Environmental association identifies candidates for tolerance to low temperature and drought

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Abstract

Barley (*Hordeum vulgare* ssp. *vulgare*) is cultivated from the equator to the Arctic Circle. The wild progenitor species, *Hordeum vulgare ssp. spontaneum*, occupies a relatively narrow latitudinal range (~30 - 40˚ N) primarily at low elevation (< 1,500 m). Adaptation to the range of cultivation has occurred over ~8,000 years. The genetic basis of this adaptation is amenable to study through environmental association. Using genotyping from 7,864 SNPs in 803 barley landraces, we performed mixed model association analysis relative to bioclimatic variables and analysis of allele frequency differentiation across multiple partitions of the data. Using resequencing data from a subset of these landraces, we tested for linkage disequilibrium (LD) between SNPs queried in genotyping and SNPs in neighboring loci. Six loci previously reported to contribute to adaptive differences in flowering time and abiotic stress in barley and six loci previously identified in other plant species were identified in our analyses. In many cases, patterns of LD are consistent with the causative variant occurring in the immediate vicinity of the queried SNP. The identification of barley orthologs to well characterized genes may provide new understanding of the nature of adaptive variation and could permit a more targeted use of potentially adaptive variants in barley breeding and germplasm improvement.

Keywords

cold, drought, adaptation, barley, allele frequency differentiation, mixed model association

Introduction

Cultivated species typically undergo adaptation to very distinct climates as they are disseminated from their region of origin (Gaut et al., 2018). These bouts of adaptation are most extreme for widely cultivated species such as barley and wheat, which are grown from the equator to the Arctic Circle.

Loci that contribute to adaptive phenotypes have typically been identified using top-down approaches (Ross-Ibarra et al., 2007). A phenotype is measured and quantitative trait locus (QTL) mapping or association (also known as linkage disequilibrium or LD) mapping is used to identify genetic variants correlated with the phenotype. Bottom-up approaches that identify genetic evidence of local adaptation or genomic signatures of selection have rarely been used to move from initial analysis to fully characterized genes (Morrell et al., 2012) (for an exception, (see Comadran et al., 2012)).

Genes identified in top-down approaches necessarily contribute to measurable trait variation, but additional evidence is required to determine if loci identified have played a role in adaptation (Kantar et al., 2017; Ross-Ibarra et al., 2007). For example, the loss of inflorescence shattering in Asian rice was mapped to two loci (Konishi et al., 2006; Li et al., 2006). Only one locus, *qSH1,* shows evidence of selection in both the *japonica* and *indica* subspecies of rice, suggesting that only *qSH1* played a direct role in domestication of both subspecies (Zhang et al., 2009).

Wild barley (*Hordeum vulgare* ssp. *spontaneum*), the progenitor of cultivated barley (*Hordeum vulgare* ssp. *vulgare*), occurs primarily within a limited latitudinal range of 30 - 40˚ N (Harlan and Zohary, 1966). The geographic range of wild barley is bisected by the Zagros Mountains, with peaks of 4,400 m, but wild barley is largely limited to sites at < 1,500 m (Zohary et al., 2012).

Barley was domesticated from its wild progenitor ~10,000 - 12,000 years ago. Domestication occurred at least twice (Morrell and Clegg, 2007) and involved genetic contributions from across the geographic range of wild barley (Poets et al., 2015b). The dissemination of cultivated barley beyond the initial centers of origin began ~2,000 years after domestication (Willcox, 2002). Barley landraces and modern cultivars are the result of pre-

historic adaptation to growing conditions in Eurasia, North Africa, and much more recently, to Australia and the New World. In Eurasia, the process occurred as humans adopted an agropastoral lifestyle and spread from the Fertile Crescent into a variety of geographic regions. This included cultivation in regions with cooler and more mesic climates in Europe (Pinhasi et al., 2005) as well as drier climates in North Africa and Central Asia (Harris and Gosden, 1996). Barley is frequently produced at high elevations in East Africa, Asia, and Europe and remains among the most important crops in Nepal and Tibet, where it is grown at elevations up to 4,700 m.

The adaptation of cereals such as barley and wheat to northern latitudes or dry climatic conditions involved changes in vernalization requirements (Yan et al., 2006; Dawson et al., 2015), growth habit (Turner et al., 2005; Zakhrabekova et al., 2012; Dawson et al., 2015), and flowering time (Comadran et al., 2012; Dawson et al., 2015). Wild species adapted to Mediterranean climates typically grow over winter and flower in the spring. This is known as a winter growth habit. Under cultivation, winter annuals such as barley and wheat have been adapted to colder climates through spring planting. This is known as spring growth habit. Spring planting can make cultivation possible at higher latitudes but also increases exposure to frost and freezing conditions (Visioni et al., 2013).

The genetic basis of vernalization and flowering time adaptation in barley has been explored extensively and multiple genes have been cloned (see Hansson et al., 2018). There are also numerous mapping studies and a smaller number of functional studies that have identified regions of the genome associated with cold tolerance in barley (Francia et al., 2004; Hayes et al., 1993; Reinheimer et al., 2004; Skinner et al., 2006; Tondelli et al., 2006; Visioni et al., 2013). Among genes involved in cold tolerance, only the CBF gene cluster has been extensively characterized (Knox et al., 2010), as collecting cold tolerance phenotypes that go beyond “winter survival” is challenging (Visioni et al., 2013). Drought tolerance in barley has also been extensively explored, but the genetic basis of tolerance remains poorly characterized (Honsdorf et al., 2014).

Approaches for the identification of genetic variants contributing to environmental adaptation must discriminate between the effects of selection and neutral evolutionary processes (Rellstab et al., 2015). Demographic effects acting on populations impact the entire genome, whereas selection alters allele frequencies at individual loci (Cavalli-Sforza, 1966). Lewontin

and Krakauer (1973) proposed an approach to identify variants subject to differential selection between populations based on allele frequency differences, measured by F-statistics. F-statistic-based comparisons suffer from several weaknesses, including a high expected variance in F_{ST} values (Nei and Maruyama, 1975) and the often arbitrary nature of the partitioning of populations (Lotterhos and Whitlock, 2014). If informative population partitions are defined, F_{ST} measures can identify loci subject to strong differential selection (Beaumont and Balding, 2004). Another approach used to identify the genetic basis of environmental adaptation is mixed-model association analysis. This approach explicitly controls for population structure and treats bioclimatic variables, such as average temperatures and rainfall, as "phenotypes" (Eckert et al., 2010; Yoder et al., 2014; Rellstab et al., 2015).

Here, we present allele frequency (F_{ST}) outlier and mixed-model association analyses applied to a geographically diverse collection of barley landraces genotyped with the barley 9K Illumina Infinium iSelect Custom Genotyping BeadChip (Comadran et al., 2012) to identify loci potentially involved in cold and drought tolerance. For the F_{ST} outlier analysis, we focus on partitions of the sample that distinguish unique growth conditions. These include latitude, elevation, and spring versus winter growth habit. To identify the factors that contribute most to allele frequency differentiation, we also calculated F_{ST} for a longitudinal comparison, a contrast reported in previous studies (Morrell and Clegg, 2007; Poets et al., 2015b; Saisho and Purugganan, 2007). We address the following questions: 1) Which of the comparisons explains the largest portion of allele frequency differentiation?, 2) How many previously reported cold temperature and drought tolerance-related loci show evidence of contributing to climatic adaptation?, 3) Do barley orthologs for genes associated with adaptive phenotypes show evidence of contribution to environmental adaptation in our sample?, 4) Given the LD expected in a self-fertilizing species, how frequently are SNPs identified in our analyses in the proximity of potentially causative loci? For this final question, we make use of exome capture resequencing from a sample of 62 landraces drawn from the larger panel. This permits a direct estimate of LD between SNPs identified in our broader panel of accessions and variants in a window surrounding each locus.

We identified a total of six barley genes previously reported to be involved in either cold or drought tolerance, or in flowering time. Furthermore, our analyses identified six additional barley orthologs of genes characterized as contributing to these traits in other plant species. A

slight relaxation of the empirical cutoff for outlier F_{ST} values identified an additional four characterized barley genes and six orthologs from other plants. Considering both allele frequency outlier and bioclimatic association analyses, we identified 282 barley genes not previously reported to be associated with environmental adaptation. Comparisons of LD between SNPs in genotyping and resequencing suggest that roughly a quarter of the genes we identified on the basis of SNP genotyping are strong candidates for association due to the relatively low gene density in barley.

Materials and Methods

Plant Materials

We use 803 accessions of barley identified as landraces based on passport data from a core collection within the United States Department of Agriculture, National Small Grain Collection (Muñoz-Amatriaín et al., 2014). The 803 individuals were collected from Europe, Asia, and North Africa. These cover the range of barley cultivation in human pre-history (Pinhasi et al., 2005; Poets et al., 2015b; Willcox, 2002). Barley growth habits describe planting times. Spring growth habit is most common, and constitutes 617 (76.8%) of our sample. The balance of the sample includes 142 (17.7%) winter accessions, 16 (2.0%) facultative accessions that can be planted for spring or winter growth, and 28 accessions (3.5%) of unknown growth habit. Barley can also be divided into the ancestral two-row inflorescence type and the more dense six-row type. Our sample includes 542 (67.5%) accessions of six-row barley, 219 (37.3%) two-row accessions, with the balance of 42 accessions of unknown row type. The reported geographic coordinates for each accession were manually confirmed to identify potentially inaccurate locations, and landraces with highly doubtful locations were filtered out (Table S1). The elevations of collection locations were inferred from the NASA Shuttle Radar Topographic Mission (SRTM) 90 m data (http://www.cgiar-csi.org/) on Oct 7, 2015 using the getData ‘raster’ R function (Hijmans et al., 2016).

Genotyping data

All samples were genotyped using the 7,864 SNPs on the 9K Illumina Infinium iSelect Custom Genotyping BeadChip (Comadran et al., 2012) genotyping platform (henceforth referred

to as 9K SNPs). The SNPs are distributed across the seven chromosomes of the diploid barley genome. Because of the relatively large size of the barley genome, the SNP panel includes ~ 1 SNP per 648 kb in the 5.1 Gb genome (Consortium, 2012). For more details on the SNP discovery panel see the description in Comadran et al. (2012; 2015b). Cultivated barley is 99% self-fertilizing (Bothmer, 1992; Wagner and Allard, 1991), and thus the number of unique chromosomes sampled is roughly equal to sample size. The genotyped dataset was filtered for monomorphic SNPs and SNPs with > 20% missingness (Supplemental dataset 1). We culled SNPs in complete LD for comparative analyses, maintaining the SNPs with lower missingness (Supplemental dataset 2).

Estimating crossover relative to physical distance

We identified the physical position of 9K SNPs relative to the barley reference genome (Mascher et al., 2017) (Supplemental dataset 3; Methods S1). The crossover rate in cM/Mb was estimated using SNP physical positions relative to genetic map positions (Muñoz-Amatriaín et al., 2011). A Python script for this calculation and an R script for Locally Weighted Scatterplot Smoothing (LOESS)(Kono et al., 2018) are included in the project repository https://github.com/MorrellLAB/Env_Assoc.

Sample differentiation

We estimated the degree of differentiation among individuals by principal components analysis (PCA). PCA was performed using the SmartPCA program from the EIGENSOFT package (Patterson et al., 2006) with SNP data converted from VCF using PLINK 1.90 (Chang et al., 2015).

Exome resequencing data

We generated exome resequencing from 62 landrace accessions from a randomly chosen subset of landraces in the core collection.This includes 37 six-row spring and 25 two-row spring accessions (Table S2). DNA was extracted from leaf tissue collected from a single plant using a standard 2X CTAB isolation protocol (Saghai-Maroof et al., 1984). The exome resequencing was performed using the NimbleGen exome capture design for barley (Mascher et al., 2017) followed by Illumina 125 bp paired-end resequencing at the University of Kansas Medical

Center Genome Sequencing Facility, Kansas City, KS. The data were processed using publicly available software integrated with bash scripts in the 'sequence_handling' workflow (PJ et al., 2018). Details are in Methods S1. Variant calling is similar to that reported by Kono et al. (2016), with parameters specified in Methods S1.

Heterozygosity, SNP diversity, and SNP annotation

Observed heterozygosity was calculated by PLINK 1.90 with the flag '--het'. The R package ape (Paradis et al., 2004) was used to calculate average percent pairwise difference (Manhattan distance) between accessions. SNPs in coding and noncoding sequences and in amino acid changing positions within genes were identified using ANNOVAR (Wang et al., 2010) with gene models provided by Mascher et al. (2017) (Supplemental dataset 4).

Bioclimatic and geographic variables

"WorldClim" bioclimatic data at a resolution of 2.5 minutes were downloaded on 07 Oct 2015 using the getData 'raster' R function (Hijmans et al., 2016) in the R statistical language (R Core Team, 2017). The latitude, longitude, elevation, and BIO1 to BIO19 values of the collection locations for each landrace are given in the phenotype data file (Supplemental data 5). Environmental variables can be divided into three categories, geographic factors, temperature, and precipitation. The latitude, longitude, and elevation were classified as geographic factors, BIO1 to BIO11 were classified as temperature, and BIO12 to 19 were classified as precipitation. To identify the relationship among the 22 variables given our sample locations, we performed Independent Components (ICs) analysis using the icaimax 'ica' function from the ica-package in R (Bell and Sejnowski, 1995). ICs are conceptually similar to principal component summaries of data; however, we found that using the top three ICs appears to capture the cold temperature trend better than using the top three PCs (Table S3). Details of IC interpretation and comparison to Bioclim variables are reported in Methods S1.

Environmental association mapping

To identify associations between genotypes and environmental variables, we used a mixed linear model (Zhang et al., 2010) implemented in the Genome Association and Prediction Integrated Tool (GAPIT) R package (Lipka et al., 2012). We used the genotyping data to infer

the population structure by principal component analysis within GAPIT, and used the first three principal components to control for structure in the mixed linear model. Estimated pairwise distance among samples did not identify individuals with close kinship (95% of comparisons had pairwise distance > 0.43 based on the Manhattan distance between accessions) (Figure S1), and thus kinship was not included in the mixed model. We excluded SNPs with minor allele frequency (MAF) ≤ 0.01 from association analysis. We applied the Benjamini-Hochberg false discovery rate (FDR) correction. We report both adjusted *p*-values and FDR with an FDR threshold ≤0.25.

F_{ST} estimation

To compare allele frequency differentiation in partitions of the data we calculated F-statistics (Wright, 1949) for individual SNPs using the measure of Weir and Cockerham (1984) as implemented in the R package HierFstat (de Meeûs and Goudet, 2007). The F_{ST} analysis considered five partitions of the data, which were elevation, high latitude, low latitude, longitude, and growth habit.

The elevation comparison used a threshold of 3,000 m to delineate high elevation accessions. This includes accessions from the European Alps, the Caucasus, Himalayan, Hindu Kush Mountain regions, and the Ethiopian Plateau. Since wild barley typically grows below 1,500 m (Zohary et al., 2012), we also compared the allele frequency at three elevations: below 1,500 m, 1,500m - 3,000m, above 3,000 m.

We compared allele frequencies at two latitudinal ranges: (1) within the wild range of the species (30˚ N – 40˚ N) versus landraces at latitudes higher than 40˚ N (high latitude), and (2) within the wild range of the species (30˚ N – 40˚ N) versus landraces at latitudes lower than 30˚ N (low latitude). High latitude includes the northern extent of the range of wild barley and extends across Eurasia from the Central Iberian Peninsula to the Northern Japanese Archipelago. Low latitude includes the southern extent of the range of wild barley. For landraces, this extends from northwestern Africa to just south of the Japanese Archipelago. We also compared low and high latitude versus the wild range in a single comparison.

For a longitudinal comparison, we divided the sample at 48˚ E, roughly through the Zagros Mountains, which coincides with the major axis of population structure in wild barley

(Fang et al., 2014). The final comparison was spring versus winter growth habit, with assignment based on USDA passport information.

To account for differences in sample size among partitions of the data (Table S4) (Bhatia et al., 2013), we used resampling with equalized sample numbers and 10 iterations without replacement. F_{ST} estimates for each SNP were averaged across 10 iterations and outliers were identified at the 99th percentile of the distribution. To calculate the *p*-value for each F_{ST} value we performed 1,000 permutations. The details can be found in Methods S1.

Identification of previously reported loci related to cold, drought tolerance, and flowering time

A literature search was used to identify genes previously reported to contribute to plant flowering time and cold or drought tolerance. Google Scholar searches were performed with the terms “cold OR freezing OR drought tolerance” or “flowering time” and “plant” or “barley” (Table S5-7). For each publication with these key words in the title or abstract, we looked for evidence that individual genes reported to contribute to flowering time, cold, or drought tolerance. The protein-coding sequence (CDS) of identified genes were used as the query or subject in BLASTN against the barley high-confidence CDS in May 2016 on the IPK Barley BLAST Server (Mascher et al., 2017). Barley genes and their interval information were extracted if the combined “Score,” “Identity,” “Percentage,” and “Expectation” produced the overall highest ranking and the “Query length” was >100 bp. In the event of identical scores, all highest ranked hits were extracted.

The BEDOPS ‘closest-features’ function (Neph et al., 2012) was used to compare the locations of SNPs and gene intervals. Specifically, if the SNPs were located in the gene interval or 10 kb up- or downstream of the closest genes, we considered those SNPs as identifying the closest gene.

LD around SNPs

For each 9K SNP identified in environmental association analysis or among F_{ST} outliers, we calculated LD with surrounding SNPs called from exome capture resequencing data. We focused on 200 kb windows, 100 kb upstream and downstream of the queried SNP. When the queried SNP was also genotyped by exome capture, this SNP was used for the LD analysis. If

the queried SNP was not present in exome capture, we extracted the SNPs called from exome-capture sequencing data surrounding the physical position of the queried SNP. Then we performed the LD analysis using the proximate SNP with a MAF similar to the queried SNP. We filtered out SNPs using a MAF threshold of 1% for all of the SNPs called from the exome-capture resequencing data. For LD analysis, filtering of variants could be anti-conservative, thus for this analysis we removed SNPs with ≥ 50% missing data. We used the R package LDheatmap (Shin et al., 2006) to calculate r^2 (Lewontin, 1988).

Inference of ancestral state

The ancestral state for each SNP from both 9K (Supplemental data 6) and resequencing datasets (Supplemental data 7) was inferred using whole genome resequencing data from *Hordeum murinum* ssp. *glaucum* (Kono et al., 2018) with the programs ANGSD and ANGSD-wrapper (Korneliussen et al., 2014; Durvasula et al., 2016;). We chose *H. murinum* ssp. *glaucum* for ancestral state inference because phylogenetic analyses have placed this diploid species in a clade relatively close to *H. vulgare* (Jakob et al., 2004). Previous comparison of Sanger and exome capture resequencing from the most closely related species, *H. bulbosum*, identified substantial shared polymorphism, resulting in ambiguous ancestral states (Morrell et al., 2013). Methods are detailed in Kono et al. (2018). Both minor and derived allele frequencies were calculated using a Python script.

Haplotype analysis for individual genes

To assess evidence for functional diversity in the immediate vicinity of SNPs identified in our analysis, we examined haplotype-level diversity in loci that flanked associations. We used exome capture resequencing from the panel described above. Overlapping SNP genotyping was extracted from SNP calls in a variant call format (VCF) file using ‘vcf-intersect‘ from vcflib (https://github.com/vcflib/vcflib). Missing genotypes were imputed using PHASE (Stephens et al., 2001; Stephens and Scheet, 2005) with PLINK 1.9 (Chang et al., 2015) used to convert the VCF format into PHASE format. Homozygotes were treated as haploid and heterozygotes were treated as diploid samples for haplotype identification.

Results

Summary of genotyping and resequencing data

After quality filtering of the genotyping and resequencing data and exclusion of landrace accessions without discrete locality information, our SNP genotyping dataset includes 5,800 SNPs in 784 accessions (Figure S2; Table S1; Supplemental dataset 2). Quality filtering of genotyping data resulted in the removal of 352 SNPs with > 20% missingness. A map of our sampled accessions is shown in Figure S3. The exome resequencing includes 482,714 SNPs in 62 samples (Figure S2; Table S2; Supplemental dataset 8). The site frequency spectrum for SNPs in both panels is shown in Figure S4. Average inbreeding coefficients estimated from SNP genotyping data and exome resequencing data are 0.996 (±0.025) and 0.981 (±0.008) respectively.

Environmental association and F_{ST} outliers

We performed independent components (IC) analysis on the 19 bioclimatic variables to identify the subset of climate variables that best summarizes the range of environments occupied by barley landraces (Supplemental dataset 9). We identified 32 SNPs with FDR ≤ 0.25 in environmental association with the first three ICs. Loosening FDR to ≤ 0.3 or ≤ 0.4 identified an additional 45 SNPs, or 77 in total. The ICs constitute a somewhat extreme summary of the bioclimatic variables, as the first three ICs included only eight bioclimatic variables (Table S3). The eight variables are not closely correlated to other bioclimatic variables (Figure S5). Limiting the analysis to ICs potentially excludes some of the bioclimatic signal associated with the remaining variables. Thus we also examined each of the bioclimatic and geographic variables independently. The environmental association with bioclimatic and geographic variables and three ICs identified 155 SNPs in significant associations (with FDR ≤ 0.25) (Figure S6; Table S8).

For both elevation and latitude, we calculated a single F_{ST} value with the samples divided into three groups (Table S4; Table S9; Figure S7). We also calculated an F_{ST} for low and middle elevations relative to high elevation (Table S4; Table S9; Figure S7). While F_{ST} values for pairwise comparisons including many barley genes previously associated with adaptive phenotypes (see below), the single F_{ST} values fail to identify these candidate loci.Thus we

focused reporting of outlier results on the two level comparisons (Supplemental dataset 10). F_{ST} comparisons for elevation, latitude, and growth habit identified 203 outliers (using F_{ST} values in the upper 2.5% as the threshold) as the threshold) (Figure 1; Figure S6; Table S10). Considering both the environmental association and F_{ST} comparisons, we identified a total of 349 unique SNPs putatively associated with environmental adaptation in our genotyping panel (Figure S6).

Environmental associations and F_{ST} outliers shared nine SNPs in 11 annotated genes. The only characterized gene found in both analyses is *HvPhyC* in barley. For details regarding overlapping results see Table S11.

Previously reported loci associated with environmental adaptation

Changes in flowering time and drought or cold tolerance are putatively adaptive traits for a cultivated species that has experienced a dramatic expansion in latitudinal range. Our results found four of the 57 genes previously identified in barley as associated with flowering time, two of the 33 genes associated with cold tolerance, and none of the 13 genes associated with drought tolerance (Table 1; Table 3) with the F_{ST} threshold set at top 1%. However, we found six genes previously identified in barley as associated with flowering time, four genes associated with cold tolerance, and none of the 13 genes associated with drought tolerance (Table 1), among the 2.5% of F_{ST} values.

The seven loci found associated with flowering time (using F_{ST} values in the upper 2.5% of F_{ST} values) include four loci identified as F_{ST} outliers. *HvPhyC* (Nishida et al., 2013) and *HvPpdH1* (Jones et al., 2008; Turner et al., 2005) occur among the upper 1% of F_{ST} values (Table 2; Table 3). *HvELF3 (Esp1L/eam8)* (Boden et al., 2014) and *HvPpd-H2* (*HvFT3*) (Casao et al., 2011) are included at the more liberal threshold of F_{ST} values in the upper 2.5% (Table 2). Environmental association identified two additional flowering time loci, *HvPRR1* (*HvTOC1*) (Ford et al., 2016) and *HvVrn-H1* (*HvAP1*) (Cockram et al., 2007), among the 155 outliers at FDR of 0.25 (Table 2).

We also identified four loci previously reported as contributing to cold adaptation in barley, using F_{ST} values in the upper 2.5% (Table 3). This includes *HvCbf4B* (Stockinger et al., 2007) and *HvICE2* (Skinner et al., 2006) as F_{ST} outliers for the low latitude, elevation and growth habit comparisons at the top 1% threshold (Table 2). The upper 2.5% threshold for F_{ST}

includes two additional characterized loci, *HvDhn8* (Choi et al., 1999) and *HvSS1* (Barrero-Sicilia et al., 2011) (Table 2).

A further six loci identified as F_{ST} outliers in the top 1% of values or in environmental associations in our barley panel had been previously associated with loci contributing to flowering time or cold or drought tolerance in other plant species (Table 2). This includes one flowering time-related locus characterized in *Arabidopsis thaliana, AtCOP1* (Xu et al., 2016), which was identified as an F_{ST} outlier in the top 1% threshold. Two loci related to cold tolerance were also identified. Wheat locus *TaWCI16* involved in freezing tolerance (Sasaki et al., 2013) and the rice (*Oryza sativa*) locus *OsiSAP8,* which has been associated with cold, drought, and salt stress response (Kanneganti and Gupta, 2008). *TaWCI16* was identified in environmental association with "minimum temperature of coldest month (BIO6)." *OsiSAP8* was identified by a SNP in the upper 1% of F_{ST} values. While no previously identified drought tolerance loci from barley were detected in our analysis, we find evidence of contributions from three loci previously characterized in three other plant species as associated with drought tolerance (Table 2). One of these genes was identified on the basis of environmental association while the other eight involved F_{ST} comparisons; only two of the SNPs were included in the upper 1%. In the top 2.5% of values, we found an additional six genes previously characterized in two other plant species as associated with drought tolerance (see Table 2 for details). The identification of multiple characterized loci between upper 2.5% and 1% of F_{ST} is indicative of the trade-off between false discovery and false negative rate in empirical scans for adaptive variation (see for example Teshima et al., 2006)

Relative differentiation among partitions of the sample

Comparison of average F_{ST} values provide a means of determining the factors that contribute most to differentiation in barley landraces. Average F_{ST} was highest for the longitude comparison with mean genome-wide F_{ST} = 0.123 (± 0.13) (Table S9; Supplemental data 10). A primary partitioning of barley populations by longitude, reported as eastern and western populations, has been reported previously (Morrell and Clegg, 2007; Poets et al., 2015b; Saisho and Purugganan, 2007). The second highest average F_{ST} was for elevation at 0.089 (± 0.10) (Table S9; Supplemental data 10). The three-level comparison of F_{ST} from 0 - 1,500 m, 1,501 -

3,000 m, and >3,000 m resulted in a slightly lower average F_{ST} = 0.0826 (± 0.0745) (Table S9; Figure S7; Supplemental data 10). A three-level comparison of latitude with comparisons above and below the range of wild barley (see Materials & Methods for details) was similar to elevation with average F_{ST} = 0.087 (±0.082) (Table S9; Figure S7; Supplemental data 10). Pairwise comparisons of the wild range to high latitude, wild to low latitude, and plant growth habit as either spring or winter barley resulted in much lower average F_{ST} values (Table S9).

F_{ST} outliers from geographic patterns and growth habit

We focused on comparisons most directly linked to climatic differentiation in F_{ST} outliers. We obtained results from the two-level comparisons for high and low latitude, elevation, and growth habit. The upper 1% of F_{ST} values from each comparison yielded 55 outlier SNPs for a total of 203 SNPs (Figure 1; Figure S6; Table S10). The comparisons tend to identify unique SNPs. There is overlap of four SNPs in the low and high latitude comparisons and seven SNPs between elevation and growth habit, but other overlaps were not detected (Figure S8). Winter barleys are less frequently grown at higher latitudes and elevations due to harsh winter weather conditions, and indeed winter barleys from these locations are relatively uncommon in the sample, thus constraining the comparisons (Table S1). The elevation comparison identified the largest number of previously characterized loci including *HvPhyC*, *HvI CE2*, and *OSiSAP8* (Figure 1).

SNPs with the most extreme F_{ST} values for elevation, growth habit, and latitude comparisons formed very distinctive geographic patterns. Each comparison with the highest F_{ST} values occurred with SNPs that fall within genes that are annotated, but uncharacterized. The highest F_{ST} from the high latitude comparison occurred at SNP 12_30191 with F_{ST} = 0.484 (*p*-value = 0). The ancestral allele dominates within the wild barley geographic range for this SNP, whereas the derived allele is more prevalent in higher latitude regions (Figure 2a; Supplemental data 6; Supplemental data 10). The highest F_{ST} from the low latitude comparison is for the SNP SCRI_RS_153793 with F_{ST} = 0.504 (*p*-value = 0). The ancestral state for the SNP predominates within the geographic range of wild barley and higher latitudes, whereas the derived allele is more prevalent in lower latitudes (Figure S9a; Supplemental data 6; Supplemental data 10). The highest F_{ST} between samples from elevation comparison is for SNP 12_20648 with F_{ST} = 0.594 (*p*-value = 0). This SNP's ancestral allele occurs at high elevations, such as the Himalaya

Mountains, while the derived allele tends to occur at lower elevation (Figure 2b; Supplemental data 6; Supplemental data 10). The highest F_{ST} between samples from the growth habit comparison was for SNP SCRI_RS_134850 with F_{ST} = 0.390 (*p*-value = 0) (Figure S9b; Supplemental data 6; Supplemental data 10). The SNP SCRI_RS_134850 is significantly negatively correlated with the growth habit with rho = -0.367 (*p*-value < 2.2×10^{-16}, Spearman ranking correlation) and thus the SNP is predictive of growth habit. The ancestral SNP state is C, and the derived state is T. The CC genotype is observed in 60.5% of winter barley while TT is observed in 88.2% of spring barley (Figure S9b; Supplemental data 6; Supplemental data 10).

Environmental association to bioclimatic variables

We identified 155 unique SNPs significantly associated with at least one environmental factor with the threshold of FDR < 0.25 (Figure S6; Table S8). All of the *p*-values and Benjamini-Hochberg FDR-values were reported in Supplemental data 9.

We found 81 SNPs associated with precipitation (variables BIO12 to BIO19) and 51 with temperature (all variables from BIO1 to BIO11) for individual environmental variables (Figure S6). We also identified 47 SNPs associated with geographic variables (latitude, longitude, and elevation), and 32 associated with independent components (top three independent components calculated from BIO1 to BIO19 values after standardization for each BIO variable, called ICs) (Figure S6). Another finding includes 47 cases where individual SNPs were associated with more than one environmental variable (Figure S10). But more generally, as with the F_{ST} comparisons, the environmental variables tend to associate with unique sets of SNPs (Figure S10). The largest proportion of unique SNPs were found for precipitation (33.55%), followed by geographic variables (18.71%), temperature variables (18.06%), and then ICs (1.29%) (Figure S10). The aggregated independent components generally did not identify novel variants.

Minor allele frequency of identified SNPs

The SNPs identified by environmental association and F_{ST} have higher MAF than the average MAF across the full SNP data set (0.262 with standard deviation of ± 0.140). SNPs with significant environmental associations have an average MAF = 0.251 (with standard deviation of ± 0.137). The F_{ST} outliers have an average MAF = 0.330 (± 0.101). While MAF limits the

potential to associate genotype to phenotype for association analysis; the relatively large sample represented here does not suffer from the major limitation of detection. The high MAF contrasts with expectations that adaptive variants for less frequently occupied habitats, such as high elevation sites of cultivation, should be relatively uncommon (average MAF = 0.240 (± 0.091) of outliers from elevation comparison). A relatively low MAF might be expected under models where adaptive variants in a particular environment exhibit antagonistic pleiotropy, and thus confer lower fitness away from habitats in which they are adaptive(Tiffin and Ross-Ibarra, 2014).

SNP density and LD near focal SNPs

As previously reported, SNP density is highest on chromosome arms and lower in pericentromeric regions (Mascher et al., 2017; Muñoz-Amatriaín et al., 2015). This trend is particularly evident for 9K SNPs (Figure 3b; and Figure S2), and is broadly consistent with lower SNP density in genomic regions with lower observed rates of crossover (Muñoz-Amatriaín et al., 2011). Exome capture density is also lower in pericentromeric regions, such that 51,567 SNPs are detected in 1.560 Gb in pericentromere regions (33 SNPs/Mb) versus 431,147 SNPs in 3.02 Gb (143 SNPs/Mb) on chromosome arms.

We compared LD at queried SNPs to the surrounding region for 358 SNPs identified by environmental association analysis or as F_{ST} outliers. We observed 89.3% of these SNPs in exome capture resequencing. The remaining 10.7% of the queried SNPs were replaced by proximal SNPs with similar MAF. The replaced SNPs had an average MAF of 0.035 (±0.005) and were on average 32.9 kb (± 31.5 kb) away from the physical position of the queried SNPs (Figure S11). LD for 123 (34.4%) of these with an $r^2 > 0.45$ (90th percentile) were limited to SNPs within the same gene (Figure S12a & b; Table 5). Detectable LD with flanking loci is limited in pericentromeric regions because the locus tested is often the only annotated gene within the 200 kb window (Figure 3c). For an additional 212 (59.2%) SNPs, LD extends well beyond the locus where the initial association was identified (Figure S12 c & d). For 23 SNPs (6.4%) there was either no LD with the focal SNPs or no SNPs identified in the 200kb window around the focal SNP (Table 4). These results indicate that the potential to identify individual

loci that contribute to adaptive phenotypes is impacted by recombination rate variation and gene density across the genome (Figure 3b, Figure S2).

Putative structural variation

An examination of F_{ST} outliers prior to LD filtering identified 15 SNPs with F_{ST} of ~0.40 for the elevation comparisons. All occur on chromosome 5H at 663.25 cM based on the consensus genetic map(Muñoz-Amatriaín et al., 2011). These SNPs span a physical distance of 133.7 Mb (Table S12). The minor allele frequencies of these SNPs are very similar (0.354 - 0.361) as expected based on F_{ST} values, with minor alleles occurring in the same individuals in almost all cases. All 15 SNPs are in nearly complete LD. The region that contains the SNPs is between 131.2 Mb and 265.0 Mb of chromosome 5H, and overlaps with a region identified as a putative chromosomal inversion in wild barley (Fang et al., 2014). The SNPs that Fang et al. (2014) associated with the putative inversion occur between 126.7 Mb and 305.5 Mb. Evidence for an inversion in wild barley was based on elevated F_{ST} values, extended LD, and enrichment for environmental associations. Fang et al. (2014) reported a similar pattern on chromosome 2H in wild barley at positions that correspond to 267.3 Mb to 508.7 Mb. We found less evidence of allele frequency differentiation on 2H than in wild barley; observing two SNPs which span ~494 Kb with F_{ST} = 0.33 in our sample of landraces (Table S12).

Haplotype analysis at individual genes

Environmental association results identified a SNP, SCRI_RS_137464, significantly associated with mean “temperature of wettest quarter(BIO8)” (p-value = 8.56 x 10^{-4}), which is in the *HvPRR1/HvTOC1* gene (Figure 4a). *TOC1* is an important component of the circadian clock in *Arabidopsis.* It conveys crucial function in the integration of light signals to control circadian and morphogenic responses, which is closely related to flowering time (Más et al., 2003). *HvPRR1/HvTOC1* is the ortholog of *TOC1* in *Arabidopsis thaliana*, and has a high level of sequence similarity and conservation of diurnal and circadian expression patterns when compared to *TOC1* in *Arabidopsis* (Campoli et al., 2012). Exome capture re sequencing data identified 48 SNPs including SCRI_RS_137464 in *HvPRR1/HvTOC1*. Five SNPs at the locus annotate as nonsynonymous SNPs and are the most obvious candidates to contribute to functional variation. Four of these are in the last exon of the gene (Figure 4b & c). Five SNPs

within *HvPRR1/HvTOC1* have relative strong LD with SCRI_RS_137464 (r^2 >0.45) (Figure 4 a &b). Resequencing identified 20 haplotypes with no obvious geographic pattern (Figure 4c).

Environmental association of both "temperature of coldest month (BIO6)" and "mean temperature of the coldest quarter (BIO11)" identified an association on chromosome 3H with SNP 11_10380 (*p*-value = 4.95×10^{-4}). The SNP is in the barley gene *HORVU3Hr1G030150.1*, which is an ortholog of the wheat gene *WCI16* (*Wheat Cold Induced 16*) (Sasaki et al., 2013) (Figure S13). The derived alleles for genotyped SNPs at this locus are much more common in landrace barley than in wild lines. In previous published wild barley genotyping data(Fang et al., 2014) showed the minor allele at 11_10380 occurs in four accessions with geographic provenance information. Those accessions occur at an average of 1460 m - near the upper end of the elevational range for wild barley. Estimated derived allele frequencies differ considerably in wild barley and landraces, at 0.0072 and 0.13 respectively. The 200 Kb window surrounding the SNP contains one gene in addition to *HvWCI16* (Figure S13a). *TaWCI16* encodes a putative transcription factor involved in stomata development. It represents a novel class of late embryogenesis abundant (LEA) proteins in response to cellular dehydration and is involved in freezing tolerance (Sasaki et al., 2013). *TaWCI16* was shown to improve freezing and cold temperature tolerance in wheat when transformed into *Arabidopsis thaliana* (Sasaki et al., 2013). There were six SNPs identified using exome capture sequencing from 61 landraces which includes 11_10380. Three of six SNPs, including 11_10380, are in noncoding sequence (Figure S13b). Of the three SNPs observed in coding regions, one is a nonsynonymous change at nucleotide position 119. This changes from valine to leucine which have similar properties. There is no evidence of LD between this SNP and others within a 200 Kb window (Figure S13a). Exome capture resequencing identified eight haplotypes, with three of the five being relatively common. Seven haplotypes predominate at lower elevation and lower latitude, with two of those occurring most frequently.(Figure S13c).

Environmental association analysis suggested that the SNP SCRI_RS_235243 significantly (*p*-value= 3.62×10^{-4}) associated with "precipitation of driest months (BIO14)" hit the barley gene *HORVU1Hr1G008120.1.* This is an ortholog that produces dehydroascorbate reductase (*DHAR*; EC 1.8.5.1) in *Arabidopsis thaliana* and bread wheat. It is one of two important enzymes functioning in the regeneration of ascorbate (AsA) which plays a role in protection against oxidative stress (Eltayeb et al., 2006; Osipova et al., 2011). The 200 Kb

window surrounding the genotyped SNP SCRI_RS_235243 contains four genes in additional to *DHAR,* which includes two with exome capture sequence coverage (Figure S14a). Previous results suggest that over expression of *DHAR* can protect plants against drought, salt, and polyethylene glycol-induced stress in tobacco and bread wheat (Eltayeb et al., 2006; Osipova et al., 2011). Resequencing identified 53 SNPs in our panel, including SCRI_RS_235243. This encompassed 28 SNPs in noncoding regions, 14 synonymous, and 11 nonsynonymous (Figure S14 b & c). SCRI_RS_235243 is one of nine nonsynonymous SNPs in the first exon of the *DHAR* gene (Figure S14 b & c). Six SNPs are in high LD with SCRI_RS_235243 ($r^2 > 0.45$), all are noncoding variants within *DHAR* (Figure S14b & c). The derived variant at SCRI_RS_235243 occur within two haplotypes (Figure S14c) that occur in high latitude regions.

A putative causative variant is not immediately apparent for all three of the loci described. However, as the loci *HvPRR1/HvTOC1* and *DHAR* demonstrate; barley landraces are frequently segregating for an abundance of potentially functional variants.

Discussion

Comparative analysis in a geographically broad collection of Old World barley landraces allowed us to detect environmental associations to bioclimatic variables and identified allele frequency differentiation at six loci with prior evidence of contribution to climatic adaption in barley (Table 1; Table 2). This includes well characterized loci that contribute to flowering time, cold or drought adaptation in barley including *HvCbf4B* (Stockinger et al., 2007), *HvICE2* (Skinner et al., 2006), *PhyC* (Nishida et al., 2013), *HvPpd-H1 (HvPRR37)* (Jones et al., 2008; Turner et al., 2005), and *HvVrn-H1* (*HvAP1*) (Cockram et al., 2007). All of these loci have been shown to alter phenotypes that are potentially associated with adaptation across the very broad geographic range of cultivation.

Orthologs that potentially played an adaptive role

We found six loci identified as F_{ST} outliers or in environmental associations that had previously been identified as contributing to flowering time, cold or drought stress in other plant species (Table 2). This includes one flowering time-related locus characterized in *Arabidopsis thaliana*, *AtCOP1* (Xu et al., 2016), which was identified as photomorphogenic repressors and regulates flowering time(Lau and Deng, 2012). Two loci related to cold tolerance were

identified. This included wheat locus *TaWCI16* involved in freezing tolerance (Sasaki et al., 2013) and the rice (*Oryza sativa*) locus *OsiSAP8* which has been associated with cold, drought, and salt stress response (Kanneganti and Gupta, 2008). *TaWCI16* was induced during cold acclimation in winter wheat (Sasaki et al., 2013). *OsiSAP8* can be induced by multiple-stresses including heat, cold, salt, desiccation, submergence, wounding, heavy metals, and the stress hormone abscisic acid (Kanneganti and Gupta, 2008). For drought tolerance, we identified nine orthologs characterized in five other plant species (Table 2). For example, over-expression of the wheat expansin gene *TaEXPA2* improved seed production and drought tolerance in transgenic tobacco plants (Chen et al., 2016).

Why did previously identified genes go undetected in our study?

The genetic basis of flowering time in barley has been explored extensively and multiple genes have been cloned (see Hansson et al., 2018). However, relatively few cold or drought tolerance-related genes have been characterized or cloned (Honsdorf et al., 2014; Visioni et al., 2013). Based on the literature search, we identified 57 flowering time and 33 cold tolerance-related genes in barley (Table S5; Table S6). Our analyses found ~10% of flowering time and ~12% of cold tolerance-related genes (Table 1). We did not identify any of the 13 previously reported drought tolerance-related genes (Table 1).

Why were more previously identified genes not detected? Not every gene was genotyped by the 9K SNPs and many genes genotyped are represented by a single SNP or a small number of SNPs (Table 1). Those SNPs have average MAF of 0.30 (±0.11). As in standard association mapping, the SNPs genotyped need to occur in LD with a causative variant (Balding, 2006); even SNPs in close physical proximity can occur on alternate haplotypes and have limited LD (Nordborg and Tavaré, 2002). For flowering time, 29 of 57 genes were genotyped by at least one SNP. Nine genes were genotyped by two or more SNPs (Table 1). All but one of these genes, *HvVrn-H3/HvFT1*, was found in our analysis (Table S13). All nine of these genes have been identified in previous geographic comparisons in barley (Muñoz-Amatriaín et al., 2014; Russell et al., 2016). Among genes identified by multiple SNPs is *HvPpd-H1/HvPRR37*, a key regulator of flowering (Jones et al., 2008; Turner et al., 2005). This gene was genotyped by eight SNPs, and with five SNPs identified as outliers in our comparisons. Previous studies have identified the *HvCEN* and *HvVrn-H2/HvZCCT-Ha/b/c* genes associated with flowering time as allele

frequency outliers (Muñoz-Amatriaín et al., 2014; Russell et al., 2016). *HvCEN* was not genotyped by any SNP in our panel. *HvVrn-H2* was genotyped by a single SNP with an F_{ST} = 0.143 in the elevation comparison, at the 75th percentile in this comparison and thus below the 99th percentile threshold. In summary, genes identified through top-down approaches are generally identified in our comparison if they are represented by a sufficient number of SNPs (Table S13). Genes can contribute to trait variation without having played a role in previous rounds of adaptation (Kantar et al., 2017; Ross-Ibarra et al., 2007). However, given current SNP densities it is premature to conclude that any of the absent loci did not contribute to adaptation in barley.

Comparison to previous studies

Three of the loci we identified as contributing to adaptive differentiation in Old World landraces were previously reported as F_{ST} outliers. They contribute to geographic differentiation in barley breeding populations in in North America (Poets et al., 2015a). This included *HvCbf4B*, *HvPpd-H1*, and *HvVrn-H1*. *HvCbf4* . They were also found as an F_{ST} outlier and in association with temperature adaptation in comparisons of wild barley populations in the Old World (Fang et al., 2014).

We focused on SNP comparisons, but also found evidence that a large chromosomal inversion has contributed to elevational adaptation in barley. On chromosome 5H, 15 SNPs have F_{ST} of ~0.40 in the elevation comparison. All occur at the consensus genetic map position of 663.25 cM. Fang et al. (2014) characterized the region as a putative chromosomal inversion that differs in frequency between the eastern and western portions of the geographic range of wild barley. Recent studies have identified putative chromosomal inversions that contribute to elevation and temperature gradients in teosinte and maize (Fang et al., 2012; Hufford et al., 2012; Pyhäjärvi et al., 2013), rainfall regime and annual versus perennial growth habit in *Mimulus guttattus* (Sweigart and Willis, 2003; Lowry and Willis, 2010), and temperature and precipitation differences in wild barley (Fang et al., 2014). In a close parallel to our results, an inversion on maize chromosome 3 appears to contribute to adaptation of teosinte, which occurs at relatively low elevation to highland cultivation (Wang et al., 2017).

Advantages of study design and future prospects

SNP density is a limitation in our study. With ~40,000 annotated genes in the barley genome (Mascher et al., 2017), roughly one in six genes was directly genotyped. For roughly one quarter of SNPs, there is limited LD with nearby loci (Figure 3; Figure S12). In regions of the genome with high crossover rates and higher gene density, LD can be limited beyond the locus containing the genotyped SNP (Figure 3c and Figure S2). In regions with limited crossover, gene density is also low (Figure 3b and Figure S2). LD would typically have to extend hundreds of kilobases between genotyped SNPs (Figure S12) and a causative variant at another locus to create an association. High MAF of genotyped variants may also contribute to limited LD. Common variants are typically older and have experienced more recombination, thus they can be closer to linkage equilibrium (Nordborg and Tavaré, 2002).

Our study benefits from largesample size. Russell et al. (2016) performed environmental association with 1,688,807 SNPs from exome capture resequencing in 137 cultivated samples. While the analysis identified 10 loci associated with flowering time, many other previously reported genes went undetected. This prompted the authors to suggest a lack of power owing to small sample size (Russell et al., 2016). Despite limited SNP density and the sampling of relatively common variants, our comparative analysis identified a number of previously identified barley loci and many plausible candidate loci from other plant species. Better coverage of barley gene space through exome capture or whole genome resequencing in a relatively deep panel of accessions would likely uncover a much more comprehensive set of variants contributing to environmental adaptation. This could contribute to targeted use of variants for adaptation to environmental and climatic conditions for barley breeding and germplasm improvement; with the potential to improve the understanding of loci that contribute to climatic adaptation in wheat and other cereals.

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Data Accessibility

All sequences were submitted to the NCBI SRA associated with BioProject numbers PRJNA473780 and PRJNA488050. Supporting data (Supplemental data 1-10), including a VCF file of exome capture SNPs and the full results for environmental association and F_{ST} are available at https://drive.google.com/drive/folders/1fEwSX2k-7Bbc-wZSWhmM-hCAqmhCcJwQ. Scripts for analysis and associated files are available on a project Github site at https://github.com/MorrellLAB/Env_Assoc.

Author contributions

PLM, AMP, FK, and LL designed the project. GJM generated the exome-capture resequencing data. RMT and BGS assisted with genotyping data quality control. LL, FK, CL, SRW, CC, XL, and AMP analyzed the data. LL, CL, BGS, and PLM wrote the manuscript.

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Tables and Figures

Figures:

Figure 1: F_{ST} for 9K SNPs in samples from comparisons of high latitude (a), low latitude (b), elevation (c), and growth habit (d).

Figure 2: The geographic distribution of the SNPs with high F_{ST}. (a & c) The geographic distribution of allelic types of 9K SNP 12_30191 with highest F_{ST} = 0.4839. The F_{ST} was from the high latitude (HL) comparison. (b & d) The geographic distribution of allelic types of 9K SNP 12_11529 with highest F_{ST} value of 0.6493. The F_{ST} was from elevation (E) comparison. The color bar indicates the elevations in meters. The filled pink circles indicate the derived allele, while the blue open circles indicate the ancestral allele.

Figure 3: (a) The genomic distribution of outlier SNPs identified according to the F_{ST} comparisons of elevation (below 3,000 m vs above 3,000 m), low latitude (below 30˚N vs 30-40˚N), high latitude (30-40˚N vs above 40˚N), growth habit (winter vs spring) and association analysis of 21 bioclimatic variables, which are categorized into three classes (precipitation, temperature, and geographic variables) on chromosome 3H; (B) exome capture target density (dark blue line), cross over rate in cM/Mb (purple line), the genomic distribution of SNPs

identified in the 62 barley landraces (vertical light blue lines), and 9K SNPs (red triangle) on chromosome 3H; (C) LD plots for SNPs significantly associated with at least one bioclimatic variables (bottom) on chromosome 3H. Each plot shows a 100 Kb window around the SNP. The vertical dotted lines in the upper panel indicate that those outlier SNPs are shared across different traits. For the LD plots, genotyped SNPs are at location 0, and positions upstream and downstream are listed as negative and positive values. The light blue bars are genes in 200 Kb windows surrounding the genotyped SNPs. The SNPs from the I to XII are: 11_20742, 11_10380, SCRI_RS_173916, 12_20108, 11_10601, 12_31008, SCRI_RS_173717, SCRI_RS_6793, SCRI_RS_207408, 12_10210, SCRI_RS_192360, and 12_30960.

Figure 4: (A) The linkage disequilibrium (LD) analysis of genotyped SNP SCRI_RS_137464 significantly associated with "mean temperature of the wettest quarter (BIO8)." The blue bars indicate genes in the 200 Kb window surrounding SCRI_RS_137464, the red arrow indicates the *HvPRR1/HvTOC1* (flowering time-related gene) that includes SNP SCRI_RS_137464 (B) The gene structure of *HvPRR1*/*HvTOC1* and the functional annotation of SNPs in this gene. (C) Haplotype structures of *HvPRR1*/*HvTOC1* based on the SNPs in this gene. L: low; H: high.

Tables:

Table 1: The number of barley genes detected with signals of adaptations and genotyped by 9K SNPs. The number in the parentheses is the fraction of total genes in that functional category that was genotyped or detected with signals of adaptation.

	Total	**Genotyped by ≥1 9K SNPs**	**Genotyped by ≥2 9K SNPs**	**With signals of adaptation**
Flowering time	57	29 (50.9%)	9 (15.8%)	6 (10.5%)
Cold tolerance	33	20 (60.6%)	9 (27.3%)	4 (12.1%)
Drought tolerance	13	7 (53.9%)	3 (23.1%)	0 (0%)

Table 2: Loci identified in environmental association or F_{ST} comparisons that are previously reported to contribute to flowering time, cold, and drought tolerance. Gene names are preceded by two letter prefix with the genus and specific epithet for the species where the gene was identified. This include, *At - Arabidopsis thaliana, Ta - Triticum aestivum, Os - Oryza sativa, Br - Brassica napus,* and *Sod - Saccharum officinarum.* The F_{ST} comparisons involve the following comparisons: E: elevation; LL: Low Latitude; GH: growth habit; HL: high latitude. The * indicates that the gene was identified at the 97.5% threshold but not the 99% threshold.

	Barley	**Other plants**	**Bioclimatic variables**	F_{ST}
Cold tolerance	*HvCBF4B*		-	LL
	**HvDhn8*		-	E
	HvICE2		-	E, GH
	**HvSS1*		-	E
Drought tolerance		*OsiSAP8*	-	E
		TaWCI 16	6	-
		**AtACBP2*	-	LL
		**AtIRX14*	-	GH
		**AtABF3*	-	HL
		**AtAREB1*	-	HL
		**AtERECTA*	-	HL
		**BrERF4*	-	GH
		SodERF3	-	HL
		TaEXPA2	IC1	-
		OsNAC52	-	LL
		AtCOP1	-	GH
Flowering time	**HvELF3/Esp1L/eam8*		-	HL, GH
	HvPpd-H1/HvPRR37		-	LL, HL
	**HvPpd-H2/HvFT3*		-	GH
	HvPhyC		7, 14,17, & IC2	E
	HvPRR1/HvTOC1		8	-

HvVrn-H1/HvAP1	3	-

Note:

1 = Annual mean temperature; 2 = Mean diurnal range (Mean of monthly (max temp - min temperature)); 3 = Isothermality (2/7) (* 100); 4 = Temperature seasonality (standard deviation *100); 5 = Max temperature of warmest month; 6 = Minimum temperature of coldest month; 7 = Temperature annual range (5 - 6); 8 = Mean temperature of wettest quarter; 9 = Mean temperature of driest quarter; 10 = Mean temperature of warmest quarter; 11 = Mean temperature of coldest quarter; 12 = Annual precipitation; 13 = Precipitation of wettest month; 14 = Precipitation of driest month; 15 = Precipitation seasonality (Coefficient of variation); 16 = Precipitation of wettest quarter; 17 = Precipitation of driest quarter; 18 = Precipitation of warmest quarter; 19 = Precipitation of coldest quarter; IC = Independent component.

Table 3: The number of SNPs identified by F_{ST} outlier approaches and number of previously reported genes they identify. For each comparison, 55 SNPs in total were identified as outliers. Flowering time genes had one across all categories. Drought tolerance had zero SNPs detected in all categories. HL: high latitude; LL: Low Latitude; E: elevation; GH: growth habit.

	Flowering time	**Cold tolerance**	
	SNPs	**SNPs**	**Genes**
HL	5	1	1
LL	4	2	2
E	1	1	1
GH	1	0	0

Table 4: The number of SNPs significantly associated with climatic factors and known genes they hit.

	Categories	Number	Climatic factors
Flowering time	SNPs	3	3 ,7 ,8 ,14 , and 17
	Genes	3	-
Cold tolerance	SNPs	1	6
	Genes	1	-
Drought tolerance	SNPs	1	IC1
	Genes	1	-

Note: Abbreviations for climatic factors are listed under Table 2.

Table 5: Linkage disequilibrium for all SNPs associated with environmental variables or identified as F_{ST} outliers.

	LD within a gene	Extended LD	No LD	Missing*
$\boldsymbol{F_{ST}}$	61	132	10	0
Association	62	80	7	6

Note: * indicates that there are no SNPs in the 200kb window around the target SNP.

Supplemental figures:

Methods S1

Figure S1: The distribution of the pairwise genetic distance (Manhattan distance) from 784 barley landraces.

Figure S2: Exome capture target density (dark blue line), crossover rate in cM/Mb (purple line), the genomic distribution of SNPs identified in 62 barley landraces (vertical light blue lines), and 9K iSelect SNPs (red triangle) for seven chromosomes. Crossover rates were calculated using 9K SNPs. SNP genetic positions are based on the genetic map of Muñoz-Amatriaín et al. (2011).

Figure S3: Relationship of barley landrace accessions based on principal components. PC1 and PC2 are depicted relative to a map of the landrace distribution in Africa and Eurasia.

Figure S4: The derived site frequency spectrum (SNPs with inferred ancestral state) for: (a) 2,806 SNPs from the 9K iSelect genotyped in the 784 landraces and (b) 340,260 SNPs with exome-capture resequencing data in 62 landraces. Ancestral state was based on majority state from *H. murinum spp. glaucum* resequencing mapped to the Morex assembly. For all SNPs: (c) Minor allele frequency, for 6,152 SNPs from the 9K iSelect and (d) for 482,714 SNPs from exome-capture resequencing.

Figure S5: The heat map of pairwise correlation coefficient (Pearson correlation) of 22 environmental variables.

Figure S6: The genomic distribution of outlier SNPs identified according to the F_{ST} comparisons of elevation (below 3,000 m versus above 3,000 m), low latitude (below 30˚N versus 30-40˚N), high latitude (30-40˚N vs above 40˚N), growth habit (winter versus spring) and association analysis of 21 bioclimatic variables, which are categorized into three classes (precipitation, temperature, and geographic variables) and ICs.

Figure S7: The distribution of ranked F_{ST} from two- and three-level comparisons of elevation and latitude.

Figure S8: Venn diagram for the F_{ST} outliers from the comparisons of elevation, high and low latitude, and growth habit.

Figure S9: The geographic distribution of the SNPs with high F_{ST}. (a) The geographic distribution of allelic types of 9K SNP SCRI_RS_153793 with highest F_{ST} = 0.505. The F_{ST} was from the low latitude (LL) comparison. (b) The geographic distribution of allelic types of 9K SCRI_RS_134850 with highest F_{ST} = 0.390. The F_{ST} was from growth habit (GH) comparison. The color bar indicates the elevations in meters. The filled pink circles indicate the derived allele, while the blue open circles indicate the ancestral allele.

Figure S10: Venn diagram for the candidate SNPs significant associated with three categories of environmental variables: precipitation, temperature, and geographic factors.

Figure S11: The difference between the replaced SNPs and queried SNPs not in the exome-capture data. (a) The minor allele frequency (MAF); (b) The physical distance. The replaced SNPs refer to those SNPs called based on the exome capture resequnecing data and with the similar MAF as the queried SNPs. But the queried SNPs are not in the exome capture resequnecing data.

Figure S12: LD decay plot for 200 Kb window around the significant SNPs associated with environmental variables. The blue bars underneath the x-axis are the annotated genes in the 200 Kb windows. The vertical dashed lines are candidate SNPlocations. The negative signs on the x-axis refer to positions downstream of the candidate SNP.

Figure S13: (a) The linkage disequilibrium (LD) analysis of candidate SNP SCRI_RS_137464 significant associated with ”min temperature of coldest month (BIO6)” and ”mean temperature of the coldest quarter (BIO6 and 11)”. The blue bars indicate genes in the 200 Kb window surrounding 11_10380, the red arrow indicates the WCI 16 (cold tolerance-related gene) hit by 11_10380, (b) The gene structure of *WCI 16* and the functional annotation of SNPs in this gene. (c) Haplotype structures of *WCI 16* based on the SNPs in this gene. L: low; H: high.

Figure S14: (a) The linkage disequilibrium (LD) analysis of candidate SNP SCRI_RS_235243 significantly associated with ”precipitation of driest months” (BIO14). The blue bars indicate genes in the 200 Kb window surrounding SCRI_RS_235243, the red arrow indicates the DHAR (drought tolerant-related gene) hit by SCRI_RS_235243, (b) The gene structure of DHAR and the functional annotation of SNPs in this gene. (c) Haplotype structures of DHAR based on the SNPs in this gene. L: low; H: high.

Supplemental tables:

Table S1: 784 barley landraces after removing 19 accessions from the 803 Poets et al. 2015 panel.

Table S2: Details of the exome capture data from 62 landraces.

Table S3: Summary statistics for top three independent components (IC) and principal components (PCs) calculated from 19 BIOs

Table S4: The samples size for each partitions for F_{ST} comparisons

Table S5: Known flowering time-related genes list.

Table S6: Known cold tolerance-related genes list.

Table S7: Known drought tolerance-related genes list.

Table S8: The annotation of the 155 significant SNPs identified by environmental association.

Table S9: The average and standard deviation of F_{ST} calculated by different comparisons

Table S10: 203 F_{ST} outliers from elevation, high and low latitude, and growth habit comparisons.

Table S11: The nine overlapping SNPs identified by F_{ST} outliers and association analysis approaches.

Table S12: F_{ST} outliers from elevation comparison in a putative inverted region reported by Fang et al. 2014 without culling SNPs in strong LD.

Table S13: Barley flowering time genes with signal of adaption, the numbers of SNPs genotyped those genes and the SNPs outlier by environmental association and F_{ST} outlier SNPs

Supplemental datasets:

Supplemental data 1: VCF file for the 6,152 SNPs without culling SNPs in complete LD.

Supplemental data 2: Genotype matrix with 5,800 SNPs for environmental association

Supplemental data 3: The physical positions of 9K SNPs.

Supplemental data 4: The annotations for SNPs called from exome capture resequencing data from 62 landraces.

Supplemental data 5: Phenotype matrix with 25 geographic and climatic variables for environmental association.

Supplemental data 6: Inferred ancestral status for each 9K SNP.

Supplemental data 7: Inferred ancestral status for each exome resequencing SNP from 62 landraces.

Supplemental data 8: VCF file for SNPs called from exome-capture resequencing data from 62 landraces

Supplemental data 9: All *p*-values and Benjamini-Hochberg FDR-values from the environmental associations for 25 variables.

Supplemental data 10: All *p*-values and F_{ST} from elevation, low and high latitude, longitude, and growth habit.