utilization.

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