GENOME-WIDE ASSOCIATION STUDIES FOR DROUGHT STRESS TOLERANCE IN ETHIOPIAN BARLEY (*HORDEUM VULGARE* SSP. *VULGARE*) LANDRACES IN FIELD AND CLIMATE CHAMBER CONDITIONS

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Abbreviations

ABA, Abscisic acid

AMOVA, Analysis of molecular variance

ANOVA, Analysis of variance

AFLP, Amplified fragment length polymorphism

BAC, Bacterial artificial chromosome

BLINK, Bayesian-information and linkage-disequilibrium iteratively nested keyway

CC, Climate chamber

CCn, Climate chamber control

cm, Centimeters

CRV, The central rift valley

CS, Climate chamber drought stress

DArT, Diversity arrays technology

DFL, Days to flowering

DM, Days to physiological maturity

DNA, Deoxyribo nucleic acid

DRFL, Days to reverse flowering

DZ, Debrezeit

DR, Dera

EBI, Ethiopian biodiversity institute

FC, Field control

FS, Field drought stress

Gb, giga bases

GB, Grain biomass in grams

GBS, Genotyping by sequencing

GDD, Growing degree day

gm, Gram

GWAS, Genome-wide association studies

ha, Hectares

HI, Harvest index

HL, Holetta

JKI , Julius Kuehn Institute

LD, Linkage disequilibrium

Abbreviations

lsmeans, Least square means

MAF, Minor allele frequency

Mb, Mega bases

MTA, Marker trait association

MK, Melkassa

NSdPS, Number of seeds per spike

OA, Osmotic adjustment

p, Probability value

PCoA, Principal coordinate analysis

PH, Plant height in cm

PVE, Phenotype variance explained

QTL, Quantitative trait locus

r, Pearson's correlation coefficient

ROS, Reactive oxygen species

RWC, Relative water content

SSTa , Sea surface temperature anomalies

SDTE, Summation of the daily temperature effect

SNP, Single-nucleotide polymorphism

SPAD, Relative chlorophyll content

ssp., Species

SSR, Simple sequence repeat

TKW, Thousand kernel weight in grams

WC, Water capacity

1. Background

Barley

Barley (*Hordeum vulgare* ssp. *vulgare*) is one of the earliest crops cultivated for human consumption, with its domestication dating back over 10,000 years in the Fertile Crescent—a region encompassing modern-day Iraq, Israel, Jordan, Lebanon, Palestine, Syria and southern Turkey (Zohary et al. 2012, Mascher et al. 2016, Molina-Cano et al. 2024). It is a self-pollinated, diploid (2n=2x=14) cereal crop and evolved from a wild barley progenitor *Hordeum vulgare* ssp. *spontaneum* which belongs to the family *Poaceae* in the subfamily of *Pooideae* and tribe of *Triticeae*; one of the largest groups of monocotyledonous plants (Payne 1969, Badr et al. 2000). Based on acreage it is the fourth most important cereal crop in the world next to wheat, maize, and rice (USDA 2016).

Nowadays worldwide barley production is about 47.07 million hectares (ha), with a total of 156.80 million tons harvested, and an average yield of 3.04 tons per ha (USDA 2024). In 2024 developing countries excluding EU, Russia, US, UK and Australia account for about 30% (43.5 million tons) of the total world barley harvested and 42% (19.6 million ha) of the total production area (USDA 2024). Barley grain is primarily produced for malt and animal feed. Although it was used for food in previous centuries, its use has declined in developed countries of Europe and North America. However, it remains an important crop for preparing food recipes in regions such as North Africa, Ethiopia, the Middle East, Nepal and Tibet, Peru and Chile (Grando 2005). Moreover the crop leftover (straw) is also used for animal feed in West Asia, North Africa, Ethiopia, Eritrea, Yemen, the Andes region and East Asia, which indicates a multidimensional use of barley (Akar et al. 2004).

Although the use of barley for human consumption is low compared to other cereal crops, it is a source of some crucial nutrients like upto 70% of starch (Zhu 2017), 8-13% of protein (Senarathna et al. 2024), 4-9% of β-glucan (Izydorczyk et al. 2000), 2.5-3.1% of free lipids (Fedak and Roche 1977), 4.8%, 8.6%, and 13.4% soluble, insoluble, and total dietary fiber, respectively (Bader et al. 2019) and 1.5-2.5% of total minerals (Wotango and Kanido 2024). The dietary fiber and soluble dietary fiber in barley grain is beneficial in solving problems related to constipation (Liljeberg et al. 1999, Lazaridou and Biliaderis 2007). Additionally, the fibers and β-glucans also contribute to reducing blood cholesterol and glucose levels, which ultimately helps to reduce the risk of heart and type-2 diabetes disease (Cavallero et al. 2002). Consumption of foods prepared from whole barley grain also leads to increased satiety, which may result in weight loss (Baik and Ullrich 2008). Ancient people thought that barley based foods are healthy and gave strength and stamina (Lukinac and Jukić 2022). In Ethiopia, thick porridge which is also known as '*genfo*' prepared from barley flour, spiced butter, and pepper powder is still believed to help a birth mother recover quickly and regain her strength soon (Hannig 2014).

Barley Production in Ethiopia

In Ethiopia, barley ranks fifth in annual production, area coverage, and production, after tef, maize, wheat, and sorghum. Its production accounts for 5.63% of the total cereal production, i.e. 799,127.84 ha acreage with a productivity of 2.6 tons per ha in 2021/22 (CSA 2022). It grows in a wide range of agro-ecologies from low lands drought-prone areas up to an elevation of 1500 to 3400 meter above sea level of Ethiopian highlands which have temperate type climate with an adequate amount of rainfall (Yaynu 2006).

Ethiopia is recognized as a center of diversity for barley. Its landraces have divergent population structure, with distinctive diversity from the rest of the world wide barley collections (Jørgensen 1992, Muñoz-Amatriaín et al. 2014, Milner et al. 2019). Chloroplast deoxyribonucleic acid (DNA) is important to study the evolutionary history of species as it is only inherited from the maternal parent. According to research on barley domestication using chloroplast DNA, Ethiopian landraces are very different (Orabi et al. 2007, Civáň et al. 2024). Orabi et al. (2007) did not confirm the presence of the wild barley progenitor in Ethiopia, and they suggested that it might have vanished due to intensive grazing by domestic or wild animals. The geographical isolation of the country and the presence of broad-farming systems due to diversified soil types, weather condition, climate, and landscape mainly contributed to the genetic diversification in Ethiopia (Harlan 1976, Mekonnon et al. 2015). Other social factors such as selection of genotypes based on their suitability for end-use have also influenced genetic diversity (Mulatu and Lakew 2011). Therefore, the unique features of Ethiopian barley landraces are due to collective long-term mutations, hybridization, gene recombination and natural and human selection in diversified growing conditions (Lakew and Alemayehu 2011).

Barley is mainly used as food and for preparation of local beverages in Ethiopia (Mohammed et al. 2016). Ethiopian barley producers had given it the nickname '*gebs yeehil nigus*' which means 'barley is the king of cereals', due to its suitability to prepare almost all types of traditional food recipe made from cereals. Various traditional dishes are prepared from barley such as '*injera*', '*dabo*' (bread), '*kolo*' (roasted de-hulled grain), '*genfo*' or '*tihlo*' (thick porridge), and '*beso*' (barley flour juice), as well as alcoholic beverages such as '*tella*' (local beer), '*areke*' (local gin), '*keneto*' (alcohol-free drink) that play an important role in the socio-economic and cultural life of Ethiopia (Mohammed et al. 2016).

Even though barley is only the fifth most important cereal crop in Ethiopia, this time its cultivation increases significantly in terms of production, and productivity as the country uses it as one way of poverty eradication (Rashid et al. 2015). The number of smallholder barley farmers jumped from 3.5 million in 2003/04 to 4.1 million in 2014/15 and decrease to 3.6 million in 2021/22. At the same time, production increased from 1.0 million tons in 2005 to 2.7 million tons in 2015 and slightly decrease to 2.07 million tons in 2021. Productivity increased from 1.17 metric tons per ha in 2005 to 2.18 metric tons per ha in 2019 to 2.59 metric tons per ha in 2022 (CSA 2014, CSA 2015, CSA 2019, CSA 2022).

Different constraints were reported, that challenge the production and productivity of barley in Ethiopia. Constraints such as low soil fertility, soil acidity, poor soil drainage, frost, and drought are categorized under abiotic stress (Mulatu and Lakew 2011). Additional constraints are diseases including, scald, net blotch, spot blotch and rusts as well as insect pests like aphids and barley shoot fly (Mulatu and Lakew 2011, Tadesse and Derso 2019). These are the major barley production constraints in Ethiopia Moreover, social factors like low adoption rate for improved barley technologies by farmers and low prices compared with other crops are also production limiting factors (Chilot et al. 1998).

The presence of diverse agro-ecological zones, which is mainly explained by differences in soils, topography, minimum and maximum temperature, humidity and the onset and cessation of the rainy season and the amount of rainfall, contributes to variable planting dates across the country (Tsega 2013, Mekasha et al. 2014). Such variability combined with the occurrence of unpredictable drought stress at any stage of crop development during the main cropping season creates difficulties for researchers to generate suitable technologies for barley production in different agro-ecological zones (Wosene et al. 2015). The effect of such problems becomes very serious in arid and semi-arid regions where total annual rainfall is high but irregularly distributed in the main cropping period.

Encyclopedia Britannica defines drought as a lack of sufficient rain for an extended period that causes a significant hydrologic (water) imbalance which leads to water shortages, crop damage, stream flow reduction, and depletion of ground water and soil moisture (Encyclopædia Britannica 2018). It is considered as the main crop production challenge which significantly decreases crop productivity in drought prone regions, where most of the world's resource-limited farmers reside (Nguyen et al. 1997).

Drought is becoming frequent and has a damaging effect on Ethiopian agriculture. The analysis of years' data of rainfall patterns reveals a statistically significant decrease in the frequency of severe drought at north and northeast regions of Ethiopia which experienced famine in 1983/1984. However, the occurrence of a repeated and moderate drought situation is reported in recent years (Kenawy et al. 2016, Zeleke et al. 2017). In the eastern, southern and southwestern part of Ethiopia a significant decline in annual total precipitation during the main cropping season (June-September) and the occurrence of a more frequent and intense drought was observed (Kenawy et al. 2016, Zeleke et al. 2017). Based on these studies, dryness/wetness over northern Ethiopia corresponds largely to El Niño–southern oscillation variability in both, the spring and summer rainy seasons, while the drying trend in south and southwest Ethiopia is associated with Atlantic ocean warming and sea surface temperature gradients across the western Pacific ocean.

In Ethiopia, seasonal drought caused by El Niño leads to a significant yield reduction, for example from 1997/98 El Niño season up to 25% decrease in production compared to the previous year was reported (USAID 2015). Therefore, El Niño remains a major challenge for crop production in Ethiopia (Berhane and Tesfay 2020, Amare et al. 2024). The occurrence of high sea surface temperature anomalies (SSTa) over Nino regions has a strong association with the variation of rainfall. Particularly in Ethiopia, the

Nino region's high SSTa resulted in a low amount of rainfall and high variability of rainfall patterns during the major (June–September) cropping season (Abdisa et al. 2017).

Mechanisms of Drought Stress Tolerance

Understanding the effects of drought stress on plants is vital to improve crop management and to develop drought tolerant cultivars (Chaves et al. 2003). Plants use different strategies to combat water deficiency. If plants grow in their native ecology, they will have a better potential to withstand fluctuating environmental conditions (Chaves and Oliveira 2004). During the occurrence of moisture deficit periods, plants respond to the stress by applying several physiological, morphological and molecular adaptation mechanisms to minimize the damage. Generally, these adaptation mechanisms can be classified into escape, avoidance and tolerance strategies (Turner 1986). However, during drought periods, plants do not use only one strategy for tolerance but a combination of all three strategies (Ludlow 1989).

In the drought escape strategy, plants complete their life cycle and produce seeds before the occurrence of drought stress (Chaves et al. 2003). Flowering time is the central trait that is directly related to the drought escape (Araus et al. 2003). Therefore, selection of early maturing varieties could be an effective strategy to escape from total crop failure caused by terminal drought (Kumar and Abbo 2001). However, crop productivity is generally associated with the duration of optimum growing conditions, therefore, the use of early maturing genotypes always compromises the amount of yield (Turner et al. 2001). Several flowering time governing genes related to photoperiod, vernalization, and earliness are identified in barley (Laurie et al. 1995, Snape et al. 2001, Cockram et al. 2007, Wang et al. 2010a, Fernández-Calleja et al. 2021, Cosenza et al. 2024).

In the drought avoidance strategy, plants avoid drought in one or a combination of the following two methods. In the first one they reduce water loss through clothing stomata, reducing leaf surface by rolling their leaves (Ehleringer and Cooper 1992), having narrow-leaf angles or reducing their total leaf area by producing smaller leaves and/or shedding of older leaves (Chaves et al. 2003). Shedding of older leaves has an extra advantage as it helps plants to relocate nutrients from older leaves to younger stems and leaves, which is mainly promoted by drought-induced proteases known as cysteine proteases (Khanna-Chopra et al. 1999).The second one is through maximizing water uptake by developing a high root to shoot ratio (Jackson et al. 2000). Drought-tolerant barley genotypes have been reported to exhibit reduced stomatal conductance (González et al. 1999, González et al. 2010, Hein et al. 2016, Lv et al. 2023), an increase in root to shoot ratio (Afshari-Behbahanizadeh et al. 2014), and the ability to shed older leaves (Parrott et al. 2007, Parrott et al. 2010).

In the drought tolerance strategy, plants withstand desiccation, despite the presence of low tissue water content, and are able to continue the major metabolic and physiological activities (Morgan 1984). Plants tolerate a low tissue water status by different physiological mechanisms like using high xylem resistance

through drought-induced 'air cavitation' which stabilizes water transport and gas exchange throughout the tissue and helps youngest leaves (mainly meristems) to stay alive at low water content and potential (Kursar et al. 2009).

Drought tolerance may also be related to osmotic adjustment (OA), the presence of rigid cell walls or smaller cells (Barnabás et al. 2008), and the efficient scavenging of reactive oxygen species (Sairam et al. 2000). During the onset of extreme drought stress, the accumulation of compatible solutes, or osmoprotectants, that perform as osmolytes to protect cellular function, helps plants to avoid cell damage. Additionally, amino acids (proline and citrulline), onium compounds (glycine betaine, 3-dimethylsulfonopropionate), monosaccharides (fructose), sugar alcohols (mannitol and pinitol), and di- and oligo-saccharides (sucrose, trehalose, and fructan) play a role in drought tolerance (Yokota et al. 2006). A reduction in leaf solute potential at full turgor during drought stress for wild barley (*H. maritimum*) and cultivated barley was reported by Maroua et al. (2016). As per this study, drought treatment increased the accumulation of compatible solutes (proline and soluble sugar), and the concentration of inorganic solutes (K^+ in cultivated barley and Na^+ in wild barley) in comparison to the control treatment.

Different barley genotypes show different metabolic responses to drought stress. Such variation is related to the difference in the genetic makeup , the intensity of the drought stress or the interaction of growth stage of respective genotypes with various levels of drought intensity (Rosegrant and Cline 2003). The biochemical responses of two different barley genotypes under similar drought stress conditions were tested, and an increase in the concentration of superoxide dismutase (which is used as defense against the accumulation of reactive oxygen species), ascorbate peroxidase, and catalase (which are involved in scavenging of hydrogen peroxide) were measured (Harb et al. 2015).

Drought stress was reported to increase the concentration of many root and leaf metabolites like amino acids, carbohydrates, lipids, carboxylic acid, nitrogen compounds, sugar acids, Krebs cycle acids, and many unknown metabolites, at various levels based on genotypic differences and the effect of drought compared to control plants (Swarcewicz et al. 2017). Plants exposed to drought stress also exhibit an increase in the accumulation of callose, superoxide, and chitinase content, and in the activity of acid invertase and the total amount of phenols and flavonoids, which are highly involved in defense against abiotic stresses by an improvement of the antioxidant activity (Ahmed et al. 2015).

Plant response to mitigate the effect of drought stress is governed by cascades of diverse molecular pathways which are activated by the perception of drought stress signals. Drought inducible regulatory genes such as transcription factors and protein kinases are either up or down regulated (Joshi et al. 2016, Singh et al. 2019). The expression of these genes directly affects the response of a number of traits that are involved in the mitigation of drought stress (Guo et al. 2009).

Groups of proteins related to jasmonate, metallothionein, late-embryogenesis-abundant and abscisic acid (ABA) are highly expressed during drought while the expression of proteins related to photosynthetic activities is significantly reduced. Additionally, there are functionally unknown genes which expression is affected at different levels (Li et al. 2007). Proline is one of the most important biochemical compounds that have a considerable role for the adaptation to drought (Blum 1998) as well as the pyrroline 5-carboxylate dehydrogenase, that catalyzes the conversion of proline to glutamate, in which glutamate controls the guard cells (Ayliffe et al. 2005, Qiu et al. 2020). The transcriptom analysis also revealed that reactive oxygen species (ROS) related genes are involved in the regulation of stomata response and ABA induction (Lv et al. 2023).

Therefore, to exploit genetic variation among different genotypes, traits influencing the morphological development as well as physiological and metabolic pathways during drought stress and their relation to stress-induced genes have to be analysed in detail.

Traits Involved in Drought Stress Tolerance of Barley

Plants reaction to drought is very complex, as it is due to climatic, soil and agronomic effects (Chaves et al. 2003). The response of plants under drought condition is complicated and depends on their genetic makeup, the growth stage at which stress occurred, as well as the duration and intensity of the drought stress (Rosegrant and Cline 2003). The presence of complex reactions to drought stress and the lack of understanding on how plants respond to drought, create difficulties in identifying the appropriate traits involved in drought stress tolerance and use these for crop improvement (Serraj et al. 2003). Significant variation in drought tolerance has been reported among different plant species, as well as among various cultivars of wheat (Erdei et al. 2002) and barley (Khalili et al. 2013). Barley genotypes that are able to withstand drought stress, are able to exploit different morphological, phenological, physiological and hormonal processes, which are governed by multiple genes that vary based on the environment (Fatemi et al. 2022).

The role of different phenological and physiological traits has been reported for drought tolerance among different barley varieties. Days to flowering and maturity are widely studied and are the best known phenological traits that significantly affect grain yield during drought periods (Vaezi et al. 2010, Barati et al. 2017). Reproductive organs (like the number of spikes and number of grains per spike), and physiological traits (like stomatal conductance and net photosynthesis rate), which have a positive correlation to grain yield, are significantly reduced by drought stress at the terminal growth stage (González et al. 2010, Thameur et al. 2012). Other developmental parameters like relative chlorphyll content (SPAD) value, plant height, fresh biomass weight, fresh root weight, dried biomass weight, and dried root weight, are also moderately reduced under drought stress (Zhao et al. 2009).

Physiological traits associated with water management like OA and proline, are negatively correlated with growth (Blum 1989). Water-use efficiency, carbon isotope discrimination (Farquhar and Richards

1984) and relative water content which are positively correlated with the number of tillers, number of leaves and fresh biomass weight in drought stressed barley are also considered as the most important parameters for drought tolerance (Teulat et al. 1997a, Teulat et al. 2003).

The biomass weight is directly correlated with the number of tillers and influenced by management practices and the environment (Davidson and Chevalier 1987, Křen et al. 2014). Numerous studies have demonstrated that the number of tillers is significantly reduced during drought periods, due to the reduction of the osmotic potential around the root, which delays the emergence of new tillers, and finally affects the total biomass by reducing the number of ears per square meter and the number of leaves (Davidson and Chevalier 1987, Samarah 2005, Křen et al. 2014). On the contrary, genotypes having a lower number of tillers are selected for the management of terminal drought in cereals because such genotypes are able to avoid the loss of limited soil moisture through transpiration (Duggan et al. 2005). An average of 0.02% increment for harvest index, and 7% reduction of total biomass were reported for wheat genotypes carring the tiller inhibition (*tin*) gene compared to genotypes without the *tin* gene in terminal drought stress experiments (Duggan et al. 2005).

Grain yield is the most important and most heavily drought stress affected trait. Different studies estimated different rates of grain yield reduction, but a significant loss is caused when drought occurred during the post-anthesis period (Samarah et al. 2009b). For example, the yield of tolerant and susceptible genotypes was decreased by 25% and 50 to 55%, respectively, compared to the control treatment (Li et al. 2006) whereas a reduction of 73 to 87% of grain yield due to drought stress was reported by Samarah et al. (2009b).

The length of the grain-filling period, plant height, total spike number, number of seeds per spike, tiller number, kernel weight, dried biomass, and harvest index decreased significantly as the severity of drought stress increased (Thameur et al. 2012). These traits are positively correlated with grain yield (González et al. 2010, Thameur et al. 2012).

Although how plants recognize drought stress is under investigation, it is assumed that roots play a major role in signal transduction, and ABA is considered the primary plant hormone governing drought stress response (Raghavendra et al. 2010). ABA is one of the major hormones involved in the adaptation and regulation of plant growth during drought stress. It helps to control water loss by managing stomata closure by promoting a loss of turgor around the stomatal guard cells and induces expression of other stress-related genes.

The Barley Genome and Molecular Marker Development

The barley genome is about 5.1 giga bases (Gb) and has seven chromosomes (Mayer et al. 2011). The genome size of *Hordeum* species were estimated as from 6.85 to 10.67 pg for diploids (2n = 2x = 14) and up to 29.85 pg for hexaploid (2n = 6x = 42) species (Jakob et al. 2004). Chromosome 2H is the

largest, followed by chromosomes 5H, 3H, 7H, 4H, 6H and 1H and the genome has a structural similarity with the hexaploid wheat (*Triticum aestivum*) A, B, and D sub-genomes (Mayer et al. 2011).

Repetitive sequences are reported to constitute a significant portion of the barley genome, with estimates of 70% by Rimpau et al. (1980), and 80% by The International Barley Genome Sequencing (2012). Rimpau et al. (1980) reported short unique sequences interspersed with tandem repeated sequences account for about 50 to 60% of the barley genome. In contrast, Mascher et al. (2017) reported about 3.7 Gb (80.8%) of the assembled sequence as derived from transposable elements, while 65.3 Mb (1.4%) are identified as annotated coding sequences. In a recent barley pangenome study, the clustering of orthologous gene models resulted in 40,176 orthologous groups, with 55% of these groups belonging to the core genome (Jayakodi et al. 2020). The barley genes are interrupted by introns which are spliced out during the transcription process. Based on a study of the total of known genes only 22% of the genes are possessing a single exon while the rest carries multiple exons, with an average of 11 exons per gene, and 1.94 transcripts per gene (Simpson et al. 2018).

In 2012 the International Barley Genome Sequencing Consortium published a partly assembled an ordered physical, genetic and functional sequence of barley (The International Barley Genome Sequencing 2012). The physical map of the barley genome comprised 9,265 bacterial artificial chromosome (BAC) contigs which covers 4.98 Gb of the genome size.

Different methods were also used to exploit the genomic resource for barley. The GenomeZipper adopted reference genomes obtained from Brachypodium (B*rachypodium distachyon*), rice (*Oryza sativa*), and sorghum (*Sorghum bicolor*) (Mayer et al. 2011). Furthermore, the Illumina 9K SNP chip, designed to deliver a standardized, high-throughput genotyping platform, was developed using RNA sequence polymorphisms from 10 diverse barley varieties (Comadran et al. 2012). In contrast, GBS (genotyping by sequencing) offers greater flexibility and higher-density output, e.g. 34,000 mapped SNPs were identified by this approach (Poland et al. 2012). POPSEQ is an advanced genomic approach that enhances barley genome studies by enabling ultra-high-density genetic mapping and anchoring approximately 90% of the assembled genome sequence (Mascher et al. 2013). Based on this findings, Ariyadasa et al. (2014) developed a high-quality physical map using fingerprinted BAC libraries and anchored it to a genetic map using 2 million SNPs identified through POPSEQ (Mascher et al. 2013). The GenomeZipper and POPSEQ technologies were evaluated on all chromosomes for known loci and provided 97.8% and 99.3% accuracy, respectively (Silvar et al. 2015). Moreover, using 15,622 BAC assemblies; 17,386 high confidence and 21,175 low confidence genes were reported (Muñoz-Amatriaín et al. 2015).

In 2015, a web-based tool to access the genome of barley was designed, which facilitates an easy access to the physical map of barley for the research community (Colmsee et al. 2015).

In 2017, the 50k SNP chip was developed for barley (Bayer et al. 2017) using published exome capture data which were combined with the SNPs of the barley 9k iSelect chip. The markers were developed based on an accurate assembly of 87,075 sequenced BACs (Mascher et al. 2017) and more than 80,000 sequenced BAC clones (Beier et al. 2017) in which a total of 39,734 high-confidence genes (Mascher et al. 2017) and 2,637 SNPs (Beier et al. 2017) were identified. Finally, 49,267 SNPs were obtained from which 42,316 SNPs were from exome capture data and 6,951 SNPs were from the 9k iSelect chip. The highest number of SNPs was mapped on chromosome 5H (7758) followed by 2H (6932), 3H (6480), 7H (6194), 6H (5228), 4H (5043) and 1H (4681).

In previous studies the barley genome was studied using a single reference genome assembly (The International Barley Genome Sequencing 2012, Mascher et al. 2017), which lacks to capture all variations in the barley genome. Therefore, the barley pangenome was developed. The first barley pan-genome assemblies were constructed using 20 barley landraces, cultivars and wild barley of which two were Ethiopian landraces (Jayakodi et al. 2020). Using pairwise comparisons of 19 chromosome-scale assemblies against the Morex reference genome, Jayakodi et al. (2020) identified 1,586,262 present-absent variations (PAVs), ranging in size from 50 bp to 999,568 bp, and revealed a significant enrichment of low-frequency variations. The second pan-genome assembly was conducted using 76 barley landraces including three Ethiopian landraces, cultivars and a wild barley. This study successfully retrived 142,521,520 SNPs and 15,420,245 indels. It was shown that some of them are involved in important traits such as disease resistance, kernel number, and tillering capacity (Jayakodi et al. 2024).

Genome Wide Association Studies in Barley

Association mapping is a powerful genetic approach used to map the relationship between genetic markers and phenotypic traits across diverse populations. The method involves genotyping a population, collecting phenotypic data, and developing statistical models to account for population structure and kinship, thereby avoiding false positive/negative associations. It is widely used to accelerate marker-assisted breeding in crops, as described by Langridge and Fleury (2011), and Singh and Singh (2015). Two types of association mapping are mainly used in crop research. The first one is candidate gene association mapping which is based on the detection of polymorphism in a specific gene controlling the phenotypic variation (Zhu et al. 2008). The second one is genome-wide association study (GWAS), which used to scan the whole genome to find polymorphisms and predict their association with complex traits. It is considered as a promising technology as it uses new high-throughput genotyping technologies with minimized cost (Ersoz et al. 2007, George and Cavanagh 2015). The technology provides an alternative method for biparental QTL mapping and has already been extensively applied in barley to map different QTLs (Igartua et al. 1999, Kraakman et al. 2004, Kraakman et al. 2006, Inostroza et al. 2008, Pswarayi et al. 2008, Comadran et al. 2009, Beattie et al. 2010, Massman et al. 2010, Comadran et al. 2011, Sun et al. 2011, Pasam et al. 2012, Rode et al. 2012, Visioni et al. 2013, Novakazi et al.

2019, Capo-chichi et al. 2021, Makhtoum et al. 2022) and major genes (Ivandic et al. 2003, Stracke et al. 2007, Cockram et al. 2008, Fricano et al. 2009, Matthies et al. 2009a, Matthies et al. 2009b, Stracke et al. 2009, Haseneyer et al. 2010, Fazlikhani et al. 2019, Pidon et al. 2021).

GWAS exploits historical genetic recombination and/or natural genetic variation in non-related sets of accessions to identify quantitative traits influencing phenotypic variation. It is based on linkage disequilibrium (LD) that is the non-random co-segregation of alleles at different loci (Ersoz et al. 2007). The statistical analysis behind is used to measure the degree of association of a marker at a locus with a phenotypic trait. A marker trait association (MTA) is present when a marker, that affects the measurable trait, is in LD with this trait (Ersoz et al. 2007, George and Cavanagh 2015). LD can be affected by different reasons such as genetic drift which could happen over generations, natural or artificial selection within populations, and population admixture (Remington et al. 2001, Flint-Garcia et al. 2003).

Different kinds of markers, including AFLPs (Amplified fragment length polymorphism), SSRs (Simple sequence repeat), and SNPs (Single-nucleotide polymorphism) can be used for association mapping (Madhusudhana 2015). AFLP markers can be easily developed for all organisms, but with limited power to distinguish heterozygous from homozygous genotypes, as they are dominant markers (Mueller and Wolfenbarger 1999). Whereas SSR markers are polymorphic but need some work to be develop and can be only transferred from closely related species (Vinod 2011). After the completion of the human genome project, SNP markers become a very popular source of genetic variation. SNPs are biallelic, more polymorphic than SSR markers and present in the whole genome (Vinod 2011, Mammadov et al. 2012). Currently, SNPs markers are vastly used for GWAS.

The first GWAS application in a crop was in maize for identifying genes responsible for encoding fatty acid desaturase (Beló et al. 2008). However, contrasting results were obtained by the use of different genetic markers. For instance, the application of Diversity Arrays Technology (DArT) markers (Lex et al. 2014) and Illumina GoldenGate Bead Array Technology (Rode et al. 2012) in barley identified varying numbers of associations for specific traits, with only a limited number of common associations shared between the two methods.

Association mapping was used in barley to identify MTAs for tolerance to leaf rust and barley yellow dwarf virus using SSR and Illumina iSelect markers (Perovic et al. 2013), QTLs associated with resistance to the spot form of net blotch (Afanasenko et al. 2015, Tamang et al. 2015, Novakazi et al. 2019), powdery mildew resistance (Genievskaya et al. 2023). Furthermore, GWAS was used to identify to identify MTAs associated with root and shoot traits under well-watered and stressed conditions (Reinert et al. 2016) as well as MTAs for chlorophyll content and days to flowering during drought stress (Elbasyoni et al. 2022). The study by Mamo et al. (2014) explored the genetic variation in Ethiopian/Eritrean barley landraces, focusing on grain zinc and iron concentrations as well as kernel weight. Additionally, Bellucci et al. (2017) investigated grain yield, straw related parameters in selected winter barley genotypes. Both studies identified significant marker associations related to these traits.

Screening of Asians barley genotypes for salt tolerance also identified a vast number of QTLs associated with salt tolerance (Sbei et al. 2014).

The development of the 50k iSelect SNP chip for barley helped to increase the marker density and improve the diagnosis of important QTL regions associated with different traits. Association mapping using this chip e.g. revealed markers related to undesired fertility restoration (Bernhard et al. 2019). The genetic relationship between different traits including the number of tillers, spike row types and photoperiod determining flowering time were studied and the result indicated the presence of more overlapping QTLs between the number of tillers and flowering time than for number of tillers and spike row type (Haaning et al. 2019). MTAs with spike productivity traits (Rozanova et al. 2023) were also studied. Several SNP markers responsible for resistance to different isolates of spot blotch disease were also identified by Novakazi et al. (2019).

The first GWAS on drought tolerance in barley used 816 markers to study yield traits under drought and optimal conditions, identifying a few MTAs explaining a low phenotypic variance (Varshney et al. 2012). Young seedlings of different barley genotypes grown under optimum and deficit moisture condition were used to identify MTAs for developmental, physiological and metabolic traits. The results revealed some QTLs associated with leaf senescence under moisture deficit condition (Wehner et al. 2015). QTLs influencing leaf water content and water use efficiency under drought stress conditions were also reported by Wehner et al. (2016b). In the study two genes involved in drought stress tolerance (*GAD3* and *P5CS2*) and one leaf senescence controlling gene (*Contig7437)* were identified. A drought stress experiment conducted under controlled water conditions also reported MTA for water use efficiency, relative water content (RWC), and photosynthesis-related traits (Wójcik-Jagła et al. 2018).

Back cross population developed using two Spanish landraces having better adaptation to arid environment and elite cultivars were used to identify QTL region related with grain yield and other agronomic traits using the 50k SNP chip. The result revealed that grain yield is mainly improved due to the presence of alleles from elite cultivars whereas parameters like flowering time, kernel weight and green area coverage are increased by alleles obtained from landraces (Monteagudo et al. 2019). MTAs related to root system architecture, shoot and root biomass traits under control and drought stress conditions were also identified by Khodaeiaminjan et al. (2023)

QTL for Drought Stress Tolerance

Landraces and wild species of barley are a useful source of variation to identify molecular markers (Kishore et al. 2016). Marker based introgression of the appropriate genes from these sources will improve yield stability. The advancement of molecular marker technologies has transformed the genetic analysis of plants and eases the identification of genes or QTL (Tester and Langridge 2010). Using this technology, genetically important QTLs involved in plant developmental, physiological and metabolic

processes during the drought period were investigated. The method also encourages breeders to use marker-assisted selection for genetic improvement (Baum et al. 2007).

Adaptation of genotypes for drought conditions could be assessed based on their variation on major developmental parameters. According to Forster et al. (2004), the common parameter of variation include plant height, spike row type-controlling genes, and variation in flowering time which are either controlled by photoperiod and/or vernalization genes. These major developmental genes are most likely to exhibit a pleiotropic effect on multiple other genes (Araus et al. 2003). The QTL regions governing the control of these traits were also reported to have an association with dehydrins which play a role in adaptation to drought stress and rubisco activase which are directly involved in photosynthesic activity (Forster et al. 2004). A pleiotropic effect for QTLs controlling plant height on chromosome 3H with days to flowering, grain yield, and biomass yield was also reported (Baum et al. 2003). Additionally close to the known barely flowering time gene (*Ppd-H1*), numerous traits associated with plant height and kernel length were reported by Gordon et al. (2020).

Chlorophyll has a vital role in photosynthesis, and its content is significantly reduced in the occurrence of drought stress (Zahra et al. 2023). QTLs controlling the chlorophyll content were studied by different researchers. Two QTL regions responsible for the development of functional chloroplasts at the post-flowering stage were identified on chromosome 2H. These, account for about 15% and 9% of the phenotypic variance during drought (Guo et al. 2008). QTLs related to the leaf chlorophyll content on chromosomes 1H, and 4H under drought stress conditions were also identified (Obsa et al. 2016). The SPAD readings are directly correlated with the chlorophyll content, and QTLs linked to this trait were identified on chromosomes 5H and 7H by Liu et al. (2015), and Wehner et al. (2015). Moreover, associations of the chlorophyll content with the net photosynthetic rate, stomatal conductance, flag leaf area, and leaf nitrogen content were reported by Liu et al. (2015). QTLs associated with leaf wilting during drought stress were identified on chromosome 2H and 5H (Fan et al. 2015). An increase in tolerance to leaf wilting due to the introgression of exotic alleles from *H. vulgare ssp. spontaneum* was also observed, and those QTL were mapped on chromosome 1H, 2H, 3H, and 4H (Sayed et al. 2012). QTLs involved in minimizing water loss through promoting leaf rolling were identified on chromosomes 2H and 3H (Obsa et al. 2016).

Important QTL regions influencing early maturity and kernel weight were identified that are correlated to grain yield (Cuesta-Marcos et al. 2009, Nadolska-Orczyk et al. 2017). As earlines accelerates fast remobilization of nutrients from source to sink, this trait may be used as a valuable option in breeding programs (Long et al. 2003). An exotic QTL located on chromosome 4H of wild barley (*H. vulgare* ssp *spontaneum*) was reported to infulence kernel weight (Honsdorf et al. 2017). The QTL analysis study conducted by Niu et al. (2022) identified QTLs at the tillering, flowering, and grain-filling development stages explaining 9% to 38% PVE values.

There are plenty of genes that are up- or down-regulated during drought stress, though most of these genes are usually highly expressed during the drought period. The particular roles of many stress-induced genes are unknown (Cattivelli et al. 2002). However Dehydrins that play a role in drought stress were reported to be highly expressed during drought stress (Close 1997, Cattivelli et al. 2002). Furthermore, a higher proline content during drought stress and associated QTLs were reported (Sayed et al. 2012). Besides this, QTLs associated with osmotic adjustment, RWC, carbon isotope discrimination, and water use efficiency were detected in different chromosome regions (Teulat et al. 1998, Teulat et al. 2001, Teulat et al. 2002, Diab et al. 2004).

Objectives

Drought stress is a major factor for the failure of crop productivity in Ethiopia. Although Ethiopia is considered as the center of diversity for many crops, the country has failed to exploit the existing genetic resources for a modern crop improvement program to tackle the existing problems. Barley is grown from the warm and drought-prone areas at an elevation of about 1500 up to 3400 meter above sea level with an adequate amount of rainfall and temperate climate conditions. Thus, genome-wide association studies in Ethiopian barley landraces were conducted using the barley 50k iSelect SNP array and phenotypic data from field and growth chamber experiments with these aims:

- To study genetic diversity in Ethiopian barley landraces derived from different geographic origins and agro-ecological zones (**Publication 2.1**).
- To identify drought-tolerant barley genotypes from Ethiopian landrace collections (**Publication 2.2**).
- To identify genomic regions involved in drought stress response in barley using genome-wide association genetics studies (**Publication 2.2**).
- To identify the major environmental factors influencing flowering time of Ethiopian barley under different climate conditions (**Publication 2.3**).
- To identify MTAs associated with flowering time in Ethiopian barley genotypes and associated QTLs (**Publication 2.3**).

2. Original papers

Publication 2.1) Teklemariam, S. S., K. N. Bayissa, A. Matros, K. Pillen, F. Ordon and G. Wehner (2022). "The genetic diversity of Ethiopian barley genotypes in relation to their geographical origin." PLOS ONE 17(5): e0260422. DOI: https://doi.org/10.1371/journal.pone.0260422.

Abstract

Ethiopia is recognized as a center of diversity for barley, and its landraces are known for the distinct genetic features compared to other barley collections. The genetic diversity of Ethiopian barley likely results from the highly diverse topography, altitude, climate conditions, soil types, and farming systems. To get detailed information on the genetic diversity a panel of 260 accessions, comprising 239 landraces and 21 barley breeding lines, obtained from the Ethiopian biodiversity institute (EBI) and the national barley improvement program, respectively were studied for their genetic diversity using the 50k iSelect single nucleotide polymorphism (SNP) array. A total of 983 highly informative SNP markers were used for structure and diversity analysis. Three genetically distinct clusters were obtained from the structure analysis comprising 80, 71, and 109 accessions, respectively. Analysis of molecular variance (AMOVA) revealed the presence of higher genetic variation (89%) within the clusters than between the clusters (11%), with moderate genetic differentiation (PhiPT=0.11) and five accessions were detected as first-generation migrants using Monte Carlo resampling methods. The Mantel test revealed that the genetic distance between accessions is poorly associated with their geographical distance. Despite the observed weak correlation between geographic distance and genetic differentiation, for some regions like Gonder, Jimma, Gamo-Gofa, Shewa, and Welo, more than 50% of the landraces derived from these regions are assigned to one of the three clusters.

Key words: Ethiopian barley, landraces, population structure, genetic variation

Zusammenfassung

Äthiopien gilt als ein Zentrum der Diversität für Gerste. Die genetische Vielfalt der äthiopischen Gerste ist wahrscheinlich auf die sehr unterschiedliche Topographie, Höhenlage, Klimabedingungen, Bodentypen und Anbausysteme zurückzuführen. Um detaillierte Informationen über die genetische Vielfalt zu erhalten, wurde ein Panel von 260 Akzessionen, bestehend aus 239 Landsorten und 21 Gerstenzuchtlinien, die vom äthiopischen Institut für Biodiversität (EBI) bzw. aus nationalen Gerstenzüchtungsprogrammen stammen, mit Hilfe des 50k iSelect Single Nucleotide Polymorphism (SNP)-Arrays auf ihre genetische Vielfalt untersucht. Insgesamt wurden 983 hochinformative SNP-Marker für die Struktur- und Diversitätsanalyse verwendet. Aus der Strukturanalyse ergaben sich drei genetisch unterschiedliche Cluster, die 80, 71 bzw. 109 Akzessionen umfassten. Die Analyse der molekularen Varianz (AMOVA) ergab eine höhere genetische Variation (89 %) innerhalb der Cluster als zwischen den Clustern (11 %), mit mäßiger genetischer Differenzierung (PhiPT=0,11), und fünf Akzessionen wurden als Migranten der ersten Generation unter Verwendung von Monte-Carlo-Resampling-Methoden entdeckt. Der Mantel-Test zeigte, dass der genetische Abstand zwischen den Akzessionen kaum mit ihrer geografischen Entfernung zusammenhängt. Trotz der beobachteten schwachen Korrelation zwischen geografischer Entfernung und genetischer Differenzierung wurden für einige Regionen wie Gonder, Jimma, Gamo-Gofa, Shewa und Welo mehr als 50 % der aus diesen Regionen stammenden Landrassen einem der drei Cluster zugeordnet.

Schlüsselwörter: Äthiopische Gerste, Landrassen, Populationsstruktur, genetische Variation

Introduction

Barley (*Hordeum vulgare* L.) ranks fifth in the acreage and production of cereals after tef, maize, wheat, and sorghum in Ethiopia. It accounts for 5.63% of the total cereal production (811,782.08 hectares (ha)) with a productivity of 2.18 ton/ha in 2018/19 (CSA 2019). It is a widely adapted crop, cultivated from drought prone lowlands of 1,500 meters above sea level to highlands of Ethiopia with an altitude of 3,400 meters above sea level with adequate moisture (Yaynu 2006). Most of the barley acreage is located in the altitude range of 2,400 to 3,400 meter above sea level in the northern and central part of the country (Asfaw 2000). In Ethiopia, barley is an important cereal crop grown by smallholder farmers for subsistence with limited capacity for modern agricultural practices, and in areas where soil fertility, drainage conditions, and topography are not suitable to produce other crops (Lakew et al. 1997). It is cultivated in two seasons; '*meher'*, which is the major rainy season (June to October) in which diverse genotypes are grown, and '*belg'* with less amount of rain (late February to early July) in which most early maturing varieties are grown (Bekele et al. 2005). The total amount of barley production in '*meher'* is by far exceeding the one in '*belg'*, which covered 84.5% of the total area of production and 93.0% of the total yearly barley harvest in 2013/14 (CSA 2014).

The origin and domestication of barley believed to be the 'Fertile Crescent' (Badr et al. 2000). Ethiopia is recognized as a center of diversity for barley, as it is cultivated in a wide range of agro-ecology zones for centuries, and its landraces have exhibited distinct genetic diversity from the rest of the world's barley collections (Jørgensen 1992, Muñoz-Amatriaín et al. 2014, Milner et al. 2019).Using chloroplast SSR markers between Ethiopia/ Eritrean landraces and wild barley (*H. vulgare* ssp. *spontaneum*) revealed that wild barley might not be an ancestor for barleys of Ethiopia/ Eritrea and that these landraces might have different domestication path (Orabi et al. 2007). The presence of diversified and distinct genetic features have been explained by geographical isolation of the country from other barley growing regions for long periods together with the occurrence of diverse soil types, climate conditions, elevation, and landscape, which affect the type of farming system practices (Harlan 1976, Mekonnon et al. 2015). One study indicated that Ethiopian barley population structure depends on the farming system, elevation, and barley row types (Samberg et al. 2013). Additionally, social factors like a preference of genotypes suited for different use also contributed significantly to the diversification (Mulatu and Lakew 2011). Therefore, it was suggested that the diversity in Ethiopian barley landraces came due to a combination of long period accumulation of distant mutations, gene recombination, hybridization, natural selection, and human preference in a highly diversified agro-ecological environment (Lakew and Alemayehu 2011).

The genetic resources of Ethiopian landraces are still rich and well maintained, as a report indicated that 95% of the Ethiopian smallholder farmers use landraces as the major seed source (CSA 2018b, CSA 2018a). Although barley is an inbreeding species with less than 5% of outcrossing, an increased rate of outcrossing was reported in Ethiopia, which is probably related to abiotic stress or variable

environmental conditions (Abay et al. 2008). Barley landraces at hand of farmers are genetically highly variable (Backes et al. 2009, Tanto Hadado et al. 2010), as farmers mainly focus to maintain morphologically uniform seeds than genetically uniform seeds, thus, sampling from smaller plots of farmers' land may result in a collection of highly genetically diversified seeds (Asfaw 2000).

Traditionally, farmers classified barley landraces based on kernel type as hulled, hull-less, and partially hulled barley (Asfaw 2000). Additionally, participatory research on durum wheat landraces revealed that farmers also considered yield, quality related to end-use products, and tolerance to different abiotic and biotic stresses like drought and diseases for the classification and selection of landraces (Mancini et al. 2017). Ethiopian barley landraces are particularly diverse in morphological appearance (Abebe 2010, Muhe and Assefa 2011) and bio-chemical composition, e.g. different hordein polypeptide patterns (Asfaw 1989, Demissie and Bjørnstad 1997) as well as anthocyanin coloration on seed coats, leaf sheath and stems (Eticha et al. 2010a).

The genetic structure of a population is influenced by variation in geographical collection distance, presence of geographical barriers like wetlands, mountains and gorges, as well as by the compatibility of genotypes to cross to each other. Besides this, the genetic structure is also due to the presence of barriers on the human local population over a long period of time (Slatkin 1987).

Application of molecular tools improved the efficiency and precision of analysis of genetic relatedness in different crop species, as they helped to decipher whether the morphological, chemical, traditional and geographical classifications are in consistence to molecular structural analysis (Collard et al. 2005). Different kinds of markers, i.e. AFLPs (amplified fragment length polymorphism), SSRs (simple sequence repeat), and SNPs (single nucleotide polymorphism) were used for genetic analysis of different cultivars, breeding lines and related species of barley (Powell et al. 1997, Thiel et al. 2003, Assefa et al. 2007, Sreenivasulu et al. 2008, Owen et al. 2019, Tanaka et al. 2019). Currently, SNP markers are commonly used to study genetic variation, as they are more abundant, have a higher throughput performance than other markers, and enable to scan whole genomes thereby facilitating the association of markers with traits of interest (Vinod 2011, Mammadov et al. 2012). The development of a 50k iSelect SNP array by (Bayer et al. 2017) further enhanced the genetic exploration with accurate physical positions of the markers and detailed gene annotation.

The presence of genetic divergence between populations can be studied using Nei's genetic distance (Nei 1972). Genetic abundance or richness within a population can be explored using the Shannon index (Shannon 1948, Good 1953), whereas the variability within a population can be studied using heterozygosity indices (Nei 1973). The fixation index (FST) is widely used to investigate the genetic distance between populations (Wright 1978, Cockerham and Weir 1993). As described by (Whitlock and McCauley 1999) indirect estimation of net migration rate using FST, might be true. Therefore, Monte Carlo resampling methods (Paetkau et al. 2004) are better suited to study first-generation migrants, while the gene flow between genotypes from different geographic locations can be studied

using the Mantel test (Mantel 1967). The neighbor-joining tree method is used to graphically demonstrate the distance between different genotypes based on their genetic background (Saitou and Nei 1987).

Several studies on the genetic distance of Ethiopian landraces using different molecular markers were conducted. Distinctive genetic features of Ethiopian landraces compared to other barley collections were reported, although a minimum genetic distance between different Ethiopian landraces was detected using RFPLs (restriction fragment length polymorphism) markers (Bjørnstad et al. 1997). Another study revealed the presence of different levels of the allelic richness and genetic diversity in relation to altitude using seven SSR markers (Tanto Hadado et al. 2010). (Abebe and Léon 2013) also revealed a poor population structure for landraces collected from different regions of the country using 15 SSR markers. Genetic diversity studies of Ethiopian barley genotypes in relation to different world barley collections were also conducted using SSR (Orabi et al. 2007), and AFLP markers (Badr et al. 2000) and the findings suggested Ethiopia as a second center of barley domestication.

Therefore, the aims of this study were, (i) to investigate the genetic diversity of Ethiopian barley landraces, and (ii) to analyses the role of the geographic origin, and defined agro-ecological zones in the formation of genetic structure using a highly informative 50k iSelect SNP array (Bayer et al. 2017). The outputs of the study will support the strategic collection and exploitation of existing barley genetic resources, to improve the livelihood of the subsistence farmers through strategic utilization of genetic resources available on the hand of smallholder farmers.

Materials and methods

Plant materials

A panel of 260 Ethiopian barley accessions was analyzed in this study (S1 Table). The 239 landrace accessions were obtained from the Ethiopian Biodiversity Institute (EBI). These were collected from diverse agro-ecological zones and represent different geographical regions of Ethiopia. The geographical locations in which the landraces were collected are shown in Fig 1, which is constructed using the GPS data of the collection area using the free ArcGIS online web program (https://www.arcgis.com) provided by Esri (ArcGIS_Online 2020). Additionally, 21 barley breeding lines were obtained from the national barley improvement program of the Holetta Agricultural Research Center (HARC).

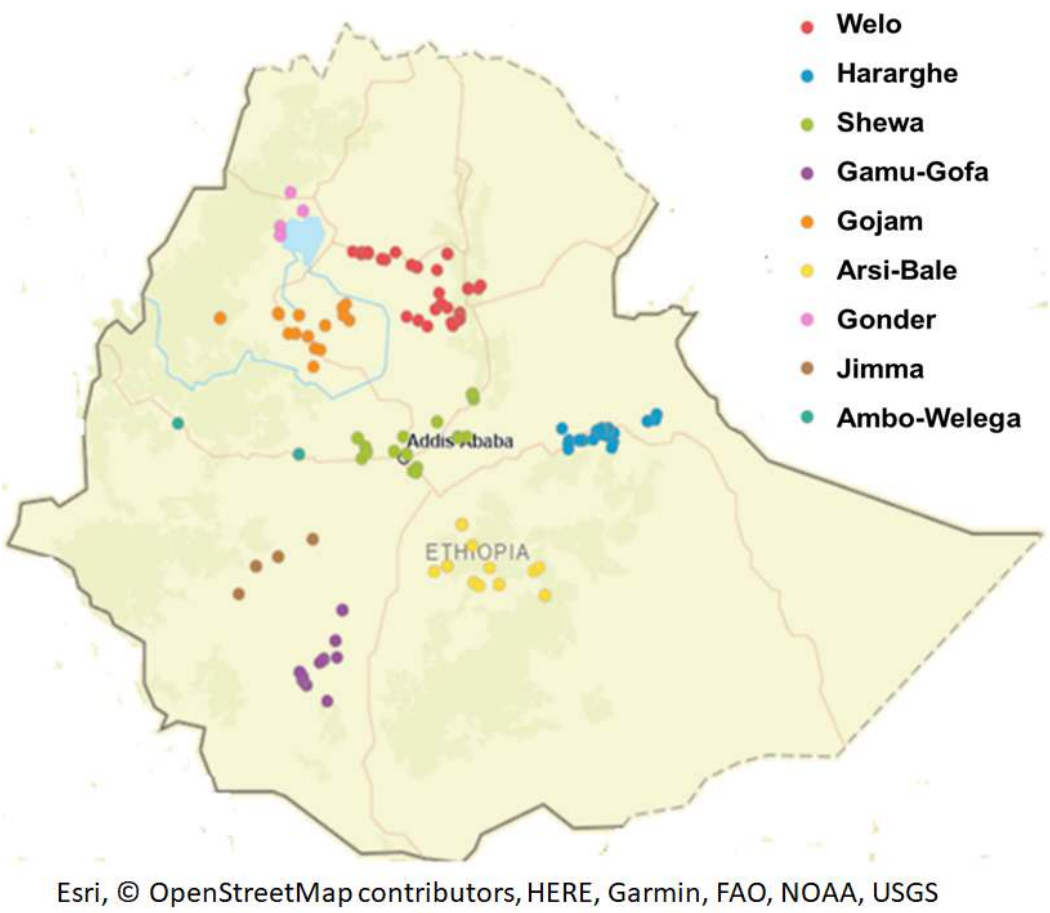


Fig. 1 Ethiopian barley landrace accessions grouped by their geographical collection areas. Ethiopian boundary and geo-positions are indicated. Filled circles represent the 239 Ethiopian landraces collected at sometimes overlapping positions. Geographical positions are also detailed in S1 Table. The map was constructed using the online ArcGIS software suite vs. 10.8.1 provided by Esri.

Genotyping

Three seeds from each of the 260 accessions were grown in the greenhouse at day (16h)/ night (8h) temperatures of 20-22°C/17-19°C as described by (Wehner et al. 2016a) in multipot trays filled with Einheitserde ED73 soil containing 14% N, 16% P2O5 and 18% K2O in kg/m3 (H. Nitsch & Sohn GmbH & Co. KG, Germany). When plants had grown to the two to three leaf stage, leaf samples with an approximate size of 300 mg were taken from a single plant for genotyping. The genomic DNA was extracted using a modified CTAB (cetyltrimethylammonium bromide) method (Stein et al. 2001) and genotyped using the barley Illumina 50k iSelect SNP array (Bayer et al. 2017) at TraitGenetics GmbH, Gatersleben, Germany.

An initial set of 40,387 markers was successfully extracted from genotyping. 10,644 SNP markers were obtained, after removing all monomorphic markers and imputation using Beagle (Browning and Browning 2009) followed by final filtering using thresholds of 5% missing values, 3% minor allele

frequency, and 12.5% heterozygous SNPs. A total of 983 highly informative markers were kept, using the software PLINK 1.9 (http://www.cog-genomics.org/plink/1.9/) (Chang et al. 2015), which uses the markers physical distances as well as pair wise linkage disequilibrium (LD) between adjacent markers to prune-in SNPs in strong LD, with unbiased representation along the genome.

Population Structure

The 983 highly informative SNP markers were used for population structure and genetic diversity analysis. The population structure was calculated using the Structure software v.2.3.4 (Pritchard et al. 2000). Computation of Bayesian statistical models was conducted by the Markov Chain Monte Carlo (MCMC) method based on 50,000 iterations following discard of 50,000 "burn-in" iterations. The web-based Structure Harvester software v0.6.94 (http://taylor0.biology.ucla.edu/structureHarvester/) (Earl and vonHoldt 2012) was used to identify the best probable number of subpopulation (k-value) according to (Evanno et al. 2005). From the best k-value, out of 10 replications the replication with the highest likelihood (mean LnP(K)) value was used as an inferred population cluster. The estimated membership coefficient of each accession was used to assign it to different clusters estimated by STRUCTURE based on the highest inferred cluster values. Principal coordinate analysis (PCoA) was applied to plot the population structure using the DARwin 5.0 software (Perrier and Jacquemoud-Collet 2006) based on the SNP matrix data.

Genetic Diversity

The 983 highly informative SNP markers were used for genetic diversity analysis. AMOVA was performed based on the number of genetically distinct clusters obtained from the structure analysis. Information about genetic variation within and between clusters based on PhiPT (analogue of fixation index (FST)) were obtained from the analysis using the GenAlEX 6.5 software plugin for Excel (Peakall and Smouse 2012). The neighbor-joining tree, which is constructed based on the genetic distance of accessions (Saitou and Nei 1987), was created using the DARwin 5.0 software (Perrier and Jacquemoud-Collet 2006) to graphically demonstrate the presence of genetic distance between the subpopulations.

The genetic variance within and between clusters was calculated using the following formulas:

$$PhiPT = \frac{AP}{(WP + AP)}$$

Where *PhiPT* is the genetic differentiation within and between clusters; *AP* is the estimated variance among clusters, and *WP* is the estimated variance within clusters.

Detection of first-generation migrants was conducted by converting the structure file to an 'Fstat' file using "PGDSpider 2.1.1.5" program (Lischer and Excoffier 2011), and analyzed by the GeneClass2.0.h software (Piry et al. 2004) using Monte Carlo resampling methods (Paetkau et al. 2004).

Genetic diversity indices i.e. Shannon's information index (I), expected heterozygosity (He), unbiased expected heterozygosity (uHe), and percentage of polymorphic loci (PPL) were also calculated using frequency based analysis in the GenAlEX software (Peakall and Smouse 2012). Additionally, the Mantel test, which is used to estimate the gene flow by correlating the genetic distance with the spatial distance, i.e. GPS data in our case, was performed to get information on the genetic divergence across the geographical distance using the GenAlEX software (Peakall and Smouse 2012).

Results

SNP analyses

From 43,461 scorable SNPs markers of the 50k iSelect SNP array [36]; 40,387 (92.9%) SNPs markers were successfully extracted in this experiment. However, 19,028 (47.1%) markers were immediately removed as monomorphic markers. From the remaining 21,355 markers, 10,767 SNPs markers (26.7% of the extracted set of markers) were removed by filtering for 3% minor allele frequency. Out of the 10,644 SNP markers, which were obtained after filtering, the highest number of markers was located on chromosome 2H (1857), and the least markers on chromosome 4H (1174). Similarly, for the 983 highly informative markers the highest number of markers was obtained for chromosome 2H (185), and the least for chromosome 4H (89) (Fig 2). The distribution of the markers revealed that most markers in the centromeric region were pruned-out, and the majority of the highly informative markers is located in the telomeric regions of all seven chromosomes (S1 Fig).

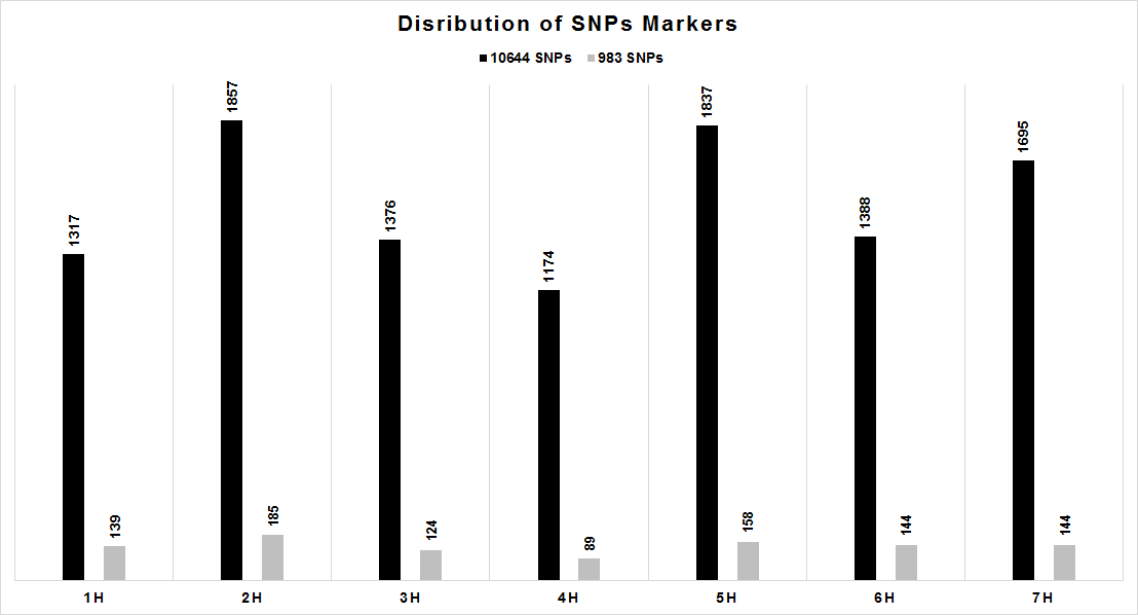


Fig. 2 Distribution of filtered (10,644) and highly informative (983) SNPs across the barley chromosomes.

Population structure analysis

Analysis of the population structure based on 983 SNP markers identified the best probable number of the subpopulation based on k-value at K=3, which therefore has been selected as an optimal number of inferred genetically defined clusters (Fig 3 a and b). According to the three genetically distinct clusters, cluster 1 consists of 80 accessions (30.8%), cluster 2 consists of 71 accessions (27.3%) and cluster 3 consists of 109 accessions (41.9%) out of the total of 260 accessions (Table 1). The average membership coefficient of the geographically defined populations indicated that Welo and Shewa population can be explained by cluster 1 and 2, respectively; whereas Gonder, Gamo-Gofa, and Jimma population were explained by cluster 3 (Table 2). When each member of a geographically defined population was re-assigned based on their highest probability value of the inferred clusters, 56% and 66% of Welo and Shewa accessions were clustered in genetically distinct cluster 1 and 2, respectively. Similarly, 88%, 86%, and 71% of Gonder, Gamo-Gofa, and Jimma accessions were grouped in the genetically distinct cluster 3, respectively (Table 1). Furthermore, 75% of the Ambo-Welega population was also assigned to cluster 3, but the low number of accessions has to be taken into account. Principal coordinate analysis (PCoA) indicated that PCoA1 and PCoA2 explained 5.87% and 4.88% of the variation, respectively. Despite these values being rather low, the high genetic variation within the set of accessions is reflected by the inferred three clusters (Fig 3c).

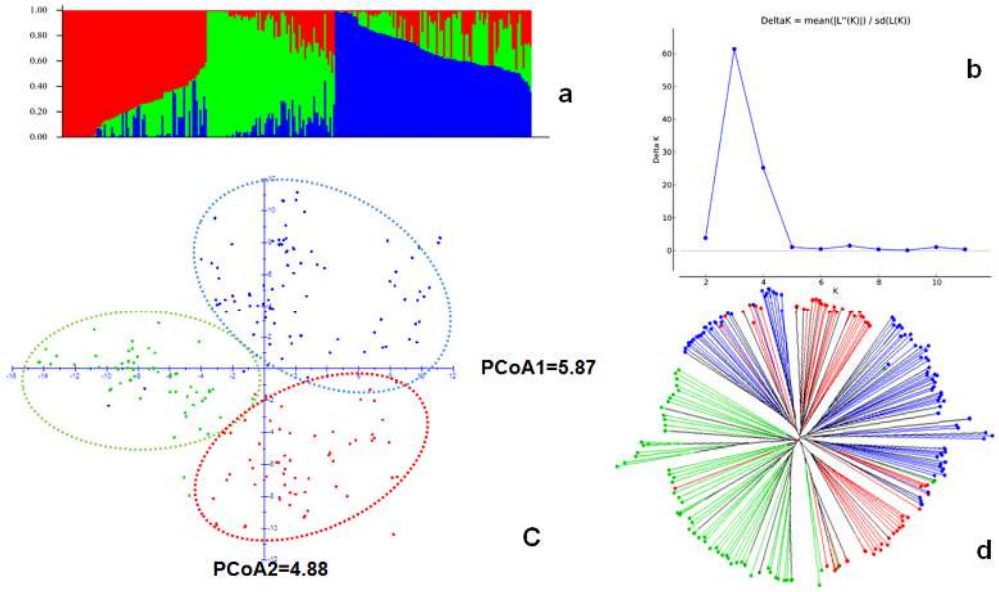


Fig. 3 Population structure analysis for the 260 Ethiopian barley accessions. a) bar plot for estimated population structure of 260 Ethiopian barley accessions based on inferred three clusters (red = cluster 1, green = cluster 2, and blue = cluster 3); b) Structure harvester Delta K (ΔK) = 3; c) results of principal coordinate (PCoA) analysis, accessions were assigned based on their highest probability of inferred clusters; and d) weighted neighbor-joining tree for the structured subpopulations (red = cluster 1, green = cluster 2, and blue = cluster 3).

Table 1:- Distribution of the Ethiopian barley accessions grouped by their geographical origin and based on the three genetically distinct clusters.

Geographically defined subpopulations	Total accessions	Percentage of accessions in genetically distinct clusters					
		Cluster 1		Cluster 2		Cluster 3	
		Number	%	Number	%	Number	%
Gonder	8	1	12.5	0	0.0	7	87.5
Arsi-Bale	19	4	21.1	4	21.1	11	57.9
Shewa	38	6	15.8	25	65.8	7	18.4
Ambo-Welega	4	0	0.0	1	25.0	3	75.0
Gojam	28	6	21.4	14	50.0	8	28.6
Welo	59	33	55.9	13	22.0	13	22.0
Gamo-Gofa	28	2	7.1	2	7.1	24	85.7
Jimma	7	1	14.3	1	14.3	5	71.4
Hararghe	48	20	41.7	5	10.4	23	47.9
HARC	21	7	33.3	6	28.6	8	38.1
Total	260	80	30.8	71	27.3	109	41.9

Table 2:- Average membership coefficient of Ethiopian geographically defined subpopulations based on the three genetically distinct clusters.

Geographically defined subpopulations	Total accessions	Average membership coefficient of the subpopulations in the three genetically distinct clusters		
		K1	K2	K3
Gonder	8	0.182	0.210	0.609
Arsi-Bale	19	0.202	0.355	0.444
Shewa	38	0.254	0.564	0.181
Ambo-Welega	4	0.221	0.421	0.359
Gojam	28	0.208	0.475	0.316
Welo	59	0.516	0.272	0.212
Gamo-Gofa	28	0.133	0.261	0.606
Jimma	7	0.145	0.256	0.600
Hararghe	48	0.437	0.161	0.402
HARC	21	0.334	0.376	0.290
Total	260	0.326	0.329	0.344

Analysis of molecular variance (AMOVA)

AMOVA analysis was conducted based on the three genetically distinct clusters obtained through the analysis of population structure. The results revealed that variation within a cluster was accounting for higher variation (89%) than the variation among clusters (11%). The genetic differentiation was moderate (PhiPT = 0.11) with statistical significance at $p < 0.001$ (Table 3). Based on a Monte Carlo resampling model, five accessions with p<0.01 were detected as first-generation migrants. Three of these derived from the genetically distinct cluster 1. Out of these two and one are likely to be immigrant from the genetically distinct clusters 2 and 3, respectively. The remaining two are from the genetically distinct cluster 2 andthese are likely to be immigrants from genetically distinct cluster 1 and 3 (S2 Table).

Table 3:- Analysis of molecular variance (AMOVA) for the Ethiopian barley accessions for the three genetically distinct clusters; PhiPT values for the total population.

Source	Degree of Freedom	Sum of square	Mean square	Estimated variance	Percentage of variation	PhiPT
Among Populations	2	6,576.5	3,288.2	35.3	11%	0.11**
Within Populations	257	73,143.0	284.6	284.6	89%	
Total	259	79,719.5		319.9	100%	

** p-value < 0.001

Genetic diversity

The study of the genetic diversity indices of the three genetically distinct clusters indicate, that cluster 3 is more diverse than the other two clusters with values of I=0.47, He=0.31, uHe=0.31, PPL=99.1%, followed by cluster 2 (I=0.43, He=0.28, uHe=0.28, PPL=95.9%) while cluster 1 is the least divers one (I =0.39, He=0.26, uHe=0.26, PPL=88.2%) (Table 4).

Table 4:- Genetic diversity indices for the genetically distinct clusters.

Genetically distinct clusters	N	I	He	uHe	PPL
1	80	0.39	0.26	0.26	88.2%
2	71	0.43	0.28	0.28	95.9%
3	109	0.47	0.31	0.31	99.1%

"N" for number of observations, "I" for Shannon's information index, "He" for expected heterozygosity, "uHe" for unbiased heterozygosity, and "PPL" for percentage of polymorphic loci

Based on the results of pairwise PhiPT value, there is a moderate genetic differentiation between the subpopulations. The results indicate that the variation between genetically distinct cluster 1 and 2 is relatively larger (0.13) than between the other populations (S3 Table).

The Mantel test, which is used to demonstrate the presence of spatial population structure indicated that the accessions were poorly structured, based on the GPS data of sampling with an R-squared value of 0.019 (Fig 4).

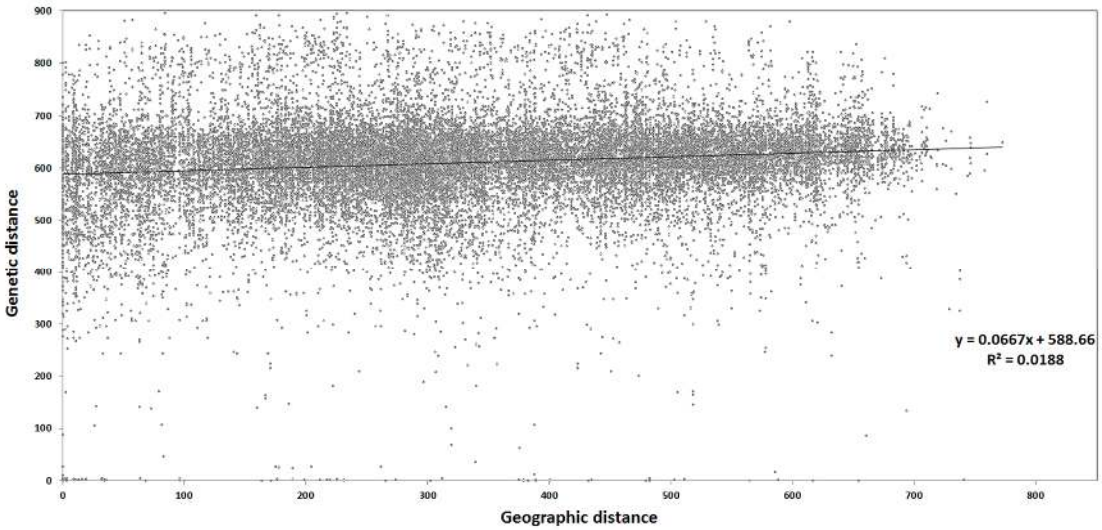


Fig. 4:- Mantel test for the 239 landraces based on the relationship between the genetic distance and the geographic distance based on GPS data.

Discussion

From 43,461 scorable SNPs markers of the 50k iSelect SNP array (Bayer et al. 2017); the final number of SNPs markers (10,644) in our study was quite small compared to previous reports of 39,733 SNPs (Darrier et al. 2019); 33,818 SNPs (Novakazi et al. 2019) and 37,242 SNPs (Cope et al. 2020). This may be explained by the under-representation of Ethiopian genotypes during the development of the 50k SNP array (Bayer et al. 2017).

The distribution across the barley genome of the SNPs markers obtained after filtering was compared with the one of the 50k SNP array (Bayer et al. 2017). The genome regions containing the first and the second highest number of SNP markers were on chromosome 5H (8123) and chromosome 2H (7227) for the 50k SNP array development, whereas in our study the first and second highest representation were recorded on chromosome 2H (1857) and chromosome 5H (1837). The two genome regions with the least number of SNP markers were chromosome 1H (4828) and chromosome 6H (5441) for the 50k SNP array, while in this study chromosome 4H (1174) and chromosome 1H (1317) were least represented. Therefore, we considered the distribution of SNP markers along the seven barley chromosomes as similar with the 50k SNP array. A total of 983 highly informative markers, located in the telomeric regions of all seven chromosomes (S1 Fig), were kept for the population structure analyses.

According to the average membership coefficient, the predefined Welo and Shewa subpopulations were classified as genetically distinct in cluster 1 and 2, respectively (Table 1). By the ratio of accessions assigned in each cluster, accessions from Gonder, Gamo-Gofa and Jimma, predefined as subpopulations, appeared to be represented by cluster 3 (Table 2). Similarly, the average membership coefficient of the Gonder, Gamo-Gofa, and Jimma (Table 1) populations clearly suggested that they are members of cluster 3. Abebe et al. (2015a) reported that landraces obtained from Shewa, Gonder, and Gojam have had minimum admixture, whereas landraces obtained from Arsi-Bale, Harerghe, and Welo were showing the highest ratio of admixture. Accordingly, in our study, landraces from Gonder, and Shewa were grouped in cluster 1 and 3 respectively; and Arsi-Bale and Harerghe were not defined by any cluster (Table 2).

Estimation of the population structure along the geographical and agro-ecological arrangement gives an important view on the pattern of population structure. In Ethiopia, studies conducted on different cereal crops highlighted the presence of higher genetic variation within geographical locations and altitude ranges for barley (Demissie et al. 1998, Abebe and Léon 2013, Abebe et al. 2013), durum wheat (Mengistu et al. 2016, Alemu et al. 2020, Negisho et al. 2021), and sorghum (Ayana et al. 2000). Similarly, the presence of minimum geographical structure was observed using the Mantel test in this study (Fig 4). This may be due to the fact that accessions from distantly located regions, i.e. Gonder, Jimma and Gamo-Gofa are grouped in cluster 3. Further analysis of AMOVA based on the agro-ecological zones of the accessions as a predefined subpopulation provided only 3% variation between agro-ecologies (S4 Table), although the variation between genetically distinct clusters was 11% (Table 3). On the contrary, previous genetic diversity studies on Ethiopian barley landraces suggested that the landraces' population structure is dependent on the altitudinal gradient; which is mainly used for the classification of Ethiopian agro-ecologies (Kebebew et al. 2001, Tanto Hadado et al. 2009, Tanto Hadado et al. 2010, Samberg et al. 2013), but of which a minimum of variation explained was found in the current study (S4 Table).

The influence of altitudinal gradient on population structure was reported as important by (Kebebew et al. 2001, Tanto Hadado et al. 2009, Tanto Hadado et al. 2010, Samberg et al. 2013), In contrast other studies (Demissie et al. 1998, Abebe and Léon 2013, Abebe et al. 2013, Abebe et al. 2015a) reported its influence as minimum for the formation of population structure. Although our study demonstrated the impact of altitudinal gradient as minimum, we carefully examined the previous studies, and the accessions' passport data; to strengthen our findings.

Previous studies (Kebebew et al. 2001, Tanto Hadado et al. 2009, Tanto Hadado et al. 2010, Samberg et al. 2013), that reported the influence of altitudinal gradient on population structure, were conducted using either one or two provinces of Ethiopia; whereas studies conducted based on landraces collected from several provinces (Demissie et al. 1998, Abebe and Léon 2013, Abebe et al. 2013, Abebe et al. 2015a), revealed the minimum impact of altitude gradient in the formation of genetic clusters. Therefore,

the presence of less province representation in these samples might have influenced the outcome of the results.

To proof the impact of the altitude gradient in specific province, Shewa and Welo subpopulations were selected, as they can be defined by cluster 2 and 1, respectively (Table 1). The landraces of these locations were classified based on their sources of agro-ecological zones, and in both locations 25 (66%) and 32 (52%) of the total landraces are from 'Cool moist mid highlands (M4)' agro-ecology zone, respectively (S1 Table). These M4 landraces were further assigned into the three genetically distinct clusters; and 16 (64%) and 17 (53%) of the M4 landraces of Shewa and Welo were assigned into cluster 2 and,1 respectively. This is less than the total amount of these populations assigned to cluster 2 and 1, respectively (Table 1). This confirms less importance of agro-ecological zone for the formation of a structured population.

As mentioned by (Backes et al. 2009, Tanto Hadado et al. 2010), the landraces at hand of Ethiopian farmers are genetically variable. Similarly, when seed increase of the EBI accessions was conducted morphologically different plants were observed in 19 EBI accessions, which finally resulted in 39 different accessions of the study material (S1 Table). The presence of population genetic structure in their descendant accession was evaluated, the result revealed the descendants of 6 of 19 EBI accessions codes (32%) are assigned into different genetic cluster (S1 Table), which indicate the presence of uneroded genetic structure even at the farmer level. If such level of genetic structures is available at the farmer hand, the presence of higher level of genetic structures is expected within the altitudinal gradient.

Therefore, as mentioned by (Abebe et al. 2015a) the climate condition have weak association with structured populations of Ethiopian barley landraces, but in the contrary to (Abebe et al. 2015a) study the geographic locations slightly contributed to the variation among the structured populations in our study.

The overall population genetic differentiation (PhiPT) value is (0.11) indicating the presence of moderate differentiation between the genetically clustered subpopulations (Wright 1978). Similarly, the pairwise PhiPT value between clusters ranges from 0.10 between cluster 1 and 3 to 0.13 between cluster 1 and 2 (S3 Table). The presence of moderate genetic differentiation between the different genetically distinct clusters hints to the exchange of adaptive traits among them (Slatkin 1987, Manel et al. 2003). A total of five accessions was detected as first-generation migrants in the study, i.e. 1.92%. Although genetically distinct cluster 3 did not have any immigrants in its population, there are two accessions originally migrated from this cluster to cluster 1 and cluster 2 (S2 Table), which indicates that the direction of migration from this cluster to others is more frequent than others (Boessenkool et al. 2009). Similarly, the pairwise PhiPT indicated that cluster 3 has the least value to differentiated genetically from other genetically distinct (S3 Table), as it possessed extra shared markers in migrate population (Boessenkool et al. 2009).

The presence of weak geographical or agro-ecological structure for Ethiopian barley landraces (Demissie et al. 1998, Abebe and Léon 2013, Abebe et al. 2013) may be explained by the exchange of important adaptive genetic traits between different genetically distinct clusters. The 21 breeding lines used in the study are proportionally distributed in the three clusters (Tables 1 and 2), which is also an indicator, that the national breeding program is introducing important adaptive traits from landraces in new varieties. The Mantel test (Fig 4) also revealed the presence of higher gene flow between the farthest locations, which may contribute to a wider adaptation of Ethiopian landraces.

From the three genetically distinct clusters, cluster 1 is explained by the Welo predefined subpopulation. (Shewayrga and Sopade 2011) described that around the Welo location barley is an important crop, and farmers conserve the landraces for different reasons, such as for their suitability to use it for short and long rainy seasons (maturity), yield potential, tolerance to water logging, frost and low soil fertility, social preference (taste and visual appearance), and storability. Furthermore, barley is also used as a main dish (to prepare injera, and bread) in this area, and a special dish and beverage (tihlo and korefe), which are exclusively prepared from barley, are commonly consumed in this area (Shewayrga and Sopade 2011). Thus, another assumption for the formation of this genetically clustered population may be related to the landraces quality to prepare staple food as well as special dishes and beverages.

Cluster 3 mainly contains landraces from Gamo-Gofa, and the production of barley in Gamo-Gofa is mainly on highlands with an altitude higher than 2,500 meter above sea level (Samberg et al. 2010). Such highland topographies are characterized by having low road access to connect with nearest commercial cities. As a result, the diversity in such areas will be kept unchanged. Accordingly, studies suggested an increased market access in the community contributing to an increase in crop diversity (Gabre-Madhin 2001, Nagarajan and Smale 2007). In our study, the presence of low market access likely contributed to the grouping of 86% of Gamo-Gofa accessions in cluster 3. Although farmers varieties selection criteria in Gamo-Gofa are similar to other locations, barley is not served as main dish in the region and usually used to prepare special dishes and beverage (local beer) during a festive holiday and special occasions (Arthur 2014). We therefore assume that the farmers selection criteria for varieties may be based on the end use of the product, and consequently landraces in cluster 3 might be related with such quality traits.

Shewa is located in the central part of Ethiopia, with best road facilities, and high consumer demand. Farmers usually produce barley for home consumption and market; and (Eticha et al. 2010b) reported that farmers produce barley as it is adapted very well comparing to other cereal crops to the low fertility soil in this region. Barley is used in this region to prepare local liquor and local beers, which have great demand for market. Additionally farmers produce suitable landraces to prepare the main dish (injera) (Eticha et al. 2010b). A significant reduction in the number of farmer's varieties comparing to the previous time was reported in Shewa (Megersa 2014) due to socio-economic and environment related reasons. Such genetic erosion may not just be a recent history in the region, but might also be present in

the previous decades, which is ultimately narrowing the genetic bases of the landraces in this area. The result obtained from weighted neighbor-joining tree (Fig 3d) and the pairwise PhiPT (S3 Table) indicated that cluster 2 derived from slightly different predecessor families, in comparison to cluster 1 and 3 which are closer related. Therefore, the remaining landraces around Shewa with a narrow genetic base may be mostly related to cluster 2 (Fig 3d, Table 2).

Cluster 3 is a diverse cluster based on the results of genetic indices (Table 4). 86% of accessions from Gamo-Gofa are assigned to this cluster and (Negassa 1985) also described that landraces obtained from Gamo-Gofa region have higher diversity index compared to other regions. On the contrary, landraces from Gonder, which are also grouped in cluster 3, have been described for having least diversity in that study.

Conclusions

Genetic structure and diversity of 260 Ethiopian barley landraces, comprising 239 accessions from EBI, and 21 barley breeding lines of the national barley improvement program of the HARC, were investigated based on data obtained from the barley 50k iSelect SNP array (S5 Table). The presence of higher rates of monomorphic markers with minor allele frequency less than three seems characteristic for Ethiopian barley accessions compared with other barley collections from the world. AMOVA revealed the existence of high genetic diversity within genetically distinct populations in comparison to the genetic diversity between genetically distinct populations. This may be due to the minimum geographical structure of landraces and the presence of higher gene flow between accessions originated from distant geographic locations. The use of barley for different food recipes and beverages may also play a role in the genetically clustered population structure as (Mulatu and Lakew 2011) described the use of different barley types for different purposes by the society of different regions. However, further analysis based on the nutritional quality of each landrace in specific geographical locations may be required. Our results will support the strategic collection and exploitation of the existing genetic resources of Ethiopian barley landraces, and will help improving farm management of subsistence farmers through the dedicated utilization of genetic resources in the near future.

Publication 2.2) Teklemariam, S. S., K. N. Bayissa, A. Matros, K. Pillen, F. Ordon and G. Wehner (2023). "Genome wide association study of Ethiopian barley for terminal drought stress tolerance under field and climate chamber conditions." CEREAL RESEARCH COMMUNICATIONS. DOI: https://doi.org/10.1007/s42976-023-00472-5.

Abstract

In order to detect markers for drought stress tolerance, field experiments in Ethiopia were conducted for three years at two naturally drought-prone locations and two optimum moisture locations using 239 Ethiopian barley landraces and 21 barley breeding lines. Furthermore, a climate chamber experiment applying drought stress at different water regimes (70% soil water capacity (WC) for control and 20% WC for drought stress conditions) after flowering was conducted for selected 196 accessions. Results revealed reduced grain biomass by 47% and 80% under field and climate chamber conditions, respectively, as well as significantly ($p < 0.05$) reduced days to maturity, plant height, in both experimental designs. Based on 10,644 SNP markers, GWAS was conducted to identify marker trait associations (MTA) for drought stress tolerance. For days to maturity, relative chlorophyll content, plant height, number of seeds per spike, thousand kernel weight, and harvest index under field and climate chamber drought stress treatments, 58 significant MTAs were identified. In total. 41.4% of the MTAs were located on chromosome 2H, of which one is very close to the *Ppd-H1* flowering locus. These findings underpin the importance of this genome region for drought tolerance. Another MTA on chromosome 1H was detected for days to maturity under field drought stress treatment in the vicinity of the known flowering time *ELF3* gene. Additionally, 13 and 3 Ethiopian landraces that tolerate severe and moderate drought stress in climate chamber and field experiments were identified, respectively, using drought indices. The results highlight the tolerance of Ethiopian landraces to different levels of drought stress as well as their potential to be considered in future barley improvement programs.

Keywords: Ethiopian barley, landraces, drought stress, drought tolerance, marker-trait-association

Zusammenfassung

Um Marker für Trockenstresstoleranz zu finden, wurden in Äthiopien drei Jahre lang Feldversuche an zwei Standorten mit natürlicher Trockenheit und zwei Standorten mit optimaler Feuchtigkeit mit 239 äthiopischen Gersten-Landrassen und 21 Gerstenzuchtlinien durchgeführt. Darüber hinaus wurde ein Klimakammerexperiment durchgeführt, bei dem nach der Blüte ausgewählter 196 Akzessionen Trockenstress bei unterschiedlichen Wasserregimen (70 % Bodenwasserkapazität (WC) für die Kontrolle und 20 % WC für Trockenstressbedingungen) angewendet wurde. Die Ergebnisse zeigten eine um 47 % bzw. 80 % verringerte Kornbiomasse unter Feld- und Klimakammerbedingungen sowie signifikant ($p < 0,05$) reduzierte Tage bis zur Reife und Pflanzenhöhe in beiden Versuchsanordnungen. Auf der Grundlage von 10.644 SNP-Markern wurde eine GWAS durchgeführt, um Marker-Merkmal-Assoziationen (MTA) für Trockenstresstoleranz zu identifizieren. Für die Tage bis zur Reife, den relativen Chlorophyllgehalt, die Pflanzenhöhe, die Anzahl der Samen pro Ähre, das Tausendkorngewicht und den Ertrag unter Trockenstress-Behandlungen im Feld und in der Klimakammer wurden 58 signifikante MTAs identifiziert. Insgesamt. 41,4 % der MTAs befanden sich auf Chromosom 2H, wovon einer sehr nahe am *Ppd-H1*-Blütenlocus liegt. Diese Ergebnisse unterstreichen die Bedeutung dieser Genomregion für die Trockentoleranz. Ein weiterer MTA auf Chromosom 1H wurde für die Tage bis zur Reife unter Trockenstress im Feld in der Nähe des bekannten *ELF3*-Gens für die Blütezeit entdeckt. Darüber hinaus wurden anhand von Trockenheitsindizes 13 bzw. 3 äthiopische Landsorten identifiziert, die in Klimakammer- und Feldversuchen Trockenstress tolerieren. Die Ergebnisse verdeutlichen die Toleranz äthiopischer Landsorten gegenüber Trockenstress sowie ihr Potenzial, in zukünftigen Gerstenzüchtungsprogrammen berücksichtigt zu werden.

Schlüsselwörter: Äthiopische Gerste, Landrassen, Trockenstress, Trockentoleranz, Marker-Merkmals-Assoziation

Introduction

Crop production across Ethiopia is widely variable and highly reliant on weather conditions, which mainly vary due to the altitudinal gradient and the amount of rainfall (Gezie 2019). Almost all smallholder farmers in Ethiopia do not have supplementary irrigation facilities to ensure high yields during drought periods (Mendes and Paglietti 2015). Therefore, farmers' choice of specific crop and cultivar depends on the amount of rainfall received at that specific location (Elisabeth 2004, Asrat et al. 2010). The majority of smallholder farms (59%) in Ethiopia are in the highlands and are based on cereal farming systems with adequate rainfall, whereas farm areas in the drought-prone highlands account for 26% of the total area cultivated (Taffesse et al. 2012). Drought prone areas do not only experience low annual rainfall but also high rainfall variability (Mersha 1999).

Barley is a major staple food in the extreme highlands of Ethiopia (Shewayrga and Sopade 2011), but it is also grown in diverse agro-ecologies. It is used to prepare various food recipes (Shewayrga and Sopade 2011, Arthur 2014). Barley small-holder farmers tend to plant diversified landraces to minimize risks caused by harsh weather conditions (Di Falco and Chavas 2009). Out of the total of cereal producing farmers in Ethiopia, 24.5% are engaged in barley production, and the production covers 7.8% and 6.4% of the total cereal production area and cereal harvested yield, respectively (CSA 2019).

Ethiopia is a recognized global center of genetic diversity for barley (Vavilov 1951, Harlan 1992), and the genetic diversity is mainly influenced by the diversification of soils, climate, altitude and topography, different farming systems, together with the geographical isolation of the country for long periods (Harlan 1976, Mekonnon et al. 2015). Ethiopian barley landraces have high potential for drought tolerance and landraces collected from wide geographical locations of the country have demonstrated differences in earliness (Engels 1991), and higher yield stability with a comparative grain yield gain than improved cultivars (Abay and Bjørnstad 2009, Wosene et al. 2015). Therefore, Ethiopian landraces may use multiple drought tolerance mechanisms to alleviate the different levels of drought stress.

Genome-wide association studies (GWAS) were initially used in human genetics to identify marker trait associations (MTAs) for the trait of interest (Risch and Merikangas 1996) and nowadays, it is widely used in plant genetics and breeding to identify quantitative trait loci (QTLs) for important traits (Ingvarsson and Street 2011). Many GWAS were conducted to identify genes controlling flowering time in plants, such as maize (Andersen et al. 2005, Camus-Kulandaivelu et al. 2006, Salvi et al. 2007), Arabidopsis (Olsen et al. 2004, Zhao et al. 2007), and barley (Kraakman et al. 2006), which is one of the significant traits influencing tolerance to drought stress.

GWAS were also specifically used to investigate drought tolerance in barley. Although only a few MTAs with limited phenotypic variation were reported by Varshney et al. (2012), in the succeeding studies, significant QTLs that influence the phenotype as well as the physiological and metabolic activities of juvenile barley genotypes grown under drought stress conditions were identified by Wehner

et al. (2015), Wehner et al. (2016b), and Xiong et al. (2023). QTLs associated with seed germination (Thabet et al. 2018), water use efficiency, relative water content, and photosynthesis-related traits (Wójcik-Jagła et al. 2018), internode length and flag leaf length (Jabbari et al. 2018), spikelet organs, and number of grain per spike (Thabet et al. 2020) were identified under drought conditions. Candidate gene associated with drought tolerance that influence grain yield and associated traits (Li et al. 2022), and drought triggered environmental signals (Elbasyoni et al. 2022) were also reported. Post flowering drought experiment also revealed QTLs influencing drought tolerance in barley (Afsharyan et al. 2023).

Backcross populations developed from highly drought-tolerant Spanish landraces and elite cultivars (Monteagudo et al. 2019), and recombinant line derived from thermo-tolerant 'Otis' cultivar and Golden Promise cultivar (Ajayi et al. 2023) were used to investigate QTLs for grain yield and related traits using the 50k iSelect chip (Bayer et al. 2017). The result concluded that QTLs positively contributing for drought tolerance were obtained from landraces and thermo-tolerant 'Otis' cultivar.

Because Ethiopia is a center of diversity for barley and its production is in diverse eco-geographic environments, Ethiopian landraces appear to be suitable for identifying QTL for drought tolerance. The presence of molecular genetic diversity in Ethiopian barley was reported by Demissie et al. (1998), Abebe and Léon (2013), Abebe et al. (2013), and Teklemariam et al. (2022), but their drought tolerance potential was not studied using a high density marker like the 50k iSelect chip. Thus, this study aims at identifying drought tolerant barley genotypes in Ethiopian landrace collections and their respective QTLs, and QTL regions for selected traits influencing drought stress tolerance using 50k iSelect chip by applying GWAS.

Materials and methods

Field Experiment

260 barley accessions, including 239 barley landraces provided by the Ethiopian Biodiversity Institute and 21 barley breeding lines provided by the Holetta National Barley Improvement Program, were used for field experiments conducted for three years (2016–2018) at four locations. Field stations were at two naturally optimum moisture locations (Holetta and Debrezeit), which were designated as field control (FC) treatments and two naturally drought prone locations (Melkassa and Dera) which were assigned as field drought stress (FS) treatments. FS experiments were conducted in the central rift valley (CRV) of Ethiopia, and Kassie et al. (2013) reported that the longer dry spell in the CRV starts in September which coincides with the post flowering development cycle of barley (Figure S1a-b). Because above-average rainfall was observed in all research locations during the 2017 cropping season, no drought stress appeared, and all data from 2017 were excluded from the analyses. Furthermore, the 2018 Debrezeit data were excluded from the analyses due to the presence of poor germination at this site.

Field trials were conducted in an alpha-lattice design with three replications. Fifty seeds of each accession were sown in a single row of 1 m in length and 20 cm between rows (Figure S1a-d). The

geographic coordinates, soil type, altitude, seasonal temperature, and rainfall of each research experimental location are provided in Supplementary Table S1.

Climate Chamber Experiment

The climate chamber experiments were conducted at the Julius Kühn Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Resistance Research and Stress Tolerance, Quedlinburg, Germany with 196 accessions. These were selected by the least-square means (lsmeans) of grain biomass of 2016 drought stress locations and 2017 Holetta (control treatment) by three drought indices namely, drought susceptibility index, tolerance index, and yield reduction index, as described by Asgarinia et al. (2017). The results were used to cluster the 260 accessions into seven clusters, using SAS 'proc cluster' procedure (SAS Institute 2019), and 75.4% of accessions were randomly selected from each cluster to accommodate a set of 196 accessions used for climate chamber experiments.

Pots with size of 15 * 15 * 20 cm were filled with 1,500 g of soil (Einheitserde ED73; H. Nitsch & Sohn GmbH & Co. KG, Germany). The water capacity (WC) of the soil was determined by analyzing the weight difference between fully hydrated soil to oven dried soil according to Paech and Simonis (1952) as described in Wehner et al. (2016a). Three plants were grown per pot and the experiment was arranged in a split plot design with two replications. Pots were watered up to 70% WC until flowering, then the pots under drought treatment were kept at 20% WC, which was considered as climate chamber drought stress (CS) treatment and pots under optimum watering treatment were watered up to 70% WC hereafter assigned as climate chamber control (CCn) treatment (Figure S1e-h).

The climate chamber was kept at 13/11 hours day/night photoperiod, with 18/14 ºC day/night temperature during the vegetative growth stage and 22/16 ºC day/night after flowering, and 25/18 ºC day/night during the maturation period.

During the experimental period, the accessions were evaluated for several physiological parameters. Days to flowering (DFL), which was recorded at Zadoks' stages 58 (Z58) in field experiments and at Zadoks' stages 49 (Z49) in the climate chamber (Zadoks et al. 1974). Relative chlorophyll content (SPAD), which was measured using the SPAD-502 Plus instrument (Minolta, Co., Ltd., Japan) as described in Wehner et al. (2016a), in the field experiments, the mean measurement of three selected plants and five measurements per flag leaf a week after DFL were recorded, whereas in the climate chamber experiment, the mean measurement of three plants and five measurements per flag leaf a week after 20% WC of CS treatment were recorded. Measurements for both CCn and CS treatments were taken on the same day. Days to physiological maturity (DM), which were recorded at Zadoks' stages 90 (Z90) (Zadoks et al. 1974). Plant height in cm (PH), the measurement was taken after physiological maturity (in centimeters), in field experiments, it was the mean value of five plants, while in climate chamber experiments, it was the mean value of three plants. Number of seeds per spike (NSdPS), which was the mean number of seeds per spike of ten plants in field experiments, while in the climate chamber experiment, it was the total number of harvested seeds divided by the total number of spikes. Thousand

kernel weight in grams (TKW); Grain biomass in grams (GB), which represents the total harvested grains of a plot in grams. Harvest index (HI), which was the total grain biomass of ten plants in grams divided by the oven dry biomass of ten plants in grams for field experiments, while for the climate chamber experiment, the total grain biomass of three plants in grams was divided by the oven dry biomass of three plants in grams.

Statistical analyses

Statistical analysis of phenotypic data was performed with the statistics package SAS 9.4 software (SAS Institute 2019). The procedure '*proc mixed*' was used for ANOVA and estimation of least square means (*lsmeans*) of traits analyzed. The model was fitted to the trait of interest as dependent variable; accessions and watering treatments were considered as fixed effects, while year, location, replication, and blocks were used as random effects. The effect of accessions interaction with treatment was also included as a fixed effect to estimate lsmeans of each trait.

The variance components and heritability (h^2) of traits were analyzed using '*lme4*' R-package (Bates et al. 2014, R Core Team 2019), for experiments conducted for more than one year. Genotype by environment interaction and genotype by year interaction were included as random factors. Variance of genotype (σ^2_g), genotype × environment (σ^2_{gl}), genotype × year (σ^2_{gj}), residual variance components (σ^2), replication (*rep*), number of location (*nl*), and number of years (*ny*) were estimated, and broad sense heritability was calculated as:

$$h^2 = \sigma_g^2/(\sigma_g^2 + \frac{\sigma_{gl}^2}{nl} + \frac{\sigma_{gj}^2}{ny} + \frac{\sigma^2}{nl * ny * rep})$$

The Harmonic mean (HM) drought index was used in this study to identify more stable genotypes in moderate drought stress treatment (Clarke et al. 1992, Guttieri et al. 2001, Akçura et al. 2011). HM was also used to identify the most stable accessions in field trials, whereas the drought susceptible index (DSI) was used to identify drought tolerant accessions in severe drought condition like in the climate chamber experiment (Fernández 1992).

$$HM = [\frac{2 * (Yp * Ys)}{Yp + Ys}]$$

$$DSI = [1 - \left(\frac{Ys}{Yp}\right) / [1 - \left(\frac{\bar{Y}s}{\bar{Y}p}\right)]$$

Abbreviations represent grain biomass under drought stress treatment (*Ys*), grain biomass under control treatment (*Yp*), total grain biomass mean under drought stress treatment ($\bar{Y}s$), and total grain biomass mean under control treatment ($\bar{Y}p$).

Genotyping

The markers from the 50k SNP iSelect chip (SGS Trait Genetics) were filtered for <5% missing values, >3% minor allele frequency, and <12.5% heterozygous SNPs resulting in a set of 10,644 SNP markers, which were used for genomic clustering in Teklemariam et al. (2022). Using these, the presence of three distinct subpopulation structure was demonstrated in Teklemariam et al. (2022) using STRUCTURE analysis (Pritchard et al. 2000), principal coordinate analysis (Perrier and Jacquemoud-Collet 2006) and neighbor-joining dendrogram (Saitou and Nei 1987). BLINK, utilizes the linkage disequilibrium information to enhance statistical power (Huang et al. 2018).

The linkage disequilibrium (LD) between all pairs of SNP markers within one chromosome was estimated using pairwise LD calculation of 'genetics', 'Ldheatmap' and 'trio' R packages (Shin et al. 2006, Warnes et al. 2013, R Core Team 2019), at 0.1 critical squared allele frequency correlation (r^2) value (Voss-Fels et al. 2015, Oyiga et al. 2018).

Genome Wide Association Studies (GWAS)

A final set of 10,644 highly polymorphic SNP markers with their physical position as well as lsmeans of phenotypic data were used to conduct GWAS. Furthermore, population structure ("PCA.total = 3") was used as correction factor. The analysis was conducted using R v.4.1.2 software (R Core Team 2021) and the "Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK)" model (Huang et al. 2018).

Markers with a false discovery rate (FDR) of adjusted p-value < 0.05 (Benjamini and Hochberg 1995) were considered as significant. Significant markers obtained for each trait in the workflow were analyzed to be linked based on each chromosome LD value on which they were detected, and in case the significant markers were linked, the marker with the smallest p-value was selected to represent the peak marker of this QTL. The identified QTLs were graphically presented using MapChart 2.32 software (Voorrips 2002). The functional annotation was analyzed by BARLEYMAP (https://floresta.eead.csic.es/barleymap) (Cantalapiedra et al. 2015) against the Morex genome v2 (Mascher et al. 2017), and genes located on the MTA positions were searched in each query and considered as candidate genes in the study.

Results

Phenotypic variation on drought stress tolerance

The presence of genetic diversity in the 260 Ethiopian genotypes was reported in Teklemariam et al. (2022). In accordance with this result, the summary of analyzed data also revealed the presence of extensive variation among the Ethiopian barley study panel for different traits (Table 1). The least coefficient of variance (CV) was observed for DM (9.4-11.9%) across all treatments and experiments. The highest CV was observed for NSdPS in FS (64.6%) and FC (64.1%), while GB (78.0%) and HI (110.8%) had the highest CV in CCn and CS, respectively (Table 1).

The mean values for all traits under drought stress were smaller than the mean values under control conditions, except for DFL in the climate chamber experiment, likely because of CS and CCn plants were watered at 70% WC until flowering (Figure S1e-h and Table 1). The highest reduction was observed for GB, which showed 47.4% and 79.8% reduction for FS and CS treatments compared to FC and CCn treatments, respectively (Table 1). NSdPS (41.4%) and PH (32.6%) of field experiments showed a high reduction compared to other traits. Reduction of SPAD (71.2%) and HI (57.9%) of climate chamber experiments was also high. The smallest reduction was observed for TKW (4.2%) and NSdPS (1.0%) for field and climate chamber experiments, respectively (Table 1). As accessions were kept on 20% WC and in controlled environment during CS treatment, the percentage of reduction of most traits was higher in the climate chamber experiment than in the field experiments. The heritability of traits in the FS treatment ranged from h^2 = 48.8-86.1%; while for FC the heritability ranged from h^2 = 12.4-90.7% (Table 1). The heritability for GB under FC and FS treatments was 41.0% and 76.4%, respectively, whereas the heritability of NSdPS and HI in FC was the least (Table 1).

Table 1: Descriptive statistics for field experiments for the three years and for the climate chamber experiments for the two replications.

Exp.	Cond	Trait	Mean	Standard deviation	Minimum	Maximum	LSD ($p<0.05$)	Coefficient of variation	Heritability	*Reduction	Number of MTAs
Field	FC	DFL	70.2	10.2	41.0	98.0	9.56	14.5	90.7%		NA
		SPAD	36.08	7.39	4.30	63.30	5.94	20.48	48.7%		2
		DM	109.4	11.6	80.0	155.0	13.24	10.6	31.5%		2
		PH	108.8	15.1	62.0	154.6	17.20	13.9	50.7%		3
		GB	72.4	37.7	0.3	246.2	41.29	52.0	41.0%		6
		NSdPS	21.8	13.9	1.2	75.7	15.26	64.1	24.1%		5
		TKW	40.7	10.3	0.0	96.8	10.35	25.2	69.9%		5
		HI	0.28	0.09	0.00	1.00	0.07	31.57	12.4%		0
	FS	DFL	63.1	12.1	37.0	113.0	8.31	19.1	86.1%	10.1%	NA
		SPAD	31.09	11.18	3.10	64.00	4.99	35.97	43.9%	13.8%	2
		DM	86.2	8.1	63.0	127.0	6.21	9.4	64.9%	21.3%	5
		PH	73.3	13.9	36.0	116.0	11.14	19.0	53.1%	32.6%	3
		GB	38.1	23.2	0.3	153.6	16.40	60.9	76.4%	47.4%	3
		NSdPS	12.8	8.3	1.0	51.6	6.43	64.6	48.8%	41.4%	5
		TKW	39.0	11.3	0.0	104.6	7.58	28.9	76.0%	4.2%	3
		HI	0.23	0.12	0.00	2.00	0.08	51.54	72.7%	17.9%	2
Climate chamber	CCn	DFL	107.5	22.3	70.0	210.0	40.17	20.8			NA
		SPAD	30.2	10.6	1.1	59.5	17.23	35.3			1
		DM	163.0	15.4	118.0	214.0	22.95	9.4			2

		PH	103.9	12.8	64.7	158.0	21.4	12.4			1
		GB	4.6	3.6	0.0	18.2	5.87	78.0			1
		NSdPS	9.6	6.0	0.3	44.9	9.2	62.3			0
		TKW	39.5	9.6	5.0	66.3	15.7	24.2			5
		HI	0.19	0.13	0.00	0.50	0.16	68.6			1
	CS	DFL	111.4	22.4	74.0	207.0	34.92	20.1		-3.%	NA
		SPAD	8.7	5.0	0.8	45.8	7.60	57.0		71.2%	2
		DM	142.0	17.0	109.0	204.0	29.97	11.9		12.9%	3
		PH	97.6	11.4	53.7	140.0	17.7	11.7		6.06%	3
		GB	0.9	0.7	0.0	3.4	1.10	71.8		79.8%	4
		NSdPS	9.5	7.0	0.3	39.3	12.2	74.5		1.0%	4
		TKW	31.1	10.2	3.3	59.8	19.1	32.9		21.3%	4
		HI	0.08	0.09	0.00	0.50	0.12	110.8		57.9%	15

Remark: DFL = days to flowering, DM = days to maturity, PH = plant height in centimeter, GB = grain biomass in gram, NSdPS = number of seeds per spike, TKW = thousand kernel weight in gram, HI = harvest index, FC = field experiments in two naturally optimum moisture locations, FS = field experiments in two naturally drought prone locations, CCn = climate chamber watering control treatment (70% WC), CS = climate chamber drought stress treatment (20% WC), LSD = least significant difference, *Reduction= ((mean of optimum – mean of deficit) / mean of optimum) *100, “NA” MTA not analysed; FC and FS data of 2017 were not included as no drought stress appeared in FS locations; and 2018 Debrezeit data were excluded from FC analyses due to the presence of poor germination data at the site.

13 accessions namely, B145.1, B191.1, B56.2, B5.1, B205, B202, B242, B213, B6, B160.2, B112.2, B143, and FTCG-20 revealed DSI < 0.4 in climate chamber experiments. Three accessions namely, FTCG-17, B185, and B137, were selected based on their stable GB in both FC and FS as they exhibited HM > 77.0. Among the top 50 accessions selected based on the results of DSI and HM, 7 overlapping accessions, namely B185, B191.1, FTCG-2, B7, B34, FTCG-13, and B260, were identified, whereas B191.1 was the only overlapping accession when the top 20 accessions from both indices were considered (Supplementary Table S2).

A highly significant genotype effect ($p < 0.001$) was observed for all traits in field and climate chamber experiments (Table 2). The interaction between accessions and treatment was also significant ($p < 0.05$) for all traits except for DM of field experiments and DFL, DM, PH, NSdPS, and TKW in climate chamber experiments. The difference between drought stress and control treatments was found to be significant ($p < 0.05$) for all traits except DFL, SPAD, TKW, and HI in field experiments, and NSdPS in climate chamber experiments (Table 2).

Table 2: ANOVA result with F-value and P-value for the analyzed variables in field and climate chamber experiments.

Trait	**Effects**	**Field experiment**		**Climate chamber experiment**	
		F-value	**P-value**	**F-value**	**P-value**
DFL	Accessions	19.46	< 0.001	4.27	< 0.001
	Treatment	0.52	0.470	4.73	0.030
	A X T	1.76	< 0.001	0.79	0.969
SPAD	Accessions	3.07	< 0.001	2.43	< 0.001
	Treatment	0.62	0.432	1952.73	< 0.001
	A X T	1.33	< 0.001	1.41	0.002
DM	Accessions	5.63	< 0.001	3.57	< 0.001
	Treatment	3.84	0.050	502.62	< 0.001
	A X T	0.94	0.745	0.97	0.599
PH	Accessions	3.55	< 0.001	3.02	< 0.001
	Treatment	4.46	0.035	77.56	< 0.001
	A X T	1.16	0.046	0.78	0.976

GB	Accessions	3.78	< 0.001	2.03	< 0.001
	Treatment	16.76	< 0.001	490.70	< 0.001
	A X T	2.12	< 0.001	1.48	0.001
NSdPS	Accessions	7.01	< 0.001	2.78	< 0.001
	Treatment	4.39	0.036	0.73	0.394
	A X T	2.09	< 0.001	0.78	0.965
TKW	Accessions	6.96	< 0.001	1.71	< 0.001
	Treatment	0.29	0.589	150.31	< 0.001
	A X T	1.68	< 0.001	1.17	0.111
HI	Accessions	2.04	< 0.001	6.00	< 0.001
	Treatment	0.04	0.835	474.19	< 0.001
	A X T	1.37	< 0.001	1.60	< 0.001

Remark: DFL = days to flowering; SPAD = relative chlorophyll content; DM = days to maturity; TKW = thousand kernel weight; NSdPS = number of seeds per spike, GB = grain biomass, PH = average plant height; HI = harvest index; A x T = accessions and treatment interaction. Additionally, data of 2017 were not included as no drought stress appeared in field naturally drought prone locations; and 2018 Debrezeit data were excluded from FC analyses due to the presence of poor germination data at the site.

Pearson's correlation coefficient (r) revealed a very strong positive correlation between DFL and DM of FC and FS treatment, with $r = 0.87$ and $r = 0.81$, $p < 0.001$, respectively (Table 3). In accordance, the highest correlation coefficients were observed for the climate chamber experiment between DFL and DM for CS ($r = 0.85$, $p < 0.001$) and CCn ($r = 0.72$, $p < 0.001$), as well as between HI and GB ($r = 0.83$, $p < 0.001$) for CS and CCn (Table 4). DFL, GB, NSdPS and HI of FS were found to be correlated with all traits (Table 3). DFL, GB and HI of CS were correlated with all traits except SPAD, and SPAD of CS was not correlated with any of the traits (Table 4).

Table 3: Pearson's correlation coefficient (r) analysis results for the field experiment; above the diagonal boxes for FC treatment, below the diagonal boxes for FS treatment.

		Control treatment							
		DFL	**SPAD**	**DM**	**PH**	**GB**	**TKW**	**NSdPS**	**HI**

Drought stress treatment	**DFL**		0.63***	0.87***	0.10 ^{ns}	0.04 ^{ns}	0.15*	0.14*	-0.28***
	SPAD	0.60***		0.59***	0.15*	0.19**	0.15*	0.21***	-0.06 ^{ns}
	DM	0.81***	0.57***		0.16**	0.12*	0.22***	0.11 ^{ns}	-0.23***
	PH	-0.18**	-0.04 ^{ns}	-0.08 ^{ns}		0.43***	0.42***	0.05 ^{ns}	0.03 ^{ns}
	GB	-0.69***	-0.42***	-0.58***	0.41***		0.39***	0.15*	0.25***
	TKW	-0.35***	-0.09 ^{ns}	-0.26***	0.28***	0.29***		-0.49***	-0.15*
	NSdPS	-0.25***	-0.19**	-0.21***	0.20**	0.48***	-0.31***		0.42***
	HI	-0.70***	-0.45***	-0.61***	0.15*	0.76***	0.21***	0.45***	

Remark: DFL = days to flowering; SPAD = relative chlorophyll content; DM = days to maturity; PH = average plant height; GB = grain biomass, TKW = thousand kernel weight; NSdPS = number of seeds per spike, HI = harvest index; *** significant at $p < 0.001$, ** significant at $p < 0.01$, * significant at $p < 0.05$, and ^{ns} for non-significant. Additionally, data of 2017 were not included as no drought stress appeared in field naturally drought prone locations; and 2018 Debrezeit data were excluded from FC analyses due to the presence of poor germination data at the site.

Table 4: Pearson's correlation coefficient (*r*) analysis results for the field experiment; above the diagonal boxes for CCn treatment, below the diagonal boxes for CS treatment.

		Control treatment							
		DFL	**SPAD**	**DM**	**PH**	**NSdPS**	**GB**	**TKW**	**HI**
Drought stress treatment	**DFL**		-0.08 ^{ns}	0.72***	-0.28***	-0.42***	-0.55***	-0.44***	-0.70***
	SPAD	-0.09 ^{ns}		0.05 ^{ns}	0.16*	0.36***	0.28***	0.21**	0.20**
	DM	0.85***	-0.02 ^{ns}		-0.25***	-0.34***	-0.42***	-0.46***	-0.58***
	PH	-0.22**	0.12 ^{ns}	-0.26***		0.29***	0.40***	0.47***	0.34***
	NSdPS	-0.41***	0.11 ^{ns}	-0.36***	0.18*		0.57***	0.36***	0.67***
	GB	-0.46***	-0.02 ^{ns}	-0.37***	0.21**	0.80***		0.29***	0.83***
	TKW	-0.22***	-0.12 ^{ns}	-0.07 ^{ns}	0.20**	0.14*	0.37***		0.34***
	HI	-0.53***	-0.03 ^{ns}	-0.47***	0.14 ^{ns}	0.65***	0.83***	0.30***	

Remark: DFL = days to flowering; SPAD = relative chlorophyll content; DM = days to maturity; PH = average plant height; GB = grain biomass, TKW = thousand kernel weight; NSdPS = number of seeds per spike, HI = harvest index; *** significant at $p < 0.001$, ** significant at $p < 0.01$, * significant at $p < 0.05$, and ^{ns} for non-significant.

DM was strongly negatively correlated $-0.21 \leq r \geq -0.61$, $p < 0.001$ with most traits but not correlated with PH and positively correlated $r = 0.57$, $p < 0.001$ with SPAD in FS. Similarly, DM was also strongly and negatively correlated $-0.26 \leq r \geq -0.37$, $p < 0.001$ with most traits but not correlated with TKW and SPAD in CS (Table 2 and Table 3). Therefore, the correlations suggest that DM is the most important trait to identify drought tolerance in Ethiopian barley accessions rather than NSdPS, as there was a reasonable reduction (21.3% in FS and 12.9% in CS) compared to their respective control treatment and significant difference was observed due to drought stress (Table 1 and Table 2).

Results obtained in climate chamber experiments of the 196 accessions were also correlated with field experiments. Positive correlations ($p < 0.001$) were detected except for SPAD of FS and CS (Table 5). The highest correlation coefficient between the drought treatments was observed for DFL ($r = 0.57$) while the smallest significant correlation coefficient was for PH ($r = 0.24$). Correlation coefficients of DM, GB and HI were ($r = 0.46$, $r = 0.40$, and HI $r = 0.47$), respectively (Table 5).

Table 5: Pearson's correlation coefficient (r) analysis of similar traits between 196 corresponding accessions of field drought stress and climate chamber drought stress as well as for field control treatment and climate chamber control treatment.

	Drought stress treatments	**Control treatment**
Traits	***r* of traits**	***r* of traits**
DFL	0.57***	0.38***
SPAD	0.11^{ns}	0.13^{ns}
DM	0.46***	0.17*
PH	0.24***	0.22**
NSdPS	0.52***	0.38***
TKW	0.38***	0.18*
GB	0.40***	0.11^{ns}
HI	0.47***	0.24***

Remark: DFL = days to flowering; SPAD = relative chlorophyll content; DM = days to maturity; PH = average plant height; GB = grain biomass, TKW = thousand kernel weight; NSdPS = number of seeds per spike, HI = harvest index; *** significant at $p < 0.001$, ** significant at $p < 0.01$, * significant at $p < 0.05$, and ^{ns} for non-significant.

For the control treatments, HI ($r = 0.24$), NSdPS ($r = 0.38$), and DFL ($r = 0.38$) of CCn were positively correlated ($p < 0.001$) with their corresponding traits estimated in FC, while PH ($r = 0.22$) at ($p < 0.01$), and DM ($r = 0.18$) and TKW ($r = 0.18$) at ($p < 0.05$) were also found to be positively correlated but GB and SPAD were not correlated, with their respective traits in CCn and FC (Table 5).

Genome wide association studies (GWAS)

The overall LD decay value at r^2 of 0.1 was 3.06 Mb. Chromosome 3H showed the largest decay of 5.08 Mb, while chromosome 1H and 6H showed the lowest decay of 2.33 and 2.31 Mb respectively (Table 6).

Table 6: Linkage disequilibrium (LD) decay values of Ethiopian barley landraces across the seven chromosomes based on ($r^2 = 0.1$).

Chromosome	LD decay (Mb)
1H	2.33
2H	3.57
3H	5.08
4H	3.82
5H	2.62
6H	2.31
7H	3.82
Over all	3.06

The Quantile-quantile (QQ) plots demonstrated that the majority of GWAS results were found to be efficiently fitted to the BLINK model (Figure S2). GWAS identified a total of 92 MTAs, based on false discovery rate (FDR) adjusted p-values < 0.05 (Benjamini and Hochberg 1995). In summary, 23, 35, 23, and 11 MTAs were identified in FS, CS, FC, and CCn, respectively (Supplementary Table S3). These correspond to 84 QTLs, of which 23, 23, 27, and 11 were detected in FC, FS, CS and CCn, respectively, based on the LD values of each chromosome. As 83 of 84 (98.8%) QTLs were represented by just one SNP marker (Supplementary Table S3), the main focus will be on MTAs in this study. Overall, the most MTAs were detected for HI (18), GB (14) and TKW (17); while the lowest number was detected for SPAD (7), and specifically, no markers were detected for HI in FC and NSdPS in of CCn (Table 1). The highest number of 17 MTAs in drought stress treatments was detected for HI (CS = 15 and FS = 2), though 9 of the 15 CS MTAs were clustered on chromosome 2H between 711.75-712.33 Mb and represented by one QTL, followed by 9 MTAs for NSdPS (FS = 5 and CS = 4) (Table 1). The least number of 4 MTAs were recorded for SPAD (FS = 2 and CS = 2) (Table 1).

Common markers detected in this study, which represent two or more MTAs were “JHI-Hv50k-2016-19711”, “JHI-Hv50k-2016-31649”, “JHI-Hv50k-2016-73780”, “JHI-Hv50k-2016-108079”, and “JHI-Hv50k-2016-281531”. Apart from the marker “JHI-Hv50k-2016-31649” on chromosome 1H at 427.69 Mb, all markers revealed at least one association under drought stress treatments (Table 7, and Supplementary Table S3). The only common marker detected between field experiments (TKW of FC) and climate chamber experiments (DM of CCn; and, HI and GB of CS) was “JHI-Hv50k-2016-73780” that is located on chromosome 2H at 29.85 Mb (Table 7 and Supplementary Table S3).

MTAs or QTLs that are at a locus of the chromosome with a physically distance less than the chromosome's LD value are considered as linked; there were 17 loci carrying 50 linked MTAs in this study (Supplementary Table S3). In the drought stress treatments 32 of the 50 linked MTAs (FS = 12 and CS = 20) were detected distributed along 16 different loci, while the remaining 18 linked MTAs (FC = 10 and CCn = 8) were detected in control conditions, which correspond to 13 different loci. Regarding drought stress treatments, the highest number of linked MTAs was detected for HI (15), which were distributed over six different loci that represent six QTLs followed by NSdPS, which had five linked MTAs, distributed across five different loci (Supplementary Table S3).

Few results of GWAS are in loci close to known flowering genes *Ppd-H1* and *ELF3*, which are on chromosome 2H at 29.1 Mb and chromosome 1H at 556.9 Mb respectively, and barley row type-determining genes *Vrs1* and *Vrs2*, which are on chromosome 2H at 652.0 Mb and chromosome 5H at 562.55 Mb respectively (Table 7 and Supplementary Table S3). The correlation analysis showed the presence of strong associations of DFL with all traits in FS and with all but not SPAD in CS (Table 3 and 4), therefore the flowering determining genes may have a significant effect on different traits. The influence of six and two rowed barley types on the number of fertile kernels, grain yield stability, and kernel weight was described in Kandic et al. (2019), as well as the fact that the critical period to determine the grain number is much earlier than the flowering time, can be used to explain the involvement of the row type determining gene in drought stress treatments.

Table 7: Summary of QTLs associated with traits under control and drought stress treatments of field and climate chamber experiment in Ethiopian barley landraces.

SNP	Chr	Position (Mb)	MAF	Effect	PVE (%)	LOD	Trt	Trait	Gene Ontologies	Annotation description
JHI-Hv50k-2016-16677	1H	27.68	0.39	-0.59	0.86	6.03	FC	DM		
JHI-Hv50k-2016-16885	1H	28.89	0.41	0.97	1.31	6.53	FS	PH	GO:0005524 GO:0006468 GO:0030247 GO:0004672 GO:0005509 GO:0005515	receptor-like protein kinase 1
JHI-Hv50k-2016-18027	1H	36.45	0.40	0.90	1.78	7.50	FC	SPAD	GO:0005515 GO:0008270	E3 SUMO-protein ligase SIZ1
BOPA2_12_10314	1H	46.54	0.48	-1.05	1.89	5.28	FC	PH	GO:0004357 GO:0006750 GO:0042398	Glutamate--cysteine ligase B, chloroplastic
JHI-Hv50k-2016-19711	1H	72.46	0.36	-0.70	1.25	5.41	FS	SPAD	GO:0005515 GO:0008270	ankyrin repeat-containing protein 2
JHI-Hv50k-2016-19711	1H	72.46	0.36	0.01	2.34	8.97	FS	HI	GO:0005515 GO:0008270	ankyrin repeat-containing protein 2
JHI-Hv50k-2016-22927	1H	311.10	0.13	3.93	13.12	6.61	CS	PH	GO:0000398 GO:0005681	Thioredoxin-like protein 4A
BOPA2_12_31134	1H	357.29	0.23	-3.90	2.23	5.81	CS	TKW	GO:0015098 GO:0015689 GO:0016021	Major facilitator superfamily protein
JHI-Hv50k-2016-26918	1H	369.36	0.10	6.53	26.30	6.09	CCn	DM	GO:0005515	Leucine-rich repeat family protein
JHI-Hv50k-2016-31649	1H	427.69	0.09	1.73	6.44	7.69	CCn	GB		RNA recognition motif-containing protein
JHI-Hv50k-2016-31649	1H	427.69	0.09	0.06	13.65	6.49	CCn	HI		RNA recognition motif-containing protein
JHI-Hv50k-2016-31649	1H	427.69	0.09	4.03	2.57	4.80	CCn	TKW		RNA recognition motif-containing protein
JHI-Hv50k-2016-54103	1H	547.73	0.45	-0.84	2.33	7.57	FS	DM		N-terminal protein myristoylation
JHI-Hv50k-2016-57491	1H	556.67	0.40	4.70	4.50	6.95	CS	DM	GO:0005488 GO:0005515 GO:0005524	TATA-binding protein-associated factor 172
JHI-Hv50k-2016-58119	1H	557.95	0.10	3.20	5.64	8.17	CS	NSdPS		structural maintenance of chromosomes 5
SCRI_RS_173604	2H	13.27	0.38	-0.02	0.00	5.13	CS	HI		Peptidase S24, S26A, S26B, S26C family protein
JHI-Hv50k-2016-67587	2H	15.46	0.04	7.67	5.42	7.46	CCn	TKW		unknown function
JHI-Hv50k-2016-72079	2H	25.15	0.27	-1.00	5.21	7.30	FC	DM	GO:0016020 GO:0055085 GO:0005215 GO:0006810 GO:0006811	Cation-chloride cotransporter 2
JHI-Hv50k-2016-73780	2H	29.85	0.15	6.74	18.66	7.20	CCn	DM		Symplekin

JHI-Hv50k-2016-73780	2H	29.85	0.15	-0.34	8.32	10.95	CS	GB		Symplekin
JHI-Hv50k-2016-73780	2H	29.85	0.15	-0.06	10.01	18.59	CS	HI		Symplekin
JHI-Hv50k-2016-73780	2H	29.85	0.13	1.59	3.19	5.70	FC	TKW		Symplekin
JHI-Hv50k-2016-73929	2H	30.19	0.13	-2.84	7.08	8.96	CS	NSdPS		
SCRI_RS_110647	2H	38.25	0.18	-3.21	2.22	6.43	CCn	TKW	GO:0043565 GO:0003700 GO:0006351 GO:0006355	transcription factor-related
JHI-Hv50k-2016-77166	2H	45.57	0.12	5.40	29.40	6.59	CCn	PH	GO:0003824 GO:0004553 GO:0005975	alpha-galactosidase 2
SCRI_RS_176159	2H	48.45	0.36	-0.02	0.00	4.82	CS	HI		
SCRI_RS_152206	2H	68.79	0.38	0.92	1.72	5.52	FS	PH	GO:0019430 GO:0045454 GO:0055114 GO:0004791 GO:0005737 GO:0016491	Thioredoxin reductase
JHI-Hv50k-2016-82131	2H	79.85	0.03	5.94	18.21	9.53	FS	TKW	GO:0003779	Stomatal closure-related actin-binding protein 1
JHI-Hv50k-2016-83709	2H	92.21	0.47	0.67	0.91	6.27	FS	NSdPS	GO:0008152 GO:0016787	unknown protein; Has 1524 Blast hits to 1298 proteins in 225 species: Archae - 9; Bacteria - 84; Metazoa - 474; Fungi - 184; Plants - 98; Viruses - 17; Other Eukaryotes - 658, Haloacid dehalogenase-like hydrolase (HAD) superfamily protein (source: NCBI BLink).
JHI-Hv50k-2016-87462	2H	120.87	0.03	0.41	4.30	4.78	CS	GB	GO:0005488	Exportin-T
JHI-Hv50k-2016-93122	2H	432.38	0.48	-1.26	1.22	6.82	FC	NSdPS		unknown function
JHI-Hv50k-2016-102161	2H	620.32	0.15	-1.56	1.94	5.78	FC	TKW		undescribed protein
JHI-Hv50k-2016-108079	2H	654.17	0.20	1.62	1.90	5.67	FC	NSdPS	GO:0003677 GO:0043565	multiprotein bridging factor 1A
JHI-Hv50k-2016-108079	2H	654.17	0.20	-2.24	3.75	10.04	FC	TKW	GO:0003677 GO:0043565	multiprotein bridging factor 1A
JHI-Hv50k-2016-108079	2H	654.17	0.20	-2.21	1.73	8.07	FS	TKW	GO:0003677 GO:0043565	multiprotein bridging factor 1A
JHI-Hv50k-2016-110148	2H	674.26	0.06	4.33	5.03	5.33	CCn	TKW	GO:0009081 GO:0003824 GO:0004084 GO:0008152	Branched-chain-amino-acid aminotransferase 6
SCRI_RS_115690	2H	676.78	0.04	8.53	27.28	15.07	FS	TKW		Acyl-CoA thioesterase family protein
SCRI_RS_139831	2H	702.54	0.28	-2.87	5.58	5.68	CS	PH	GO:0000105 GO:0004424	imidazoleglycerol-phosphate dehydratase

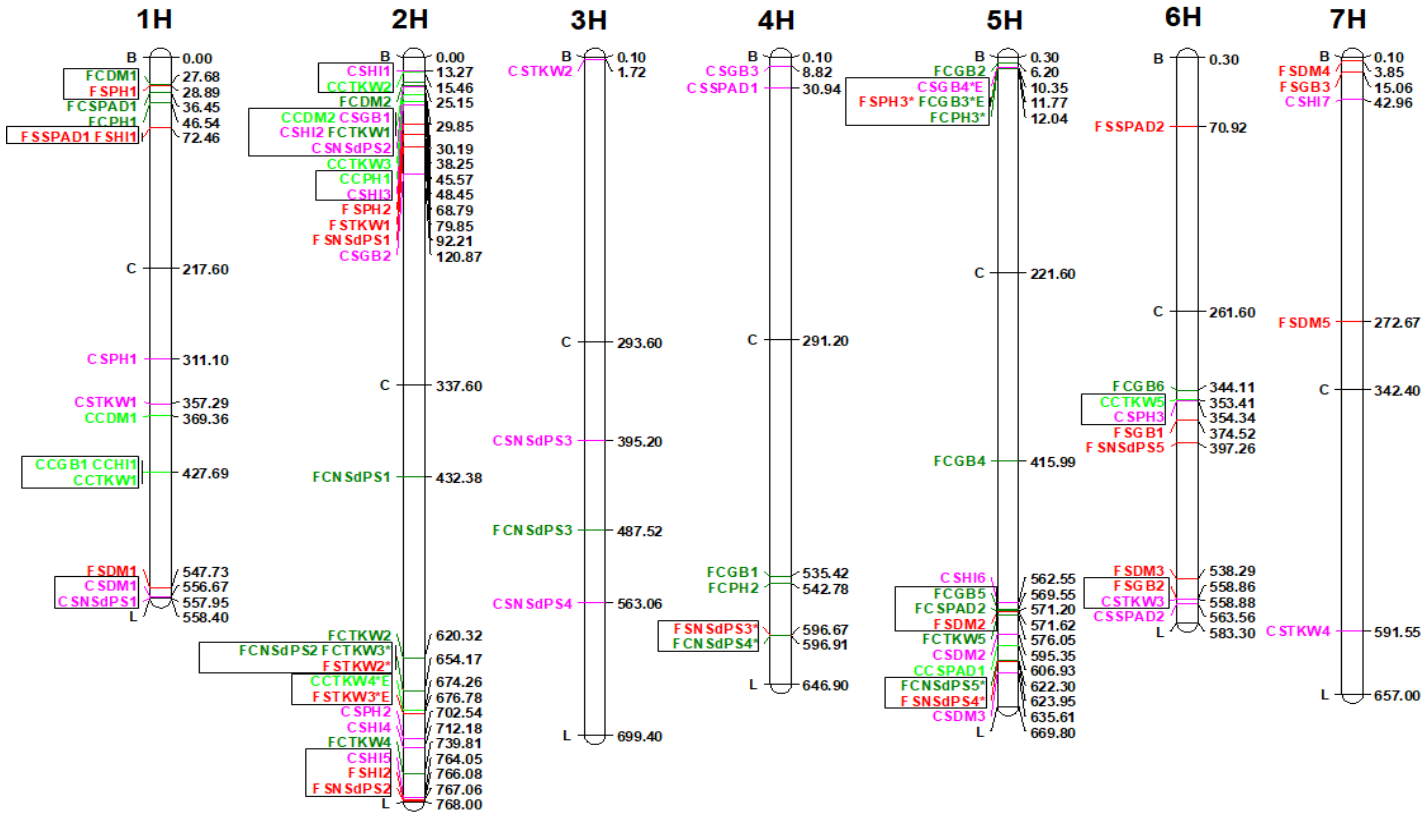
JHI-Hv50k-2016-122549	2H	712.18	0.38	-0.02	0.00	5.17	CS	HI	GO:0016620 GO:0055114 GO:0004491 GO:0008152 GO:0016491	Aldehyde dehydrogenase family 9 member A1-B
JHI-Hv50k-2016-134459	2H	739.81	0.03	2.84	13.79	4.80	FC	TKW		unknown function
SCRI_RS_156075	2H	764.05	0.37	-0.02	0.39	5.44	CS	HI		translocon at the outer membrane of chloroplasts 64-V
SCRI_RS_150519	2H	766.08	0.07	-0.02	6.39	7.01	FS	HI	GO:0008270	RING, FYVE, PHD zinc finger superfamily protein
JHI-Hv50k-2016-147953	2H	767.06	0.06	1.29	3.22	4.68	FS	NSdPS	GO:0043565 GO:0003700 GO:0006355 GO:0008270	GATA transcription factor 26
JHI-Hv50k-2016-149558	3H	1.72	0.46	-3.30	1.70	6.74	CS	TKW		
SCRI_RS_189167	3H	395.20	0.24	-2.23	4.39	8.18	CS	NSdPS		Protein of unknown function (DUF1644)
JHI-Hv50k-2016-183028	3H	487.52	0.32	1.17	2.02	5.57	FC	NSdPS	alpha, beta-Hydrolases superfamily protein, undescribed protein	
JHI-Hv50k-2016-192692	3H	563.06	0.22	-1.59	2.50	5.72	CS	NSdPS		double-stranded-RNA-binding protein 4
JHI-Hv50k-2016-228946	4H	8.82	0.11	0.30	7.88	9.63	CS	GB	GO:0005515	Transducin, WD40 repeat-like superfamily protein
JHI-Hv50k-2016-232915	4H	30.94	0.16	1.73	15.47	6.50	CS	SPAD	Cysteine proteinases superfamily protein, cathepsin B	
JHI-Hv50k-2016-252004	4H	535.42	0.38	-3.84	0.71	5.64	FC	GB		Cell cycle checkpoint protein RAD17
SCRI_RS_166159	4H	542.78	0.49	1.43	3.42	8.12	FC	PH	GO:0016020 GO:0005215 GO:0006810 GO:0006857	Protein NRT1, PTR FAMILY 8.3
JHI-Hv50k-2016-260329	4H	596.67	0.43	-1.06	2.06	9.38	FS	NSdPS		
JHI-Hv50k-2016-260339	4H	596.91	0.46	-1.50	1.41	7.74	FC	NSdPS	GO:0043565 GO:0003677 GO:0003700 GO:0006355	Homeobox-leucine zipper protein family
JHI-Hv50k-2016-279138	5H	6.20	0.03	-10.99	7.58	6.22	FC	GB		Oxidase
JHI-Hv50k-2016-281261	5H	10.35	0.07	-0.26	3.55	5.25	CS	GB	GO:0005488	ARM repeat superfamily protein
JHI-Hv50k-2016-281531	5H	11.77	0.18	1.26	2.95	6.96	FS	PH	GO:0016021 GO:0055085	Cation, calcium exchanger 1
JHI-Hv50k-2016-281531	5H	11.77	0.18	6.39	4.44	9.77	FC	GB	GO:0016021 GO:0055085	Cation, calcium exchanger 1
JHI-Hv50k-2016-281652	5H	12.04	0.03	-2.81	10.93	5.34	FC	PH	GO:004353	Disease resistance protein (CC-NBS-LRR class) family, OJ000126_13.6 protein
SCRI_RS_180821	5H	415.99	0.21	5.03	1.61	7.31	FC	GB		

JHI-Hv50k-2016-320912	5H	562.55	0.04	0.05	9.48	6.87	CS	HI		unknown function
JHI-Hv50k-2016-322900	5H	569.55	0.24	4.63	6.31	6.31	FC	GB	unknown function, Major facilitator superfamily protein	
JHI-Hv50k-2016-323226	5H	571.20	0.30	0.80	1.98	5.41	FC	SPAD	GO:0006633 GO:0031177	acyl carrier protein 3
JHI-Hv50k-2016-323294	5H	571.62	0.32	0.54	1.40	5.06	FS	DM	GO:0003700 GO:0006355 GO:0043565	ABSCISIC ACID-INSENSITIVE 5-like protein 2
JHI-Hv50k-2016-325133	5H	576.05	0.03	-2.71	9.75	5.37	FC	TKW	GO:0009733	SAUR-like auxin-responsive protein family, undescribed protein
JHI-Hv50k-2016-334019	5H	595.35	0.07	-9.42	22.09	8.50	CS	DM	GO:0043565 GO:0003700 GO:0005634 GO:0006355	Heat stress transcription factor A-2a
JHI-Hv50k-2016-338412	5H	606.93	0.34	3.62	24.05	7.45	CCn	SPAD		N-terminal protein myristoylation
JHI-Hv50k-2016-345216	5H	622.30	0.47	-1.25	1.55	5.94	FC	NSdPS		unknown protein; BEST Arabidopsis thaliana protein match is: unknown protein
JHI-Hv50k-2016-346162	5H	623.95	0.28	0.76	1.32	5.54	FS	NSdPS	GO:0004824 GO:0005524 GO:0005737 GO:0006418 GO:0006430 GO:0000166 GO:0003676 GO:0004812	Lysine--tRNA ligase
JHI-Hv50k-2016-349520	5H	635.61	0.03	-10.35	33.12	6.35	CS	DM		Triacylglycerol lipase SDP1
JHI-Hv50k-2016-388506	6H	70.92	0.14	1.06	1.54	6.25	FS	SPAD	GO:0016491 GO:0055114	Apoptosis-inducing factor homolog A
JHI-Hv50k-2016-397691	6H	344.11	0.39	-4.31	1.53	5.33	FC	GB		
JHI-Hv50k-2016-398315	6H	353.41	0.31	3.02	3.64	6.76	CCn	TKW	GO:0008270	TNF receptor-associated factor 6
JHI-Hv50k-2016-398334	6H	354.34	0.18	-3.79	7.36	5.92	CS	PH	GO:0008152 GO:0016491	Dehydrogenase, reductase SDR family member 7B
JHI-Hv50k-2016-400135	6H	374.52	0.14	4.96	4.42	7.53	FS	GB	GO:0004654 GO:0006396 GO:0006402 GO:0000175 GO:0003676 GO:0003723	polyribonucleotide nucleotidyltransferase, putative
JHI-Hv50k-2016-402539	6H	397.26	0.26	-0.77	1.35	5.53	FS	NSdPS	GO:0055114	alcohol dehydrogenase 1
JHI-Hv50k-2016-416678	6H	538.29	0.43	-0.79	1.98	5.72	FS	DM	GO:0016491 GO:0055114	1-aminocyclopropane-1-carboxylate oxidase
JHI-Hv50k-2016-422808	6H	558.86	0.03	7.59	6.72	5.91	FS	GB	GO:0003676 GO:0005634	CCR4-NOT transcription complex subunit 7
JHI-Hv50k-2016-422853	6H	558.88	0.04	9.68	21.04	9.43	CS	TKW	GO:0005515	Pentatricopeptide repeat-containing protein
JHI-Hv50k-2016-424617	6H	563.56	0.25	-1.52	16.81	7.31	CS	SPAD	GO:0008270 GO:0016491 GO:0055114	Alcohol dehydrogenase

SCRI_RS_621	7H	3.85	0.30	0.79	1.69	6.91	FS	DM	GO:0003677	myb-like transcription factor family protein, undescribed protein
JHI-Hv50k-2016-450056	7H	15.06	0.08	-5.39	5.42	6.20	FS	GB	GO:0003993 GO:0005524 GO:0006468 GO:0030247 GO:0004672 GO:0005509 GO:0005515	Acid phosphatase 1, undescribed protein, Protein kinase family protein
JHI-Hv50k-2016-460705	7H	42.96	0.33	0.02	1.09	4.96	CS	HI	GO:0006355 GO:0046983 GO:0003677 GO:0003700 GO:0005634	MADS-box transcription factor 5, Retrotransposon protein, putative, unclassified
JHI-Hv50k-2016-478809	7H	272.67	0.07	-1.09	2.86	5.81	FS	DM		Disease resistance protein RPM1
BOPA2_12_30335	7H	591.55	0.47	-2.76	1.28	5.26	CS	TKW	GO:0000413 GO:0003755 GO:0006457	Peptidyl-prolyl cis-trans isomerase B

Remark: Chr = Chromosome; MAF = minor allele frequency; PVE(%)= Percentage of phenotype variance explained; LOD = logarithm of odds; Trt = treatment (FS = field drought stress; CS = climate chamber drought stress; FC = field control treatment and CCn = climate chamber control treatment);Trait (SPAD = relative chlorophyll content; DM = days to maturity; PH = Average plant height; GB = grain biomas, TKW = thousand kernel weight; NSdPS = number of seeds per spike, HI = harvest index); GO term = Gene Ontologies

Fig. 1*:* Genetic map showing identified QTLs of Ethiopian barley landraces for drought tolerance traits under field and climate chamber conditions (the physical distance in Mb).



Remark: C = centromere region of the chromosome; B and L = known start and stop position of genome based on (Mascher et al. 2017); QTL in dark green font = FC; red font = FS; light green font = CCn; pink font = CS; QTLs inside boxes were linked; 1H to 7H for barley chromosome, the first letter "F" and "C" designated for field and climate chamber experiment, respectively; the second letter designated for treatments "C" for control treatment and "S" for drought stress treatment; the rest letter for variables "DM" for days to maturity, "GB" for grain biomass, "NSdPS" for number of seeds per spike, "PH" for plant height, "TKW" for thousand kernel weight, "HI" for harvest index; "*" constitutive QTLs, "*E" constitutive QTLs but across treatments of climate chamber and filed experiments.

Linked QTLs or MTAs that are detected for the same trait in both control and drought stress treatments are considered constitutive. “JHI-Hv50k-2016-108079” was the only constitutive marker found on chromosome 2H at 654.17 Mb for TKW in FC and FS (Table 7 and Supplementary Table S3). Furthermore, there were two constitutive MTAs detected between the same traits of climate chamber and field experiment treatments. The first one is located on chromosome 2H (674.26–676.78 Mb) for TKW in CCn and FS, while the second one is for GB in CS and FC mapped on 5H (10.35-11.77 Mb) which also possessed constitutive markers for PH in FC and FS.

The MTAs on chromosome 2H (764.05-766.08 Mb) were the only overlapping MTAs identified for the same trait (HI) of drought stress treatment in the field and climate chamber experiment (Table 7, Fig. 1, and Supplementary Table S3).

Both experiments' drought stress treatments revealed a higher LOD values for MTAs than the control treatments. The first and the third highest LOD values were observed at the locus of multiple significant marker trait associations on chromosome 2H at 29.85 Mb for HI (LOD = 18.6), and GB (LOD = 11.0) of CS. There were also other MTAs at this locus, namely for NSdPS (LOD = 9.0), DM (LOD = 7.2) and TKW (LOD = 5.7) of the CS, CCn, and FC, respectively (Table 7). The MTA with the second highest LOD = 15.1 was found on chromosome 2H at 676.78 Mb for TKW in FS. In LD with this MTA, there is a constitutive MTA associated with TKW in CCn at 674.26 Mb with LOD = 5.3 (Table 7, and Fig. 1). The highest LOD value for a locus carrying MTAs only in drought stress treatments was observed on 2H at 79.85 Mb for TKW (LOD = 9.5) (Table 7). In general, the LOD values of MTAs under CS were higher than FS (Table 7).

The highest marker effect was observed for FC of GB (-10.99), and CS of DM (-10.35) both from chromosome 5H at 6.20, and 635.61 Mb respectively; followed by CS of TKW (9.68) and DM (-9.42); and FS of TKW (8.53) on chromosome 6H, 5H and 2H at 558.88, 595.35, and 676.78 Mb, respectively; while the least effect (-0.02 to 0.06) was observed for all significant markers observed for HI (Table 7, and Supplementary Table S3).

Six out of the seven highest PVE values were observed from the climate chamber experiment. DM of CS (33.12%), PH of CCn (29.40%), and TKW of FS (27.28) were the three highest on chromosome 5H at 635.61 Mb and 2H at 45.57 and 676.78 Mb, respectively, while PVE values close to zero were recorded from HI of CS on chromosome 2H at 13.27, 48.45, and 712.18 Mb; and from the field experiment GB (0.71%) and DM (0.86%) of FC and NSdPS of FS (0.91%), were with the least PVE values on chromosome 4H at 535.42 Mb, 1H at 27.68 Mb, and 2H at 92.21 Mb, respectively (Table 7 and Supplementary Table S3).

On chromosome 2H the highest number of significant MTAs was assigned (36) with the highest LOD values, while on chromosome 3H the least number of MTAs (4) was detected (Supplementary Table S4). MTAs detected for CS were found on all chromosomes, whereas for FS on all except chromosome

3H. Furthermore, the number of MTAs detected in FS and CS on chromosomes 1H, 2H, 6H, and 7H were either equal or higher than the number of MTAs detected in FC and CCn (Fig. 1). On chromosomes 2H, 6H and 1H, 16 out of 23 detected MTAs for FS are located, while on chromosomes 2H, 1H and 5H, 25 of the 35 detected MTAs in CS were detected (Supplementary Table S4).

Discussion

The amount of rainfall reduction during the main cropping season of the FS experimental locations in Ethiopia reached up to 40% compared with the FC locations (Supplementary Table S1). The FS experiments were conducted in the central rift valley (CRV) of Ethiopia, where the pattern of rainfall through the main cropping season is erratic, unpredictable, and variable compared to other locations in Ethiopia (Adimassu et al. 2014). The extended dry spell starts in general in September (Kassie et al. 2013). Bekele et al. (2016), and Bekele et al. (2019) also predicted that the pattern on the onset of the dry spell would persist in CRV at least until the end of the century.

Thus, drought stress experiments in the CRVs help in the identification of early flowering accessions that are capable of completing their life cycle before the onset of the dry period (Chaves et al. 2003). Medium-flowering accessions can also tolerate post-flowering drought stress through minimizing water loss, increasing water uptake through high root biomass, osmotic adjustment or storage of different compatible solutes (Ehleringer and Cooper 1992, Jackson et al. 2000, Yokota et al. 2006, Barnabás et al. 2008), instead of late-flowering accessions which suffer most. In the climate chamber experiments, the drought tolerance potential of all accessions was evaluated at 20% WC after flower initiation (Figure S1e-h) which enabled us to assess the potential of all accessions, including late maturing ones, unlike in the field experiment.

From Ethiopian barley landrace collections, 13 accessions were selected as tolerant to severe drought stress in the climate chamber experiment, while three landrace accessions were selected based on their stable grain biomass performance in field experiments. One accession (B191.1) was found in both experiments in the top 20 accessions (Supplementary Table S2). Nevertheless, general grain biomass reduction due to drought stress was observed, i.e. 47.4% and 79.8% in FS and CS treatments, respectively (Table 1). According to Samarah et al. (2009a), drought resulted in a 73% to 87% grain yield reduction in barley, whereas Li et al. (2006) reported 25% grain yield reduction for tolerant genotypes and 50% to 55% grain yield reduction for susceptible genotypes; thus, the observed grain biomass yield reduction is consistent with previous reports.

Drought stress tolerance is considered a complex trait, especially, when the genotypes are evaluated for GB, as GB and other important agronomic traits are polygenic traits and also influenced by epistasis (Blum 2011). GB reduction by drought stress treatments was reported to be strongly correlated with different agro-physiological traits. For example, a negative correlation with DFL and DM (Vaezi et al. 2010); positive correlation with number of spikes and NSdPS, SPAD, stomatal conductance and net

photosynthesis rate (González et al. 2010, Thameur et al. 2012); negative correlation with number of tillers and biomass weight (Teulat et al. 1997b, Teulat et al. 2003) under drought stress conditions were reported. Except for SPAD, which was conversely correlated with GB in FS (r = -0.42, $p < 0.001$) and revealed no correlation in CS (Tables 2 and 3), our findings on drought treatments were in line with those already reported.

An acceptable rage of heritability was observed for most traits in FS treatment (Table 1). Such results are the basis to explore the influence of genetic variation on drought tolerance and enabled us to exploit the power of GWAS to detect important genomic regions associated with drought stress tolerance. Previous drought stress studies reported 80% and 64% of heritablity by Wiegmann et al. (2019), and Thabet et al. (2020) for grain yield, respectively. Our observed heritability of 76.4% for GB of FS is also in a similar range (Table 1).

Previous studies on GWAS for drought tolerance of barley detected different numbers of QTL across the barley genome. 44% of QTLs related to drought tolerance were identified on chromosomes 2H and 3H by Zhang et al. (2017) using meta-analysis; 52% and 54% of significant markers were located on chromosomes 2H and 3H in studies by Mora et al. (2016) and Gudys et al. (2018), respectively. In other studies, the most significant markers were reported for chromosomes 2H and 4H (Wójcik-Jagła et al. 2018); and 5H (Wehner et al. 2015). In our study, on chromosome 2H 24 out of 58 (41.4%) markers associated with traits under drought stress were detected (Supplementary Table S4). Therefore, chromosome 2H was the most important for drought tolerance of Ethiopian barley landraces, not only because it had the most MTAs, but also because it had the highest LOD values (Table 7).

Genes that are expressed constantly in different environments are considered constitutive genes. Mostly, such genes are expressed at an intermediate level and provide immediate response when the environment is changing. However, to acquire an optimal level of protein from adaptive genes, the optimal environmental signal is needed (Geisel 2011). Therefore, constitutive types of genes are important to withstand mild drought stress. In this study, a pair of six constitutive QTLs distributed across five loci were detected on chromosomes 2H (2), 4H (1), and 5H (2) (Fig. 1). The intensity of drought stress in FS was moderate compared with CS. As a result, 4 out of 6 constitutive QTLs were identified in FC and FS for TKW (1), PH (1) and NSdPS (2). The remaining two constitutive QTLs were on chromosome 5H at 10.35-12.04 Mb, for GB in FC and CS, the other for TKW in CCn and FS on chromosome 2H (Fig. 1).

The constitutive QTLs for the complex trait of GB were identified in FC and CS on chromosome 5H at 10.35-11.77 Mb (Table 7 and Fig.1). The identification of such QTLs is important as the level of stress in CS was very high compared to FS, and another constitutive QTL was observed at this locus for PH as it was detected in FC and FS (Supplementary Table S3).

The study identified 87 significant genes, 51 of which were associated with at least one Gene Ontology (GO) term (Supplementary Table S3). GO term enrichment analysis was conducted using the Singular Enrichment Analysis tool in GO analysis toolkit and database for agricultural community, AgriGO v2.0 (https://systemsbiology.cpolar.cn/agriGOv2/index.php) (Tian et al. 2017); and, showed only one enriched GO terms based on a Bonferroni adjusted p-value; however, using the non-adjusted method, a total of six significant GO terms were found (Supplementary Table S5).

Three GO terms were associated with biological processes, whereas two and one were associated with molecular function, a cellular component, respectively. Specifically, the GO terms in the biological process are GO:0010468 for regulation of gene expression, GO:0060255 for regulation of macromolecule metabolic process, and GO:0019222 for regulation of metabolic process; GO terms in the molecular function are GO:0003700 for transcription factor activity, and sequence-specific DNA binding, and GO:0003677 for DNA binding, while in the cellular component GO:0005634 was in the nucleus (Supplementary Table S5).

Except for chromosomes 3H and 7H, on which no linked QTLs were detected, other chromosomes carry at least two linked QTLs (Supplementary Table S3). One of the loci on 2H at 29.85-30.19 Mb contains five linked QTLs (3=CS, 1=CCn, and 1=FC) for different traits (HI, GY, NSdPS, DM, and TKW) (Table 7 and Fig. 1); which indicates the importance of the locus to govern multiple traits. Gordon et al. (2020) also detected the association of multiple traits such as heading date, plant height, and kernel length on chromosome 2H at 27.2-29.8 Mb in field drought stress experiments. Such effects may be due to the tight linkage of genes or the pleiotropic effect of a single gene (Hall et al. 2006). Close to these MTAs, the known barely flowering time gene (*Ppd-H1*) is located on chromosome 2H at 29.1 Mb (Cockram et al. 2007, Mascher et al. 2017). Different studies also mentioned the importance of this locus as it carries a constitutive QTL for biomass accumulation in greenhouse experiments (Dhanagond et al. 2019), besides flowering time QTL and other grain yield related traits (Ogrodowicz et al. 2017, Thabet et al. 2018) in field and greenhouse experiments.

The locus on 2H (764.05-766.08 Mb) is the only overlapping locus for the same trait of (HI) in CS and FS. In LD with this locus is an MTA at 767.06 Mb for NSdPS in FS also detected (Fig. 1 and Supplementary Table S3). Overlapping MTAs between FS and CS are one of the possible indicators for the presence of similar drought tolerance mechanisms in both experiments. Although there were 17 loci, that revealed linked QTLs detected in this study, only three of them, which were mapped on chromosomes 2H (764.05-767.06 Mb) for HI, 5H (10.35-11.77 Mb) for GB and PH, and 6H (558.86-558.88 Mb) for TKW and GB which also had the highest effect values, were detected in CS and FS, respectively (Fig. 1 and Supplementary Table S3). This may be due to the fact, that drought stress experiments conducted in pots do not represent actual field conditions, as pot experiments typically favor cultivars that are sensitive to ABA and give minor credit to drought tolerance mediated through high osmotic adjustment and a deep root system (Blum 2011). Furthermore, in our study at the FS

locations, early flowering accessions were mostly favored over late flowering accessions, which may have efficient grain-filling capacity (Figure S1a-d).

Several studies reported different significant QTLs and candidate genes, that were close to some of the identified MTAs in our study. For example, one of the constitutive MTAs reported here for NSdPS in FS and FC was detected on chromosome 5H at 622.30-623.95 Mb. Close to these MTAs, a QTL associated with grain yield and biomass was reported by Al-Abdallat et al. (2017) and Mora et al. (2016), respectively in field experiments. Furthermore, on chromosome 5H at 623.5 Mb, *HvHsfA2e*, and *Dhn9* genes, which are expressed in response to heat stress (Mikołajczak et al. 2022) and drought stress (Graether and Boddington 2014, Banerjee and Roychoudhury 2016), respectively, are located and known to be associated to biomass development during a drought stress period in Dhanagond et al. (2019). Although the reported QTL was mapped on chromosome 5H at 617.1 Mb, an association with the number of seeds and spikelets per main spike were reported by Ogrodowicz et al. (2017).

The constitutive QTLs for TKW in field experiments were mapped on chromosome 2H at 654.17 Mb. At 652.0 Mb the row type determining gene *Vrs1* is located. *Vrs2* is also mapped on chromosome 5H at 564.4 Mb. In a close proximity to the *Vrs2* locus at 562.55 Mb on chromosome 5H, an MTA for HI in CS was detected in our study. Furthermore, a QTL associated with biomass recovery after drought stress in a greenhouse experiment was reported near to the *Vrs2* locus in Dhanagond et al. (2019), and Al-Abdallat et al. (2017) also reported a QTL associated with awn length in a drought-prone location. Six-rowed barley has more florets than two-rowed barley, allowing to produce more seeds per spike. Concordantly in our study, a strong and positive correlation of NSdPS and GB was observed (Table 3 and Table 4). Dodig et al. (2018) have shown the absence of clear differences in drought tolerance in two row type of barley but observed that six-rowed barley demonstrated a better grain yield stability while two-rowed barley showed better drought tolerance as well as better kernel weight during a defoliated drought treatment. Furthermore, Kandic et al. (2019) revealed that six-rowed barley has higher percentage of grain yield reduction than two-rowed barely in a defoliated drought stress field experiment. Therefore, the difference in row type resulting in a change in GB and TKW in our study, ultimately contributed to the difference in HI in CS treatment.

An MTA on chromosome 4H at 8.82 Mb was also identified for GB in CS (Table 7). QTLs at LD with this locus were reported to be associated with hectoliter weight and dry biomass in drought stress treatments conducted in field and greenhouse experiments (Mora et al. 2016, Pham et al. 2019), which had a positive correlation with grain yield in these studies. In FS, one MTA with GB was mapped on chromosome 7H at 15.1 Mb (Table 7). Although the identified QTLs were out of LD, Pham et al. (2019) reported an association with relative growth rate in a greenhouse drought experiment on barley at 10.2Mb. The MTA on chromosome 7H, at 3.9 Mb was found to be associated with DM in FS (Table 7). A candidate gene known as *DWARF 3* (*HvD3*) was reported very close to this MTA in a greenhouse conducted experiment (Alqudah et al. 2018, Pham et al. 2019), that has a strong correlation with

environmental response and a significant association with agronomic, physiological traits and leaf blade as well as grain yield.

ELF3, also known as eam8 was mapped on chromosome 1H at 556.9Mb (Faure et al. 2012, Zakhrabekova et al. 2012, Mascher et al. 2017), and it promotes the transition from the vegetative development to the reproductive stage. *HvCMF6a* and *HvCMF6b*, which have similar effect like *ELF3* were also mapped on chromosome 1H located closely at 558.2 Mb (Cockram et al. 2012, Mascher et al. 2017). In our study, MTA associated with DM in FS and CS was detected on chromosome 1H at 547.73 and 556.67 Mb, respectively. DM had a strong correlation with DFL (r = 0.81, and 0.85, $p < 0.001$ for FS and CS respectively), and genes associated with DFL may have a role in DM. Additionally, an MTA for NSdPS was detected on chromosome 1H at 557.95 Mb in CS. In field and greenhouse experiments Hu et al. (2018), and Pham et al. (2019) discovered QTL close to these loci, which have an influence on drought stress tolerance or play a role in the increase of grain yield related traits.

MTAs with the highest PVE values were on chromosome 5H and 2H at 635.61 and 676.75 Mb for CS of DM and FS of PH with annotation of 'triacylglycerol lipase SDP1' and 'acyl-CoA thioesterase family protein', respectively (Supplementary Table S3). Perlikowski et al. (2022) explained the storage of triacylglycerol in the chloroplast during drought helps to prevent the storage of toxic fatty acids in monocots.

In rice, the overexpression of rice acyl-CoA thioesterase was reported to increase grain weight by 47% by improving the grain filling rate (Zhao et al. 2019). The presence of a significant positive association of plant height with leaf length and area of flag leaf to the fourth leaves was reported by Du et al. (2019), and in our study, the presence of a significant positive association between PH and GB in FS was observed (Table 3), which may be associated with the remobilization of important nutrients to grain during drought stress period. Additionally, maize acyl-CoA-binding proteins were reported to be expressed during drought stress, and the overexpression of the protein improves drought tolerance (Zhao et al. 2019).

Physiological maturity is an important stage at which the maximum GB and seed number are attained, and the crop stops further growth (Calderini et al. 2000). In our study, the importance of the trait was also demonstrated as it was strongly and negatively correlated ($p < 0.001$) with most other traits analysed (Table 3 and Table 4) in FS and CS. The occurrence of drought significantly reduced the time to maturity (Table 1). A number of annotations associated with DM in FS and CS were identified in this study. 1-aminocyclopropane-1-carboxylate oxidase (ACO) was one of these identified in FS (gene id of '*HORVU6Hr1G079640'*) mapped on chromosome 6H at 538.29 Mb (Supplementary Table S3). This protein was considered as a rate-limiting enzyme in ethylene production (Houben and Van de Poel 2019), and a study demonstrated that the ratio between ABA and ethylene has a significant role in differentiating between drought tolerant and susceptible seedlings of wheat genotypes (Valluru et al. 2016).

In general, different QTLs that influenced drought stress tolerance of barley were reported by different studies (Hu et al. 2018, Thabet et al. 2018, Dhanagond et al. 2019, Pham et al. 2019, Thabet et al. 2020), which are located in the vicinity of those detected QTLs in this study. Furthermore, QTLs were also reported around the same genome regions that influenced different traits by and Wehner et al. (2015), Al-Abdallat et al. (2017), Ogrodowicz et al. (2017), Abdel-Ghani et al. (2019), Sallam et al. (2019).

GWAS have been used to investigate agro-physiological important traits in barley (Wehner et al. 2015, Wehner et al. 2016b, Wójcik-Jagła et al. 2018, Thabet et al. 2020, Elbasyoni et al. 2022, Li et al. 2022, Afsharyan et al. 2023, Xiong et al. 2023), but the presence of a high false positive rate result was the major challenge (Tibbs Cortes et al. 2021). Beside the application of different p-values correction methods like FDR (Benjamini and Hochberg 1995) and Bonferroni methods, different GWAS models were developed to improve the computational power and efficiency. The multi-locus GWAS model of the multi-locus mixed model (MLMM) (Segura et al. 2012) had better performance than the single-locus GWAS models. Fixed and random model circulating probability unification (FarmCPU) (Liu et al. 2016) and its modified version (BLINK) had better performance than MLMM (Tibbs Cortes et al. 2021). The GWAS result obtained from this study had less false-positive result as the analysis was conducted using the BLINK model. However, to utilize the significant MTAs in the breeding program, validation of markers is required, as it ensures the marker's reliability and helps to evaluate its performance before applying them for crop improvement (Rawat 2023).

Conclusion

The drought stress experiments were conducted in naturally drought-prone areas of Ethiopia and in the climate chamber at 20% WC after flowering. The occurrence of drought reduced all the analysed traits, except DFL in the climate chamber, but the highest reduction was exhibited for GB in both experiments, which indicates that a small effect of drought in each trait can result in a high grain biomass penalty. The accession B191.1 was the only accession found to be tolerant to drought stress in the top 20 of both experiments based on HM and DSI drought indices. Strong positive correlation coefficient ($p < 0.001$) between GB and HI in CS and FS also demonstrated the potential of the landraces in future breeding programs. The presence of an acceptable level of heritability in most traits in FS rather than in FC, and a better correlation coefficient between FS and CS than FC and CCn, suggested a better adaptability of Ethiopian genotypes to different levels of drought. Using GWAS, 58 marker trait associations (23 for FS and 35 for CS) influencing drought tolerance in Ethiopian barley landraces were identified. The study found that DM was strongly associated with TKW, NSdPS, PH, SPAD, and HI traits of FS and CS treatments, indicating the importance of DM for drought tolerance. Chromosome 2H was considered most important, as it possessed the highest number (7 and 17) of MTAs for FS and CS, respectively, MTAs with the highest LOD values, as well as a locus with multiple overlapping MTAs (Fig. 1, Table 7, and Supplementary Table S3). The presence of only one overlapping MTA between a trait obtained in FS and CS, could be due to the pot experiments favouring more ABA sensetive genotypes (Blum

2011). MTAs that were close to known flowering genes such as *Ppd-H1* and *ELF3*, as well as the barley row type determining locus *Vrs1* and *Vrs2* were identified. In general, this study provides an insight into the drought tolerance potential of Ethiopian barley landraces and identifies important genome regions with potential candidate genes. However, additional research will be required to validate the detected MTAs.

Publication 2.3) Teklemariam, S. S., K. N. Bayissa, A. Matros, K. Pillen, F. Ordon and G. Wehner (2024). "Genetic analysis of flowering time of Ethiopian barley accessions under field and climate chamber conditions." Agronomy 2024, 14, 3031. https://doi.org/10.3390/agronomy14123031.

Abstract

The flowering time is one of the traits strongly influencing grain yield. In barley, the flowering time is mostly determined by the photoperiod, vernalization, and timely rainfall. As Ethiopia is located near the equator, the photoperiod and vernalization have a minimum effect on barley, but rainfall and temperatures are major challenges. In this study, 260 Ethiopian barley accessions were evaluated for flowering time at four different locations in Ethiopia in three years. Additionally, a set of 196 accessions was evaluated in climate chambers with corresponding environmental parameters. According to the results, the sum of the daily temperature of growing days strongly influenced the flowering time. The mean flowering time of the warmer Melkassa location was 15, 7, and 4 days earlier than of the cooler Holetta location and the less warm Debrezeit and Dera locations, respectively. On the other hand, the flowering time in the climate chamber was delayed by 52 and 37 days than that at the Melkassa and Holetta locations, respectively; its lowest average daily temperature (18 °C), compared to Melkassa (28.0 °C) and Holetta (22.1 °C), might be the reason. GWAS identified MTAs on chromosomes 5H at 571.62 to 572.54 Mb as strongly associated with the flowering time at warm locations (Melkassa, Dera, and Debrezeit); MTAs on chromosome 2H at 25.1 and 29.3 Mb turned out to be associated with the flowering time at Holetta and in the climate chamber, respectively. Important factors that influence the flowering times of Ethiopian barley landraces and associated SNP markers are identified in this study, which might be useful to consider in future barley breeding programs.

Keywords: barley; Ethiopian landraces; flowering time; growing degree days; temperature effect; GWAS

Zusammenfassung

Die Blütezeit ist eines der Merkmale, die den Kornertrag stark beeinflussen. Bei Gerste wird die Blütezeit hauptsächlich durch die Photoperiode, die Vernalisation und rechtzeitige Niederschläge bestimmt. Da Äthiopien in der Nähe des Äquators liegt, haben die Photoperiode und die Vernalisation nur minimale Auswirkungen auf die Gerste, aber Niederschläge und Temperaturen stellen eine große Herausforderung dar. In dieser Studie wurden 260 äthiopische Gerstenakzessionen an vier verschiedenen Standorten in Äthiopien in drei Jahren auf ihren Blühzeitpunkt hin untersucht. Zusätzlich wurde eine Gruppe von 196 Akzessionen in Klimakammern mit entsprechenden Umweltparametern untersucht. Die Ergebnisse zeigen, dass die Summe der täglichen Temperatur der Wachstumstage die Blütezeit stark beeinflusst. Die mittlere Blütezeit am wärmeren Standort Melkassa war 15, 7 bzw. 4 Tage früher als am kühleren Standort Holetta und an den weniger warmen Standorten Debrezeit und Dera. Andererseits verzögerte sich die Blütezeit in der Klimakammer um 52 bzw. 37 Tage gegenüber den Standorten Melkassa und Holetta. GWAS identifizierte MTAs auf Chromosom 5H bei 571,62 bis 572,54 Mb als stark mit der Blütezeit an warmen Standorten (Melkassa, Dera und Debrezeit) assoziiert; MTAs auf Chromosom 2H bei 25,1 und 29,3 Mb erwiesen sich als mit der Blütezeit in Holetta bzw. in der Klimakammer assoziiert. In dieser Studie wurden wichtige Faktoren identifiziert, die die Blütezeit der äthiopischen Gersten-Landrassen beeinflussen, sowie die dazugehörigen SNP-Marker, die bei zukünftigen Gerstenzuchtprogrammen berücksichtigt werden könnten.

Schlüsselwörter: Gerste; äthiopische Landrassen; Blütezeit; Wachstumsgradtage; Temperatureffekt; GWAS

Introduction

In Ethiopia, barley ranks fifth in terms of production area and total yield harvested; in 2018/19, 811,782.08 hectares of land were cultivated and an average of 2.18 tons per hectare was harvested, which accounts for 5.63% of the total cereal production (CSA 2019). It is the most adapted cereal crop, growing in a wide range of agro-ecologies, from low lands of drought-prone areas at 1500 m above sea level (masl) to the highlands of Ethiopia at 3400 masl (Yaynu 2006). The main purposes of barley are to prepare different types of food and local beverages (Mohammed et al. 2016).

Flowering is the most important stage in plant development, which significantly contributes to environmental adaptation and, ultimately, to grain yield (Royo et al. 2018, Göransson et al. 2019). Therefore, the complex trait flowering time is considered one of the key interventions in barley (*Hordeum vulgare* L.) breeding programs (Alqudah and Schnurbusch 2017) and is of prime importance for improving yield and yield components (Esparza Martínez and Foster 1998, Cuesta-Marcos et al. 2009).

Despite the fact that genes associated with barley's flowering pathways are not quite similar in the northern and south part of the hemispheres (Pham et al. 2020), it is difficult to pinpoint a gene that regulates flowering time, even in the northern hemisphere (Cockram et al. 2007). However, several studies identified genes associated with the length of photoperiod (*Ppd-H1* and *Ppd-H2*, which are located on chromosomes 2HS and 1HL, respectively), vernalization requirements (*Vrn-H1*, *Vrn-H2*, and *Vrn-H3* that are mapped on chromosomes 5HL, 4HL, and 7HS, respectively), and earliness per se (*ELF3*, *eps2S*, *sdw1*, *eps3L*, and *HvPHYC*, which are mapped on chromosomes 1H, 2H, 3H, 3H, and 5H, respectively); these are believed to be the most important genes (Laurie et al. 1995, Snape et al. 2001, Cockram et al. 2007, Kikuchi and Handa 2009, Maurer et al. 2015, Fernández-Calleja et al. 2021, Cosenza et al. 2024).

The dominant *Ppd-H1* allele enhanced earlier flowering during the long photoperiod days than the recessive *ppd-H1* allele (Turner et al. 2005, Hemming et al. 2008, Fernández-Calleja et al. 2021). Because the dominant *Ppd-H2* allele is found in spring and winter barley genotypes, *Ppd-H2* is often described as a complex gene (Casao et al. 2011, Fernández-Calleja et al. 2021); as a result, it is very difficult to categorize barley genotypes based on this flowering gene. *Vrn-H1* is the major regulatory gene for vernalization in barley (Trevaskis et al. 2007). The winter genotypes with the *vrn-H1* allele require prolonged exposure to cold temperatures in order to complete the transition from the vegetative stage to the flowering development stage in a timely way (Trevaskis et al. 2003, Fernández-Calleja et al. 2021). On the other hand, the deletion or insertion in the first intron of the *Vrn-H1* gene significantly affects the length of cold exposure (Fu et al. 2005, Cockram et al. 2007, Hemming et al. 2008), which benefited the wide adaptation of barley to different environments (von Bothmer et al. 2003, Cockram et

al. 2011). Winter barley types have the dominant *Vrn-H2* allele, which has a high expression during the winter period, which helps to enhance flower induction (Trevaskis et al. 2003).

Although the importance of environmental cues like day length (photoperiod) and an extended exposure to cold temperature (vernalization) are described to determine the flowering time in barley, these factors may not be important for Ethiopian barley landraces. Being located near the equator (between 3.3° and 14.9° N of the latitude), there is an insignificant difference between the day and night length throughout the year and there is no cold winter. Rather, thermal time (growing degree-days (GDD) [°C d]) may influence the optimal time of flowering in Ethiopian barleys (Slafer et al. 2003, Borràs-Gelonch et al. 2010, Alqudah et al. 2014). GDD is calculated as the sum of the temperatures required to reach a certain developmental stage (McMaster and Wilhelm 1997, Miller et al. 2001). A range from 756 to 1382 GDD from seedling emergence to the heading of barley was reported by Juskiw et al. (2001), and, Ibrahim et al. (2018), while the GDD for anthesis was reported in a range from 705 to 966 by Juskiw et al. (2001).

Although Ethiopian barley landraces are characterized as spring types (Saisho et al. 2011), a low frequency of lines respond to minimal vernalization temperatures (<4 °C), as previously observed by Knüpffer et al. (2003). In the Tibetan plateau, over 4000 masl, the winter varieties of barley grew very well, despite the dominance of spring varieties in this region (Knüpffer et al. 2003, Yang et al. 2010). As a result, the requirement of the low vernalization of few Ethiopian landraces may be related to the adaptation of landraces at extremely high altitudes in Ethiopia. According to Tsehaye et al. (2012), Ethiopian landraces also exhibit strong responses to long days, and flower as early as 4–5 weeks after sowing.

Tsehaye et al. (2012) reported that Ethiopian landraces possess the *Ppd-H2* allele, which responds to short photoperiods (SD), also known as “non-inductive SD conditions”. This allele utilized the GA pathway to induce flowering under SD conditions, in which the flowering time is controlled by *SOC1* (*SUPRESSOR OF OVEREXPRESSION OF CO 1*) and *LFY* (*LEAFY*) genes (Kikuchi and Handa 2009). The presence of the *Ppd-H2* allele in barley could also be related to the requirement for milder winter temperatures, which was reported for Mediterranean barley genotypes (Casao et al. 2011). Tsehaye et al. (2012) also detected QTLs related to *HvFT4* (57.9 Mb) on chromosome 2H, *HvFT2* (101.6 Mb) on chromosome 3H, *HvFT5* (609.4 Mb) on chromosome 4H, *HvCO3* (358.1 Mb) on chromosome 5H, *HvCO2* (488.4 Mb) and *HvCO5* (357.5 Mb) on chromosome 6H, as well as *HvCO8* (50.1 Mb) in proximity to *HvCO1* (*Vrn-H3*) on chromosome 7H, which are mapped based on the Morex genome v2 reference (Mascher et al. 2017). Caproni et al. (2023) also reported that the flowering of Ethiopian landraces is associated with the *Vrn-H1* and *FRIGIDA* of chromosome 5H. It has been documented that *FRIGIDA*, also known as *FRI*, influences the adaptation of *Arabidopsis* during high temperatures and drought stress (Lovell et al. 2013).

Hemming et al. (2009) reported that Ethiopian barley landraces carry *HvVRN1-1*, *HvVRN1-2*, *HvVRN1-4*, *HvVRN1-5*, and *HvVRN1-8*, which have different deletions in intron-I of the *HvVRN1* allele. Of these

types, only lines carrying the *HvVRN1-1* allele type are reported to take longer than 100 days (d) from sowing to flowering, whereas all other types were reported to flower in less than 60 d in Mediterranean conditions. Wang et al. (2010b) reviewed that a mutation in the promoter region of *HvVRN1* or a deletion in the first intron are responsible for the reduced requirement of vernalization.

The drought stress experimental sites used in the studies of Teklemariam et al. (2023) had a higher minimum and maximum temperature compared with optimal moisture sites. This resulted in an accelerated flowering time at the drought stress locations by 10% compared to naturally optimal moisture locations. Similarly, in that study, the flowering time in climate chamber experiments was delayed by 39 and 46 d compared to naturally optimal moisture and drought stress treatments in field experiments, respectively (Teklemariam et al. 2023). These results highlighted the diversity of the set of 260 Ethiopian landrace accessions regarding flowering time and their potential for further genomic analysis for this trait.

Therefore, this study is focused on exploring environmental factors associated with flowering time as well as the identification of marker trait associations (MTAs) in the barley genome that influence the flowering time of Ethiopian barley in different agro-ecologies as well as in controlled climate chamber conditions, using genome wide association studies (GWAS).

Material and Methods

Experiment Setup

The experiments were conducted during the major growing season in Ethiopia (June-September), at four locations, i.e., Holetta, Debrezeit, Melkassa, and Dera for three years (2016–2018), hereafter named as HL, DZ, MK, and DR, respectively. Due to a poor stand in DZ during 2018, these data were omitted from further analysis.

The climate chamber experiment was conducted in 2018 at the Julius Kühn Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Resistance Research and Stress Tolerance, Quedlinburg, Germany, which will be designated as CC in this study. The altitude, seasonal temperature, rainfall, and soil characteristics of the field research sites are described in Table 1.

Table 1. Summary of seasonal weather, altitude, and soil characteristics of experimental locations during the experimental period (2016–2018).

Characteristics	Holetta	Debrezeit	Melkassa	Dera
Altitude (masl)	2400	1900	1550	1620
Maximum temperature (°C) *	21.7	25.3	28.2	26.2
Minimum temperature (°C) *	8.7	12.5	15.1	14.9

Rainfall (mm) *	732.4	470.3	546.7	381.6
Soil type	Nitosol	Vertisol	Hypo Calcic andosol/fulvisols	Calcic fulvic regosol
Soil texture	Clay	Clay	Clay loam to Clay	Clay loam
Soil pH (H_2O method)	5.48–5.90	6.23–7.14	7.22–7.55	7.04–8.10
Organic matter (%)	2.03–4.41	1.26–2.63	1.78–3.14	2.08–3.95
Cation exchange capacity (cmol/100 g)	19.11–33.18	35.19–48.15	21.63–32.28	27.30–37.17
Exchangeable sodium (%)	0.01–0.46	0.01–0.48	0.02–1.47	0.02–0.57
Bulk density (g/cm^3)	1.12–1.37	1.15–1.45	1.1–1.34	1.29–1.34

* The cropping season was from June to September for Holetta and from July to September for others.

A total of 260 barley accessions were included in the field experiments, of which 239 accessions were provided by the Ethiopian Biodiversity Institute and 21 barley breeding lines were provided by the Holetta National Barley Improvement Program. Detailed information about the plant material is presented in Teklemariam et al. (2022). The accessions were evaluated using an alpha-lattice design comprising three replications. Each replication comprised 20 incomplete blocks of 13 experimental plots, as described in Teklemariam et al. (2023).

For the CC, a set of 196 accessions was selected. Pots with a size of 15 × 15 × 20 cm^3 were filled with 1500 g of substrate (Einheitserde ED73, H. Nitsch & Sohn GmbH & Co. KG, Kreuztal, Germany). In each pot, three plants were grown. The experiment was performed using a complete randomized design with four replications. All pots were watered up to 70% of the soil water capacity (WC), as described in Teklemariam et al. (2023). A photoperiod of 13/11 h day/night was applied; the temperature of the climate chamber was 18/14 °C day/night during vegetative growth and increased to 22/16 °C when five to ten pots started to flower.

Data Collection

In the field, each experimental plot was evaluated for days to flowering (DFL), i.e., when approximately 50% of the main inflorescence emergence was completed in Zadoks' stages 58 (Z58). In the CC, due to some accessions being unable to fully complete ear emergence, the time when 50% of the main spike awn was visible in Zadoks' stages 49 (Z49) was considered as DFL (Zadoks et al. 1974).

A few accessions did not flower during the experimental period in certain locations and CC. Therefore, days to reverse flowering (DRFL) was considered for data analysis. DRFL was calculated by subtracting the last maturity date of an experimental plot at a specific location from the flowering date. Accessions

which did not flower during the experimental period have "0" values, whereas early flowering accessions have the maximum value for DRFL. To calculate DRFL, the maximum growing days of 155, 136, 123, 124, and 232 were used for HL, DZ, MK, DR, and CC respectively.

Metrological data, including daily minimum and maximum temperature and daily total rainfall, were recorded for each location from the first day of sowing to the maturity of the last plot. The cardinal temperatures, which consist of base temperature (T_b), optimal temperature (T_o), and ceiling temperature (T_c) were used to analyze the optimal growth as well as chilling requirements of Ethiopian landrace accessions. T_b, T_o, and T_c cardinal temperatures for optimal barley growth have been reported to be 0 °C, 21–28 °C, and 35 °C, respectively (Ellis et al. 1988, Cao and Moss 1989, Tamaki et al. 2002). According to Chujo (Chujo 1975), cited in Porter and Gawith (Porter and Gawith 1999)), an optimal chilling temperature (T_{oc}) of 3.8–6.0 °C, base chilling temperature (T_{bc}) of −1.3 °C, and ceiling chilling temperature (T_{cc}) of 15.7 °C were reported.

When the daily maximum temperature was between T_b and T_o, the day was considered optimal for barley growth; however, when it was below T_b or exceeded T_c, the day was considered suboptimal for barley growth, further plant development halted, or the plant died. The daily chilling temperature requirement was met when the daily minimum temperature was between T_{bc} and T_{oc}; when the daily minimum temperature was higher than T_{cc} or lower than T_{bc}, the chilling temperature requirement was deemed unmet, and accessions that required vernalization delayed or failed to flower in a timely way.

Based on each day's maximum and minimum temperature, the proportion of optimal growing days (Ellis et al. 1988, Cao and Moss 1989, Tamaki et al. 2002) and days that met the chilling temperature requirement (Chujo 1975) was calculated.

Usually, growing degree days (GDDs) (in °C d) are calculated based on the daily mean temperature when it exceeds 0 °C. However, in this study, the daily temperature effect was calculated according to Yin et al. (2005a), and the DFL of each accession was converted to the sum of the daily temperature effect (SDTE) by the summation of each daily temperature effect "*g(T)*" from sowing to flowering.

$$g(T) = \left(\frac{T_c - T}{T_c - T_o}\right)\left(\frac{T - T_b}{T_o - T_b}\right)^{\frac{T_o - T_b}{T_c - T_o}}$$

where *g(T)* is the daily temperature effect, *T* is the daily mean temperature, T_b (0 °C) is the barley base temperature, T_o (21 °C) is the barley optimal temperature, and T_c (35 °C) is the barely ceiling temperature according to and, Ellis et al. (1988), Cao and Moss (1989), Tamaki et al. (2002).

Statistical Analyses

The statistical analysis of flowering data was performed with the statistics package SAS 9.4 software (SAS Institute 2019). The analysis of DFL, DRFL, and SDTE, as well as average, minimum and maximum temperature was conducted using the *proc means* procedure. Additionally, the procedure *proc*

mixed was used for the ANOVA and estimation of least-square means (*lsmeans*) for DFL, DRFL, and SDTE. The model was fit with selected parameters as the dependent variable; accessions and locations were fixed effects, while year, replication, and blocks were random effects. The effect of accessions' interaction with location was also included as a fixed effect to estimate the *lsmeans* of each variable.

As parameters were evaluated at one location over different years, repeatability (r^2) was used to evaluate the adaptability and stability of the accessions. The '*lme4*' R package (Bates et al. 2014, R Core Team 2019) was applied to compute the variance components and repeatability (r^2) of traits recorded for more than one year. The variance in accession (σ^2_G), residual variance components ($\sigma^2 e$), number of replications (*rep*), and number of years (*ny*) were used to calculate repeatability as follows:

$$r^2 = \sigma_G^2/(\sigma_G^2 + \frac{\sigma_e^2}{ny \times rep})$$

The frequency and regression analysis was conducted using Microsoft excel 2016 software. The frequency of days above the optimal temperature (21 °C) as well as the frequency of chilling days that fulfill the respective chilling temperature requirement were analyzed for each year and location and compared with the average flowering time as well as with the flowering response. Moreover, the regression analysis between the frequency of days above the optimal temperature with the average DFL of a specific location as well as the frequency of chilling days with the percentage of non-flowered plots was also conducted.

The Pearson correlation coefficient analysis between DFL and DRFL, as well as DFL and SDTE, was conducted with the statistics package SAS 9.4 software (SAS Institute 2019) using the *proc corr* procedure.

Genome Wide Association Studies (GWAS)

A set of 10,644 highly polymorphic SNP markers (Teklemariam et al. 2023) as well as the *lsmeans* of phenotype data of DRFL and SDTE with the correction factor of population structure ("PCA.total = 3") were used to conduct GWAS using the Bayesian information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK)" model (Huang et al. 2018) in R v.4.1.2 software (R Core Team 2021). Benjamini and Hochberg (1995) considered markers significant if they surpass a false discovery rate (FDR) adjusted *p*-value of <0.05, and this standard was used in this study.

Significant markers obtained from the GWAS analysis were further analyzed using the LD decay value of each chromosome, as reported in Teklemariam et al. (2023), and if the SNP markers were within the LD decay value, the marker with the smallest *p*-value was selected to represent the respective QTL. The logarithm of the odds (LOD) was calculated for the significant markers with a "–log (*p*-value)". The significant QTLs were mapped using MapChart 2.32 software (Voorrips 2002), and the functional annotation of significant markers was analyzed by BARLEYMAP

(http://floresta.eead.csic.es/barleymap, accessed on 18 April 2024) (Cantalapiedra et al. 2015) against the Morex genome v2 (Mascher et al. 2017).

Results

Analysis of Weather Conditions

The highest maximum mean temperature during the flowering time evaluation period was observed at MK (28.2 °C), while the lowest minimum mean temperature was observed at HL (7.9 °C). In CC, the lowest mean maximum temperature (20.4 °C) and the highest mean minimum temperature (15.2 °C) compared with all field experiment locations was observed. The presence of a very high coefficient of variation for the minimum temperature across all field locations indicated the presence of a wider range of minimum temperatures (Table S1).

In all experimental locations, the maximum temperature was below T_c and above T_b. (Table S1). The HL and CC experiments were carried out at an optimal temperature (0 to 28 °C) throughout the experimental period, followed by DZ, at which 95% of experimental days were in the optimal temperature range. MK had the least optimal growth temperature, with only 46% of the days falling in the range of optimal growth conditions (Figure 1).

According to defined chilling requirement criteria (Chujo 1975), HL had the highest percentage (27%) of chilling days, while CC and DR had the lowest (0.0%). When the effective vernalization temperature increased from 6 to 10 °C, 78% of growing days at HL could be defined as chilling days, while the lowest number of days was observed at CC and DR, with 0 and 3% of days, respectively (Figure 1).

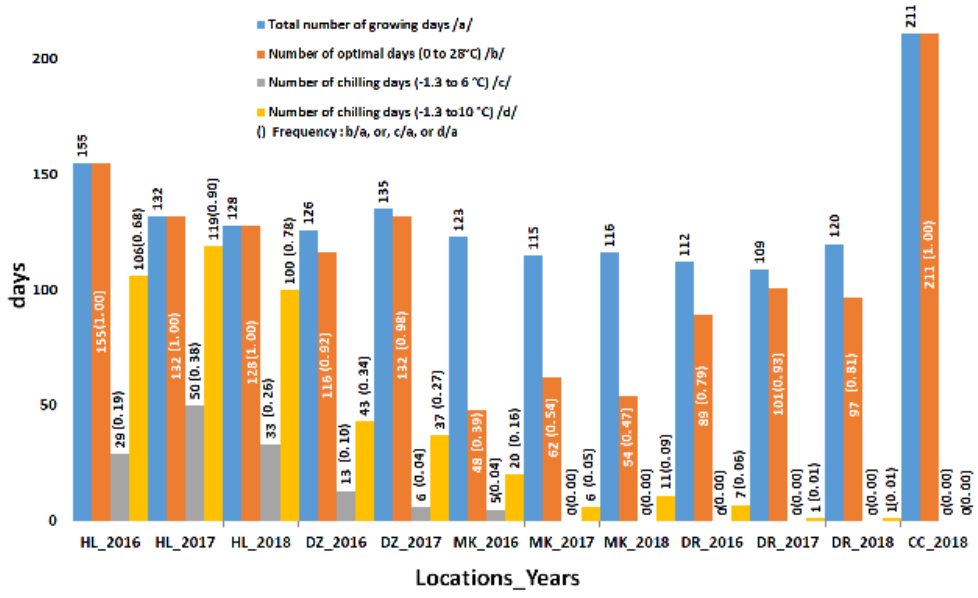


Figure 1. The number of growing days, days with optimal temperatures (0 to 28 °C), and days with chilling temperature (−1.3 to 6 °C; and −1.3 to 10 °C), along with their corresponding frequency values. Experimental locations are DZ = Debrezeit, DR = Dera, HL = Holetta, MK = Melkassa, and CC = climate chamber.

Evaluation of Flowering Status

The phenotypic diversity in the 260 Ethiopian barley landrace accessions during the time of flowering was observed in each trial. In the field experiments, the highest mean of DFL was recorded at HL (72.4 d), which was 8, 11, and 15 d longer than in DZ, DR, and MK, respectively. The mean DFL of the CC experiment was 109.5 d, which was on average 42% or 46 d longer than the average DFL (63.8 d) of all field locations, at which the least difference was observed in HL (37 d), while the highest variation was in MK (52 d). Moreover, the CC experiment was also the one with the highest standard deviation as well as coefficient of variance compared with all field locations, followed by DZ and MK (Table 2).

Table 2. Descriptive statistics for days to flowering, days to reverse flowering, and summation of daily temperature effect.

	Location	Mean	Std Dev	Min.	Max.	Variance	Std Error	CV	LSD	Repeatability
DFL	DZ	64.1	10.5	41.0	97.0	111.0	0.27	16.4	6.7	92.5
	DR	61.2	9.2	39.0	101.0	84.6	0.19	15.0	5.5	93.3
	HL	72.4	8.0	50.0	98.0	64.8	0.17	11.1	3.4	96.4
	MK	57.6	9.6	38.0	105.0	93.0	0.20	16.7	5.4	93.4
	CC	109.5	22.5	70.0	210.0	506.6	0.82	20.6	21.2	-
DRFL	DZ	71.7	11.4	0.0	95.0	130.4	0.29	15.9	7.6	85.5
	DR	60.6	14.7	0.0	85.0	215.4	0.30	24.2	8.9	93.3
	HL	82.5	8.2	0.0	105.0	67.7	0.17	10.0	3.6	96.0
	MK	64.5	12.4	0.0	86.0	154.9	0.26	19.3	7.9	90.1
	CC	119.6	28.9	0.0	162.0	834.8	1.03	24.2	27.6	-
SDTE	DZ	62.8	10.3	40.3	95.0	106.5	0.26	16.4	6.5	92.5
	DR	60.5	9.0	38.7	99.8	81.4	0.19	14.9	5.4	93.3
	HL	63.0	7.2	43.8	85.7	51.7	0.15	11.4	3.0	96.4
	MK	57.2	9.6	37.8	105.3	91.6	0.20	16.7	5.3	93.3
	CC	100.8	22.0	63.2	199.7	484.3	0.80	21.8	20.8	-

Legend: Experimental locations are DZ = Debrezeit, DR = Dera, HL = Holetta, MK = Melkassa, and CC = climate chamber; Std Dev = standard deviation, Min. = minimum value, Max. = maximum value, Std Error = standard

error, CV = coefficient of variation, LSD = the least significant difference; traits analyzed are DFL = days to flowering, DRFL = days to reverse flowering, and SDTE = sum of daily temperature effect. * Reduction calculated for reduction percentage with the following formula: (mean of DFL—mean of SDTE)/mean of DFL) × 100.

When the DFL data were converted to DRFL, the highest CV was recorded for DR and CC. This is due to few accessions in DR and CC that did not flower in contrast to other locations (Figure 2). Although the result of SDTE seems in harmony with DFL, the mean SDTE difference between each field location was the smallest compared with DFL except for HL, where the SDTE value was reduced by 13% compared to DFL, while the lowest reduction was observed at MK, DR, and DZ in the range of 0.6 to 2.0% (Table 2).

Flowering (Z58) at MK began at the 6th week after sowing (WAS), while it started on the 7th WAS at DR and DZ. The latest flowering response under field conditions was observed at HL (8th WAS). Most of the accessions at MK, DZ, and DR flowered on the 9th WAS, while it was on the 10th WAS at HL. The flowering pattern in the CC, in contrast, differed from field experiments, in which Z49 was started on the 11th WAS and the peak flower response was noted on the 15th WAS (Figure 2).

The highest proportion of non-flowering plots (3.5%) was observed for DR and the highest flowering response was recorded for HL (Figure 2; Table S2). The presence of such a low flowering response at DR could also be associated with the presence of drought stress at this location (Teklemariam et al. 2023). Although the longer dry spell at DR and MK started in the month of September (Kassie et al. 2013, Bekele et al. 2016, Bekele et al. 2019), accessions at MK flowered on average 4 days earlier than at DR (Table 2), which could be associated with the presence of a higher day temperature than at DR (Table S1).

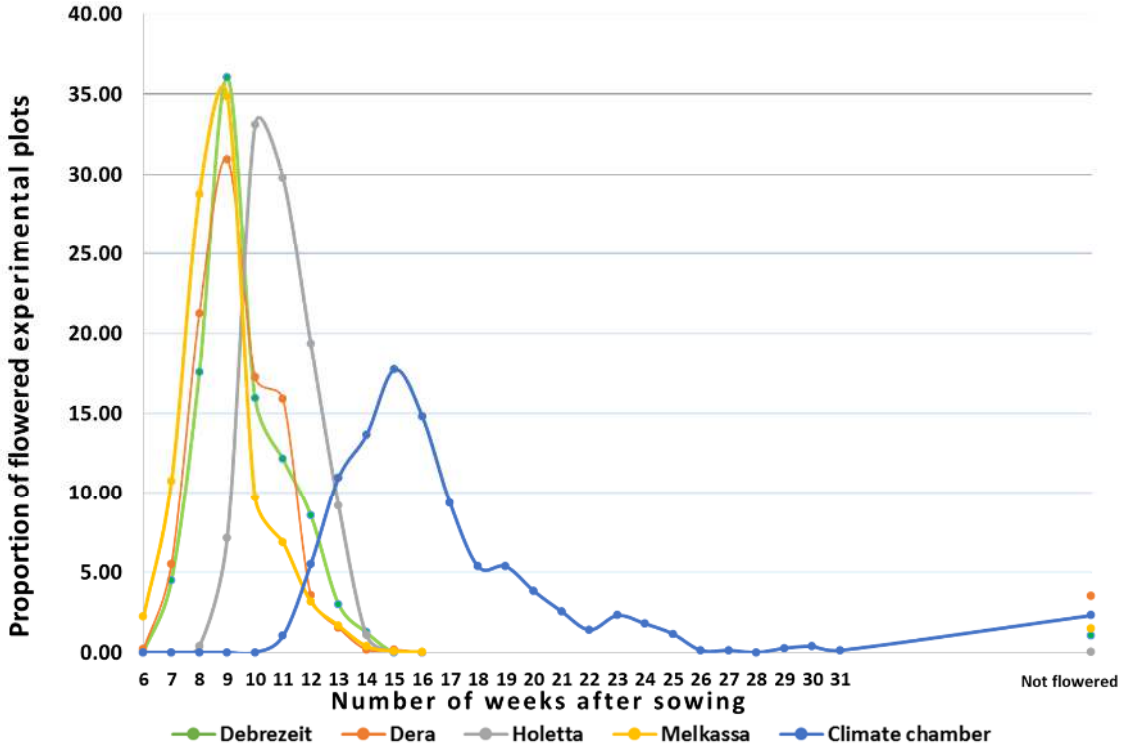


Figure 2. Percentage (proportion) of flowering plots in each experimental field location (Z58) and for climate chamber experimental pots (Z49) throughout the experimental period (weeks).

Repeatability

The repeatability of DFL, DRFL, and SDTE parameters was high in all field experimental locations. The least observed repeatability of DFL was for DZ (92.5%) and the highest was for HL (96.4%). The highest repeatability for DRFL was for HL (96.0%), and the least was for DZ (85.5%), while for SDTE the highest was for HL (96.4%) and the lowest was for DZ (92.5%). The repeatability of CC was not analyzed, as the experiment was conducted for one year only (Table 2). The presence of a high repeatability as well as variation among accessions provided an excellent intervention point to study the genetics of the flowering time in Ethiopian accessions in detail.

Correlation and Regression Analysis

The DFL of Ethiopian barley landraces was observed to be very strongly and positively associated with most agronomic traits investigated during the drought stress experiment conducted at the field and climate chamber experiments (Teklemariam et al. 2023).

The Pearson correlation coefficient (r) of DFL and DRFL among the field experiment locations was positive and highly significant ($p < 0.001$). The highest correlation for DFL was between DR and MK ($r = 0.92$, $p < 0.001$), while the lowest was between DR and HL ($r = 0.82$, $p < 0.001$). For DRFL, the highest was between DR and MK ($r = 0.93$, $p < 0.001$) and the lowest was between DR and HL ($r = 0.80$, $p < 0.001$) (Table 3). Although a positive and significant correlation ($p < 0.001$) was observed for both DFL and DRFL in CC and field trials, the r value was much lower than the r values observed

between field sites. The smallest *r* value of DFL between CC and field trials was found for HL ($r = 0.49$, $p < 0.001$) and the highest for MK ($r = 0.57$, $p < 0.001$), while for DRFL, the highest ($r = 0.55$, $p < 0.001$) and the lowest ($r = 0.44$, $p < 0.001$) correlations were found for MK and HL, respectively (Table 3). This might be associated with the presence of the highest values for standard deviation, variance, and CV for both variables in CC compared to all field sites (Tables 2, 3 and S1).

Table 3. Pearson correlation coefficient (*r*) between the field and climatic chamber experiments using Ethiopian barley accessions. Days to flowering are shown above the diagonal cells, while correlations for days to reverse flowering are listed below the diagonal cells; *** significant at $p < 0.001$.

		DFL				
		CC	**DZ**	**DR**	**HL**	**MK**
DRFL	CC		0.53 ***	0.55 ***	0.49 ***	0.57 ***
	DZ	0.50 ***		0.88 ***	0.89 ***	0.91 ***
	DR	0.52 ***	0.86 ***		0.82 ***	0.92 ***
	HL	0.44 ***	0.89 ***	0.75 ***		0.85 ***
	MK	0.55 ***	0.89 ***	0.93 ***	0.80 ***	

Legend: Experimental locations are DZ = Debrezeit, DR = Dera, HL = Holetta, MK = Melkassa, and CC = climate chamber; traits analyzed are DFL = days to flowering, and DRFL = days to reverse flowering.

The maximum temperature was recorded above T_b (0 °C) in all experimental locations, while a chilling temperature below T_{bc} (−1.3 °C) was observed only for 4 d at HL during 2016. The minimum temperature was also above T_{bc} in all locations (Table S3). Therefore, a frequency below 1 for optimal growth conditions was due to the presence of above T_o (28 °C), which ultimately shortened the average flowering days in DZ, DR, and MK compared to HL (Figures 1 and 3a). Similarly, a frequency below 1 for chilling days was primarily due to the presence of temperatures above T_{oc} (6 °C or 10 °C), which appears to be associated with the flowering of all accessions or with the lowest non-flowering proportion of accessions in each location (Figures 1 and 3b,c).

The regression analysis indicated that the daily maximum temperature explained about 32% of flowering time in Ethiopian accessions (Figure 3a). Additionally, the presence of an optimal chilling temperature below 6 °C contributed about 56% to complete flowering of all accessions, while in the case that the optimal chilling temperature was assumed to be below 10 °C, its contribution increased to 65% (Figure 3b,c).

Analysis of Variance (ANOVA)

The ANOVA results for DFL, DRFL, and SDTE of the four field locations revealed significant variation ($p < 0.001$) between locations, accessions, as well as the interaction of accessions and locations. The presence of significant variation between accessions of CC was also observed (Table 4).

Based on the *lsmeans* of accessions at each location, the 20 earliest and the 20 latest flowering accessions were sorted. The common number of accessions obtained in the drought-prone locations (MK and DR) was 13/20 for the latest and 14/20 for the earliest flowering accessions. Similarly, 15/20 of the earliest and 11/20 of the latest flowering accessions were common for the optimal moisture locations (DZ and HL). Overall, only nine out of twenty of the earliest and four out of twenty of the latest common flowering accessions were found in all field trial locations. The CC had only two out of twenty common late-flowering accessions with drought-prone locations and no common earliest accessions with all field experiment locations (Table S4). The mean DFL values of every accession in the CC were found to be higher than the mean DFL values of every other accession in the MK, DZ, DR, and HL sites, with the exception of 3, 8, 10, and 10 of the earliest accessions in the CC (Table S4).

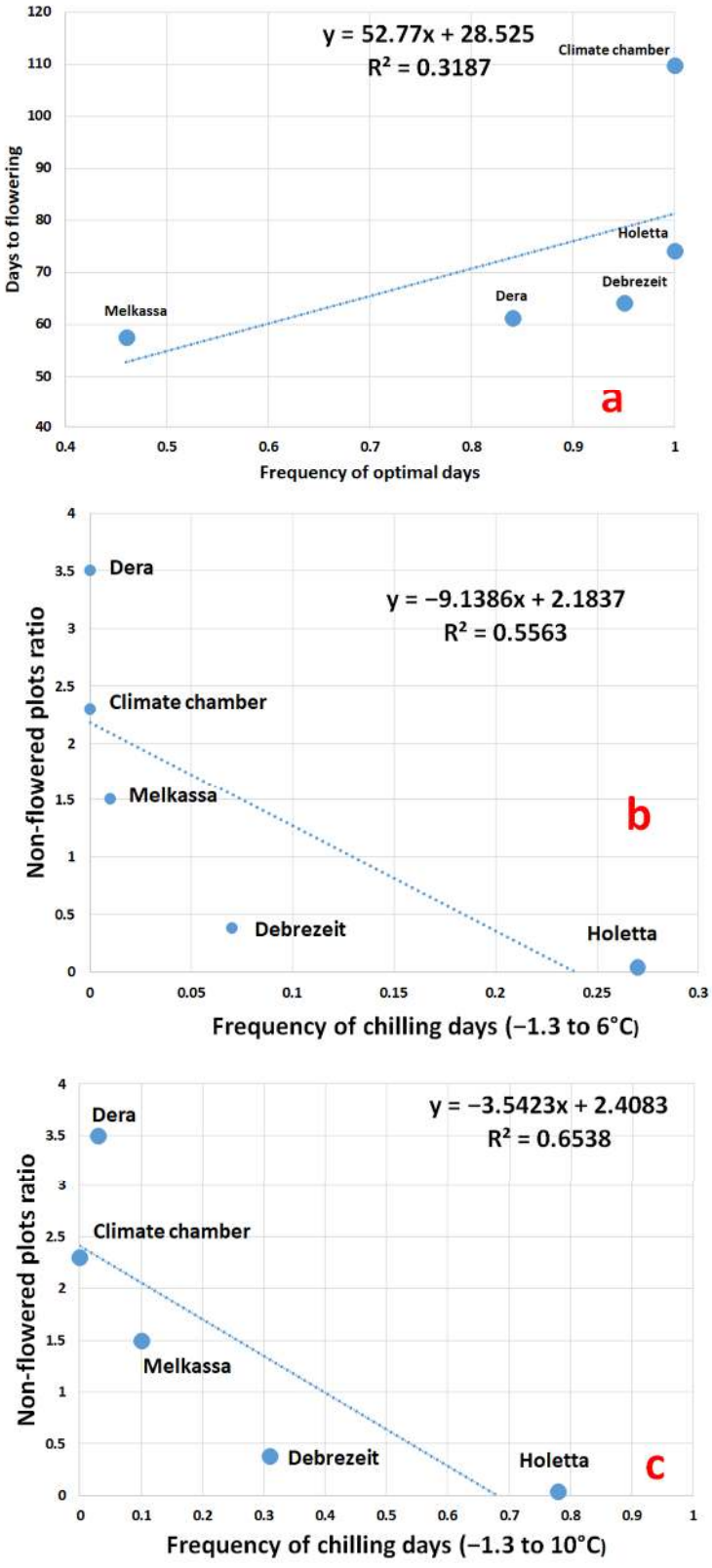


Figure 3. Regression analysis between daily temperature and flowering proportion: (a) based on optimal temperature (T_o); (b) based on optimal chilling temperature requirement (T_{bc} = 6 °C); and (c) based on optimal chilling temperature requirement (T_{bc} = 10 °C).

Table 4. F values from ANOVA of flowering time of field and climate chamber experiments and morphological parameters of field experiments.

Experiment	Effects		DRFL	DFL	SDTE
Field	Accessions (A)	F value	34.24 ***	40.96 ***	40.00 ***
		DF	259	259	259
	Location (L)	F value	3429.55 ***	2759.16 ***	388.63 ***
		DF	3	3	3
	A X L	F value	2.25 ***	1.72 ***	1.85 ***
		DF	777	777	777
Climate chamber	Accessions	F value	4.76***	4.63 ***	4.64 ***
		DF	195	195	195

Legend: Experimental locations are from field (Debrezeit, Dera, Holetta, and Melkassa), and climate chamber; traits analyzed are DRFL for days to reverse flowering, DFL for days to flowering, and SDTE for sum of daily temperature effect. DF stands for degree of freedom; and "***" indicate significance at *p*-value < 0.001 probability level.

Genome Wide Association Study Analysis of Flowering Time Traits

The presence of three distinct structural populations and the LD decay values of each chromosome have already been described in and, Teklemariam et al. (2022), Teklemariam et al. (2023).

The correlation analysis between one location of SDTE and DFL indicated that the two parameters were similar, as the *r* values were 1 (Table S5). A GWAS analysis was therefore conducted for SDTE and DRFL. Combined multiple-year data of each location and one-year data from the CC were used during the analysis. Most of the GWAS results were found to be well-fitted to the BLINK model, as seen by the Quantile–quantile (QQ) plots (Figure S1). Based on FDR values, 40 marker trait associations (MTAs) were identified, of which 23 MTAs were for DRFL, while 17 MTAs were for SDTE (Tables 5 and S6). When the detected MTAs were further assigned to putative QTLs based on the LD decay value of each chromosome (Teklemariam et al. 2023), a total of 39 QTLs were detected, of which 22 QTLs were for DRFL and 17 QTLs for SDTE. Since all QTLs, except one for DRFL of CC on chromosome 4H 3.6–4.1 Mb, were represented by one MTA (Tables 5 and S6), the findings of this study will center on MTAs.

For each barley chromosome, at least two associations with a flowering time of Ethiopian barley's accession were detected, despite the presence of variations in the number of MTA distributions between chromosomes. The chromosome 5H had the highest number of MTAs (9), while chromosome 7H had the least (2) (Table S7).

MTAs with the highest three LOD values are on chromosome 6H at 658.9 Mb (14.5) for DRFL at MK, chromosome 2H at 29.3 Mb (11.2) for SDTE at CC, and chromosome 1H at 59.3 Mb (10.2) for SDTE at MK (Table S6). The three highest marker effect values were recorded at CC, for MTAs on chromosome 3H at 643.2 Mb for DRFL (−14.85), at 662.66 Mb for SDTE (−13.06) and DRFL (16.46), and on chromosome 1H at 427.7 Mb for DRFL (10.46). The presence of the highest standard deviations, variance, and LSD values in the CC (Table 2), could potentially contribute to the highest marker effect values in the CC (Tables 5 S6). MTAs with the highest percentage of phenotype variance explained (PVE) values are on chromosome 6H at 558.9 Mb for DRFL of MK (15.39) and on chromosome 2H at 678.2 Mb for SDTE of CC (9.22) (Tables 5 and S6).

There were three common markers that were detected for at least more than one parameter or location (Table S6). The "JHI-Hv50k-2016-323294" marker, which was located on chromosome 5H at 571.62 Mb, was detected for the SDTE of MK and DR and DRFL of DZ, DR, and MK. The remaining two were the "JHI-Hv50k-2016-18950" marker on chromosome 1H at 59.3 Mb detected for the SDTE of MK and DRFL of DR, and the "JHI-Hv50k-2016-73570" marker on chromosome 2H at 29.3 Mb detected for SDTE and DRFL of CC (Tables 5 and S6).

Although there were no common markers detected between the field and CC, a QTL was detected on chromosome 3H at 4.1–4.6 Mb for the DRFL of DR and CC (Figure 4 and Table S6). Four QTLs were overlapped between the SDTE and DRFL of CC on chromosome 1H-4H, while two QTLs were detected between DR and MK on chromosome 1H and 2H; the rest were between CC and DR on chromosome 3H, and between DZ, MK, and DR on chromosome 5H (Figure 4 and Table S6).

Table 5. List of MTAs for flowering time of Ethiopian barley landraces under four field experimental locations in Ethiopia and climate chamber using BLINK models.

No	SNP	Chr	Pos (Mb)	MAF	LOD	Effect	PVE (%)	Loc	Trait	QTL	Gene Ontologies	Annotation Description
1	JHI-Hv50k-2016-12926	1H	13.73	0.32	5.2	−1.14	0.45	MK	SDTE	MKSDTE1	GO:0005488	Armadillo/beta-catenin-like repeat protein
2	SCRI_RS_116548	1H	24.03	0.08	6.5	−1.59	1.49	HL	SDTE	HLSDTE1		Unknown protein; located in endomembrane system; BEST *Arabidopsis thaliana* protein match is an unknown protein
3	JHI-Hv50k-2016-18950	1H	59.28	0.08	6.7	−4.26	2.70	DR	DRFL	DRDRFL1	GO:0016887 GO:0042626 GO:0055085 GO:0005524 GO:0006810 GO:0016021	Lipid A export ATP-binding/permease protein MsbA
4	JHI-Hv50k-2016-18950	1H	59.28	0.08	10.2	3.17	4.27	MK	SDTE	MKSDTE2	GO:0016887 GO:0042626 GO:0055085 GO:0005524 GO:0006810 GO:0016021	Lipid A export ATP-binding/permease protein MsbA
5	JHI-Hv50k-2016-26918	1H	369.36	0.10	5.9	6.32	4.83	CC	SDTE	CCSDTE1	GO:0005515	Leucine-rich repeat family protein
6	JHI-Hv50k-2016-26982	1H	371.70	0.31	7.3	7.72	1.91	CC	DRFL	CCDRFL1		Abscisic acid receptor *PYR1*
7	JHI-Hv50k-2016-31649	1H	427.69	0.09	6.8	10.46	4.16	CC	DRFL	CCDRFL2		RNA recognition motif-containing protein
8	JHI-Hv50k-2016-72079	2H	25.15	0.27	6.2	−1.28	2.81	HL	SDTE	HLSDTE2	GO:0016020 GO:0055085 GO:0005215 GO:0006810 GO:0006811	Cation-chloride cotransporter 2
9	JHI-Hv50k-2016-73570	2H	29.31	0.19	7.6	−9.32	2.12	CC	DRFL	CCDRFL3		Undescribed protein
10	JHI-Hv50k-2016-73570	2H	29.31	0.19	11.2	8.27	5.27	CC	SDTE	CCSDTE2		Undescribed protein
11	JHI-Hv50k-2016-111156	2H	678.19	0.03	5.1	−8.63	9.22	CC	SDTE	CCSDTE3	GO:0006486 GO:0016757	Hexosyltransferase
12	JHI-Hv50k-2016-147628	2H	766.08	0.07	6.0	−2.57	2.34	MK	SDTE	MKSDTE3	GO:0008270	*RING/FYVE/PHD* zinc finger superfamily protein
13	JHI-Hv50k-2016-148133	2H	767.33	0.07	8.2	5.06	5.79	DR	DRFL	DRDRFL2		
14	JHI-Hv50k-2016-151505	3H	4.06	0.14	4.6	3.18	0.81	DR	DRFL	DRDRFL3		
15	JHI-Hv50k-2016-151820	3H	4.57	0.11	5.6	−9.19	1.97	CC	DRFL	CCDRFL4	GO:0043531	Disease resistance protein
16	JHI-Hv50k-2016-198140	3H	596.97	0.16	5.0	4.64	2.16	CC	SDTE	CCSDTE4	GO:0004553 GO:0005975 GO:0030246	Beta-galactosidase 10

17	JHI-Hv50k-2016-206858	3H	643.17	0.04	6.0	−14.85	2.66	CC	DRFL	CCDRFL5	GO:0005515 GO:0008270	RING/U-box superfamily protein
18	JHI-Hv50k-2016-213204	3H	662.66	0.03	5.6	16.46	3.55	CC	DRFL	CCDRFL6		UV-stimulated scaffold protein A homolog
19	JHI-Hv50k-2016-213207	3H	662.66	0.03	6.3	−13.06	4.77	CC	SDTE	CCSDTE5		
20	SCRI_RS_188420	3H	681.79	0.29	7.1	1.21	1.75	HL	DRFL	HLDRFL1	GO:0051861 GO:0005737 GO:0017089 GO:0046836	Kinesin-like protein/Silicon efflux transporter
21	JHI-Hv50k-2016-227500	4H	3.24	0.09	5.9	−6.18	3.53	CC	SDTE	CCSDTE6		Chromosome 3B, genomic scaffold, cultivar Chinese Spring
22	JHI-Hv50k-2016-227517	4H	3.57	0.09	4.7	8.32	2.98	CC	DRFL	CCDRFL7		Unknown function
23	JHI-Hv50k-2016-227778	4H	4.06	0.11	4.7	7.67	2.44	CC	DRFL	CCDRFL7		Very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase
24	SCRI_RS_188340	4H	471.65	0.39	6.7	6.48	1.22	CC	DRFL	CCDRFL8		Vacuolar protein-sorting-associated protein 37 homolog 2
25	JHI-Hv50k-2016-259986	4H	595.53	0.04	5.6	−4.54	4.75	MK	DRFL	MKDRFL1		
26	JHI-Hv50k-2016-281308	5H	10.63	0.04	5.4	3.04	1.73	DZ	SDTE	DZSDTE1	GO:0005515	F-box protein/undescribed protein
27	JHI-Hv50k-2016-310148	5H	510.24	0.25	5.6	2.33	1.10	DR	DRFL	DRDRFL4		Ribosomal L5P family protein
28	JHI-Hv50k-2016-323294	5H	571.62	0.32	5.7	−1.69	1.26	DZ	DRFL	DZDRFL1	GO:0003700 GO:0006355 GO:0043565	ABSCISIC ACID-INSENSITIVE 5-like protein 2
29	JHI-Hv50k-2016-323294	5H	571.62	0.32	5.6	−1.46	0.85	MK	DRFL	MKDRFL2	GO:0003700 GO:0006355 GO:0043565	ABSCISIC ACID-INSENSITIVE 5-like protein 2
30	JHI-Hv50k-2016-323294	5H	571.62	0.32	5.9	1.20	0.76	MK	SDTE	MKSDTE4	GO:0003700 GO:0006355 GO:0043565	ABSCISIC ACID-INSENSITIVE 5-like protein 2
31	JHI-Hv50k-2016-323294	5H	571.62	0.32	8.6	1.78	1.38	DR	SDTE	DRSDTE1	GO:0003700 GO:0006355 GO:0043565	ABSCISIC ACID-INSENSITIVE 5-like protein 2
32	JHI-Hv50k-2016-323571	5H	572.54	0.36	4.9	1.98	0.44	DR	DRFL	DRDRFL5	GO:0042176 GO:0000502 GO:0005515 GO:0030234/GO:0006468 GO:0004672 GO:0005515 GO:0005524	26S proteasome non-ATPase regulatory subunit 3 homolog A/Leucine-rich receptor-like protein kinase family protein

33	JHI-Hv50k-2016-345406	5H	623.06	0.30	6.6	1.64	0.51	DZ	DRFL	DZDRFL2	GO:0005515	U3 small nucleolar RNA-associated protein 18 homolog
34	JHI-Hv50k-2016-367393	5H	668.61	0.25	4.6	−3.91	1.01	CC	SDTE	CCSDTE7	GO:0043531	Disease resistance protein
35	JHI-Hv50k-2016-378408	6H	19.55	0.35	5.0	−2.19	0.42	DR	DRFL	DRDRFL6		Unknown function
36	JHI-Hv50k-2016-397916	6H	348.23	0.03	5.2	5.73	3.67	DR	DRFL	DRDRFL7		GDP-L-galactose phosphorylase 2
37	JHI-Hv50k-2016-410857	6H	499.35	0.50	6.5	5.71	1.22	CC	DRFL	CCDRFL9		Unknown function/undescribed protein
38	JHI-Hv50k-2016-422808	6H	558.86	0.03	14.5	9.14	15.39	MK	DRFL	MKDRFL3	GO:0003676 GO:0005634	CCR4-NOT transcription complex subunit 7
39	JHI-Hv50k-2016-449688	7H	14.61	0.24	4.9	1.30	0.66	MK	SDTE	MKSDTE5	GO:0008152 GO:0016758	UDP-Glycosyltransferase superfamily protein
40	JHI-Hv50k-2016-457708	7H	32.79	0.34	7.2	1.64	0.95	DR	SDTE	DRSDTE2	GO:0003676 GO:0003677	Unknown function

Legend: SNP = names of SNP marker associated; Chr = chromosome; Loc = locations (HL = Holetta, DZ = Debrezeit, DR = Dera, MK = Melkassa, and CC = climate chamber); Trait = traits (DRFL= days to reverse flowering; and SDTE = sum of daily temperature effect); MAF = minor allele frequency; PVE (%) = percentage of phenotype variance explained; LOD is logarithm of odds calculated as LOD = −log (*p*-value); Gene Ontologies and Annotation description are based on Morex genome v2 [29].

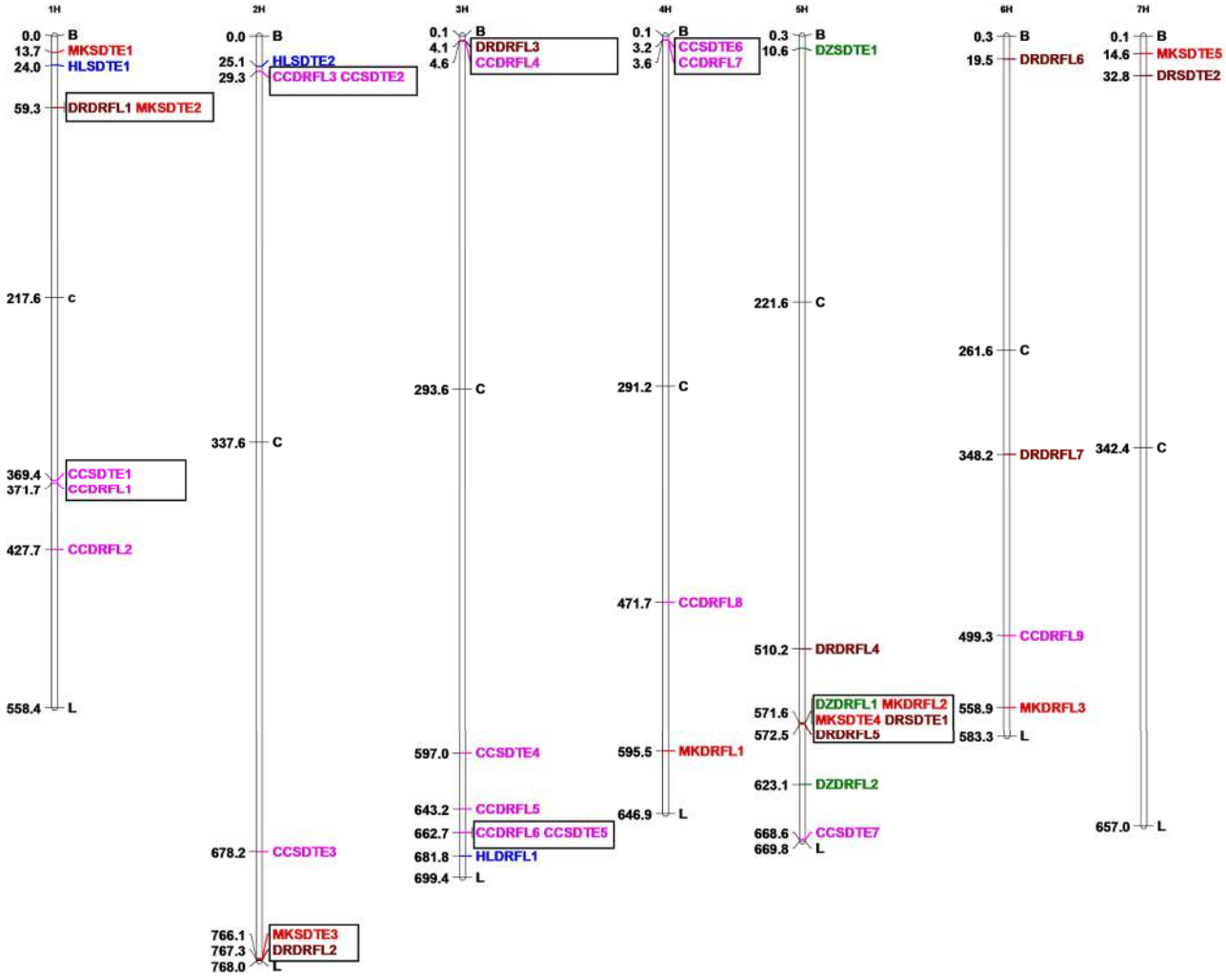


Figure 4. Genetic map showing significant QTLs of Ethiopian barley landrace accessions associated with days to reverse flowering (DRFL) and summation of daily temperature effect (SDTE). The physical distance in Mb; C = centromere region of the chromosome; B and L = known start and stop position of chromosomes based on (Mascher et al. 2017); QTL in **blue** font for Holetta, **green** font for Debrezeit, **red** font for Melkassa, **brown** for Dera, and **purple** font for climate chamber experimental location detected using the BLINK model.

Discussion

Phenotypic Variation on Flowering Time

The flowering time is an important and complex trait that is controlled by a number of genetic networks and environmental signals (Cockram et al. 2007, Cosenza et al. 2024). The flowering time of Ethiopian barley accessions was evaluated in this study at different locations of Ethiopia that had varying daily temperatures and annual precipitation. The study was also conducted in the climate chamber that had a constant day and night temperature. In previous research, Tsehaye et al. (2012) also studied the flowering time on Ethiopian barley landraces in both long and short days and reported the role of major flowering genes.

The flowering pattern of a worldwide barley collection was studied in Japan at the same field location for 20 seasons (Sato et al. 2020). According to the results, the genotype accounted for the majority of the variation in flowering time, with the geographic origin being second. The interaction between genotype with environment and geographic origin with environment explained about 90% and 55% of the variation in flowering time, respectively.

Although Ethiopian barleys in the Sato et al. (2020) study formed a genetically distinct cluster, they did not show a different flowering pattern compared with other geographic origins. However, about 25% of Ethiopian materials in the study were included in the group of Japanese local materials based on their stable flowering pattern performance in varying environments across different seasons. The insensitivity of Ethiopian materials to the photoperiod was explained as the reason for their stable flowering pattern in that study.

Altitude and atmospheric temperature typically have an inverse relationship. Although there was a significant difference in minimum temperature across the different altitudes of barley growing areas in Ethiopia, the coldest months of the year (October to January) do not fall in either of the two cropping seasons: the main season '*meher'*, which is from June to September, and the minor season '*belg'*, which is from February to April. Therefore, in both cropping seasons, the landraces were assumed to be not exposed to the vernalization temperature requirement for flower initiation. As a result, Ethiopian barleys are considered as spring types that did not require strong vernalization temperatures and a critical photoperiod length to flower (Saisho et al. 2011).

Our experiments conducted at four locations in Ethiopia with different environmental conditions (Table 1) and in the climate chamber also confirmed that accessions are independent from the requirement of a strong vernalization temperature, but there is a significant difference in the flowering time (Table 4 and Figure S2). Notably, the flowering time of the accessions appear to be more dependent on the thermal time (growing degree-days (GDD) [°C d]) (Figure 3a), which refers to the summation of the total temperature required to reach the flowering stage (McMaster and Wilhelm 1997, Miller et al. 2001).

Global warming is a major concern for sustainable agricultural production in the world, and Solomon (2007) forecasted that global temperatures will increase by 1.8 to 4.0 °C at the end of this century. The lowest altitude (1550 masl) and warmest location in our study is MK, while the highest altitude (2400 masl) and coldest location is HL (Table 1). The average temperature variation between DR and MK, DZ and DR, and HL and DZ is about 1.1 °C, 1.7 °C, and 3.7 °C, respectively (Table 1). Therefore, the field experiment in these four locations enables us to predict how global warming would affect barley production in Ethiopian conditions.

Nevo et al. (2012) studied the global warming effect on flowering patterns of 10 wild barley ancestral populations from the 1980s with their descendant populations from 2008. The result indicated that the descendant population flowered earlier than the ancestral population. A subsequent study by Qian et al. (2020) suggested that the fixation of SNP/Indels in major flowering regions of the descendant populations contributed to early flowering in the changing environment.

The flowering time of the latest accessions groups was more affected by the variation in temperature in the four experimental locations, as only four of twenty common accessions between them were found, in contrast to nine of twenty common accessions in the earliest accessions groups (Table S4). Moreover, accessions at MK flowered earliest, while they flowered latest at HL. Previous studies conducted in Ethiopia also demonstrated that the mean flowering time at Sheno (2800 masl) was 84.6 d (Tsehaye et al. 2012), while it was 87.3 d at Ankober (2970 masl) (Yigzaw 2021), and barley landraces were reported to mature between 7 and 9 months in the extreme highlands of Ethiopia (>3500 masl) (personal communication).

Hemming et al. (2012) revealed barley genotypes flowering earlier at 25 °C than at 15 °C. Karsai et al. (2008) grew barley genotypes at a constant day temperature of 9 to 18 °C and at a 2 °C lower night temperature and demonstrated that a 1 °C increase in temperature resulted in a 5.2 d earlier flowering time. White et al. (2011), and, Dixon et al. (2019) also reported a significant shortening of the flowering time in wheat when the ambient temperature increased.

In addition, the presence of fluctuating temperatures of 18/16 °C day/night delayed flowering time by up to 71 d in spring-type barley genotypes compared with a constant temperature of 18 °C (Karsai et al. 2008). In a 20-season research project conducted at the same location in Japan, the Ethiopian barley genotypes' flowering time ranged from 145 to 165 (Sato et al. 2020). The DFL of accessions in the CC was delayed for an average maximum and minimum of 52 and 37 d in comparison to MK and HL, respectively (Table 2), which is lower than the 71 d reported by Karsai et al. (2008), while the observed range of DFL (70–210 d) was wider than the findings from the field study of Sato et al. (2020).

In addition to the primary factors like the photoperiod and vernalization, additional critical factors may be required for the timely flowering of barley (Bernier and Périlleux 2005). Hence, for the Ethiopian accessions, critical alternative factors might be the presence of a wide range of day/night temperature

variation and/or ambient daily maximum temperatures of >21 °C. In contrast to the CC, where temperature fluctuation between day and night was 4 °C and showed a significantly delayed flowering time, our field experiments showed a temperature fluctuation of >10 °C on the majority of days (Table S3).

The negative influence of higher atmospheric temperature on the grain yield of barley was discussed in different studies using long photoperiods (Ejaz and von Korff 2016, Ochagavía et al. 2022). Morphological parameters like plant height, spike length, and flag leaf length were reported to be influenced by elevated temperatures over the optimal range (Zahn et al. 2023). According to Zhu et al. (2023), during early barley development, elevated temperature promotes plant length; in the later developmental stage, plants grown at an elevated temperature become shorter compared to plants grown at an optimal temperature. However, the elevated temperature has at the same time insignificant effects on leaf length and leaf width. The effect of atmospheric temperature on some morphological parameters of Ethiopian barley accessions was also assessed in our study using correlation analysis. The findings revealed that the experimental location's atmospheric temperature difference has a significant effect on both the plant height and flag leaf length, as accessions at warm locations (MK and DR) had been shorter in plant height and flag leaf length compared with the cold location HL. However, it has less of an effect on spike length (Table S8).

Earliness is one of the drought tolerance mechanisms, in which genotypes escape the adverse drought stress and complete the lifecycle before the onset of a longer dry period (Chaves et al. 2003). and, Forster et al. (2004), Vaezi et al. (2010), Barati et al. (2017) demonstrated the negative association of the flowering time with grain yield during a drought stress period. Our previous study also confirmed a strong negative correlation of DFL with grain biomass in drought stress treatments (Teklemariam et al. 2023). Compared with other experimental locations, MK and DR exhibited the highest frequency of days above the optimal average daily temperature (21 °C), with values of 0.69 and 0.37, respectively (Table S3), which favor accelerated vegetative growth, and benefited early flowering accession to complete their lifecycle before the occurrence of a drought spell.

The developmental transition of barley from the vegetative stage to the reproductive stage is significantly influenced by drought stress (Su et al. 2013, Gol et al. 2017). The presence of adverse drought conditions at MK and DR seriously affected flowering. This can be witnessed by the presence of a very low ratio of non-flowering plots during 2017, when a very good distribution of rainfall was observed at each location compared with other seasons (Tables S2 and S4).

In the climate chamber experiment, most accessions had difficulties to complete the transition to flowering from Z49, i.e., awn tipping to Z58, i.e., spike emergence (Figure S3). While such an effect was also observed in both water treatments, it was more pronounced in the drought stress treatment (86% of the plot failed to transit to Z58) than in the optimal moisture treatment (30% of the plot failed the transition) (Table S9). A quartile analysis was used to further analyze the failure of the Z49–Z58

transition. The highest percentage of early flowering accessions (88% and 24%) from the first quartile of control and drought stress treatments, respectively, completed the flowering stage transition. In the subsequent quartiles, the percentage of completed flowering stage transitions decreased gradually, and the lowest percentage was found for the latest flowering accessions in the fourth quartile (21% and 4%), respectively, for control and drought stress treatments (Table S9). The effect of drought stress on the developmental transition of barley genotypes from the vegetative stage to the reproductive stage was also discussed in Su et al. (2013), and, Gol et al. (2017).

The highest correlation coefficient between flowering time and days to maturity (DM) of the drought stress treatments and control treatments in both the field and climate chamber experiments was reported in Teklemariam et al. (2023). The correlation between drought stress and control treatments for corresponding accessions was analyzed. The DM of the climatic chamber drought stress treatment has a significant correlation with the DM of DR and MK at $p < 0.001$ ($r = 0.42$ and $r = 0.49$), respectively. In contrast, the DM of the CC control treatment exhibited the lowest correlation coefficient with the DM of HL ($r = 0.15$) compared to the DM of all field locations, although it had a significant correlation at $p < 0.05$ (Table S9). The low correlation coefficient between the cool and naturally optimal moisture location (HL) and CC control treatment, compared to warm and naturally optimal moisture (DZ) and drought-prone (DR and MK) locations, indicates that in addition to an optimal soil moisture content, the atmospheric conditions of the cool agro-ecologies of Ethiopia play a role in determining the morphological growth parameters.

Yin et al. (2005a) reported an average of 66.09 SDTE for two varieties; and the range of 57 to 80 SDTE was reported for 94 barley recombinant breeding lines (Yin et al. 2005b). In our study, the mean SDTE was 57.2 to 63.0 in field trial locations, while the mean of the CC was 100.8 (Table 2).

Flowering Time Associated Marker under Different Environments

Chromosome 2H, 5H, and 7H were identified as important chromosomes, which are strongly associated with the flowering time of barley (Wang et al. 2010b, Digel et al. 2016, Mikołajczak et al. 2016, Khahani et al. 2019). Yin et al. (2005b) also identified chromosome 1H, 2H, and 3H as important chromosomes associated with barley SDTE. In our study, most MTAs for flowering time were detected on chromosome 5H, but also on chromosome 1H, 2H, and 3H. (Tables S6 and S7; and Figure 4).

In our study, the detected MTAs corresponded to 35 genes, of which, for 19, at least one Gene Ontology (GO) term (Table S6) was found. A GO term enrichment analysis was conducted using "The Singular Enrichment Analysis tool in GO analysis toolkit and database for the agricultural community, AgriGO v2.0" (https://systemsbiology.cpolar.cn/agriGOv2/index.php, accessed on 26 July 2024) (Tian et al. 2017). Based on both the Bonferroni adjusted *p*-value and the non-adjusted method, no significantly enriched GO term was identified. The absence of enriched significant GO terms in our study could be associated with the identified GO terms, may not be properly annotated with the corresponding terms (Glass and Girvan 2014).

The dominant flowering gene of barley *'Ppd-H1'* promotes flowering under longer days, while plants with the recessive *ppd-H1* allele remain vegetative during long days (Cockram et al. 2007, Hemming et al. 2008, Alqudah et al. 2014). The MTAs for the SDTE and DRFL were found in the CC on chromosome 2H at 29.3 Mb (Tables 5 and S6). Upstream of '*Ppd-H1'* at 21.6 Mb, the '*GBM1035'* marker, which is identified with a genebank ID of '*AL500260'* on https://wheat.pw.usda.gov/GG3/ (accessed on 22 May 2023), promotes flowering in wild barley *H. spontaneum* (von Korff et al. 2010). The MTA for the SDTE of HL, which has cool weather conditions with adequate rainfall and a longer growing season (4 months), was identified between the *'Ppd-H1'* gene and the '*GBM1035'* marker at 25.1 Mb (Table 5 and Figure 4). Caproni et al. (2023) reported an MTA around this flowering gene in the barley growing highlands of Ethiopia, with an elevation of 2000–2400 masl.

Cosenza et al. (2024) reported the presence of a cluster of QTLs around *Ppd-H1* from 22.3 to 31.3 Mb, which are associated with flowering time and plant height; they suggested that these QTLs might have a small effect on the control of this trait. Therefore, the reported MTAs at 25.1 Mb in HL and 29.3 Mb in the CC might have a related effect on determining the flowering time.

Some of the telomere regions of chromosomes have significant MTAs with the flowering time of Ethiopian barley, as significant MTAs were found close to the telomere regions of chromosomes 2H, 3H, 4H, and 5H (Figure 4). Alqudah et al. (2014) also reported significant MTAs associated with different flowering time-related traits on similar telomeres regions of chromosomes for photoperiod-sensitive genotypes; however, it was not reported for non-sensitive genotypes. Significant MTAs at the telomere of 3H were also reported in Kikuchi et al. (2009).

The only locus that overlapped between the field and CC experimental locations was found on chromosome 3H, for DR and CC, at 4.1 to 4.6 Mb (Table 5). Alqudah et al. (2014) reported the association of a marker for awn tipping (Z49) at 4.2 Mb of chromosome 3H. Kikuchi et al. (2009) also reported a marker known as '*HvMFT1*', which was mapped at 2.4 Mb of chromosome 3H, which has a poor association with flowering time. A distinct difference in the temperature conditions between the CC and field locations (Table S2) may account for the few overlapping loci, and this is also explained by the lower correlation coefficient (*r*) value between the CC and field locations compared to the *r* value between field locations (Table 3). Teklemariam et al. (2023) also reported a few overlapping MTAs between the field and CC for different traits in drought stress experiments.

An MTA was found for DRFL on chromosome 1H at 427.7 Mb, which has an association with the flowering time in the CC experiment (Table 5 and Figure 4). He et al. (2019) identified the *HvPAF* (*HORVU1Hr1G058630*) gene close to this locus at 427.9 Mb, which is associated with a phytochrome-A-associated F-box protein and has a role in light perception and signaling in the flowering pathway. This MTA was also identified in Teklemariam et al. (2023) for grain biomass, harvest index, and thousand kernel weight in a climate chamber experiment at an optimal moisture treatment.

The drought stress during the vegetative developmental stage of barley revealed that genotypes that carry the recessive *ppd-H1* allele were observed to significantly delay flowering time in comparison to wild-type *Ppd-H1* allele (Gol et al. 2021). On the other hand, during high ambient temperatures, recessive *ppd-H1* barley was observed to accelerate the flowering time with the background *Vrn-H1* allele (Ejaz and von Korff 2016, Ochagavía et al. 2022).

The major genes that differentiate winter barley from spring barley are mapped on chromosome 5H as '*Vrn-H1'* at 599.1 Mb (Fu et al. 2005, Cockram et al. 2007) and a rice ortholog flowering gene, '*HD6-5H'*, mapped at 531.6 Mb (Roongsattham et al. 2006). The circadian clock gene,'*HvPRR95'*, was mapped at 565.15 Mb, and reported to be expressed during the evening of a long day, while its expression is reduced due to osmotic stress (Habte et al. 2014); and Ford et al. (2016) also reported an increasing trend of *HvPRR95* gene expression with the increase in temperature.

In our study, MTAs, which determine the flowering time at the warm location of DZ, DR, and MK, were consistently detected on chromosome 5H at 571.6 to 572.5 Mb with a gene ID of '*HORVU5Hr1G084260*' and a protein description of "abscisic acid-insensitive 5-like (*AbI5*) protein 2" for MTA at 571.6 Mb (Table S6). Thiel et al. (2021) also describe the role of this gene in flower organ formation. The *Arabidopsis* ortholog gene '*AT4G35900*' promotes flower initiation in the wild-type allele, while it delays the flowering time in the mutant (Wigge et al. 2005). Additionally, Finkelstein (2006) explained that *AbI5* delayed flower initiation by prohibiting lateral root formation through ABA-dependent nitrate inhibition. The association of this locus with grain biomass at an optimal moisture condition as well as days to maturity of drought condition was reported for the field experiment in Teklemariam et al. (2023).

Moreover, due to the difference in day length between the Australian and European environments, *HvPRR95* was reported to have a significant role compared to *Ppd-H1* by Pham et al. (2020), and also reported to have an effect on elevated temperature (Ford et al. 2016). Therefore, the consistent detection of MTAs close to the *HvPRR95* circadian clock gene could be associated with the presence of the significant day length between the Ethiopian environment and high latitude regions and/or the higher temperature at the warmer locations of DZ, DR, and MK.

A significant MTA on chromosome 5H for the CC was detected at 668.6 Mb for the STDE (Table 5 and Figure 4). Upstream of this MTA, *FRIGIDA*, which is known to have pleiotropic effects for environmental adaptation during drought stress (Lovell et al. 2013), was mapped at 662.6 Mb by Mascher et al. (2017). Caproni et al. (2023) also reported the effect of this MTA on the flowering time of Ethiopian barley landraces.

Specifically, the flowering time of Ethiopian barley landraces were studied at the molecular level by Tsehaye et al. (2012), and, Caproni et al. (2023). In our study, the GWAS analysis was used to identify MTAs that have a direct influence on the flowering time of Ethiopian barley; however, to utilize these

MTAs as breeding targets of barley's flowering time, validations of MTAs in a controlled environment will be required to develop high-resolution markers.

Conclusions

The vernalization temperature had no effect on the flowering time of Ethiopian barley landraces, as expected. This study identified GDDs as the major influencing factor in determining the flowering response in Ethiopian barley accessions. The presence of warm atmospheric temperatures accelerated the accession's vegetative growth, resulting in the shortest number of days to flowering in MK, DR, and DZ. The early flower response of the accessions in all field experiment locations compared to the CC may be associated with the presence of an optimal day temperature of >21 °C and a fluctuating temperature >10 °C between day/night. Furthermore, because all accessions consistently flowered at HL, few landraces might require mild chilling temperatures of at least 11 to 13 °C to complete flowering. The locus on chromosome 5H at 571.6 to 572.5 Mb has a strong association with the flowering time in warm areas, while the two loci on chromosome 2H at 25.1 Mb and 29.3 Mb could have an influence on the flowering time at the colder HL location and the CC, respectively. While this study provided broad insights into factors (GDD, >21 °C daily temperature, and extended rainfall) affecting the flowering time of Ethiopian barley accessions and identified significant MTAs, more research in controlled environments with distinct variations in all possible factors and detailed research on the interaction between the identified MTAs and known major flowering genes will pinpoint the exact factors related to Ethiopian barley landrace accession flowering times.

3. General discussion

Genetic diversity and influencing parameters

Many domesticated crops, such as coffee (*Coffea arabica*), tef (*Eragrostis tef*), enset (*Ensete ventricosum*), noug (*Guizotia abyssinica*), mustard (*Brassica carinata*), and anchote (*Coccinia abyssinica*) are known to originate from Ethiopia. Additionally, the country is known for its center of diversity for different field crops, including barley, sorghum, durum wheat, finger millet, faba bean, linseed, sesame, safflower, chickpea, lentil, cowpea, fenugreek, and grass pea (Ethiopian Biodiversity Institute 2023).

Barley is placed as the fourth most important cereal crop in the world after maize, rice, and wheat. Ethiopian barley genotypes are distinguished from the global barley collection by their distinctive nature (Jørgensen 1992, Muñoz-Amatriaín et al. 2014, Milner et al. 2019). Phenotypically, Ethiopian barley varieties exhibit distinct hordein polypeptide patterns and anthocyanin pigmentation on seed coats, leaf sheaths, and stems (Asfaw 1989, Demissie et al. 1998, Eticha et al. 2010a). Ethiopian farmers still hold diversified landraces in their hands (van Leur and Gebre 2003, Sansaloni et al. 2020), as they utilize these based on end-use products, maturity, and different agronomic preferences and yield.

The distinctiveness of Ethiopian barley genotypes is also confirmed in this study, as 69% of 43,461 scorable SNPs markers were removed either as monomorphic or for being less than 3% minor allele frequency. One of the purposes of genetic diversity studies is to exploit unique alleles for breeding programs. In this respect, the presence of a unique genetic makeup in the Ethiopian barley study panel in constrat to the world barley was observed in this study.

Utilizing the percentage of polymorphic loci (PPL), one may predict the general genetic diversity pattern, and smaller values indicate the presence of a low level of variation, which is also associated with less adaptation to various biotic and abiotic stresses (Reed and Frankham 2003, Spielman et al. 2004). In general, a high PPL is observed in all genetically distinct clusters; however, relatively cluster 1 has the least (88%) (Publication 2.1, Table 4). Cluster 1 comprises 41% of the Welo landraces (33 out of 80). The presence of the smallest value of percentage of polymorphic loci in cluster 1 may be explained either by the very unique socio-economic situation of the Welo location or the different geographical topography of the area, which is characterized by uneven terrain, chain mountains, and sharp cliffs (Hassen 2021). These factors ultimately limit the exchange of plant materials.

Abebe (2010) found that the least genetic diversity was associated with the presence of specific abiotic stress in the region, as abiotic stress limits the option of genetic selection. Landraces from each geographic region were collected at least from two different agro-ecologies, with the exception of Gonder landraces, which were collected from moist highland agro-ecology (Publication 2.1 S1 Table). However, the agro-ecological related stresses have a minor effect on the formation of genetic structure

as accessions originated from different agro-ecological zones are evenly assigned to the three clusters (Publication 2.1, Table S1).

The presence of high genetic variation within Ethiopian barley landraces was reported previously (Demissie et al. 1998, Abebe and Léon 2013, Abebe et al. 2013). Tanto Hadado et al. (2009) studied the landrace diversity using spike morphological traits. The results revealed the presence of significant genetic variation between the growing seasons, namely *'meher'* (the main cropping season, which extends from June to October) and '*belg'* (the short cropping season, which extends from late February to early July), and altitude classes. Similarly, the molecular genetic diversity study by Abebe et al. (2015b) demonstrated that climate variation contributed significantly more to the formation of population structure than geographic distance, as previously reported by Abebe and Léon (2013). However, in our study, the formation of population structure seems to be more influenced by geographic region than climate conditions (Publication 2.1 Table 1, and Table S1). Moreover, the presence of uneroded genetic diversity in the hands of farmers was also observed in our study, as six of the 19 EBI accessions decedents clustered in different genetically distinct clusters (Publication 2.1 Table S1).

Climate variation was reported to inlfuence the genetic structure in Ethiopian barley landraces (Tanto Hadado et al. 2009, Abebe et al. 2015b). The altitude gradient classification is similar to the climate classification, in which a gradient below 1500 meter above sea level (masl), is considered *'kola'*, which is characterized by a daily mean temperature higher than 20°C and short duration erratic rainfall; an altitude between 1500 and 2000 masl is considered *'woyna dega*' with a daily mean temperature between 16 and 20°C with adequate rainfall; and there is '*dega'* with an altitude gradient greater than 2000 masl with a daily mean temperature below 16°C with extended rainfall (Huffnagel 1961). In addition to this, the choice of landrace was also influenced by the season and farm soil type, as '*belg'* sown landraces have to be harvested before the beginning of the main cropping season, and farm areas with Vertisol soil type often experience relay cropping in which early-maturing cereal crops are planted first, followed by chickpea or grass pea (Minta et al. 2014). In these farming systems, farmers always choose early-maturing genotypes over late ones.

Furthermore, classification based on climate or altitudinal gradient is in most cases associated with the duration of the cropping season. The drought experiment conducted at two drought-prone locations and in climate chambers revealed that days to maturity is the most important trait to determine the level of drought stress tolerance in Ethiopian barley landraces (Publication 2.2, Table 3, and Table 4). Moreover, the ANOVA result revealed a significant difference at $p<0.05$ for the interaction of genotypes and environment for all tested parameters except DM and DFL in the field experiments (Publication 2.2, Table 2), indicating the presence of plasticity in some landraces. Plasticity is important as it helps the plant to respond quickly in fluctuating environments (Pigliucci 2005, Laitinen and Nikoloski 2018), but it is also a challenge as it renders selection difficult (Yan and Frégeau-Reid 2018).

The 21 barley breeding lines collected from HARC are the most heterogeneous collection, as they are collected for different agronomic, physiological, and food quality traits.

Generally, based on altitude gradient and season-based classification, Ethiopian landraces can be divided into two groups: short-duration and medium- or long-duration landraces, and a lack of distinct structure for landraces collected from different agro-ecologies might be associated with a lack of information for which cropping season respective landraces are used.

Traits, loci and genome regions associated to drought tolerance

It is obvious that drought stress has an effect on grain yield, and in our experiment, the maximum effect was observed on grain biomass (Publication 2.2_Table 1). However, one of the objectives of the drought stress experiment is to find genotypes with an acceptable grain yield under drought stress conditions.

Genotypes with high grain yield due to early maturity and better values of spike-related parameters are the most preferred in the selection of drought tolerant varieties by Ethiopian farmers (Abay et al. 2008, Mancini et al. 2017, Semahegn et al. 2021). Additionally, farmers choose early-maturing genotypes for the *'belg'* season and relay cropping. Consequently, it is not unexpected that the most important traits affecting Ethiopian genotypes' tolerance to drought stress are DFL and/or DM.

Mascher et al. (2017) explored the diversity of barley in 48 elite spring barley cultivars and reported the presence of diversity across the seven genomes of spring barley. One of the regions with the highest diversity is on chromosome 1H near the '*ELF3'* flowering gene, which is mapped at 556.9 Mb. At this locus MTAs related to DM and NSdPS of CS are identified (Publication 2.2, Table 8).

Apart from the MTA close to the *ELF3* region, MTAs for CS have been detected close to the flowering gene '*PpdH1'* and the barley row-determining gene '*Vrs2'*, which are located on chromosome 2H at 29.1 Mb and chromosome 5H at 564.4 Mb, respectively (Publication 2.2, Table 8), and were also reported to be diverse by Mascher et al. (2017).

Interestingly, QTL regions, mostly reported to be associated with drought tolerance by different studies (Al-Abdallat et al. 2017, Ogrodowicz et al. 2017, Thabet et al. 2018, Dhanagond et al. 2019, Gordon et al. 2020), are repeatedly identified in climate chamber experiments rather than field experiments. This might be correlated with the fact that the growing conditions in the climate chamber experiment were more similar to previous drought stress experiments, while the climate conditions of the field experiment in Ethiopia were quit different from these.

Plant height is also found to be strongly associated with drought stress tolerance (Publication 2.2 Table 3). However, Ethiopian barely landraces were reported to grow very tall and are prone to lodging compared with exotic barley genotypes at optimum moisture environment conditions (Daba 2015). In our study also, the landraces at Holetta were very susceptible to lodging as the mean plant height was 116 cm, while plant height was reduced in drought-prone locations, as the mean values were 63cm at

Dera and 82cm at Melkassa. However, in years of extended rainfall like 2017, landraces at drought-prone areas were also observed to be affected by lodging.

The identified MTAs on chromosome 5H at 571.6-572.5 Mb were consistently detected in all field experiment locations except Holetta for flowering time and also for FS of DM and FC of SPAD and GB (Publication 2.2 Table 7, and Publication 2.3 Table 5). This highlights that the genomic region is the key locus determining the agronomic performance of Ethiopian landraces.

Khodaeiaminjan et al. (2023) reported a candidate gene on chromosome 5H at 570.4 Mb associated with the ratio of the surface of the root network and the depth of the root system between drought stress and control treatments, which improves plant performance during the drought period. However, the association of this locus with flowering time was not reported in previous experiments, and the locus was also not identified in the climate chamber experiments (Publication 2.3 Table S6).

Another important region was detected on chromosome 5H at 10.35-12.04 Mb for PH in FC and FS as well as for GB in CS and FC. In addition, in this region also flowering-related MTAs for the DZ location were detected. A significant level of arabinose, arbutin, C32 metabolites expression are reported for chromosome 5H at 10.6 Mb from the end of ear emergence to flowering and at the beginning of the shooting stage by Gemmer et al. (2021). However, they suggested the presence of a weak association with flower initiation as pre-ear emergence sampling was better suited to predict the flowering time association than post-ear emergence. An increase in expression of arbutin was also reported during drought stress in safflower (Wei et al. 2020). Similarly, an increased level of arabinose in wild tomato than in an ABA-deficient mutant line was reported during a shortage of soil water content by Živanović et al. (2020), Furthermore, a decrease in the content of C32 in drought-tolerant water melon genotypes favors leaf wax deposition under drought stress (Li et al. 2020).

The highest number of detected MTAs in our studies was on chromosome 2H (Publication 2.2, Supplementary Table 3; and Publication 2.3, Supplementary Table S6), which indicated the importance of this chromosome in drought tolerance of the Ethiopian barley landraces. Out of the identified 38 MTAs on chromosome 2H (Publication 2.2, Supplementary Table 3), 22 were for the climate chamber, of which 14 were associated with drought tolerance. Furthermore, among the six MTAs associated with flowering time on chromosome 2H, three were identified in the climate chamber experiments (Publication 2.3, Table S6).

Exploiting GWAS results for crop improvement

The effect of drought stress is highly associated with the development stage of plants and the intensity of drought stress. Dry spell is defined as a period when there is less than 1 mm of precipitation recorded in each of five consecutive days (Barron et al. 2003, Polade et al. 2014). However, the length of consecutive days could vary based on the soil type, as clay soil texture has a higher water-holding

capacity than loamy- or sandy-type soils. Similarly the effect is more severe in warm environment than cool environment (Hillel 2003, Brady et al. 2008).

The delay in the onset of the rainy season or the lack of adequate rainfall at the start of the rainy season in drought-prone locations of Ethiopia gives the farmer the option to shift from long-maturing crops like maize, barley, wheat and sorghum to short-maturing crops like mung-bean, common-bean, tef, or to other early-maturing varieties.

Drought stress that occurs at the flowering stage, also known as terminal drought, has a detrimental effect on crop production as it inhibits farmers from shifting to short-maturing crops or varieties. The viable solution is to plan ahead by planting drought-tolerant cultivars. The rainfall pattern in drought-prone locations of Ethiopia is not only minimal but also erratic, and the dry spell usually starts much earlier than in other locations (Kassie et al. 2013, Adimassu et al. 2014). The high impact of terminal drought on productivity is due to its significant reduction in kernel weight as well as the number of seeds per spike, which have a strong association with grain yield (Sallam et al. 2019).

This study identified three accessions with stable grain biomass under moderate drought stress in field experiments as well as 13 accessions with the highest drought tolerance in the severe drought stress condition of the climate chamber. However, there is only one common accesssion in the top 20 performing accessions from the moderate and severe drought stress groups (Publication 2.2 Supplementary Table 2). Nevertheless, selecting accessions for breeding enhancement based on these drought indices will be misleading as grain biomass is a complex trait. The results of these indices can vary across environments, and they tend to prioritize grain yield, overlooking genotypes with superior drought tolerance but low yield. Additionally, they often mask the contribution of secondary traits (Blum 2011, Sallam et al. 2019). Therefore, additional selection parameters are required to fully exploit the drought potential of candidate genotypes.

The genetic diversity analysis provided information on the unique genetic makeup of the Ethiopian barley collection. The GWAS experiment on drought stress and flowering time analysis identified a number of MTAs, and genomic regions associated with drought tolerance. However, summarizing the results in a way suitable to exploit the reported MTAs in a crop improvement program is crucial.

Admixture indicated the presence of a higher rate of gene flow within different populations.The Mantel test revealed the presence of a higher rate of gene flow across different geographic locations (Publication 2.1, Figure 4). Usually, an accession is assigned to the admixture population when its membership coefficient is less than 0.7 (Lehnert et al. 2017, Rufo et al. 2019). When we reassign the accessions based on this criteria, 118 landraces could be categorized as part of the admixture population (Publication 2.1, S Table 1).

Based on that, out of the 16 accessions selected based on their adaptation to moderate/severe drought stress during the drought stress experiments, 50% of them can be assigned to the admixture population.

Admixture is important as it enhances genetic diversity, introduces stress tolerance and improves crop productivity through the wider adaptability of respective genotypes (Shi et al. 2018).

The pleiotropic effect, where a single genetic locus impacts multiple traits, is a crucial consideration when exploiting GWAS , as it allows breeders to target several desirable traits simultaneously by focusing on a limited number of loci. Results of Liller et al. (2015) reported that row type-governing genes have an effect on the number of tillers as well as the number of seeds and flag leaf length. Kuczyńska et al. (2014) studied the effect of the *sdw1/denso* locus on plant height, flowering time, grain yield, and kernel weight. Flowering time-controlling *Ppd-H1*, *Vrn-H2*, and *Vrn-H3* genes were also suggested to have an effect on yield and related parameters (Wang et al. 2010b).

Sixteen different pleiotropic loci that are associated with more than one trait were detected in this study, either in the flowering time analysis or in the drought tolerance experiment. In the climate chamber experiment, the region on chromosome 1H at 427.69 Mb was associated with HI, GB, TKW, and the flowering time of CCn. Similarly, the region on chromosome 2H at 29.31–30.19 Mb was associated with DM, the flowering time of CCn, and HI, GB, TKW, and NSdPS of CS (Publication 2.2 Table 7, Supplementary Table 5, and Publication 2.3 Table S6).

In the field experiment, the region on chromosome 2H at 766.08–767.33 Mb was associated with flowering time, HI, and NSdPS of FS. Additionally, the locus on chromosome 6H at 558.86 Mb was associated with flowering time, GB, and TKW CS. Furthermore, near the *Vrs1* barley row-determining gene on chromosome 2H, QTLs were detected for TKW of FC and FS, and also near the *Vrs2* gene on chromosome 5H, an MTA associated with HI of CS was detected (Publication 2.2 Table 7, Supplementary Table 5, and Publication 2.3 Table S6).

Constitutive markers, or genes, are also important to analyze the plasticity of genotypes, as these types of genes give responses at an intermediate level of environmental change (Geisel 2011). Therefore, the reported constitutive MTAs are important to exploit the GWAS results in breeding.

Phenotypic variation explained (PVE) is used for the estimation of phenotypic variation explained by markers and is also an important criteria for choosing the most important MTAs. According to Kumar et al. (2017), in most studies, 10% for PVE was considered a threshold level to distinguish between minor and major MTAs, while in other studies 20% is considered (Pasam et al. 2012, Korte and Farlow 2013). There are four associations with a PVE value higher than 20% associated with TKW in FS and DM (2) and TKW in CS, while a total of nine MTAs with a PVE higher than 10% associated with FS (2) or CS (7) are detected in the drought stress experiments in this study (Publication 2.2, Table 7, and Publication 2.2 Supplementary Table 5). In flowering time analysis, however, only one locus had a PVE value higher than 10% (Publication 2.3, Table 5, and Publication 2.3 Table S6). This may be due to the fact that most MTAs identified during the flowering time analysis only have a minor effect as flowering

time besides the major genes is highly influenced by the environment and minor QTL (Aslibekyan et al. 2014, Kumar et al. 2017, Voss-Fels et al. 2019).

4. Summary

A genetic diversity study was conducted on a panel of 260 Ethiopian barley landraces and cultivars using the 50k iSelect SNP array. The results revealed three genetically distinct clusters. Additionally, the high proportion of monomorphic markers and the large number of markers with a minor allele frequency of less than 3% underscored the distinctiveness of the Ethiopian barley collection.

The presence of high genetic diversity within genetically distinct clusters reflects the high variation within the set of genotypes analysed. Although accessions from a few locations like Gonder, Jimma, and Shewa are clustered according to the geographic origin the Mantel test also revealed the absence of a correlation between geographic distance and the formation of population structure.

The presence of such genetic diversity in the Ethiopian barley collection was the basis to conduct field and climate chamber experiments for drought stress tolerance. For both experiments a moderate level of heritability for the traits analysed was observed. The correlations of results observed were higher between CS and FS than between CCn and FC. In summary, 16 accessions were selected either for stable yield performance under field conditions or better performance in strong drought stress in the climate chamber experiment; only B191.1 accession was found common in the top 20 of the two groups.

The study also indicated DM as the most important trait for drought tolerance in the Ethiopian barely collection, as it is strongly correlated to most traits in FS and CS. Additionally, chromosome 2H was identified as the most important for drought stress in this study as a high number of MTAs, with the highest LOD and PVE values was located there.

In the study conducted at four locations in Ethiopia and the climate chamber, the flowering time of Ethiopian barely turned out to be mostly influenced by the summation of growing day temperature (GDD), as accessions in warm locations flowered earlier than in cold locations. Additionally, environmental conditions like extended optimal rainfall with optimum day and night temperatures have an influence on flowering time. All accessions in the field experiments flowered much earlier than those in the climate chamber. The GWAS revealed that the region on chromosome 5H, at 571.6 to 572.5 Mb, is an important region influencing flowering time at the warm locations of DZ, MK, and DR. In contrast, chromosome 2H at 25.1 Mb and 29.3 Mb was associated with flowering time in HL and CC, respectively.

In general, our study highlighted the diversity of barley cultivated by Ethiopian farmers. Although the study identified several MTAs with high PVE values and pleiotropic effects related to drought stress treatment and flowering time, independent validation of each MTA is required before applying these in barley breeding. The findings also recomend strategic conservation of Ethiopia’s barley genetic resources, as they are very diverese and may contribute to face global food production challenges.

5. Zusammenfassung

Zur Erfassung der genetischen Diversität wurde ein Panel von 260 äthiopischen Gerstenlandrassen und -sorten unter Verwendung des 50k iSelect SNP-Arrays analysiert. Die Analysen ergaben drei genetisch unterschiedliche Cluster. Der hohe Anteil monomorpher Marker und die große Anzahl an Markern mit einer Minor-Allel-Häufigkeit von weniger als 3 % unterstreichen die Besonderheit der äthiopischen Gerstensammlung.

Das Vorhandensein einer hohen genetischen Diversität auch inerhalb der einzelnen Cluster spiegelt die erhebliche genetische Variation des analysierten Materials wider. Obwohl die Akzessionen von einigen wenigen Standorten wie Gonder, Jimma und Shewa nach der geografischen Herkunft geclustert sind, zeigte der Mantel-Test, dass keine Korrelation zwischen der geografischen Entfernung und der Bildung einer Populationsstruktur besteht.

Das Vorhandensein dieser genetischen Vielfalt in der äthiopischen Gerstensammlung war die Grundlage für die Durchführung von Feld- und Klimakammerversuchen zur Trockenstresstoleranz. In beiden Versuchen wurde eine mäßige Heritabilität für die analysierten Merkmale festgestellt. Die beobachteten Korrelationen der Ergebnisse waren zwischen CS und FS höher als zwischen CCn und FC. Zusammenfassend konnten 16 Akzessionen im Hinblick auf eine stabile Ertragsleistung unter Feldbedingungen bzw. mit einer besseren Leistung bei starkem Trockenstress im Klimakammerexperiment identifiziert werden. Lediglich die Akzession B191.1 war unter beiden Bedingungen in de Gruppe der zwanzig besten Genotypen.

Die Studie zeigt, dass DM das wichtigste Merkmal für die Trockenstresstoleranz in der äthiopischen Sammlung ist, da es stark mit den meisten Merkmalen in FS und CS korreliert. Darüber hinaus wurde Chromosom 2H in dieser Studie als besonders bedeutend für Trockenstresstoleranz identifiziert, da auf diesem Chromosom eine große Anzahl an MTAs mit den höchsten LOD- und PVE-Werten identifiziert werden konnte.

In der Studie, die an vier Standorten in Äthiopien und in der Klimakammer durchgeführt wurde, stellte sich heraus, dass die Blütezeit der äthiopischen Sorten im Wesentlichen von der durchschnittlichen Temperatursumme der Wachstumstage (GDD) beeinflusst wird, da die Sorten an warmen Standorten früher blühten als an kühleren Standorten. Darüber hinaus zeigen die Ergebnisse, dass Umweltbedingungen wie langanhaltende optimale Niederschläge mit optimalen Tages- und Nachttemperaturen einen Einfluss auf die Blütezeit haben. Alle Akzessionen blühten in den Feldversuchen deutlich früher als in der Klimakammer. Genomweite Assoziationsstudien ergaben, dass eine Region auf Chromosom 5H (571,6 bis 572,5 Mb) von besonderer für die Blütezeit an den warmen Standorten von DZ, MK und DR ist. Im Gegensatz dazu steht eine Region von 25,1 Mb bis 29,3 Mb auf Chromosom 2H mit der Blütezeit in HL bzw. CC in Verbindung.

Summary

Generell unterstreicht die vorliegende Arbeit die genetische Vielfalt der von äthiopischen Landwirten angebauten Gerste. Obwohl in der Studie mehrere MTAs mit hohen PVE-Werten und pleiotropen Effekten für die Trockenstresstoleranz und die Blütezeit identifiziert werden konnten, ist eine unabhängige Validierung der einzelnen MTAs vor einem Einsatz in der Gerstenzüchtung erforderlich. Die Ergebnisse legen ebenfalls die strategische Erhaltung der äthiopischen genetischen Ressourcen der Gerste nah, da diese genetisch sehr divers sind und zukünftig zur Bewältigung der globalen Herausforderungen in der Nahrungsmittelproduktion beitragen können.

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7. Supplementary files

Publication 2.1) The genetic diversity of Ethiopian barley genotypes in relation to their geographical origin

Table S1. Geographical location based on GPS and agro-ecological zones of the Ethiopian barley landrace. Accessions descended from similar 'EBI accession code' are highlighted by yellow color

No	Accessions	EBI accession code	Latitude	Longitude	Geographical location	Agro-ecology zone	Genetically distinct cluster	Highest membership coefficient in the distinct genetic cluster
1	B1	202541	12.38	37.28	Gonder	Tepid moist mid highlands (M3)	3	0.589
2	B3.1	202543	12.17	36.95	Gonder	Tepid moist mid highlands (M3)	1	0.554
3	B3.2	202543	12.17	36.95	Gonder	Tepid moist mid highlands (M3)	3	0.785
4	B4.1	202545	12.05	36.95	Gonder	Tepid moist mid highlands (M3)	3	0.787
5	B4.3	202545	12.05	36.95	Gonder	Tepid moist mid highlands (M3)	3	0.563
6	B4.4	202545	12.05	36.95	Gonder	Tepid moist mid highlands (M3)	3	0.669
7	B5.1	202546	12.05	36.95	Gonder	Tepid moist mid highlands (M3)	3	0.628
8	B6	202547	7.33	39.75	Arsi-Bale	Tepid sub-humid mid highlands (SH3)	3	0.41
9	B7	202548	7.33	39.75	Arsi-Bale	Tepid sub-humid mid highlands (SH3)	3	0.875
10	B8	202550	7.28	39.83	Arsi-Bale	Cool sub-humid mid highlands (SH4)	3	0.643
11	B9.1	202553	7.3	40.12	Arsi-Bale	Tepid moist mid highlands (M3)	3	0.68
12	B9.2	202553	7.3	40.12	Arsi-Bale	Tepid moist mid highlands (M3)	3	0.83
13	B10.1	202558	7.15	40.78	Arsi-Bale	Tepid moist mid highlands (H3)	3	0.828
14	B10.2	202558	7.15	40.78	Arsi-Bale	Tepid moist mid highlands (H3)	1	0.606
15	B11	202559	7.48	40.62	Arsi-Bale	Tepid moist mid highlands (M3)	2	0.992
16	B12	202561	7.53	40.7	Arsi-Bale	Tepid moist mid highlands (M3)	2	0.924
17	B13.1	202569	7.83	39.73	Arsi-Bale	Tepid moist mid highlands (H3)	3	0.569
18	B13.2	202569	7.83	39.73	Arsi-Bale	Tepid moist mid highlands (H3)	2	0.931
19	B16.2	202575	7.53	39.98	Arsi-Bale	Tepid sub-humid mid highlands (SH3)	2	0.547
20	B17	202585	9.02	38.13	Shewa	Tepid moist mid highlands (M3)	2	0.754
21	B18	202587	9.02	38.13	Shewa	Tepid moist mid highlands (M3)	1	0.862
22	B19	202592	10.68	37.35	Gojam	Tepid moist mid highlands (M3)	2	0.763

23	B20	202593	10.68	37.35	Gojam	Tepid moist mid highlands (M3)	3	0.805
24	B21	202595	10.72	37.17	Gojam	Tepid moist mid highlands (M3)	2	0.794
25	B23.2	202597	10.72	37.17	Gojam	Tepid moist mid highlands (M3)	3	0.642
26	B25	202599	10.72	37.07	Gojam	Tepid moist mid highlands (M3)	2	0.978
27	B26.3	202602	10.98	36.93	Gojam	Cool sub-humid mid highlands (SH4)	1	0.597
28	B27	202609	10.52	37.45	Gojam	Tepid moist mid highlands (M3)	2	0.709
29	B28	202612	11.12	37.9	Gojam	Tepid moist mid highlands (M3)	2	0.655
30	B29	202613	10.97	37.87	Gojam	Tepid moist mid highlands (M3)	3	0.663
31	B30	202614	10.97	37.87	Gojam	Tepid moist mid highlands (M3)	1	0.687
32	B31.2	202615	10.97	37.87	Gojam	Tepid moist mid highlands (M3)	1	0.557
33	B32.1	202616	10.97	37.87	Gojam	Tepid moist mid highlands (M3)	1	0.595
34	B32.2	202616	10.97	37.87	Gojam	Tepid moist mid highlands (M3)	1	0.586
35	B33.1	202667	7.68	36.92	Jimma	Tepid moist mid highlands (H3)	1	0.62
36	B33.2	202667	7.68	36.92	Jimma	Tepid moist mid highlands (H3)	3	0.67
37	B34	202671	7.92	37.42	Jimma	Cool humid mid highlands (SH2)	3	0.985
38	B35	202672	7.92	37.42	Jimma	Cool humid mid highlands (SH2)	3	0.825
39	B37	202674	9.5	35.47	Ambo-Welega	Tepid sub-humid mid highlands (SH3)	2	0.909
40	B38	202680	11	36.92	Gojam	Cool sub-humid mid highlands (SH4)	3	0.872
41	B39	202682	10.5	37.53	Gojam	Tepid moist mid highlands (M3)	3	0.841
42	B41	202707	9.25	41.13	Hararghe	Tepid sub-humid mid highlands (SH3)	3	0.986
43	B42.1	202709	9.25	41.13	Hararghe	Tepid sub-humid mid highlands (SH3)	3	0.986
44	B42.2	202709	9.25	41.13	Hararghe	Tepid sub-humid mid highlands (SH3)	3	0.586
45	B43	202711	9.27	41.13	Hararghe	Tepid sub-humid mid highlands (SH3)	3	0.984
46	B45	202719	9.28	41.48	Hararghe	Tepid sub-humid mid highlands (SH3)	3	0.595
47	B46	202725	9.4	41.58	Hararghe	Tepid sub-humid mid highlands (SH3)	3	0.757
48	B48	202727	9.4	41.58	Hararghe	Tepid sub-humid mid highlands (SH3)	1	0.76
49	B49	202730	9.4	41.58	Hararghe	Tepid sub-humid mid highlands (SH3)	3	0.514
50	B50	202782	9.43	41.03	Hararghe	Warm moist lowlands (M2)	3	0.359
51	B51	202783	11.58	39.22	Welo	Cool sub-moist mid highlands (SM4)	3	0.993

52	B52	202784	11.62	38.93	Welo	Cool sub-moist mid highlands (SM4)	1	0.866
53	B53.1	202785	11.62	38.93	Welo	Cool sub-moist mid highlands (SM4)	3	0.993
54	B53.2	202785	11.62	38.93	Welo	Cool sub-moist mid highlands (SM4)	3	0.627
55	B54	202786	11.62	38.93	Welo	Cool sub-moist mid highlands (SM4)	3	0.606
56	B55	202791	11.65	38.85	Welo	Tepid sub-moist mid highland*s (SM3)	1	0.997
57	B56.2	202792	11.65	38.85	Welo	Tepid sub-moist mid highlands (SM3)	2	0.539
58	B57	202793	11.82	38.62	Welo	Cool sub-moist mid highlands (SM4)	2	0.544
59	B59.2	202795	11.27	39.25	Welo	Cool moist mid highlands (M4)	1	0.638
60	B60.1	202799	11.05	39.2	Welo	Cold moist sub-afro-alpine to afro-alpine (M5)	1	0.908
61	B60.2	202799	11.05	39.2	Welo	Cold moist sub-afro-alpine to afro-alpine (M5)	1	0.642
62	B61	202802	10.82	39.08	Welo	Tepid sub-moist mid highlands (SM3)	3	0.568
63	B62.2	202806	10.95	38.78	Welo	Cool sub-moist mid highlands (SM4)	3	0.533
64	B63	202809	11.07	39.37	Welo	Cool moist mid highlands (M4)	1	0.73
65	B65	202811	11.12	39.28	Welo	Cool moist mid highlands (M4)	2	0.856
66	B66	202812	11.12	39.28	Welo	Cool moist mid highlands (M4)	1	0.807
67	B67	202813	11.12	39.28	Welo	Cool moist mid highlands (M4)	1	0.993
68	B68	202814	10.83	39.45	Welo	Cool moist mid highlands (M4)	1	0.811
69	B69	202815	10.83	39.45	Welo	Cool moist mid highlands (M4)	1	0.631
70	B72	202821	9.15	41.12	Hararghe	Tepid sub-humid mid highlands (SH3)	1	0.618
71	B73	201823	9.22	41.12	Hararghe	Tepid sub-humid mid highlands (SH3)	3	0.62
72	B74.1	202825	9.27	41.33	Hararghe	Tepid sub-humid mid highlands (SH3)	1	0.497
73	B74.2	202825	9.27	41.33	Hararghe	Tepid sub-humid mid highlands (SH3)	1	0.721
74	B75	202826	9.27	41.33	Hararghe	Tepid sub-humid mid highlands (SH3)	1	0.996
75	B76	202827	9.27	41.33	Hararghe	Tepid sub-humid mid highlands (SH3)	1	0.996
76	B77	202828	9.27	41.28	Hararghe	Tepid sub-humid mid highlands (SH3)	1	0.795
77	B78	202829	9.27	41.28	Hararghe	Tepid sub-humid mid highlands (SH3)	2	0.522
78	B79	202830	9.27	41.28	Hararghe	Tepid sub-humid mid highlands (SH3)	1	0.808
79	B83	216917	9.52	39.22	Shewa	Cool sub-moist mid highlands (SM4)	3	0.82

80	B84	216918	9.52	39.22	Shewa	Cool sub-moist mid highlands (SM4)	3	0.787
81	B86	216920	9.32	38.73	Shewa	Cool moist mid highlands (M4)	2	0.998
82	B87	216923	9.12	38.6	Shewa	Cool moist mid highlands (M4)	3	0.645
83	B88	216924	9.12	38.6	Shewa	Cool moist mid highlands (M4)	2	0.64
84	B89	216927	8.9	38.93	Shewa	Tepid sub-moist mid highlands (SM3)	2	0.817
85	B90	216928	8.9	38.93	Shewa	Tepid sub-moist mid highlands (SM3)	2	0.984
86	B93	216931	8.85	38.87	Shewa	Tepid moist mid highlands (H3)	2	0.647
87	B94	216932	8.83	38.92	Shewa	Tepid sub-humid mid highlands (SH3)	2	0.907
88	B96	216934	9.1	38.2	Shewa	Cool moist mid highlands (M4)	2	0.995
89	B97	216935	9.1	38.2	Shewa	Cool moist mid highlands (M4)	2	0.998
90	B101	216939	9.12	38.2	Shewa	Cool moist mid highlands (M4)	2	0.932
91	B103	216941	9.12	38.2	Shewa	Cool moist mid highlands (M4)	2	0.91
92	B104	216942	9.18	38.18	Shewa	Cool moist mid highlands (M4)	2	0.542
93	B105	216944	9.18	38.18	Shewa	Cool moist mid highlands (M4)	2	0.995
94	B108	216947	9.3	38.07	Shewa	Cool moist mid highlands (M4)	2	0.997
95	B110	216949	9.3	38.07	Shewa	Cool moist mid highlands (M4)	2	0.919
96	B111	216950	9.3	38.07	Shewa	Cool moist mid highlands (M4)	2	0.923
97	B112.1	216951	9.08	38.78	Shewa	Cool moist mid highlands (M4)	2	0.998
98	B112.2	216951	9.08	38.78	Shewa	Cool moist mid highlands (M4)	2	0.903
99	B113.2	216952	9.08	38.78	Shewa	Cool moist mid highlands (M4)	3	0.516
100	B114.1	216953	9.08	37.22	Ambo-Welega	Tepid sub-humid mid highlands (SH3)	3	0.517
101	B114.2	216953	9.08	37.22	Ambo-Welega	Tepid sub-humid mid highlands (SH3)	3	0.437
102	B115.1	216954	9.08	37.22	Ambo-Welega	Tepid sub-humid mid highlands (SH3)	3	0.438
103	B116.2	216955	10.97	37.22	Gojam	Cool moist mid highlands (M4)	2	0.859
104	B118	216957	10.97	37.22	Gojam	Cool moist mid highlands (M4)	2	0.919
105	B120	216959	11.83	38	Welo	Tepid moist mid highlands (M3)	1	0.41
106	B123	216962	11.82	38.13	Welo	Cool moist mid highlands (M4)	3	0.618
107	B125	216964	11.82	38.13	Welo	Cool moist mid highlands (M4)	2	0.773
108	B126	216965	11.82	38.13	Welo	Cool moist mid highlands (M4)	2	0.575

109	B127	216966	11.82	38.13	Welo	Cool moist mid highlands (M4)	2	0.838
110	B128	216967	11.8	38.12	Welo	Cool moist mid highlands (M4)	2	0.642
111	B129	216968	11.8	38.12	Welo	Cool moist mid highlands (M4)	1	0.512
112	B130.2	216969	11.8	38.12	Welo	Cool moist mid highlands (M4)	1	0.699
113	B133	216972	11.8	38.22	Welo	Cool moist mid highlands (M4)	1	0.607
114	B134	216973	11.82	38.22	Welo	Cool moist mid highlands (M4)	2	0.953
115	B135.1	216974	11.82	38.22	Welo	Cool moist mid highlands (M4)	1	0.649
116	B135.2	216974	11.82	38.22	Welo	Cool moist mid highlands (M4)	3	0.771
117	B136	216975	10.83	37.6	Gojam	Cool moist mid highlands (M4)	3	0.694
118	B137	216976	10.83	37.6	Gojam	Cool moist mid highlands (M4)	2	0.695
119	B138	216978	11.07	37.85	Gojam	Tepid moist mid highlands (M3)	2	0.65
120	B139	216979	10.9	37.95	Gojam	Tepid moist mid highlands (M3)	1	0.464
121	B141	216981	10.9	38.95	Welo	Cold sub-moist mid highlands (SM5)	3	0.56
122	B142	216982	10.9	38.95	Welo	Cold sub-moist mid highlands (SM5)	2	0.529
123	B143	216985	10.93	36.08	Gojam	Warm moist lowlands (M2)	2	0.893
124	B144	216987	10.93	36.08	Gojam	Warm moist lowlands (M2)	3	0.782
125	B145.1	216988	10.93	36.08	Gojam	Warm moist lowlands (M2)	2	0.812
126	B146	216989	7.48	39.18	Arsi-Bale	Cool humid mid highlands (H4)	3	0.571
127	B148	216992	6.27	37.58	Gamo-Gofa	Cool sub-humid mid highlands (SH4)	2	0.701
128	B149	216993	6.27	37.58	Gamo-Gofa	Cool sub-humid mid highlands (SH4)	3	0.686
129	B151	216995	6.28	37.58	Gamo-Gofa	Cool sub-humid mid highlands (SH4)	3	0.513
130	B153	216998	6.28	37.58	Gamo-Gofa	Cool sub-humid mid highlands (SH4)	3	0.573
131	B154	216999	6.28	37.58	Gamo-Gofa	Cool sub-humid mid highlands (SH4)	3	0.53
132	B156	217002	6.53	37.75	Gamo-Gofa	Cool humid mid highlands (SH2)	3	0.685
133	B157	217003	6.53	37.75	Gamo-Gofa	Cool humid mid highlands (SH2)	3	0.568
134	B158	217004	6.53	37.75	Gamo-Gofa	Cool humid mid highlands (SH2)	3	0.583
135	B159	217008	8.12	39.58	Arsi-Bale	Cool humid mid highlands (H4)	3	0.812
136	B160.1	217009	8.12	39.58	Arsi-Bale	Cool humid mid highlands (H4)	1	0.997
137	B160.2	217009	8.12	39.58	Arsi-Bale	Cool humid mid highlands (H4)	1	0.775

138	B161	217010	12.63	37.1	Gonder	Tepid moist mid highlands (M3)	3	0.846
139	B163	217016	11.72	38.47	Welo	Cool moist mid highlands (M4)	2	0.687
140	B164	217017	11.73	38.42	Welo	Cool moist mid highlands (M4)	2	0.635
141	B165	217018	11.73	38.42	Welo	Cool moist mid highlands (M4)	2	0.396
142	B168	217173	7.55	36.6	Jimma	Tepid moist mid highlands (H3)	3	0.533
143	B169	217174	7.55	36.6	Jimma	Tepid moist mid highlands (H3)	3	0.826
144	B170	217176	7.17	36.35	Jimma	Tepid moist mid highlands (H3)	2	0.831
145	B173	223958	7.55	39.37	Arsi-Bale	Cool humid mid highlands (H4)	1	0.448
146	B174	223959	7.55	39.37	Arsi-Bale	Cool humid mid highlands (H4)	3	0.505
147	B175	223960	7.55	39.37	Arsi-Bale	Cool humid mid highlands (H4)	3	0.82
148	B176	224912	9.32	39.65	Shewa	Tepid sub-moist mid highlands (SM3)	2	0.66
149	B178	224914	9.32	39.52	Shewa	Cool sub-moist mid highlands (SM4)	2	0.528
150	B179	224915	9.3	39.53	Shewa	Cool sub-moist mid highlands (SM4)	2	0.593
151	B181	224917	9.3	39.53	Shewa	Cool sub-moist mid highlands (SM4)	1	0.975
152	B182	224918	9.3	39.53	Shewa	Cool sub-moist mid highlands (SM4)	2	0.828
153	B183.1	224919	11	39.55	Welo	Cool moist mid highlands (M4)	2	0.665
154	B183.2	224919	11	39.55	Welo	Cool moist mid highlands (M4)	2	0.678
155	B184	224920	11	39.55	Welo	Cool moist mid highlands (M4)	1	0.994
156	B185	224922	10.95	39.55	Welo	Cool moist mid highlands (M4)	1	0.998
157	B186	224923	10.95	39.55	Welo	Cool moist mid highlands (M4)	1	0.998
158	B187	224924	10.9	39.52	Welo	Cold moist sub-afro-alpine to afro-alpine (M5)	1	0.994
159	B188	224925	10.9	39.52	Welo	Cold moist sub-afro-alpine to afro-alpine (M5)	1	0.739
160	B189	224926	10.92	39.55	Welo	Cool moist mid highlands (M4)	2	0.605
161	B190	224927	10.92	39.55	Welo	Cool moist mid highlands (M4)	3	0.656
162	B191.1	224928	10.92	39.55	Welo	Cool moist mid highlands (M4)	1	0.998
163	B192	224929	10.92	39.55	Welo	Cool moist mid highlands (M4)	1	0.879
164	B193	224930	10.9	39.52	Welo	Cold moist sub-afro-alpine to afro-alpine (M5)	1	0.702

165	B194	224931	10.9	39.52	Welo	Cold moist sub-afro-alpine to afro-alpine (M5)	1	0.834
166	B196	224933	10.87	39.43	Welo	Cool moist mid highlands (M4)	1	0.995
167	B198	224935	10.87	39.43	Welo	Cool moist mid highlands (M4)	1	0.724
168	B199	224948	11.8	39.37	Welo	Cool sub-moist mid highlands (SM4)	1	0.998
169	B200	224949	11.8	39.37	Welo	Cool sub-moist mid highlands (SM4)	1	0.698
170	B201	224950	11.8	39.37	Welo	Cool sub-moist mid highlands (SM4)	1	0.998
171	B202	224954	9.83	39.75	Shewa	Cool moist mid highlands (M4)	1	0.731
172	B203	224957	9.83	39.75	Shewa	Cool moist mid highlands (M4)	2	0.557
173	B204	224958	9.9	39.73	Shewa	Cool moist mid highlands (M4)	1	0.701
174	B205	224959	9.9	39.73	Shewa	Cool moist mid highlands (M4)	2	0.527
175	B206	224960	9.9	39.73	Shewa	Cool moist mid highlands (M4)	3	0.805
176	B207	224961	9.9	39.73	Shewa	Cool moist mid highlands (M4)	2	0.609
177	B209	224963	9.9	39.73	Shewa	Cool moist mid highlands (M4)	3	0.565
178	B211	224965	9.9	39.73	Shewa	Cool moist mid highlands (M4)	3	0.987
179	B213	224967	9.9	39.73	Shewa	Cool moist mid highlands (M4)	1	0.989
180	B216.1	224970	9.9	39.73	Shewa	Cool moist mid highlands (M4)	1	0.996
181	B218	225175	6.95	37.85	Gamo-Gofa	Tepid sub-humid mid highlands (SH3)	3	0.747
182	B220	225177	6.95	37.85	Gamo-Gofa	Tepid sub-humid mid highlands (SH3)	3	0.463
183	B222	225179	6.95	37.85	Gamo-Gofa	Tepid sub-humid mid highlands (SH3)	2	0.696
184	B225	225183	6.95	37.85	Gamo-Gofa	Tepid sub-humid mid highlands (SH3)	1	0.998
185	B227	225240	11.33	39.67	Welo	Tepid moist mid highlands (M3)	2	0.664
186	B227.1	225240	11.33	39.67	Welo	Tepid moist mid highlands (M3)	3	0.978
187	B228	225241	11.33	39.67	Welo	Tepid moist mid highlands (M3)	3	0.617
188	B229	225242	11.33	39.67	Welo	Tepid moist mid highlands (M3)	3	0.606
189	B230	225243	11.33	39.67	Welo	Tepid moist mid highlands (M3)	1	0.919
190	B232	225247	11.33	39.82	Welo	Tepid moist mid highlands (M3)	1	0.92
191	B233	225248	11.37	39.85	Welo	Tepid moist mid highlands (M3)	1	0.989
192	B234	225249	11.37	39.85	Welo	Tepid moist mid highlands (M3)	1	0.731
193	B235	225265	10.27	37.43	Gojam	Tepid moist mid highlands (M3)	3	0.798

194	B237	232350	9.62	42.4	Hararghe	Warm arid lowland plains (A2)	3	0.64
195	B238	232351	9.62	42.4	Hararghe	Warm arid lowland plains (A2)	3	0.875
196	B240	232355	9.55	42.38	Hararghe	Warm arid lowland plains (A2)	2	0.511
197	B242	232358	9.53	42.27	Hararghe	Tepid moist mid highlands (M3)	1	0.637
198	B243	232359	9.53	42.27	Hararghe	Tepid moist mid highlands (M3)	1	0.821
199	B244	232360	9.53	42.27	Hararghe	Tepid moist mid highlands (M3)	1	0.538
200	B245	232362	9.25	41.77	Hararghe	Tepid sub-humid mid highlands (SH3)	1	0.863
201	B247.2	232364	9.25	41.77	Hararghe	Tepid sub-humid mid highlands (SH3)	1	0.864
202	B248	232369	9.17	41.75	Hararghe	Tepid sub-humid mid highlands (SH3)	3	0.77
203	B249	232372	9.37	41.78	Hararghe	Tepid sub-humid mid highlands (SH3)	3	0.604
204	B251	232374	9.43	41.7	Hararghe	Tepid sub-humid mid highlands (SH3)	3	0.864
205	B252	232375	9.43	41.7	Hararghe	Tepid sub-humid mid highlands (SH3)	3	0.785
206	B253	232376	9.43	41.7	Hararghe	Tepid sub-humid mid highlands (SH3)	3	0.762
207	B254	232384	9.35	41.68	Hararghe	Tepid sub-humid mid highlands (SH3)	3	0.725
208	B255	232385	9.33	41.68	Hararghe	Tepid sub-humid mid highlands (SH3)	3	0.728
209	B257	232387	9.43	41.7	Hararghe	Tepid sub-humid mid highlands (SH3)	1	0.83
210	B258	232388	9.43	41.7	Hararghe	Tepid sub-humid mid highlands (SH3)	1	0.623
211	B259	232389	9.43	41.7	Hararghe	Tepid sub-humid mid highlands (SH3)	3	0.884
212	B260	232390	9.42	41.68	Hararghe	Tepid sub-humid mid highlands (SH3)	3	0.624
213	B263	232393	9.42	41.68	Hararghe	Tepid sub-humid mid highlands (SH3)	3	0.746
214	B264	232394	9.42	41.68	Hararghe	Tepid sub-humid mid highlands (SH3)	2	0.783
215	B265	232395	9.42	41.68	Hararghe	Tepid sub-humid mid highlands (SH3)	1	0.742
216	B266	232396	9.43	41.6	Hararghe	Tepid sub-humid mid highlands (SH3)	2	0.998
217	B268	232398	9.43	41.6	Hararghe	Tepid sub-humid mid highlands (SH3)	3	0.775
218	B269	232399	9.43	41.6	Hararghe	Tepid sub-humid mid highlands (SH3)	1	0.793
219	B271	232401	9.43	41.6	Hararghe	Tepid sub-humid mid highlands (SH3)	1	0.996
220	B272	232402	9.43	41.6	Hararghe	Tepid sub-humid mid highlands (SH3)	1	0.76
221	B273	232403	9.43	41.6	Hararghe	Tepid sub-humid mid highlands (SH3)	1	0.773
222	B275	232405	9.4	41.53	Hararghe	Tepid sub-humid mid highlands (SH3)	3	0.479

223	B276.2	232406	9.35	41.6	Hararghe	Tepid sub-humid mid highlands (SH3)	2	0.804
224	B278	233023	6.25	37.55	Gamo-Gofa	Cool sub-humid mid highlands (SH4)	3	0.628
225	B279	233024	6.25	37.55	Gamo-Gofa	Cool sub-humid mid highlands (SH4)	3	0.931
226	B282	233027	6.23	37.52	Gamo-Gofa	Cool sub-humid mid highlands (SH4)	1	0.781
227	B283.1	233028	5.92	37.33	Gamo-Gofa	Tepid sub-humid mid highlands (SH3)	3	0.518
228	B283.2	233028	5.92	37.33	Gamo-Gofa	Tepid sub-humid mid highlands (SH3)	3	0.703
229	B284	233029	5.92	37.33	Gamo-Gofa	Tepid sub-humid mid highlands (SH3)	3	0.606
230	B286	233033	5.97	37.28	Gamo-Gofa	Tepid sub-humid mid highlands (SH3)	3	0.844
231	B287.1	233034	6	37.27	Gamo-Gofa	Tepid sub-humid mid highlands (SH3)	3	0.951
232	B287.2	233034	6	37.27	Gamo-Gofa	Tepid sub-humid mid highlands (SH3)	3	0.986
233	B288	233035	6	37.27	Gamo-Gofa	Tepid sub-humid mid highlands (SH3)	3	0.989
234	B290	233037	6.03	37.28	Gamo-Gofa	Tepid sub-humid mid highlands (SH3)	3	0.887
235	B292	233039	6.08	37.25	Gamo-Gofa	Tepid sub-humid mid highlands (SH3)	3	0.952
236	B293	233040	6.1	37.23	Gamo-Gofa	Tepid sub-humid mid highlands (SH3)	3	0.565
237	B295	233042	6.1	37.23	Gamo-Gofa	Tepid sub-humid mid highlands (SH3)	3	0.568
238	B296	233047	6.3	37.77	Gamo-Gofa	Cool humid mid highlands (SH2)	3	0.552
239	B299	233050	5.7	37.63	Gamo-Gofa	Warm moist lowlands (M2)	3	0.551
240	FTCG-2	FTCG-2	-	-	HARC	Holeta national barley research program (HARC)	1	0.54
241	FTCG-3	FTCG-3	-	-	HARC	Holeta national barley research program (HARC)	2	0.998
242	FTCG-4	FTCG-4	-	-	HARC	Holeta national barley research program (HARC)	1	0.848
243	FTCG-5	FTCG-5	-	-	HARC	Holeta national barley research program (HARC)	1	0.719
244	FTCG-6	FTCG-6	-	-	HARC	Holeta national barley research program (HARC)	1	0.724
245	FTCG-7	FTCG-7	-	-	HARC	Holeta national barley research program (HARC)	3	0.602
246	FTCG-8	FTCG-8	-	-	HARC	Holeta national barley research program (HARC)	1	0.609
247	FTCG-9	FTCG-9	-	-	HARC	Holeta national barley research program (HARC)	1	0.453

248	FTCG-10	FTCG-10	-	-	HARC	Holeta national barley research program (HARC)	2	0.547
249	FTCG-11	FTCG-11	-	-	HARC	Holeta national barley research program (HARC)	3	0.611
250	FTCG-12	FTCG-12	-	-	HARC	Holeta national barley research program (HARC)	2	0.546
251	FTCG-13	FTCG-13	-	-	HARC	Holeta national barley research program (HARC)	3	0.75
252	FTCG-14	FTCG-14	-	-	HARC	Holeta national barley research program (HARC)	2	0.997
253	FTCG-16	FTCG-16	-	-	HARC	Holeta national barley research program (HARC)	2	0.947
254	FTCG-17	FTCG-17	-	-	HARC	Holeta national barley research program (HARC)	3	0.596
255	FTCG-20	FTCG-20	-	-	HARC	Holeta national barley research program (HARC)	3	0.573
256	FTCG-21	FTCG-21	-	-	HARC	Holeta national barley research program (HARC)	3	0.533
257	FTCG-22	FTCG-22	-	-	HARC	Holeta national barley research program (HARC)	3	0.566
258	FTCG-23	FTCG-23	-	-	HARC	Holeta national barley research program (HARC)	3	0.602
259	FTCG-24	FTCG-24	-	-	HARC	Holeta national barley research program (HARC)	2	0.82
260	FTCG-25	FTCG-25	-	-	HARC	Holeta national barley research program (HARC)	1	0.849

Table S2. First-generation migrants from genetically distinct clusters

Genetically distinct		**Total migrants**		**Total number of migrants likely originated from genetically distinct clusters**		
Clusters	**Num. of members**	**Num.**	**Perc.**	**1**	**2**	**3**
1	**80**	3	3.75	.	2	1
2	**71**	2	2.82	1	.	1
3	**109**	0	0	0	0	.
Total	**260**	**5**	**1.92**	**1**	**2**	**2**

Remark: Num. = Number; Perc. = Percentage from total number of members

Table S3. Pairwise correlation matrix for genetic differentiation (PhiPT).

Subpopulation	**1**	**2**	**3**
1		0.13	0.10

2			0.11
3			

Table S4. Molecular variance (AMOVA) for the Ethiopian barley accessions based on the 14 defined agro-ecological zones; genetic differentiation (PhiPT) of the total population.

Source	Degree of freedom	Sum of square	Mean square	Estimated variance	Percentage of variation	PhiPT
Among Populations	13	5,607.4	431.3	7.7	3%	0.03
Within Populations	246	74,112.1	301.3	301.3	97%	
Total	**259**	**79,719.5**		**309.0**	**100%**	

Table S5. Raw 'hapmap' data file containing the 983 informative SNPs for the Ethiopian barley landraces used in the study.

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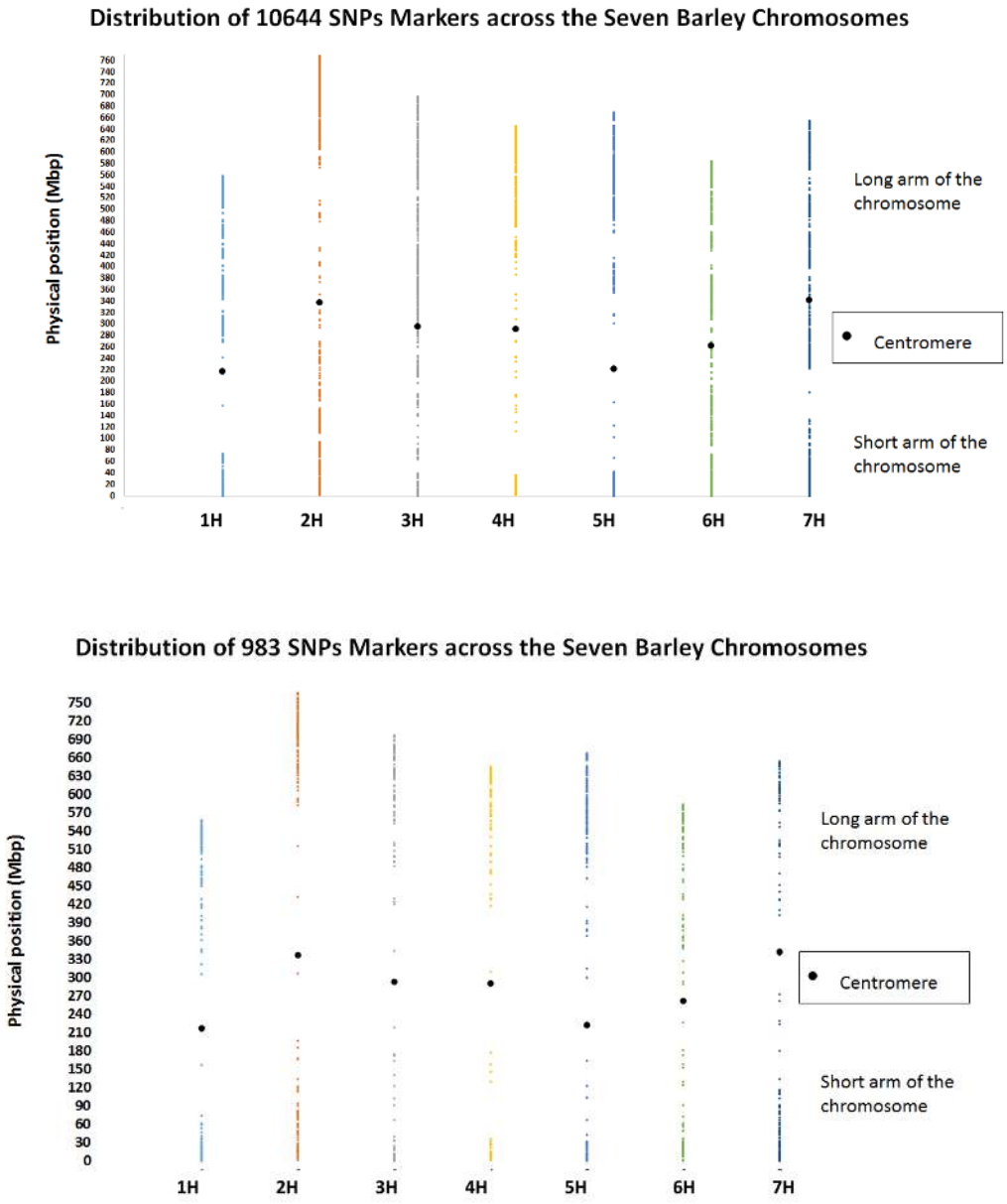

Figure S1. Physical map distribution of SNP markers across the seven barley Chromosomes.

Publication 2.2) Genome wide association study of Ethiopian barley for terminal drought stress tolerance under field and climate chamber conditions

Table S1: Climate and soil characteristics of the experimental locations in Ethiopia

Characteristics	**Control treatment locations**		**Drought stress treatment locations**	
	Holetta	**Debrezeit**	**Melkassa**	**Dera**
Geographic position	8°10′N, 38°30′E	8°44′N, 38° 58′E	8°20′N, 39° 19′E	8°24′N, 39° 21′E
Altitude (m.a.s.l)	2400	1900	1550	1620
Maximum Temperature (ºC)*	21.0	24.9	27.0	26.0
Minimum Temperature (ºC)*	10.0	12.1	15.5	11.0
Rainfall (mm)*	810	617	491	367
Soil type	Nitosol	Vertisol	Hypo calcic andosol/ fulvisols	Calcic fulvic regosol
Soil texture	Clay	Clay	Clay loam to clay	Clay loam
Soil pH	5.48-5.90	6.23-7.14	7.22-7.55	7.04-8.10
Organic carbon (%)	1.18-2.56	0.73-1.52	1.03-1.82	1.27-2.29
Organic matter (%)	2.03-4.41	1.26-2.63	1.78-3.14	2.08-3.95
Cation exchange capacity (cmol/100g)	19.11-33.18	35.19-48.15	21.63-32.28	27.30-37.17
Exchangeable sodium (%)	0.01-0.46	0.01-0.48	0.02-1.47	0.02-0.57
Bulk density (g/cm^3)	1.12-1.37	1.15-1.45	1.1-1.34	1.29-1.34

*During major cropping season from June-September for Holetta; from July to September for others

Table S2: Lists of top 50 accessions based on drought susceptible index (DSI), and harmonic mean index (HM) values of climate chamber and field experiments respectively.

Rank	**Acc**	**CSGB**	**CCnGB**	**CDSI**	**Rank**	**Acc**	**FSGB**	**FCGB**	**FHM**
1	B145.1	0.11	0.03	-3.92	1	FTCG-17	75.94	55.58	85.08
2	B191.1	1.00	0.26	-3.47	2	B185	58.76	58.10	80.99
3	B56.2	0.23	0.15	-0.68	3	B137	43.72	44.52	77.02
4	B5.1	1.64	1.19	-0.47	4	B225	45.21	46.51	75.92
5	B205	0.57	0.60	0.07	5	B191.1	51.52	56.39	74.76
6	B202	2.06	2.30	0.13	6	B115.1	58.74	64.88	73.86
7	B242	2.10	2.42	0.16	7	FTCG-2	70.89	79.08	73.80
8	B213	0.61	0.70	0.17	8	B259	51.11	58.07	73.46
9	B6	1.52	1.84	0.22	9	B7	66.67	76.12	72.84
10	B160.2	2.34	3.15	0.32	10	B187	55.82	66.01	72.75
11	B112.2	0.41	0.56	0.33	11	B276.2	48.31	57.89	72.31
12	B143	0.30	0.42	0.34	12	B186	54.79	65.89	72.14
13	FTCG-20	1.10	1.61	0.39	13	B38	36.12	43.56	72.01
14	B25	0.72	1.06	0.40	14	B268	52.29	63.79	71.97
15	B233	0.95	1.48	0.44	15	B50	66.63	81.85	71.50
16	B141	1.70	2.94	0.52	16	B181	56.70	69.83	71.49
17	B48	1.13	2.06	0.56	17	B154	53.18	66.45	71.32
18	B138	0.90	1.68	0.57	18	B52	32.94	41.38	71.32
19	B234	1.00	1.94	0.60	19	B79	57.82	72.64	71.08
20	B157	1.01	2.05	0.62	20	B128	55.29	70.39	70.31

21	B34	0.66	1.36	0.63	21	B34	41.11	52.51	69.25
22	B7	1.30	2.70	0.64	22	B76	55.56	71.87	68.89
23	B10.2	0.64	1.34	0.64	23	B174	47.96	62.18	68.08
24	FTCG-16	1.39	2.93	0.65	24	B67	39.98	51.84	67.84
25	B248	1.43	3.21	0.68	25	B83	75.13	98.08	67.83
26	B136	1.36	3.05	0.68	26	B39	63.09	82.45	67.75
27	B185	1.34	3.21	0.72	27	B77	44.47	58.46	67.69
28	B69	1.40	3.45	0.73	28	B144	52.97	69.67	67.64
29	B27	1.42	3.67	0.75	29	B279	64.79	85.88	67.55
30	B183.1	0.77	1.99	0.75	30	B237	42.68	56.71	67.27
31	FTCG-5	1.61	4.38	0.77	31	B253	54.17	72.44	66.74
32	B272	1.38	3.81	0.78	32	B114.2	60.51	80.96	66.29
33	B299	0.69	1.91	0.78	33	B65	47.51	63.81	66.04
34	B151	0.76	2.11	0.78	34	B9.2	47.90	64.35	65.98
35	B278	0.76	2.12	0.79	35	B222	51.72	69.49	65.96
36	B68	1.92	5.35	0.79	36	B173	47.11	63.63	65.83
37	B240	1.28	3.58	0.79	37	B33.2	35.57	48.98	65.64
38	B66	1.08	3.02	0.79	38	B20	42.00	57.87	65.54
39	B160.1	0.11	0.30	0.79	39	B42.1	40.39	55.77	65.34
40	B260	1.51	4.38	0.80	40	B175	48.84	67.51	65.09
41	B159	0.54	1.57	0.81	41	B123	50.11	70.00	64.78
42	B108	0.54	1.59	0.81	42	B249	36.60	51.53	64.43
43	FTCG-2	0.46	1.36	0.82	43	FTCG-13	44.22	62.45	64.41
44	FTCG-22	1.94	5.99	0.83	44	B255	31.09	43.99	64.39
45	B287.2	1.37	4.34	0.84	45	B260	56.46	80.26	64.19
46	FTCG-13	1.46	4.65	0.84	46	B32.2	57.73	82.22	64.12
47	B232	0.82	2.61	0.84	47	FTCG-23	45.61	65.04	63.99
48	B282	2.24	7.16	0.84	48	B118	56.01	80.22	63.92
49	B275	0.37	1.18	0.84	49	FTCG-7	32.64	46.86	63.10
50	B165	0.54	1.76	0.85	50	B184	46.57	67.35	63.10

Remark: Acc = names of accessions; CSGB and CCnGB = grain biomass of climate chamber drought and control treatments, respectively; FSGB and FCGB = grain biomass of field drought and control treatments, respectively. Cells with blue shading colors are overlapping accessions in the top 50.

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Table S3: Lists of significant MTAs obtained based on BLINK-GWAS analysis for 260 and 196 Ethiopia barley landrace accession in field and climate chamber experiments respectively, using 10,644 SNP markers; Listed are MTAs, candidate gene identifiers and annotations.

SNP	Chromo-some	Position	Posi-tion (Mb)	P.value	maf	Nobs	FDR Adjusted P-values	Effect	Phenotype variance explained (%)	LOD	Trait	Treatment	Experi-ment	Remark	QTL	Gene ID	Gene Ontologies	Annotation description
JHI-Hv50k-2016-16677	1H	27684392	27.68	9.33E-07	0.39	260	0.004968	-0.59	0.86	6.03	DM	Control	Field	Linked with "FSPH_QTL1"	FCDM1			
JHI-Hv50k-2016-16885	1H	28893999	28.89	2.96E-07	0.41	260	0.001578	0.97	1.31	6.53	PH	Drought	Field	Linked with "FCDM_QTL1"	FSPH1	HORVU1Hr1G011860	GO:0005524 GO:0006468 GO:0030247 GO:0004672 GO:0005509 GO:0005515	receptor-like protein kinase 1
JHI-Hv50k-2016-18027	1H	36450777	36.45	3.14E-08	0.40	260	0.000335	0.90	1.78	7.50	SPAD	Control	Field		FCSPAD1	HORVU1Hr1G013600	GO:0005515 GO:0008270 GO:0004357 GO:0006750	E3 SUMO-protein ligase SIZ1
BOPA2_12_10314	1H	46538882	46.54	5.27E-06	0.48	260	0.018698	-1.05	1.89	5.28	PH	Control	Field		FCPH1	HORVU1Hr1G015590	GO:0042398	Glutamate--cysteine ligase B, chloroplastic
JHI-Hv50k-2016-19711	1H	72458002	72.46	1.07E-09	0.36	260	0.020866	0.01	2.34	8.97	HI	Drought	Field	Linked with "FSSPAD_QTL2"	FSHI1	HORVU1Hr1G019320	GO:0005515 GO:0008270	ankyrin repeat-containing protein 2
JHI-Hv50k-2016-19711	1H	72458002	72.46	3.92E-06	0.36	260	1.14E-05	-0.70	1.25	5.41	SPAD	Drought	Field	Linked with "FSHI_QTL1"	FSSPAD1	HORVU1Hr1G019320	GO:0005515 GO:0008270	ankyrin repeat-containing protein 2
JHI-Hv50k-2016-22927	1H	311101155	311.10	2.47E-07	0.13	196	0.002629	3.93	13.12	6.61	PH	Drought	Climate		CSPH1	HORVU1Hr1G042920	GO:0000398 GO:0005681 GO:0015098 GO:0015689	Thioredoxin-like protein 4A
BOPA2_12_31134	1H	357285032	357.29	1.53E-06	0.23	196	0.005428	-3.90	2.23	5.81	TKW	Drought	Climate		CSTKW1	HORVU1Hr1G048360	GO:0016021	Major facilitator superfamily protein
JHI-Hv50k-2016-26918	1H	369364947	369.36	8.18E-07	0.10	189	0.004353	6.53	26.30	6.09	DM	Control	Climate		CCnDM1	HORVU1Hr1G049900	GO:0005515	Leucine-rich repeat family protein
JHI-Hv50k-2016-31649	1H	427687621	427.69	3.24E-07	0.09	196	0.003449	0.06	13.65	6.49	HI	Control	Climate	Linked with "CCGB_QTL1, CCTKW_QTL1"	CCnHI1	HORVU1Hr1G058590		RNA recognition motif-containing protein
JHI-Hv50k-2016-31649	1H	427687621	427.69	2.06E-08	0.09	196	0.000219	1.73	6.44	7.69	GB	Control	Climate	Linked with "CCHI_QTL1, CCTKW_QTL1"	CCnGB1	HORVU1Hr1G058590		RNA recognition motif-containing protein
JHI-Hv50k-2016-31649	1H	427687621	427.69	1.57E-05	0.09	196	0.033422	4.03	2.57	4.80	TKW	Control	Climate	Linked with "CCGB_QTL1, CCHI_QTL1"	CCnTKW1	HORVU1Hr1G058590		RNA recognition motif-containing protein
JHI-Hv50k-2016-54103	1H	547727341	547.73	2.66E-08	0.45	260	0.000283	-0.84	2.33	7.57	DM	Drought	Field		FSDM1	HORVU1Hr1G091210		N-terminal protein myristoylation
JHI-Hv50k-2016-57491	1H	556673062	556.67	1.11E-07	0.40	189	0.000591	4.70	4.50	6.95	DM	Drought	Climate	Linked with "CSNSdPS1"	CSDM1	HORVU1Hr1G094870	GO:0005488 GO:0005515 GO:0005524	TATA-binding protein-associated factor 172
JHI-Hv50k-2016-58119	1H	557950775	557.95	6.79E-09	0.10	196	2.41E-05	3.20	5.64	8.17	NSdPS	Drought	Climate	Linked with "CSDM1"	CSNSdPS1	HORVU1Hr1G095230		structural maintenance of chromosomes 5
SCRI_RS_173604	2H	13265856	13.27	7.43E-06	0.38	196	0.010644	-0.02	0.00	5.13	HI	Drought	Climate	Linked with "CCTKW2"	CSHI1	HORVU2Hr1G006330		Peptidase S24, S26A, S26B, S26C family protein
JHI-Hv50k-2016-67587	2H	15455506	15.46	3.47E-08	0.04	196	0.000369	7.67	5.42	7.46	TKW	Control	Climate	Linked with "CSHI1"	CCnTKW2	HORVU2Hr1G007580		unknown function
JHI-Hv50k-2016-72079	2H	25148431	25.15	5.07E-08	0.27	260	0.000539	-1.00	5.21	7.30	DM	Control	Field		FCDM2	HORVU2Hr1G011870	GO:0016020 GO:0055085 GO:0005215 GO:0006810 GO:0006811	Cation-chloride cotransporter 2
JHI-Hv50k-2016-73780	2H	29845026	29.85	6.29E-08	0.15	189	0.00067	6.74	18.66	7.20	DM	Control	Climate	Linked with "CSGB1, CSHI2, FCTKW1, CSNSdPS2"	CCnDM2	HORVU2Hr1G013790		Symplekin

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JHI-Hv50k-2016-73780	2H	29845026	29.85	2.57E-19	0.15	196	2.74E-15	-0.06	10.01	18.59	HI	Drought	Climate	Linked with "CCDM2, CSGB1, FCTKW1, CSNSdPS2"	CSHI2	HORVU2Hr1G013790		Symplekin
JHI-Hv50k-2016-73780	2H	29845026	29.85	1.11E-11	0.15	196	1.18E-07	-0.34	8.32	10.95	GB	Drought	Climate	Linked with "CCDM2, CSHI2, FCTKW1, CSNSdPS2"	CSGB1	HORVU2Hr1G013790		Symplekin
JHI-Hv50k-2016-73780	2H	29845026	29.85	2.00E-06	0.13	260	0.007085	1.59	3.19	5.70	TKW	Control	Field	Linked with "CCDM2, CSGB1, CSHI2, CSNSdPS2"	FCTKW1	HORVU2Hr1G013790		Symplekin
JHI-Hv50k-2016-73929	2H	30188357	30.19	1.09E-09	0.13	196	1.16E-05	-2.84	7.08	8.96	NSdPS	Drought	Climate	Linked with "CCDM2, CSGB1, CSHI2, FCTKW1"	CSNSdPS2			
SCRI_RS_110647	2H	38251144	38.25	3.73E-07	0.18	196	0.001323	-3.21	2.22	6.43	TKW	Control	Climate		CCnTKW3	HORVU2Hr1G016580	GO:0043565 GO:0003700 GO:0006351 GO:0006355	transcription factor-related
JHI-Hv50k-2016-77166	2H	45565581	45.57	2.59E-07	0.12	196	0.002757	5.40	29.40	6.59	PH	Control	Climate	Linked with "CSHI3"	CCnPH1	HORVU2Hr1G017880, HORVU2Hr1G017900	GO:0003824 GO:0004553 GO:0005975	alpha-galactosidase 2
SCRI_RS_176159	2H	48450222	48.45	1.50E-05	0.36	196	0.010644	-0.02	0.00	4.82	HI	Drought	Climate	Linked with "CCPH1"	CSHI3			
SCRI_RS_152206	2H	68787661	68.79	3.03E-06	0.38	260	0.010748	0.92	1.72	5.52	PH	Drought	Field		FSPH2	HORVU2Hr1G023170	GO:0019430 GO:0045454 GO:0055114 GO:0004791 GO:0005737 GO:0016491	Thioredoxin reductase
JHI-Hv50k-2016-82131	2H	79850092	79.85	2.98E-10	0.03	260	1.59E-06	5.94	18.21	9.53	TKW	Drought	Field		FSTKW1	HORVU2Hr1G025570	GO:0003779	Stomatal closure-related actin-binding protein 1 unknown protein; Has 1524 Blast hits to 1298 proteins in 225 species: Archae - 9; Bacteria - 84; Metazoa - 474; Fungi - 184; Plants - 98; Viruses - 17; Other Eukaryotes - 658, Haloacid dehalogenase-like hydrolase (HAD) superfamily protein (source: NCBI BLink).
JHI-Hv50k-2016-83709	2H	92206305	92.21	5.31E-07	0.47	260	0.002827	0.67	0.91	6.27	NSdPS	Drought	Field		FSNSdPS1	HORVU2Hr1G027670, HORVU2Hr1G027680	GO:0008152 GO:0016787	
JHI-Hv50k-2016-87462	2H	120872846	120.87	1.64E-05	0.03	196	0.04364	0.41	4.30	4.78	GB	Drought	Climate		CSGB2	HORVU2Hr1G031690	GO:0005488	Exportin-T
JHI-Hv50k-2016-93122	2H	432380334	432.38	1.52E-07	0.48	260	0.000811	-1.26	1.22	6.82	NSdPS	Control	Field		FCNSdPS1	HORVU2Hr1G063820		unknown function
JHI-Hv50k-2016-102161	2H	620316536	620.32	1.67E-06	0.15	260	0.007085	-1.56	1.94	5.78	TKW	Control	Field		FCTKW2	HORVU2Hr1G085840		undescribed protein
JHI-Hv50k-2016-108079	2H	654165641	654.17	9.18E-11	0.20	260	9.77E-07	-2.24	3.75	10.04	TKW	Control	Field	Linked with "FCNSdPS2, FSTKW2"	FCTKW3	HORVU2Hr1G092600	GO:0003677 GO:0043565	multiprotein bridging factor 1A
JHI-Hv50k-2016-108079	2H	654165641	654.17	2.14E-06	0.20	260	0.005692	1.62	1.90	5.67	NSdPS	Control	Field	Linked with "FCTKW3, FSTKW2"	FCNSdPS2	HORVU2Hr1G092600	GO:0003677 GO:0043565	multiprotein bridging factor 1A
JHI-Hv50k-2016-108079	2H	654165641	654.17	8.45E-09	0.20	260	3E-05	-2.21	1.73	8.07	TKW	Drought	Field	Linked with "FCTKW3, FCNSdPS2"	FSTKW2	HORVU2Hr1G092600	GO:0003677 GO:0043565	multiprotein bridging factor 1A
JHI-Hv50k-2016-110148	2H	674256816	674.26	4.72E-06	0.06	196	0.01256	4.33	5.03	5.33	TKW	Control	Climate	Linked with "FSTKW3"	CCnTKW4	HORVU2Hr1G096380	GO:0009081 GO:0003824 GO:0004084 GO:0008152	Branched-chain-amino-acid aminotransferase 6

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SCRI_RS_115690	2H	676775793	676.78	8.42E-16	0.04	260	8.96E-12	8.53	27.28	15.07	TKW	Drought	Field	Linked with "CCTKW4"	FSTKW3	HORVU2Hr1G096760		Acyl-CoA thioesterase family protein
SCRI_RS_139831	2H	702542026	702.54	2.09E-06	0.28	196	0.007415	-2.87	5.58	5.68	PH	Drought	Climate		CSPH2	HORVU2Hr1G104280	GO:0000105 GO:0004424	imidazoleglycerol-phosphate dehydratase
JHI-Hv50k-2016-122478	2H	711750288	711.75	8.12E-06	0.36	196	0.010644	-0.02	0.00	5.09	HI	Drought	Climate	Linked with marker (711.75-712.33Mb)		HORVU2Hr1G107380, CAJ32531.1	GO:0003676 GO:0003677 GO:0046983	Zinc finger BED domain-containing protein RICESLEEPER 1, transposase-related protein b-
BOPA1_1381-547	2H	711786092	711.79	9.10E-06	0.39	196	0.010644	-0.02	0.00	5.04	HI	Drought	Climate	Linked with marker (711.75-712.33Mb)		HORVU2Hr1G107460	GO:0006869 GO:0008289 GO:0055114	Non-specific lipid-transfer protein 3
BOPA1_3256-1196	2H	711931409	711.93	1.50E-05	0.36	196	0.010644	-0.02	0.16	4.82	HI	Drought	Climate	Linked with marker (711.75-712.33Mb)		HORVU2Hr1G107520	GO:0008152 GO:0016491 GO:0016620 GO:0016620 GO:0055114	Aldehyde dehydrogenase family 9 member A1-B
JHI-Hv50k-2016-122549	2H	712178173	712.18	6.71E-06	0.38	196	0.010644	-0.02	0.00	5.17	HI	Drought	Climate	Linked with marker (711.75-712.33Mb)	CSHI4	HORVU2Hr1G107550	GO:0004491 GO:0008152 GO:0016491	Aldehyde dehydrogenase family 9 member A1-B
JHI-Hv50k-2016-122550	2H	712178275	712.18	1.50E-05	0.36	196	0.010644	-0.02	0.06	4.82	HI	Drought	Climate	Linked with marker (711.75-712.33Mb)			GO:0018024 GO:0034968 GO:0005515	
JHI-Hv50k-2016-122563	2H	712194673	712.19	1.50E-05	0.36	196	0.010644	-0.02	0.00	4.82	HI	Drought	Climate	Linked with marker (711.75-712.33Mb)		HORVU2Hr1G107560	GO:0005634 GO:0008270	Histone-lysine N-methyltransferase 2A
JHI-Hv50k-2016-122570	2H	712195435	712.20	7.35E-06	0.39	196	0.010644	-0.02	0.00	5.13	HI	Drought	Climate	Linked with marker (711.75-712.33Mb)		HORVU2Hr1G107570		undescribed protein
JHI-Hv50k-2016-122609	2H	712205508	712.21	1.11E-05	0.39	196	0.010644	-0.02	0.00	4.95	HI	Drought	Climate	Linked with marker (711.75-712.33Mb)		HORVU2Hr1G107580		Amino-terminal region of chorein, A TM vesicle-mediated sorter
JHI-Hv50k-2016-122699	2H	712330524	712.33	7.35E-06	0.39	196	0.010644	-0.02	0.00	5.13	HI	Drought	Climate	Linked with marker (711.75-712.33Mb)		HORVU2Hr1G107660		Sugar transport protein 5
JHI-Hv50k-2016-134459	2H	739812941	739.81	1.57E-05	0.03	260	0.033512	2.84	13.79	4.80	TKW	Control	Field		FCTKW4	HORVU2Hr1G117200		unknown function
SCRI_RS_156075	2H	764045565	764.05	3.66E-06	0.37	196	0.010644	-0.02	0.39	5.44	HI	Drought	Climate	Linked with "FSHI2, FSNSdPS2"	CSHI5	HORVU2Hr1G126640		translocon at the outer membrane of chloroplasts 64-V
SCRI_RS_150519	2H	766082237	766.08	9.87E-08	0.07	260	0.000526	-0.02	6.39	7.01	HI	Drought	Field	Linked with "CSHI5, FSNSdPS2"	FSHI2	HORVU2Hr1G127190	GO:0008270 GO:0043565 GO:0003700	RING, FYVE, PHD zinc finger superfamily protein
JHI-Hv50k-2016-147953	2H	767057612	767.06	2.10E-05	0.06	260	0.044763	1.29	3.22	4.68	NSdPS	Drought	Field	Linked with "FSHI2, CSHI5"	FSNSdPS2	HORVU2Hr1G127370	GO:0006355 GO:0008270	GATA transcription factor 26
JHI-Hv50k-2016-149558	3H	1718034	1.72	1.81E-07	0.46	196	0.000963	-3.30	1.70	6.74	TKW	Drought	Climate		CSTKW2			
SCRI_RS_189167	3H	395203171	395.20	6.61E-09	0.24	196	2.41E-05	-2.23	4.39	8.18	NSdPS	Drought	Climate		CSNSdPS3	HORVU3Hr1G053620		Protein of unknown function (DUF1644)
JHI-Hv50k-2016-183028	3H	487517028	487.52	2.68E-06	0.32	260	0.005702	1.17	2.02	5.57	NSdPS	Control	Field		FCNSdPS3	HORVU3Hr1G063840, HORVU3Hr1G063850		alpha, beta-Hydrolases superfamily protein, undescribed protein
JHI-Hv50k-2016-192692	3H	563059876	563.06	1.90E-06	0.22	196	0.005056	-1.59	2.50	5.72	NSdPS	Drought	Climate		CSNSdPS4	HORVU3Hr1G075340		double-stranded-RNA-binding protein 4
JHI-Hv50k-2016-228946	4H	8818961	8.82	2.33E-10	0.11	196	1.24E-06	0.30	7.88	9.63	GB	Drought	Climate		CSGB3	HORVU4Hr1G003940	GO:0005515	Transducin, WD40 repeat-like superfamily protein
JHI-Hv50k-2016-232915	4H	30942713	30.94	3.17E-07	0.16	196	0.001687	1.73	15.47	6.50	SPAD	Drought	Climate		CSSPAD1	HORVU4Hr1G010390, CAC83720.1		Cysteine proteinases superfamily protein, cathepsin B

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JHI-Hv50k-2016-252004	4H	535419148	535.42	2.28E-06	0.38	260	0.004864	-3.84	0.71	5.64	GB	Control	Field		FCGB1	HORVU4Hr1G063910		Cell cycle checkpoint protein RAD17
SCRI_RS_166159	4H	542784090	542.78	7.62E-09	0.49	260	8.11E-05	1.43	3.42	8.12	PH	Control	Field		FCPH2	HORVU4Hr1G064870	GO:0016020 GO:0005215 GO:0006810 GO:0006857	Protein NRT1, PTR FAMILY 8.3
JHI-Hv50k-2016-260329	4H	596668709	596.67	4.17E-10	0.43	260	4.44E-06	-1.06	2.06	9.38	NSdPS	Drought	Field	Linked with "FCNSdPS4"	FSNSdPS3			
JHI-Hv50k-2016-260339	4H	596914866	596.91	1.81E-08	0.46	260	0.000192	-1.50	1.41	7.74	NSdPS	Control	Field	Linked with "FSNSdPS3"	FCNSdPS4	HORVU4Hr1G075180	GO:0043565 GO:0003677 GO:0003700 GO:0006355	Homeobox-leucine zipper protein family
JHI-Hv50k-2016-279138	5H	6203123	6.20	6.00E-07	0.03	260	0.001596	-10.99	7.58	6.22	GB	Control	Field		FCGB2	HORVU5Hr1G002220		Oxidase
JHI-Hv50k-2016-281261	5H	10350439	10.35	5.68E-06	0.07	196	0.020153	-0.26	3.55	5.25	GB	Drought	Climate	Linked with "FSPH3, FCGB3, FCPH3"	CSGB4	HORVU5Hr1G006220	GO:0005488	ARM repeat superfamily protein
JHI-Hv50k-2016-281531	5H	11766193	11.77	1.71E-10	0.18	260	1.82E-06	6.39	4.44	9.77	GB	Control	Field	Linked with "CSGB4, FSPH3, FCPH3"	FCGB3	HORVU5Hr1G006550	GO:0016021 GO:0055085	Cation, calcium exchanger 1
JHI-Hv50k-2016-281531	5H	11766193	11.77	1.10E-07	0.18	260	0.001171	1.26	2.95	6.96	PH	Drought	Field	Linked with "CSGB4, FCGB3, FCPH3"	FSPH3	HORVU5Hr1G006550	GO:0016021 GO:0055085	Cation, calcium exchanger 1
JHI-Hv50k-2016-281652	5H	12036348	12.04	4.61E-06	0.03	260	0.018698	-2.81	10.93	5.34	PH	Control	Field	Linked with "CSGB4, FSPH3, FCGB3"	FCPH3	HORVU5Hr1G006710, HORVU5Hr1G006720	GO:004353	Disease resistance protein (CC-NBS-LRR class) family, OJ000126_13.6 protein
SCRI_RS_180821	5H	415994200	415.99	4.93E-08	0.21	260	0.000262	5.03	1.61	7.31	GB	Control	Field		FCGB4			
JHI-Hv50k-2016-320912	5H	562554856	562.55	1.35E-07	0.04	196	0.000718	0.05	9.48	6.87	HI	Drought	Climate		CSHI6	HORVU5Hr1G080960		unknown function
JHI-Hv50k-2016-322900	5H	569549449	569.55	4.93E-07	0.24	260	0.001596	4.63	6.31	6.31	GB	Control	Field	Linked with "FCSPAD2, FSDM2"	FCGB5	HORVU5Hr1G083200, HORVU5Hr1G083210		unknown function, Major facilitator superfamily protein
JHI-Hv50k-2016-323226	5H	571195054	571.20	3.89E-06	0.30	260	0.02071	0.80	1.98	5.41	SPAD	Control	Field	Linked with "FCGB5, FSDM2"	FCSPAD2	HORVU5Hr1G084120	GO:0006633 GO:0031177 GO:0003700 GO:0006355	acyl carrier protein 3
JHI-Hv50k-2016-323294	5H	571615210	571.62	8.64E-06	0.32	260	0.018384	0.54	1.40	5.06	DM	Drought	Field	Linked with "FCGB5, FCSPAD2"	FSDM2	HORVU5Hr1G084260	GO:0043565	ABSCISIC ACID-INSENSITIVE 5-like protein 2
JHI-Hv50k-2016-325133	5H	576048979	576.05	4.27E-06	0.03	260	0.011351	-2.71	9.75	5.37	TKW	Control	Field		FCTKW5	HORVU5Hr1G086080, HORVU5Hr1G086110	GO:0009733 GO:0043565 GO:0003700 GO:0005634	SAUR-like auxin-responsive protein family, undescribed protein
JHI-Hv50k-2016-334019	5H	595347234	595.35	3.13E-09	0.07	189	3.33E-05	-9.42	22.09	8.50	DM	Drought	Climate		CSDM2	HORVU5Hr1G094380	GO:0006355	Heat stress transcription factor A-2a
JHI-Hv50k-2016-338412	5H	606928889	606.93	3.55E-08	0.34	196	0.000378	3.62	24.05	7.45	SPAD	Control	Climate		CCnSPAD1	HORVU5Hr1G098290		N-terminal protein myristoylation
JHI-Hv50k-2016-345216	5H	622298127	622.30	1.14E-06	0.47	260	0.00405	-1.25	1.55	5.94	NSdPS	Control	Field	Linked with "FSNSdPS4"	FCNSdPS5	HORVU5Hr1G105810		unknown protein; BEST Arabidopsis thaliana protein match is: unknown protein
JHI-Hv50k-2016-346162	5H	623950131	623.95	2.90E-06	0.28	260	0.007872	0.76	1.32	5.54	NSdPS	Drought	Field	Linked with "FCNSdPS5"	FSNSdPS4	HORVU5Hr1G106380	GO:0004824 GO:0005524 GO:0005737 GO:0006418 GO:0006430 GO:0000166 GO:0003676 GO:0004812	Lysine--tRNA ligase
JHI-Hv50k-2016-349520	5H	635613832	635.61	4.45E-07	0.03	189	0.001579	-10.35	33.12	6.35	DM	Drought	Climate		CSDM3	HORVU5Hr1G111180		Triacylglycerol lipase SDP1

Supplementary files

JHI-Hv50k-2016-388506	6H	70921630	70.92	5.65E-07	0.14	260	0.006016	1.06	1.54	6.25	SPAD	Drought	Field		FSSPAD2	HORVU6Hr1G022380	GO:0016491 GO:0055114	Apoptosis-inducing factor homolog A
JHI-Hv50k-2016-397691	6H	344112271	344.11	4.68E-06	0.39	260	0.00831	-4.31	1.53	5.33	GB	Control	Field		FCGB6			
JHI-Hv50k-2016-398315	6H	353413357	353.41	1.75E-07	0.31	196	0.000931	3.02	3.64	6.76	TKW	Control	Climate	Linked with "CSPH3"	CCnTKW5	HORVU6Hr1G055570	GO:0008270	TNF receptor-associated factor 6
JHI-Hv50k-2016-398334	6H	354336869	354.34	1.21E-06	0.18	196	0.00644	-3.79	7.36	5.92	PH	Drought	Climate	Linked with "CCTKW5"	CSPH3	HORVU6Hr1G055660	GO:0008152 GO:0016491 GO:0004654 GO:0006396 GO:0006402	Dehydrogenase, reductase SDR family member 7B
JHI-Hv50k-2016-400135	6H	374517574	374.52	2.93E-08	0.14	260	0.000312	4.96	4.42	7.53	GB	Drought	Field		FSGB1	HORVU6Hr1G057570	GO:0000175 GO:0003676 GO:0003723	polyribonucleotide nucleotidyltransferase, putative
JHI-Hv50k-2016-402539	6H	397260248	397.26	2.96E-06	0.26	260	0.007872	-0.77	1.35	5.53	NSdPS	Drought	Field		FSNSdPS5	HORVU6Hr1G060010	GO:0055114	alcohol dehydrogenase 1
JHI-Hv50k-2016-416678	6H	538289666	538.29	1.93E-06	0.43	260	0.005127	-0.79	1.98	5.72	DM	Drought	Field		FSDM3	HORVU6Hr1G079640	GO:0016491 GO:0055114	1-aminocyclopropane-1-carboxylate oxidase
JHI-Hv50k-2016-422808	6H	558862422	558.86	1.24E-06	0.03	260	0.004414	7.59	6.72	5.91	GB	Drought	Field	Linked with "CSTKW3"	FSGB2	HORVU6Hr1G085410	GO:0003676 GO:0005634	CCR4-NOT transcription complex subunit 7
JHI-Hv50k-2016-422853	6H	558880331	558.88	3.76E-10	0.04	196	4.00E-06	9.68	21.04	9.43	TKW	Drought	Climate	Linked with "FSGB2"	CSTKW3	HORVU6Hr1G085430	GO:0005515 GO:0008270	Pentatricopeptide repeat-containing protein
JHI-Hv50k-2016-424617	6H	563561183	563.56	4.88E-08	0.25	196	0.000519	-1.52	16.81	7.31	SPAD	Drought	Climate		CSSPAD2	HORVU6Hr1G087220	GO:0016491 GO:0055114	Alcohol dehydrogenase
SCRI_RS_621	7H	3854903	3.85	1.24E-07	0.30	260	0.000661	0.79	1.69	6.91	DM	Drought	Field		FSDM4	HORVU7Hr1G001830, HORVU7Hr1G001850	GO:0003677 GO:0003993 GO:0005524 GO:0006468	myb-like transcription factor family protein, undescribed protein
JHI-Hv50k-2016-450056	7H	15060330	15.06	6.30E-07	0.08	260	0.003352	-5.39	5.42	6.20	GB	Drought	Field		FSGB3	HORVU7Hr1G010690, HORVU7Hr1G010730, HORVU7Hr1G010740	GO:0030247 GO:0004672 GO:0005509 GO:0005515 GO:0006355	Acid phosphatase 1, undescribed protein, Protein kinase family protein
JHI-Hv50k-2016-460705	7H	42955019	42.96	1.10E-05	0.33	196	0.010644	0.02	1.09	4.96	HI	Drought	Climate		CSHI7	HORVU7Hr1G025700, HORVU7Hr1G025710	GO:0046983 GO:0003677 GO:0003700 GO:0005634	MADS-box transcription factor 5, Retrotransposon protein, putative, unclassified
JHI-Hv50k-2016-478809	7H	272670151	272.67	1.54E-06	0.07	260	0.005127	-1.09	2.86	5.81	DM	Drought	Field		FSDM5	HORVU7Hr1G060040		Disease resistance protein RPM1
BOPA2_12_30335	7H	591550949	591.55	5.51E-06	0.47	196	0.014662	-2.76	1.28	5.26	TKW	Drought	Climate		CSTKW4	HORVU7Hr1G097250	GO:0000413 GO:0003755 GO:0006457	Peptidyl-prolyl cis-trans isomerase B

Table S4: Number of detected MTAs across the barley genome (1H to 7H).

Number of detected MTAs									
	FS	**CS**	**FC**	**CCn**	**Total**	**STR**	**CON**	**STR%**	**CON%**
1H	4	4	3	4	15	8	7	13.8	20.6
2H	7	17	7	5	36	24	12	41.4	35.3
3H	0	3	1	0	4	3	1	5.2	2.9
4H	1	2	3	0	6	3	3	5.2	8.8
5H	3	4	8	1	16	7	9	12.1	26.5
6H	5	3	1	1	10	8	2	13.8	5.9
7H	3	2	0	0	5	5	0	8.6	0.0
Total	23	35	23	11	92	58	34	100.0	100.0

Remark: FS = field drought stress treatments, CS = climate chamber drought stress treatments, FC = field control treatment, CCn = climate chamber control treatment, STR = sum of FS and CS, CON = sum of FC and CC, STR% = STR percentage from total STR, CON% = CON percentage from total CON.

Table S5: GO terms enrichment of significant barley genes; detail information of the enriched corresponding GO terms and graphical diagram of significant GO.

GO term	**Ontology**	**Description**	**Number in input list**	**Number in BG/Ref**	**p-value**	**FDR**
GO:0010468	Biological Process	regulation of gene expression	6	1025	0.0360	ns
GO:0060255	Biological Process	regulation of macromolecule metabolic process	6	1057	0.0410	ns
GO:0019222	Biological Process	regulation of metabolic process	6	1076	0.0440	ns
GO:0003700	Molecular Function	transcription factor activity, sequence-specific DNA binding	6	513	0.0015	0.0250
GO:0003677	Molecular Function	DNA binding	9	1866	0.0330	ns
GO:0005634	Cellular Component	nucleus	5	771	0.0380	ns

Remark: 'ns' for non-significant based on Boferroni FDR

GO Accession: GO:0005634
Ontology: Cellular Component
Description: nucleus
Annotated/Total number in query list: 5/51
Annotated/Total number in background/reference: 771/21146

Name	**Associated traits**	**GO terms**
HORVU5Hr1G094380	CSDM	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0043565 sequence-specific DNA binding GO:0005634 nucleus GO:0006355 regulation of transcription, DNA-templated
HORVU1Hr1G042920	CSPH	GO:0000398 mRNA splicing, via spliceosome GO:0005681 spliceosomal complex
HORVU7Hr1G025700	CSHI	GO:0003700 transcription factor activity, sequence-specific DNA binding

		GO:0006355 regulation of transcription, DNA-templated GO:0046983 protein dimerization activity GO:0005634 nucleus GO:0003677 DNA binding
HORVU6Hr1G085410	FSGB	GO:0005634 nucleus GO:0003676 nucleic acid binding
HORVU2Hr1G107560	CSHI	GO:0018024 histone-lysine N-methyltransferase activity GO:0034968 histone lysine methylation GO:0005634 nucleus GO:0005515 protein binding GO:0008270 zinc ion binding

GO Accession: GO:0019222
Ontology: Biological Process
Description: regulation of metabolic process
Annotated/Total number in query list: 6/51
Annotated/Total number in background/reference: 1076/21146

Name	Associated traits	GO terms
HORVU7Hr1G025700	CSHI	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0006355 regulation of transcription, DNA-templated GO:0046983 protein dimerization activity GO:0005634 nucleus GO:0003677 DNA binding
HORVU2Hr1G127370	FSNSdPS	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0043565 sequence-specific DNA binding GO:0008270 zinc ion binding GO:0006355 regulation of transcription, DNA-templated
HORVU4Hr1G075180	FCNSdPS	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0043565 sequence-specific DNA binding GO:0003677 DNA binding GO:0006355 regulation of transcription, DNA-templated
HORVU5Hr1G094380	CSDM	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0043565 sequence-specific DNA binding GO:0005634 nucleus GO:0006355 regulation of transcription, DNA-templated
HORVU5Hr1G084260	FSDM	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0006355 regulation of transcription, DNA-templated

		GO:0043565 sequence-specific DNA binding
HORVU2Hr1G016580	CCnTKW	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0043565 sequence-specific DNA binding GO:0006351 transcription, DNA-templated GO:0006355 regulation of transcription, DNA-templated

GO Accession: GO:0060255
Ontology: Biological Process
Description: regulation of macromolecule metabolic process
Annotated/Total number in query list: 6/51
Annotated/Total number in background/reference: 1057/21146

Name	Associated traits	GO terms
HORVU7Hr1G025700	CSHI	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0006355 regulation of transcription, DNA-templated GO:0046983 protein dimerization activity GO:0005634 nucleus GO:0003677 DNA binding
HORVU2Hr1G127370	FSNSdPS	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0043565 sequence-specific DNA binding GO:0008270 zinc ion binding GO:0006355 regulation of transcription, DNA-templated
HORVU4Hr1G075180	FCNSdPS	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0043565 sequence-specific DNA binding GO:0003677 DNA binding GO:0006355 regulation of transcription, DNA-templated
HORVU5Hr1G094380	CSDM	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0043565 sequence-specific DNA binding GO:0005634 nucleus GO:0006355 regulation of transcription, DNA-templated
HORVU5Hr1G084260	FSDM	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0006355 regulation of transcription, DNA-templated GO:0043565 sequence-specific DNA binding
HORVU2Hr1G016580	CCnTKW	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0043565 sequence-specific DNA binding GO:0006351 transcription, DNA-templated GO:0006355 regulation of transcription, DNA-templated

GO Accession: GO:0010468
Ontology: Biological Process
Description: regulation of gene expression

Annotated/Total number in query list: 6/51
Annotated/Total number in background/reference: 1025/21146

Name	Associated traits	GO terms
HORVU7Hr1G025700	CSHI	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0006355 regulation of transcription, DNA-templated GO:0046983 protein dimerization activity GO:0005634 nucleus GO:0003677 DNA binding
HORVU2Hr1G127370	FSNSdPS	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0043565 sequence-specific DNA binding GO:0008270 zinc ion binding GO:0006355 regulation of transcription, DNA-templated
HORVU4Hr1G075180	FCNSdPS	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0043565 sequence-specific DNA binding GO:0003677 DNA binding GO:0006355 regulation of transcription, DNA-templated
HORVU5Hr1G094380	CSDM	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0043565 sequence-specific DNA binding GO:0005634 nucleus GO:0006355 regulation of transcription, DNA-templated
HORVU5Hr1G084260	FSDM	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0006355 regulation of transcription, DNA-templated GO:0043565 sequence-specific DNA binding
HORVU2Hr1G016580	CCnTKW	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0043565 sequence-specific DNA binding GO:0006351 transcription, DNA-templated GO:0006355 regulation of transcription, DNA-templated

GO Accession: GO:0003677
Ontology: Molecular Function
Description: DNA binding
Annotated/Total number in query list: 9/51
Annotated/Total number in background/reference: 1866/21146

Name	Associated traits	GO terms
HORVU7Hr1G025700	CSHI	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0006355 regulation of transcription, DNA-templated GO:0046983 protein dimerization activity GO:0005634 nucleus GO:0003677 DNA binding
HORVU2Hr1G127370	FSNSdPS	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0043565 sequence-specific DNA binding GO:0008270 zinc ion binding GO:0006355 regulation of transcription, DNA-templated
HORVU4Hr1G075180	FCNSdPS	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0043565 sequence-specific DNA binding GO:0003677 DNA binding GO:0006355 regulation of transcription, DNA-templated
HORVU5Hr1G094380	CSDM	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0043565 sequence-specific DNA binding GO:0005634 nucleus GO:0006355 regulation of transcription, DNA-templated
HORVU2Hr1G092600	FSDM	GO:0043565 sequence-specific DNA binding GO:0003677 DNA binding
HORVU2Hr1G107380	CSPH	GO:0046983 protein dimerization activity GO:0003676 nucleic acid binding GO:0003677 DNA binding
HORVU7Hr1G001830	FSDM	GO:0003677 DNA binding
HORVU5Hr1G084260	FSDM	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0006355 regulation of transcription, DNA-templated GO:0043565 sequence-specific DNA binding
HORVU2Hr1G016580	CCnTKW	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0043565 sequence-specific DNA binding GO:0006351 transcription, DNA-templated GO:0006355 regulation of transcription, DNA-templated

GO Accession: GO:0003700
Ontology: Molecular Function
Description: transcription factor activity, sequence-specific DNA binding
Annotated/Total number in query list: 6/51

Annotated/Total number in background/reference: 513/21146

Name	Associated traits	GO terms
HORVU7Hr1G025700	CSHI	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0006355 regulation of transcription, DNA-templated GO:0046983 protein dimerization activity GO:0005634 nucleus GO:0003677 DNA binding
HORVU2Hr1G127370	FSNSdPS	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0043565 sequence-specific DNA binding GO:0008270 zinc ion binding GO:0006355 regulation of transcription, DNA-templated
HORVU4Hr1G075180	FCNSdPS	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0043565 sequence-specific DNA binding GO:0003677 DNA binding GO:0006355 regulation of transcription, DNA-templated
HORVU5Hr1G094380	CSDM	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0043565 sequence-specific DNA binding GO:0005634 nucleus GO:0006355 regulation of transcription, DNA-templated
HORVU5Hr1G084260	FSDM	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0006355 regulation of transcription, DNA-templated GO:0043565 sequence-specific DNA binding
HORVU2Hr1G016580	CCnTKW	GO:0003700 transcription factor activity, sequence-specific DNA binding GO:0043565 sequence-specific DNA binding GO:0006351 transcription, DNA-templated GO:0006355 regulation of transcription, DNA-templated

Remark: “F” and “C” designated for field and climate chamber experiment, respectively; the second and third letter designated for treatments “Cn” for control treatment and “S” for drought stress treatment; the rest letter for variables “DM” for days to maturity, “GB” for grain biomass, “NSdPS” for number of seeds per spike, “PH” for plant height, “TKW” for thousand kernel weight, “HI” for harvest index

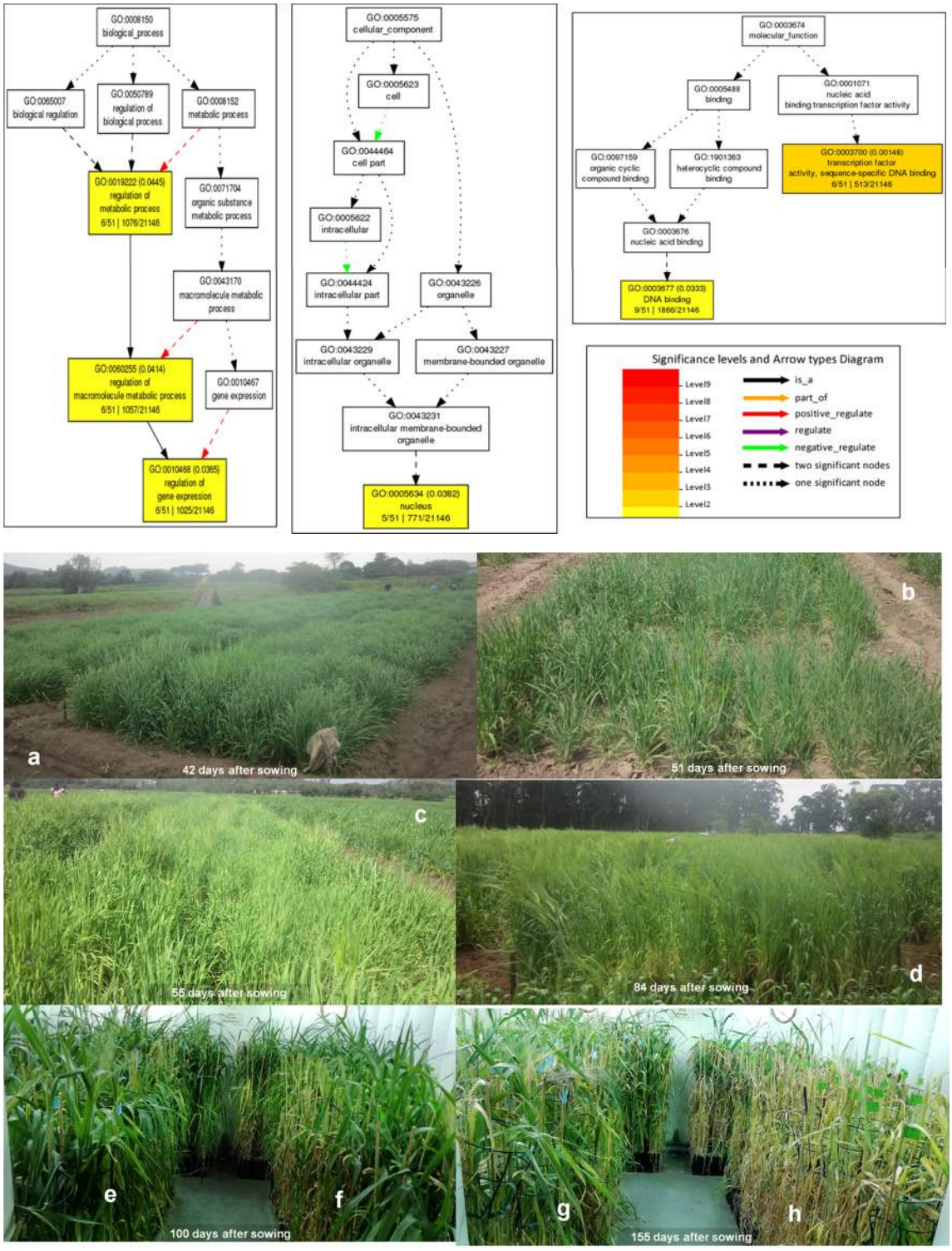

Figure S1*:* Drought stress experiments at field and climate chamber. FS (field drought stress) treatments were a = Melkassa (picture taken on 29/08/2016) and b = Dera (picture taken on 07/09/2016); FC (field control) treatments were c = Debrezeit (picture taken on 15/09/2016) and d = Holetta (picture taken on 20/09/2016); e and g = for CCn (climate chamber control) treatment kept on 70% WC; and f and h = for CS (climate chamber drought stress) treatment, as f = 20% WC treatments imposed only on few early flowering accessions, and h = 20% WC imposed on majority of accessions.

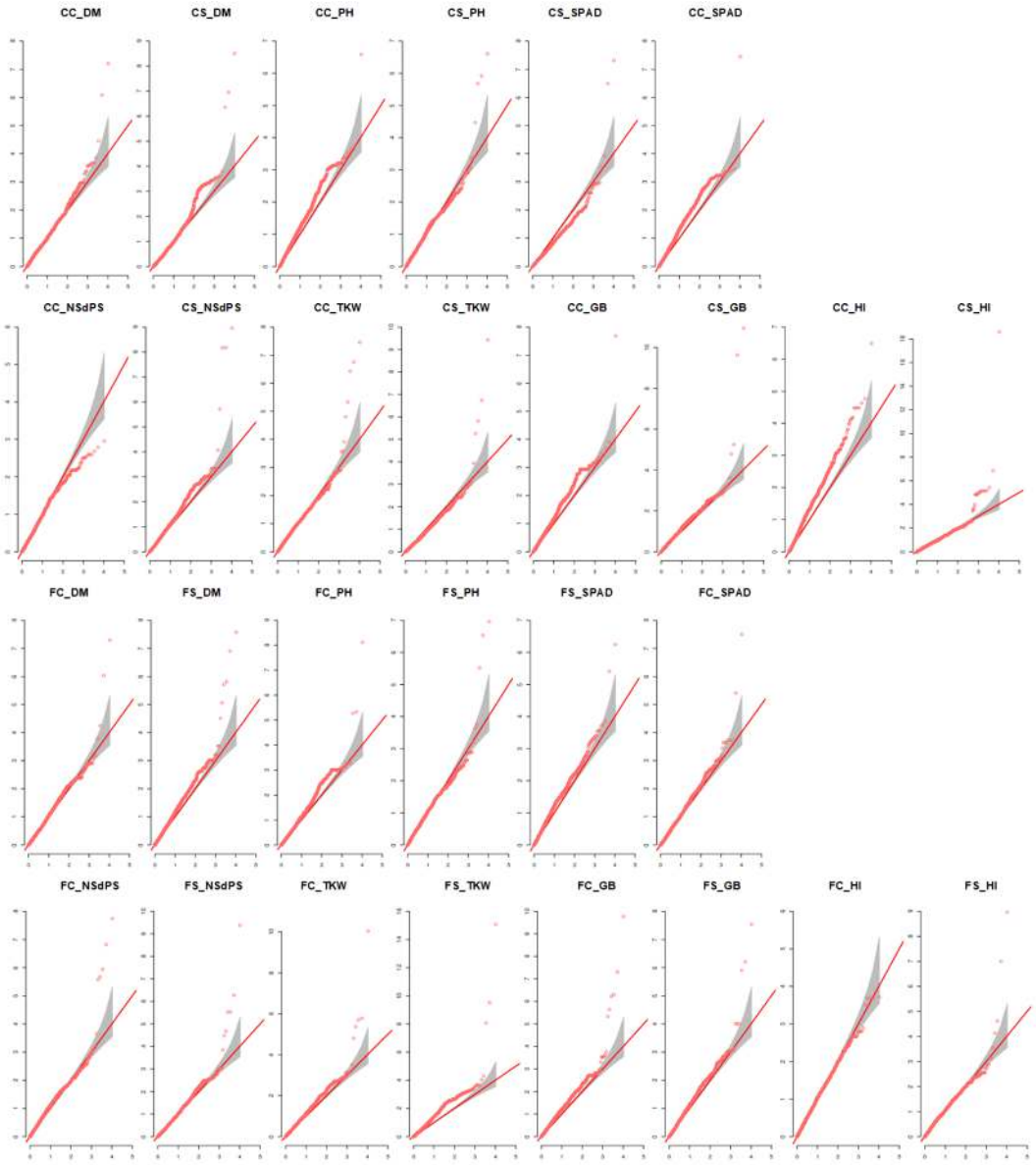

Figure S2: Quantile-quantile (QQ) plots of GWAS for barley drought tolerance using BLINK model; "CCn" for climate chamber control treatment; "CS" for climate chamber drought stress treatment; "FC" for field control treatment; "FS" for field drought stress treatment; "DM" for days to maturity, "GB" for grain biomass, "NSdPS" for number of seeds per spike, "PH" for plant height, "TKW" for thousand kernel weight, "HI" for harvest index.

Publication 2.3) Genetic analysis of flowering time of Ethiopian barley accessions under field and climate chamber conditions

Table S1: Summarized experimental location weather data. Legend: 'MaxTemp" = mean maximum temperature, "MinTemp" = mean minimum temperature, "AvTemp" = mean of daily average temperature; Std Dev = standard deviation, Minimum = minimum value, Maximum = maximum value, Std Error = standard error, CV = coefficient of variation; CC = climate chamber, DZ = Debrezeit, DR = Dera, HL = Holetta, MK = Melkassa

Location	Variable	Mean	Std Dev	Minimum	Maximum	Std Error	Variance	CV
CC	MaxTemp	20.4	1.9	18.0	22.0	0.1	3.7	9.5
	MinTemp	15.2	1.0	14.0	16.0	0.1	0.9	6.3
	AvTemp	17.8	1.4	16.0	19.0	0.1	2.1	8.1
DZ	MaxTemp	25.3	2.1	19.0	29.5	0.1	4.2	8.2
	MinTemp	11.8	3.2	3.0	16.5	0.2	10.2	27.1
	AvTemp	18.5	1.4	13.8	21.8	0.1	2.1	7.7
DR	MaxTemp	26.3	1.7	20.1	32.0	0.1	2.9	6.5
	MinTemp	15.1	2.6	6.4	22.1	0.1	6.6	17.1
	AvTemp	20.7	1.4	15.2	24.6	0.1	1.8	6.6
HL	MaxTemp	22.1	1.8	16.5	26.5	0.1	3.1	8.0
	MinTemp	7.9	2.8	-3.5	12.5	0.1	8.0	36.0
	AvTemp	15.0	1.2	9.0	18.3	0.1	1.5	8.2
MK	MaxTemp	28.2	2.1	19.2	32.5	0.1	4.4	7.4
	MinTemp	14.7	2.8	3.0	18.5	0.1	7.7	18.9
	AvTemp	21.4	1.3	14.6	24.7	0.1	1.7	6.1

Table S2: Ethiopian barley landrace flowering response at the field of the four locations from 2016–2018 and climate chamber 2018 experiment. Legend: DZ = Debrezeit, DR = Dera, HL = Holetta, MK = Melkassa, CC = climate chamber.

Locations	Year	No. of Experimental plots	No. of Plot with flowering response	No. of Plot not responded to flowering	Flowering percentage	Non-flowering percentage
DZ	2016	780	775	5	99.36	0.64
	2017	780	779	1	99.87	0.13
	Total	**1560**	**1554**	**6**	**99.62**	**0.38**
DR	2016	780	739	41	94.74	5.26
	2017	780	773	7	99.1	0.9
	2018	780	746	34	95.64	4.36
	Total	**2340**	**2258**	**82**	**96.5**	**3.5**
HL	2016	780	780	0	100	0
	2017	780	779	1	99.87	0.13
	2018	780	780	0	100	0
	Total	**2340**	**2339**	**1**	**99.96**	**0.04**
MK	2016	780	767	13	98.33	1.67
	2017	780	777	3	99.62	0.38

	2018	780	761	19	97.56	2.44
	Total	**2340**	**2305**	**35**	**98.5**	**1.5**
CC	2018	**784**	**766**	**18**	**97.7**	**2.3**

Supplementary files

Table S3: Summary weather data of experimental locations during the experimental period

Summary of 2016 to 2018 maximum temperature and climate chamber

Days	HL MX16	MK MX16	DR MX16	DZ MX16	HL MX17	MK MX17	DR MX17	DZ MX17	HL MX18	MK MX18	DR MX18	CC Max	HL AV	MK AV	DR AV	DZ AV
Total days (a)	155	123	112	126	132	115	109	135	128	116	120	210	138.3	118.0	113.7	130.5
>28 °C (b)	0	75	23	10	0	53	8	3	0	62	23	0	0.0	63.3	18.0	6.5
Frequency (b/a)	0.00	0.61	0.21	0.08	0.00	0.46	0.07	0.02	0.00	0.53	0.19	0.00	0.00	0.54	0.16	0.05
>21 °C (c)	108	123	112	122	104	114	107	125	76	115	120	121	96.0	117.3	113.0	123.5
Frequency (c/a)	0.70	1.00	1.00	0.97	0.79	0.99	0.98	0.93	0.59	0.99	1.00	0.58	0.69	0.99	0.99	0.95

Summary of 2016 to 2018 minimum temperature and climate chamber

Days	HL MN16	MK MN16	DR MN16	DZ MN16	HL MN17	MK MN17	DR MN17	DZ MN17	HL MN18	MK MN18	DR MN18	CC Min	HL AV	MK AV	DR AV	DZ AV
Total days (a)	155	123	112	126	132	115	109	135	128	116	120	210	138.3	118.0	113.7	130.5
>-1.3 °C and 10 °C< (b)	90	17	5	42	116	5	1	34	87	9	0	0	97.7	10.3	2.0	38.0
Frequency (b/a)	0.58	0.14	0.04	0.33	0.88	0.04	0.01	0.25	0.68	0.08	0.00	0.00	0.71	0.09	0.02	0.29
<-1.3 °C (c)	4	0	0	0	0	0	0	0	0	0	0	0	1.3	0.0	0.0	0.0
Frequency (c/a)	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00

Summary of 2016 to 2018 average temperature and climate chamber

Days	HL AV16	MK AV16	DR AV16	DZ AV16	HL AV17	MK AV17	DR AV17	DZ AV17	HL AV18	MK AV18	DR AV18	CC AV	HL AV	MK AV	DR AV	DZ AV
Total days (a)	155	123	112	126	132	115	109	135	128	116	121	210	138.3	118.0	114.0	130.5
>21 °C(b)	0	82	31	1	0	86	60	3	0	76	36	0	0.0	81.3	42.3	2.0
Frequency (b/a)	0.00	0.67	0.28	0.01	0.00	0.75	0.55	0.02	0.00	0.66	0.30	0.00	0.00	0.69	0.37	0.02

Summary of 2016 to 2018 rainfall temperature

Days	HL RF16	MK RF16	DR RF16	DZ RF16	HL RF17	MK RF17	DR RF17	DZ RF17	HL RF18	MK RF18	DR RF18	HL AV	MK AV	DR AV	DZ AV
Total rainfall	612.9	294.1	434.7	280.8	792	465.5	396.3	296.1	587.5	370	244.9	664.1	376.5	358.6	288.5
Number of rainy days after mean DFL (a)	19	6	8	4	23	14	15	3	11	5	2	17.7	8.3	8.3	3.5
total rainfall days (b)	84	67	52	63	61	59	49	73	57	60	61	67.33	62.00	54.00	68.00
Frequency (a/b)	0.23	0.09	0.15	0.06	0.38	0.24	0.31	0.04	0.19	0.08	0.03	0.27	0.14	0.16	0.05

Table S4: The top and bottom 20 accessions in each location for flowering (DFL = days to flowering, Acc = Accessions, CC = climate chamber, DR = Dera, DZ = Debrezeit, HL = Holetta, MK = Melkassa).

The shared top 20 are highlighted by red boxes, the shared bottom 20 are highlighted by blue boxes, and those shared between CC, MK, and DR are highlighted by purple boxes

No.	AccDZ	DZ DFL	AccHL	HL DFL	AccDR	DR DFL	AccMK	MK DFL	Acc	CC DFL
1	B9.1	46.40	B5.1	58.55	B136	46.41	B4.4	41.67	B154	75.75
2	B4.4	46.59	B4.4	58.78	B5.1	46.78	B5.1	41.78	B160.2	76.00
3	B3.2	46.62	B4.1	58.83	B4.4	47.00	B4.1	43.95	B123	80.75
4	B135.2	46.71	B3.2	59.54	B4.1	47.06	B9.1	44.38	B130.2	81.75
5	B4.1	46.75	B9.1	59.65	B9.1	47.50	B136	44.85	B196	84.25
6	B136	46.83	B29	60.61	B3.2	47.65	B227	45.85	B283.1	84.25
7	B5.1	46.92	B39	60.93	B190	47.84	B237	46.15	B242	84.50
8	B227	47.09	B23.2	61.62	B139	48.11	B29	47.17	B146	85.25
9	B237	47.79	B206	62.63	B141	48.82	B206	47.52	B200	85.75
10	B141	47.80	B237	62.82	B29	48.95	B135.2	47.74	FTCG17	88.50
11	B227.1	48.96	B135.2	62.85	B23.2	49.84	B130.2	47.80	B35	88.75
12	B216.1	49.31	B227.1	63.21	B39	50.48	B141	47.93	B161	89.00
13	B29	49.53	B227	63.23	B220	50.49	B38	47.98	B292	90.50
14	B238	50.28	B190	63.28	B38	50.54	B227.1	48.27	B57	90.50
15	B20	50.31	B136	63.63	B31.2	50.74	B31.2	48.29	B18	90.75
16	B38	50.84	B149	63.87	B287.1	50.99	B3.2	48.32	B228	90.75
17	B149	50.90	B141	64.04	B129	51.17	B173	48.66	B290	90.75
18	B190	50.98	B33.2	64.06	B144	51.53	B120	48.98	B181	91.00
19	B139	51.19	B35	64.26	B173	51.55	B129	49.06	B31.2	91.25
20	B160.2	51.28	B139	64.44	B237	51.71	B23.2	49.06	B10.1	92.00
241/177	**B118**	**76.95**	**FTCG-12**	**82.62**	**B97**	**72.94**	**B127**	**70.34**	**B142**	**134.00**
242/178	**B96**	**77.14**	**FTCG-3**	**82.67**	**B96**	**73.14**	**B78**	**71.27**	**B143**	**134.50**
243/179	**B183.1**	**77.20**	**B21**	**83.04**	**B86**	**73.20**	**B164**	**71.43**	**B60.1**	**137.24**
244/180	**B11**	**77.39**	**B145.1**	**83.16**	**B112.1**	**74.53**	**B11**	**71.53**	**B53.2**	**137.50**
245/181	**B266**	**77.43**	**B126**	**83.26**	**B56.2**	**74.57**	**B56.2**	**71.60**	**B112.2**	**138.50**
246/182	**B178**	**78.22**	**B105**	**83.28**	**FTCG-10**	**74.65**	**B179**	**71.72**	**B11**	**139.00**
247/183	**B112.1**	**78.56**	**B88**	**83.30**	**B101**	**75.03**	**B37**	**71.97**	**B264**	**140.00**
248/184	**B89**	**78.65**	**B266**	**83.35**	**B205**	**75.22**	**B101**	**72.81**	**B205**	**140.24**
249/185	**B94**	**78.82**	**B148**	**83.36**	**FTCG-9**	**75.69**	**B163**	**72.92**	**B163**	**142.00**
250/186	**B148**	**78.83**	**B138**	**83.73**	**B94**	**75.83**	**FTCG-9**	**73.61**	**B134**	**142.25**
251/187	**B53.2**	**79.45**	**B94**	**84.18**	**B127**	**75.89**	**B94**	**74.26**	**FTCG4**	**143.50**
252/188	**FTCG-3**	**79.48**	**FTCG-14**	**84.19**	**B108**	**75.95**	**FTCG-12**	**74.69**	**B178**	**143.50**
253/189	**FTCG-16**	**79.57**	**B96**	**84.39**	**B143**	**75.98**	**B134**	**74.72**	**B120**	**146.25**
254/190	**B134**	**79.86**	**B89**	**84.52**	**B105**	**78.08**	**FTCG-10**	**75.48**	**B145.1**	**147.24**
255/191	**B93**	**80.07**	**B97**	**84.94**	**B74.1**	**78.45**	**B86**	**76.09**	**B30**	**148.07**
256/192	**B74.1**	**80.65**	**B142**	**84.94**	**B163**	**79.47**	**B266**	**76.40**	**B74.1**	**150.14**

257/193	**B37**	**82.44**	**B108**	**84.96**	**B3.1**	**79.71**	**B108**	**77.07**	**B126**	**156.50**
258/194	**FTCG-12**	**83.81**	**B134**	**85.50**	**B266**	**81.44**	**B178**	**77.10**	**B78**	**160.24**
259/195	**B108**	**84.66**	**B93**	**85.88**	**B178**	**86.51**	**FTCG-3**	**79.56**	**FTCG10**	**175.19**
260/196	**B97**	**85.64**	**B78**	**88.64**	**FTCG-12**	**88.25**	**B74.1**	**80.85**	**FTCG12**	**182.64**

Remark:- Shared by all field locations of top 20 are highlighted by red, bottom 20 are highlighted with blue; shared by DZ and HL in green box while by MK and DR in red box; and shared between CC, MK and DR are highlighted by purple

Table S5: Person correlation coefficient (r) for days to flowering and sum of daily temperature effect based on 260 Ethiopian barley landraces in field experiments. Above the diagonal line correlation result for sum of daily temperature effect (SDTE); below diagonal line correlation result for days to flowering (DFL); and on the diagonal line correlation between SDTE and DFL of the same locations; *** significant at $p < 0.001$. Legend: DZ = Debrezeit, DR = Dera, HL = Holetta, MK = Melkassa, CC = climate chamber.

	DZ SDTE	**DR SDTE**	**HL SDTE**	**MK SDTE**	**CC SDTE**
DZ DFL	**1.00*** **	0.88***	0.89***	0.91***	0.53***
DR DFL	0.88***	**1.00*** **	0.82***	0.92***	0.55***
HL DFL	0.89***	0.82***	**1.00*** **	0.85***	0.49***
MK DFL	0.91***	0.92***	0.85***	**1.00*** **	0.57***
CC DFL	0.53***	0.55***	0.49***	0.57***	**1.00*** **

Supplementary files

Table S6: List of significant MTAs obtained using BLINK model analysis for GWAS analysis to detect associations with flowering time of 260 and 196 Ethiopia barley landrace accessions in field and climate chamber experiments, respectively, using 10,644 SNP markers; listed are MTA (SNP), related genomic region (QTL), candidate gene identifiers, and annotations.

Remark: SNP markers detected for more than one location or parameter are highlighted with blue color, SNP markers with distance less than the LD decay value of the chromosome are circled with bold rectangular line; QTL markers represented by more than one SNP marker are highlighted with green color.

No	SNP	Chr	Position	Position (Mb)	P.value	maf	nobs	LOD	effect	PVE (%)	FDR	Loction	Trait	Model	QTL	Locus	Gene ID	Gene Ontologies	Annotation description
1	JHI-Hv50k-2016-12926	1H	13732326	13.73	6.52E-06	0.32	260	5.2	-1.14	0.45	0.017	Melkassa	SDTE	BLINK	MKSDTE1	Locus 1	HORVU1Hr1G006950	GO:0005488	Armadillo/beta-catenin-like repeat protein
2	SCRI_RS_116548	1H	24027749	24.03	3.42E-07	0.08	260	6.5	-1.59	1.49	0.003	Holetta	SDTE	BLINK	HLSDTE1	Locus 2	HORVU1Hr1G010510		Unknown protein; LOCATED IN: endomembrane system; BEST Arabidopsis thaliana protein match is: unknown protein .
3	JHI-Hv50k-2016-18950	1H	59284684	59.28	1.88E-07	0.08	260	6.7	-4.26	2.70	0.001	Dera	DRFL	BLINK	DRDRFL1	Locus 3	HORVU1Hr1G017460	GO:0016887 GO:0042626 GO:0055085 GO:0005524 GO:0006810 GO:0016021 GO:0016887 GO:0042626 GO:0055085 GO:0005524	Lipid A export ATP-binding/permease protein MsbA
4	JHI-Hv50k-2016-18950	1H	59284684	59.28	6.27E-11	0.08	260	10.2	3.17	4.27	0.000	Melkassa	SDTE	BLINK	MKSDTE2	Locus 3	HORVU1Hr1G017460	GO:0006810 GO:0016021	Lipid A export ATP-binding/permease protein MsbA
5	JHI-Hv50k-2016-26918	1H	369364947	369.36	1.26E-06	0.10	196	5.9	6.32	4.83	0.003	Climate	SDTE	BLINK	CCSDTE1	Locus 4	HORVU1Hr1G049900	GO:0005515	Leucine-rich repeat family protein
6	JHI-Hv50k-2016-26982	1H	371702260	371.70	4.91E-08	0.31	196	7.3	7.72	1.91	0.000	Climate	DRFL	BLINK	CCDRFL1	Locus 4	HORVU1Hr1G050110		Abscisic acid receptor PYR1
7	JHI-Hv50k-2016-31649	1H	427687621	427.69	1.60E-07	0.09	196	6.8	10.46	4.16	0.001	Climate	DRFL	BLINK	CCDRFL2	Locus 5	HORVU1Hr1G058590		RNA recognition motif-containing protein
8	JHI-Hv50k-2016-72079	2H	25148431	25.15	6.14E-07	0.27	260	6.2	-1.28	2.81	0.003	Holetta	SDTE	BLINK	HLSDTE2	Locus 6	HORVU2Hr1G011870	GO:0016020 GO:0055085 GO:0005215 GO:0006810 GO:0006811	Cation-chloride cotransporter 2
9	JHI-Hv50k-2016-73570	2H	29307953	29.31	2.27E-08	0.19	196	7.6	-9.32	2.12	0.000	Climate	DRFL	BLINK	CCDRFL3	Locus 7	HORVU2Hr1G013490		Undescribed protein
10	JHI-Hv50k-2016-73570	2H	29307953	29.31	6.46E-12	0.19	196	11.2	8.27	5.27	0.000	Climate	SDTE	BLINK	CCSDTE2	Locus 7	HORVU2Hr1G013490		Undescribed protein
11	JHI-Hv50k-2016-111156	2H	678192138	678.19	8.47E-06	0.03	196	5.1	-8.63	9.22	0.016	Climate	SDTE	BLINK	CCSDTE3	Locus 8	HORVU2Hr1G097240	GO:0006486 GO:0016757	Hexosyltransferase
12	JHI-Hv50k-2016-147628	2H	766082113	766.08	9.35E-07	0.07	260	6.0	-2.57	2.34	0.004	Melkassa	SDTE	BLINK	MKSDTE3	Locus 9	HORVU2Hr1G127190	GO:0008270	RING/FYVE/PHD zinc finger superfamily protein
13	JHI-Hv50k-2016-148133	2H	767327396	767.33	5.66E-09	0.07	260	8.2	5.06	5.79	0.000	Dera	DRFL	BLINK	DRDRFL2	Locus 9			
14	JHI-Hv50k-2016-151505	3H	4058298	4.06	2.34E-05	0.14	260	4.6	3.18	0.81	0.036	Dera	DRFL	BLINK	DRDRFL3	Locus 10			
15	JHI-Hv50k-2016-151820	3H	4569804	4.57	2.37E-06	0.11	196	5.6	-9.19	1.97	0.004	Climate	DRFL	BLINK	CCDRFL4	Locus 10	HORVU3Hr1G002150	GO:0043531	Disease resistance protein
16	JHI-Hv50k-2016-198140	3H	596967250	596.97	8.97E-06	0.16	196	5.0	4.64	2.16	0.016	Climate	SDTE	BLINK	CCSDTE4	Locus 11	HORVU3Hr1G081960	GO:0004553 GO:0005975 GO:0030246	Beta-galactosidase 10
17	JHI-Hv50k-2016-206858	3H	643167221	643.17	1.12E-06	0.04	196	6.0	-14.85	2.66	0.002	Climate	DRFL	BLINK	CCDRFL5	Locus 12	HORVU3Hr1G093580	GO:0005515 GO:0008270	RING/U-box superfamily protein

18	JHI-Hv50k-2016-213204	3H	662655004	662.66	2.71E-06	0.03	196	5.6	16.46	3.55	0.004	Climate	DRFL	BLINK	CCDRFL6	Locus 13	HORVU3Hr1G099920		UV-stimulated scaffold protein A homolog
19	JHI-Hv50k-2016-213207	3H	662655695	662.66	4.92E-07	0.03	196	6.3	-13.06	4.77	0.003	Climate	SDTE	BLINK	CCSDTE5	Locus 13			
20	SCRI_RS_188420	3H	681788954	681.79	7.37E-08	0.29	260	7.1	1.21	1.75	0.001	Holetta	DRFL	BLINK	HLDRFL1	Locus 14	HORVU3Hr1G110520 /HORVU3Hr1G110530	GO:0051861 GO:0005737 GO:0017089 GO:0046836	Kinesin-like protein/Silicon efflux transporter
21	JHI-Hv50k-2016-227500	4H	3241586	3.24	1.32E-06	0.09	196	5.9	-6.18	3.53	0.003	Climate	SDTE	BLINK	CCSDTE6	Locus 15	HORVU4Hr1G001900		Chromosome 3B, genomic scaffold, cultivar Chinese Spring
22	JHI-Hv50k-2016-227517	4H	3571577	3.57	2.22E-05	0.09	196	4.7	8.32	2.98	0.024	Climate	DRFL	BLINK	CCDRFL7	Locus 15	HORVU4Hr1G002010		Unknown function
23	JHI-Hv50k-2016-227778	4H	4059978	4.06	2.22E-05	0.11	196	4.7	7.67	2.44	0.024	Climate	DRFL	BLINK	CCDRFL7	Locus 15	HORVU4Hr1G002230		Very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase
24	SCRI_RS_188340	4H	471650897	471.65	2.04E-07	0.39	196	6.7	6.48	1.22	0.001	Climate	DRFL	BLINK	CCDRFL8	Locus 16	HORVU4Hr1G056200		Vacuolar protein-sorting-associated protein 37 homolog 2
25	JHI-Hv50k-2016-259986	4H	595528294	595.53	2.40E-06	0.04	260	5.6	-4.54	4.75	0.010	Melkassa	DRFL	BLINK	MKDRFL1	Locus 17			
26	JHI-Hv50k-2016-281308	5H	10632553	10.63	3.90E-06	0.04	260	5.4	3.04	1.73	0.041	Debrezeit	SDTE	BLINK	DZSDTE1	Locus 18	HORVU5Hr1G006260/ HORVU5Hr1G006270	GO:0005515	F-box protein/ undescribed protein
27	JHI-Hv50k-2016-310148	5H	510241249	510.24	2.68E-06	0.25	260	5.6	2.33	1.10	0.010	Dera	DRFL	BLINK	DRDRFL4	Locus 19	HORVU5Hr1G067110		Ribosomal L5P family protein
28	JHI-Hv50k-2016-323294	5H	571615210	571.62	2.10E-06	0.32	260	5.7	-1.69	1.26	0.011	Debrezeit	DRFL	BLINK	DZDRFL1	Locus 20	HORVU5Hr1G084260	GO:0003700 GO:0006355 GO:0043565	ABSCISIC ACID-INSENSITIVE 5-like protein 2
29	JHI-Hv50k-2016-323294	5H	571615210	571.62	2.68E-06	0.32	260	5.6	-1.46	0.85	0.010	Melkassa	DRFL	BLINK	MKDRFL2	Locus 20	HORVU5Hr1G084260	GO:0003700 GO:0006355 GO:0043565	ABSCISIC ACID-INSENSITIVE 5-like protein 2
30	JHI-Hv50k-2016-323294	5H	571615210	571.62	1.22E-06	0.32	260	5.9	1.20	0.76	0.004	Melkassa	SDTE	BLINK	MKSDTE4	Locus 20	HORVU5Hr1G084260	GO:0003700 GO:0006355 GO:0043565	ABSCISIC ACID-INSENSITIVE 5-like protein 2
31	JHI-Hv50k-2016-323294	5H	571615210	571.62	2.74E-09	0.32	260	8.6	1.78	1.38	0.000	Dera	SDTE	BLINK	DRSDTE1	Locus 20	HORVU5Hr1G084260	GO:0003700 GO:0006355 GO:0043565 GO:0042176 GO:0000502 GO:0005515 GO:0030234/ GO:0006468 GO:0004672	ABSCISIC ACID-INSENSITIVE 5-like protein 2 26S proteasome non-ATPase regulatory subunit 3 homolog A/
32	JHI-Hv50k-2016-323571	5H	572541803	572.54	1.35E-05	0.36	260	4.9	1.98	0.44	0.024	Dera	DRFL	BLINK	DRDRFL5	Locus 20	HORVU5Hr1G084540/ HORVU5Hr1G084560	GO:0005515 GO:0005524	Leucine-rich receptor-like protein kinase family protein
33	JHI-Hv50k-2016-345406	5H	623059076	623.06	2.62E-07	0.30	260	6.6	1.64	0.51	0.003	Debrezeit	DRFL	BLINK	DZDRFL2	Locus 21	HORVU5Hr1G105970	GO:0005515	U3 small nucleolar RNA-associated protein 18 homolog
34	JHI-Hv50k-2016-367393	5H	668609323	668.61	2.40E-05	0.25	196	4.6	-3.91	1.01	0.037	Climate	SDTE	BLINK	CCSDTE7	Locus 22	HORVU5Hr1G125350	GO:0043531	Disease resistance protein
35	JHI-Hv50k-2016-378408	6H	19548058	19.55	1.02E-05	0.35	260	5.0	-2.19	0.42	0.022	Dera	DRFL	BLINK	DRDRFL6	Locus 23	HORVU6Hr1G011060		Unknown function
36	JHI-Hv50k-2016-397916	6H	348227463	348.23	5.87E-06	0.03	260	5.2	5.73	3.67	0.016	Dera	DRFL	BLINK	DRDRFL7	Locus 24	HORVU6Hr1G054830		GDP-L-galactose phosphorylase 2
37	JHI-Hv50k-2016-410857	6H	499348658	499.35	3.34E-07	0.50	196	6.5	5.71	1.22	0.001	Climate	DRFL	BLINK	CCDRFL9	Locus 25	HORVU6Hr1G071870/ HORVU6Hr1G071880		Unknown function/ undescribed protein
38	JHI-Hv50k-2016-422808	6H	558862422	558.86	3.22E-15	0.03	260	14.5	9.14	15.39	0.000	Melkassa	DRFL	BLINK	MKDRFL3	Locus 26	HORVU6Hr1G085410	GO:0003676 GO:0005634	CCR4-NOT transcription complex subunit 7
39	JHI-Hv50k-2016-449688	7H	14614292	14.61	1.25E-05	0.24	260	4.9	1.30	0.66	0.027	Melkassa	SDTE	BLINK	MKSDTE5	Locus 27	HORVU7Hr1G010370	GO:0008152 GO:0016758	UDP-Glycosyltransferase superfamily protein
40	JHI-Hv50k-2016-457708	7H	32789210	32.79	6.09E-08	0.34	260	7.2	1.64	0.95	0.000	Dera	SDTE	BLINK	DRSDTE2	Locus 28	HORVU7Hr1G022420	GO:0003676 GO:0003677	Unknown function

Table S7: Number of detected MTAs that determine the flowering time of Ethiopian barley across the barley genome and identified influential/consistently detected loci (chromosome 1H to 7H)

	Number of detected MTAs						
Chr	CC	DR	DZ	HL	MK	Total	Percent
1H	3	1	0	1	2	**7**	**9.3**
2H	3	1	0	1	1	**6**	**8.0**
3H	5	1	0	1	0	**7**	**9.3**
4H	4	0	0	0	1	**5**	**6.7**
5H	1	3	3	0	2	**9**	**12.0**
6H	1	2	0	0	1	**4**	**5.3**
7H	0	1	0	0	1	**2**	**2.7**
Total	**17**	**9**	**3**	**3**	**8**	**40**	**53.3**

Table S8: Person correlation coefficient (r) of flowering time (DFL for days to flowering), and DRFL for days to reverse flowering) of Ethiopian barley landraces with some morphological parameters (FLL for flag leaf length, PH for plant height, SL for spike length) for the individual field locations.

Legend: *** significant at $p < 0.001$, ** significant at $p < 0.01$, * significant at $p < 0.05$, and ns for non-significant

	DZ			**DR**	
	DRFL	**DFL**		**DRFL**	**DFL**
FLL	-0.04 ^{ns}	0.02 ^{ns}	**FLL**	-0.61***	0.60***
PH	0.43***	-0.43***	**PH**	-0.19**	0.23***
SL	0.29***	-0.32***	**SL**	-0.05 ^{ns}	0.05 ^{ns}
	HL			**MK**	
	DRFL	**DFL**		**DRFL**	**DFL**
FLL	-0.52***	0.52***	**FLL**	-0.28***	0.24***
PH	-0.33***	0.34***	**PH**	0.22***	-0.14*
SL	-0.08 ^{ns}	0.08 ^{ns}	**SL**	0.33***	-0.38***

Legend: Experimental locations are DZ = Debrezeit, DR = Dera, HL = Holetta, and MK = Melkassa; traits analysed are DFL = days to flowering, DRFL = days to reverse flowering, FLL = flag leaf length, PH = plant height, SL= spike length

Supplementary files

Table S9: Quartile analysis for awn tipping (Z49) and complete emergence of spike (Z58); and Person correlation analysis (r) of days to maturity for 196 Ethiopian barley landraces between the field and climatic chamber experiments using Ethiopian barley accessions.

Legend: DM = days to maturity, DZ = Debrezeit, DR = Dera, HL = Holetta, MK = Melkassa, CC = climate chamber, Dr = drought stress treatments, Con = control treatments

Quartile analysis for awn tipping (Z49) and complete emergence of spike (Z58)

		Control				Stress				
	Values	No of plots completed Z49 between the quartiles	No of plots not completed Z58 between the quartiles	Percentage of plots not completed Z58 between the quartiles	Percentage of plots completed Z58 between the quartiles	Values	No of plots completed Z49 between the quartiles	No of plots not completed Z58 between the quartiles	Percentage of plots not completed Z58 between the quartiles	Percentage of plots completed Z58 between the quartiles
Minimum value	75.0	-	-	-	-	78.0	-	-	-	-
First quartile	102.0	186	23	12.37	87.63	96.0	108	82	75.93	24.07
Second quartile/Median	113.0	88	24	27.27	72.73	105.0	88	73	82.95	17.05
Third quartile	123.0	34	14	41.18	58.82	123.0	102	92	90.20	9.80
Maximum Value	200.0	67	53	79.10	20.90	207.0	93	89	95.70	4.30
Total		375	114	30.40	69.60		391	336	85.93	14.07

Pearson Correlation analysis for days to maturity between field and climate chamber experiments

Legend: "*" and "***" indicates significant correlation at p value <0.05, and < 0.001)

CC_Dr	CC_Con	DZ	DR	HL	MK	
1	0.55***	0.39***	0.42***	0.36***	0.49***	**CC_Dr**
	1	0.24***	0.28***	0.15*	0.25***	**CC_Con**
		1	0.83***	0.78***	0.82***	**DZ**
			1	0.69***	0.81***	**DR**
				1	0.75***	**HL**
					1	**MK**

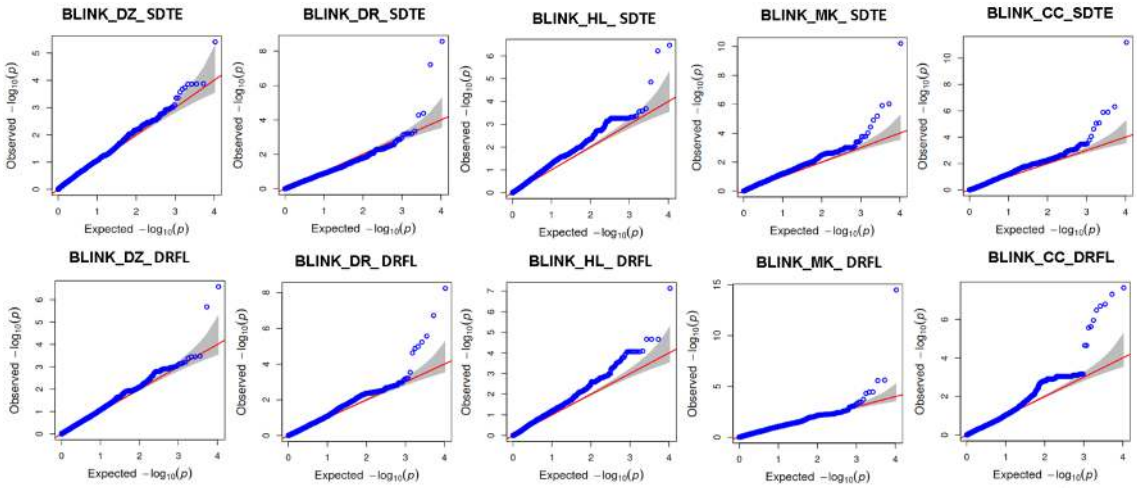

Figure S1: Quantile–quantile (QQ) plots of GWAS for barley drought tolerance using BLINK model analysis; Legend: "CC" for climate chamber; "DZ" for Debrezeit; "DR" for Dera; "HL" for Holetta; "MK" for Melkassa, "SDTE" for summation daily temperature effect; "DRFL" for days to reverse flowering.

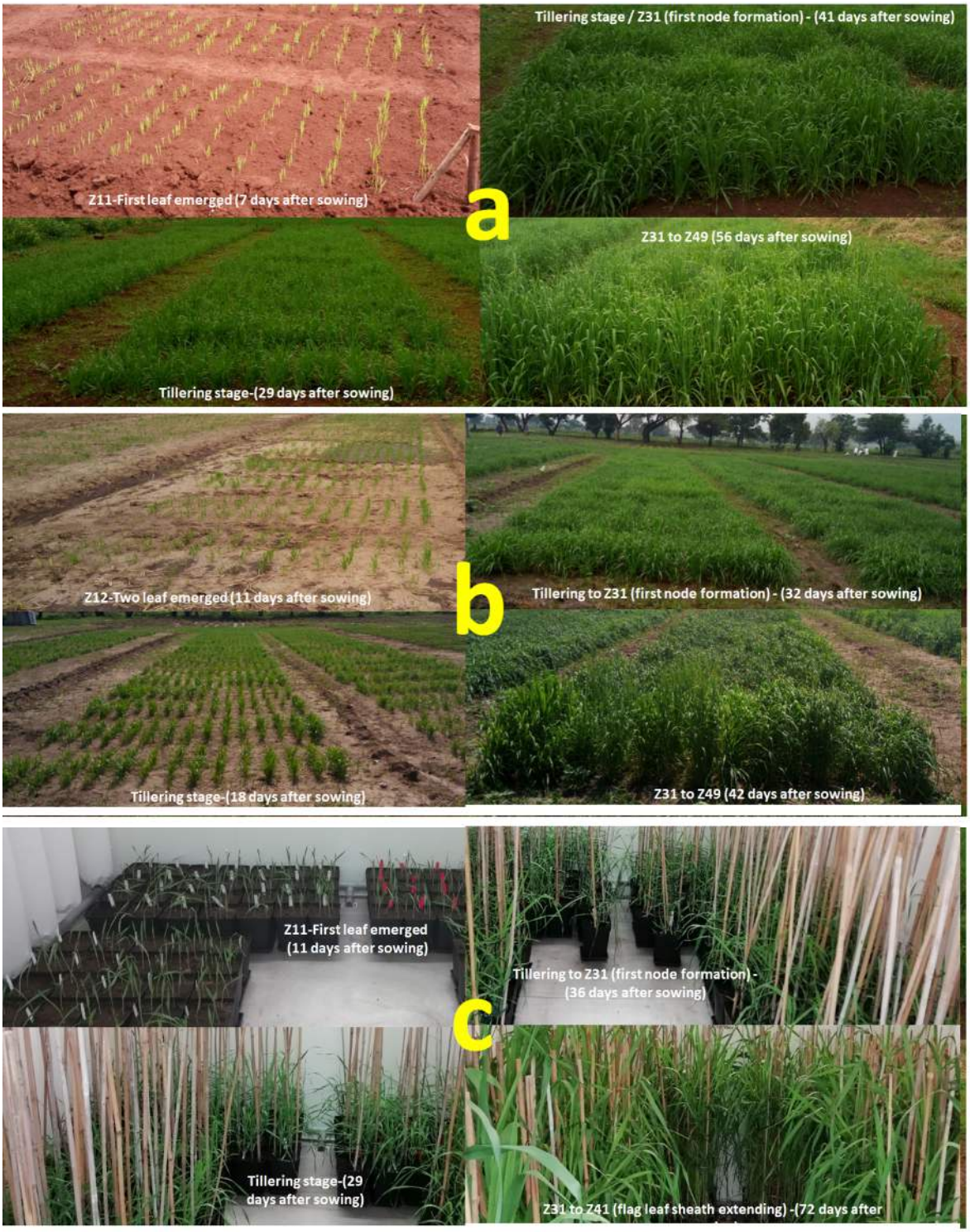

Figure S2: Morphological growth of Ethiopian barley (Z11—early germination or Z12 from two leaf emerged; Z31—first node formation and Z49—main spike awn visible) at field experiments (a = Holetta location, and b = Melkassa location) and c = climate chamber experiment.

Figure S3: Flowering of Ethiopian barley in the climate chamber (a = control plot (left) completed Z58 stage; drought stressed plot (right) failed to reach Z58 stage; b = both control (left) and stressed (right) plots failed to reach Z58 stage).

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DECLARATION

Eidstattliche Erklärung / Declaration under Oath

Ich erkläre an Eides statt, dass diese Arbeit vollständig von mir selbst und ohne fremde Hilfe verfasst worden ist. Ich habe nur die angegebenen Quellen verwendet und alle Zitate sowohl wörtlich als auch inhaltlich korrekt angegeben.

I declare under penalty of perjury that this thesis is my own work entirely and has been written without any help from other people. I used only the sources mentioned and included all the citations correctly both in word or content.

Datum/Date

Unterschrift/Signature

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