Supplemental Information

For:

Natural variation in abiotic stress responsive gene expression and local adaptation to climate in *Arabidopsis thaliana*

Jesse R. Lasky, David L. Des Marais, David B. Lowry, Inna Povolotskaya, John K. McKay, James H. Richards, Timothy H. Keitt, and Thomas E. Juenger

SUPPLEMENTAL METHODS

Climate data

Hijmans et al. (2005) created WorldClim data by spatially interpolating 1950-2000 weather station data and resolving it to 30” grid squares. The authors (Hijmans et al. 2005) estimated mean monthly minimum, mean, and maximum temperatures and mean monthly precipitation averaged across years of the time period. Furthermore, Hijmans et al. (2005) derived variables of climatic extremes and variability.

We used Climate Research Unit (CRU) humidity and temperature data to approximate vapor pressure deficit (VPD), which is the difference between water vapor partial pressure and maximum potential pressure at a given air temperature, and indicates evaporative demand on plants (Johnson and Ferrell 1983). CRU data come from 1961-1990 weather station data that were subsequently interpolated to 10’ resolution (New et al. 2002) .

We used a third database to estimate inter-annual variability in precipitation. The National Centers for Environmental Prediction (NCEP) generated Reanalysis data on a T62 grid (resolution ~ 210 km) for the years 1948-2009 (data provided by NOAA/OAR/ESRL PSD, http://www.esrl.noaa.gov/psd/) (Kalnay et al. 1996). We used monthly surface precipitation rates to calculate each calendar month’s coefficient of variation (CV) across years (Lasky et al.

2012).

Growing season climate – We previously used temperature and precipitation data to model the months of the year when accessions may be growing (climate diagram model) (Walter and Lieth 1960; Lasky et al. 2012). Putative growing months were specified as all months having abundant soil moisture and mean temperature ≥ 4ºC. We considered soil moisture to be abundant in a given month if mean precipitation (mm) ≥ 2 * mean temperature (ºC) (Walter and Lieth 1960).

We used growing season predictions to calculate growing season climate conditions for each accession, consisting of mean values of monthly precipitation, VPD, and minimum and mean temperature. We also calculated the CV of mean monthly precipitation within the growing season and the mean inter-annual CV of growing season month's precipitation.

We selected eleven climate variables we hypothesized would represent selective gradients due to drought and cold stress in order to test for SNP-climate associations. Six climate variables described the growing season: 1) mean monthly precipitation, 2) coefficient of variation (CV) of mean monthly precipitation, 3) mean VPD at mean monthly conditions, 4) mean inter-annual CV of mean monthly precipitation, 5) mean monthly mean temperature, and 6) mean monthly minimum temperature. Five variables described yearlong climate conditions: 1) CV of mean monthly precipitation, 2) isothermality (average diurnal temperature range / annual range), 3) standard deviation (SD) of monthly temperature, 4) minimum temperature of coldest month, and 5) annual temperature range.

Association studies of climate and fitness under acclimation to abiotic stress

Mixed model - We used the Efficient Mixed-Model Association (EMMA) mapping linear mixed model (Kang et al. 2008) to test SNP associations with climate and fitness in new GWAS (*i.e*. not previously published). EMMA includes a kinship random effect to attempt to control for population structure. Hancock *et al*. (2011) previously used non-parametric partial Mantel tests for SNP-climate association tests to reduce the influence of accessions occupying outlier climates. We chose not to use partial Mantel tests, which use permutations to generate null distributions, because Mantel permutations may be poor null models for data heavily influenced by spatially autocorrelated processes (e.g. climatic gradients, population structure, Raufaste and Rousset 2001; Goslee and Urban 2007; Guillot and Rousset 2013). Partial Mantel tests of climate-SNP associations by Hancock et al. (2011) generated extremely high numbers of associations with the lowest possible *p*-value, which the authors hypothesized indicates that these tests performed poorly at controlling for population structure. Instead, we used EMMA, and culled accessions from each analysis that we identified as outliers by visually inspecting climate histograms (Figures S10-S12).

In contrast to a partial Mantel results (Hancock et al. 2011), our quantile-quantile plots compared theoretical and observed test statistics (Figures S6-S9) indicate our models follow the null much more closely, albeit with small enrichments of strong associations (low p-values) as expected if a small number of loci are involved in local adaptation. For example, we found between a 1- and 7-fold enrichment of SNPs in the lower 0.001 p-value tail compared to the null expectation; in contrast to the 156- to 368-fold enrichment found by Hancock et al. (2011). The lack of a large enrichment of low p-values in our mixed model approach signifies that our model is likely controlling for the large portion of population structure that is collinear with climate (Lasky et al. 2012). Note that this approach is highly conservative because we expect that a portion of the genome-wide divergence between populations captured by the kinship matrix will be caused by local adaptation to climate.

We used EMMA to test the null hypothesis that the mean climate inhabited by accessions with one allele was equal to the mean climate inhabited by the alternative allele, while controlling for population structure (Kang et al. 2008; Yoder et al. 2014). In fitness association tests, we tested the null hypothesis that the fitness of accessions with one allele was equal to the fitness of the alternative allele. Formally stated

$$\boldsymbol{y} = \boldsymbol{X}\boldsymbol{\beta} + \boldsymbol{u} + \boldsymbol{e} \qquad \text{(eqn. 1)}$$

where *y is* the *n* x 1 vector of observed climate data for each accession (total of *n* accessions). For association mapping with fitness, $\boldsymbol{y}$ was a vector of accession fitness data. $\boldsymbol{X}$ is an n x q matrix of data for q fixed effects, consisting of intercept and SNP effects. $\boldsymbol{\beta}$ is a q x 1 vector giving the slope of the fixed effects. The $\boldsymbol{u}$ term gives the random error due to kinship

$$Var(u) = \sigma_g^2 \boldsymbol{K} \qquad \text{(eqn. 2)}$$

with $\boldsymbol{K}$ being the n x n kinship matrix. The $\boldsymbol{e}$ term gives the random error of each accession

$$Var(e) = \sigma_e^2 \boldsymbol{I} \qquad \text{(eqn. 3).}$$

The kinship matrix was constructed using identity in-state of SNPs (Kang et al. 2008). The significance of SNP-climate or SNP-fitness relationships, β_1, was assessed using t-tests, the p-values of which indicate the significance of SNP associations to climate or fitness (Kang et al. 2008).

All climate variables were scaled to have a mean of 0 and a standard deviation of 1. Survival data were arcsin square-root transformed in order to improve normality. Only SNPs with a minor allele frequency greater than 0.1 among tested accessions were analyzed in order to avoid spurious significant associations (Atwell et al. 2010).

GWAS stratified by flowering time – The study of Des Marais *et al*. (2012), which provided our drought stress gene expression data, split microarray experiments into early and late-flowering groups of accessions (in the absence of vernalization) because flowering time groups may have varied phenology and life-history that affects response to abiotic stress (McKay et al. 2003; Donohue 2005; Korves et al. 2007). We followed Des Marais *et al*. (2012) and our previous study (Lasky et al. 2012), conducting a subset of GWAS on putative early and late-flowering groups.

Because flowering time data were only available for 476 of the 1,307 accessions with genomic data, in the previous study we used data from the 476 to predict flowering time variation in the remaining accessions (Lasky et al. 2012) . Methods are described in greater detail in Lasky *et al*. (2012). We used data from 13 common garden experiments in different environments, all without vernalization (Table S10, Figure S4) (Shindo et al. 2005; Zhao et al. 2007; Atwell et al. 2010; Li et al. 2010; Kenney 2012) because vernalization accelerates the flowering of late-flowering putative winter annuals, which would have limited our ability to distinguish life history variation (Stinchcombe et al. 2004). We then used these empirical flowering time data to categorize accessions using a SNP-based model of flowering-time category for accessions lacking data.

For the flowering time model, we used candidate SNPs identified in the original flowering time association studies (Atwell et al. 2010; Li et al. 2010) and SNPs within 100 kb of *FRI* and *FLC,* two interacting genes in the vernalization-sensitivity pathway (Michaels and Amasino 1999; Stinchcombe et al. 2004; Zhao et al. 2007), giving 857 total SNPs as predictor variables (SNP data from Horton et al. 2012). We modeled flowering category with support vector machines (SVM), a type of classification model flexible enough to deal with interaction and non-linear effects. SVM based on predictor SNPs and empirical flowering time categories predicted a total of 765 early-flowering and 248 late-flowering accessions of the 1,003 accessions used in climate association mapping. We previously experimentally validated flowering time categories predicted using SVM and found our predictions were correct for 24 of 27 accessions (89%) previously lacking flowering time data (Lasky et al. 2012). Although there are limitations to predicting flowering time from genotype, the included experiments span a variety of environments and thus we believe our categories capture ecologically important flowering time variation.

Enrichment of genes with expression plasticity

We assessed whether genes having abiotic stress treatment effects (eSR) or accession by treatment effects (eGEI) on expression were more likely than randomly selected genes to have SNPs associated with signatures of selection, climate and fitness. In enrichment tests, we tested null hypotheses stating, in essence, that SNPs near eSR and eGEI genes had equal climate, fitness, and selection statistics as did SNPs from randomly chosen genes. We used a permutation enrichment test based on that of Segrè et al. (2010). The test compares the proportion of candidate genes (eSR or eGEI genes) having nearby SNPs with strong associations to the proportion of randomly selected genes having nearby SNPs with strong associations (Figure S3). Typically, existing methods link to a gene all SNPs within a window of a defined number of base pairs of the gene's coding region (e.g. Segrè et al. 2010; Cabrera et al. 2012). However, recombination rates and linkage disequilibrium (LD) in diverse genotype panels vary extensively across the Arabidopsis genome (Horton et al. 2012). Thus the information a SNP contains about variation in nearby genes a given distance away is highly heterogeneous across the genome. In order to account for this heterogeneity, we used an adaptive window based on local rates of LD to link SNP association signals to nearby genes (SNP data from Horton et al. 2012). We identified a window surrounding genes where the smoothed average minimum correlation between SNPs (Pearson's r) was greater than 0.3. We first averaged the maximum distance where $r > 0.3$ from each SNP with the two neighboring SNPs on either side. We then averaged those distances for all SNPs within 5 kb of the coding region of each gene to get the linkage window for each gene. If there were no SNPs within 5 kb, we set the linkage window for a gene as the average linkage distance for the nearest SNP.

In order to calculate the enrichment test statistic for climate and fitness associations, we first found the lowest association *p*-value among SNPs linked to each gene in the candidate list (eSR or eGEI genes). If a single SNP had the lowest p-value for multiple genes, then the SNP was only included once in the candidate list. Typically, >90% genes had a unique SNP having the lowest p-value for that gene (*i.e.* the low p-value SNP was not the low p-value SNP for an additional gene). Next, we calculated the 5th percentile of p-values for all SNPs of a particular association study. We then found the proportion of candidate SNP p-values falling below the 5th percentile and considered this proportion the observed enrichment test statistic. For direct comparisons of eSR and eGEI enrichments we calculated the difference between gene lists (eSR – eGEI) in their proportion of genes falling in the 5th percentile p-values.

We then created a null distribution by permuting gene classifications as eSR, eGEI or neither circularly around the genome 10,000 times in order to maintain LD patterns and the number of genes in each category (excluding the same genes that were excluded in analyses of microarrays described above). For each random gene set we calculated a test statistic in the same way as the observed test statistic. We then compared observed test statistics to permuted test statistics. Finally, we conducted a two-tailed permutation-based hypothesis test because gene lists might be biased toward many or few strong SNP associations to climate and fitness and selection statistics. To conduct the two-tailed test we determined the proportion of random sets with a test statistic in the tail beyond our observed statistic and doubled this proportion to get a two-tailed permutation p-value. This was the p-value for the null hypothesis that the stress responsive candidate genes co-occurred randomly with respect to strong SNP associations (Segrè et al. 2010).

Promoter motif polymorphisms from resequencing data

We quantified ABRE and DRE/CBF motifs across the 1000 bp promoters of the first 80 genomes of the 1001 Arabidopsis genomes project (Cao et al. 2011). Quantification of the number of motifs was conducted on the transcribed strand with custom perl scripts. Based on past studies, we counted ABRE type motifs only in the 5' to 3' orientation with the transcribed gene (Zhang et al. 2005; Maruyama et al. 2012; Fujita et al. 2013). In contrast, DRE/CBF type motifs have been show to be functional in both the 5' to 3' and 3' to 5' orientations (Geisler et al. 2006) and so we counted them in both directions.

For each gene set, we tested whether each motif showed differences in frequency among accessions or in variance of frequency compared to random genes. We tested the aggregated enrichment of gene lists for all motif variants of the same core using the test statistic of O’Brien (1984). The O’Brien (1984) method allows calculation of a non-parametric statistic for multivariate responses (here the multiple variants of each core motif). For each gene, we calculated the mean frequency and variance in frequency of the motif among of accessions (out of 80). We then ranked the mean frequency and variance in frequency of each gene and averaged across motif variants to get a statistic for each gene. For variance in frequency we only included motif-gene combinations where at least one accession had the motif. Because different motif variants differed widely in genome-wide occurrence, the number of genes with missing variance observations differed widely among motif variants. Thus for variance in frequency, we standardized ranks to mean zero and unit standard deviation for each motif variant in order to balance the influence of different motif variants. For each gene list, we then calculated an observed enrichment as the mean gene-level statistic. Observed statistics were compared to 10,000 circular permutations of gene list categories.

We also conducted tests of gene list enrichment with each motif variant individually. For these tests, we calculated observed enrichment as on raw frequency and variance in frequency data (in contrast to rank values used above) averaged across genes in the gene list. Observed statistics were compared to 10,000 circular permutations of gene list categories (results shown in Tables S7 & S9).

RESULTS

Climate association enrichments for combined flowering time categories

Among accessions of both flowering time categories combined, drought eGEI genes were most strongly associated with intra-annual CV of monthly precipitation ($z = 2.97$, $p = 0.0036$; Table S2). By contrast, drought eSR genes had a significantly low proportion of SNPs associated with mean monthly growing season precipitation ($z = -2.06$ $p = 0.0412$). Cold eGEI genes were most significantly enriched for SNP associations with the standard deviation of monthly temperature ($z = 2.12$, $p = 0.0316$; Table S2). Cold eSR genes, even more so than drought eSR genes, tended to have fewer associations to temperature climatic variables. Cold eSR genes had significantly fewer SNP associations with mean growing season temperature ($z = -2.29$, $p = 0.0224$) and monthly minimum growing season temperature compared to genomic controls ($z = -2.07$, $p = 0.0404$). In direct comparison with eGEI genes, eSR genes had tended to have fewer SNP associations with climate, significantly for SD of monthly temperature ($z = -2.58$, $p = 0.0064$), CV of monthly precipitation ($z = -2.41$, $p = 0.0166$) and CV of monthly growing season precipitation ($z = -2.04$, $p = 0.0406$, Table S2).

References

Atwell S, Huang YS, Vilhjalmsson BJ, et al. 2010. Genome-wide association study of 107 phenotypes in Arabidopsis thaliana inbred lines. Nature 465:627–631.

Cabrera CP, Navarro P, Huffman JE, et al. 2012. Uncovering Networks from Genome-Wide Association Studies via Circular Genomic Permutation. G3 GenesGenomesGenetics 2:1067–1075.

Cao J, Schneeberger K, Ossowski S, et al. 2011. Whole-genome sequencing of multiple Arabidopsis thaliana populations. Nat Genet 43:956–963.

Donohue K. 2005. Niche construction through phenological plasticity: life history dynamics and ecological consequences. New Phytol. 166:83–92.

Fujita Y, Yoshida T, Yamaguchi-Shinozaki K. 2013. Pivotal role of the AREB/ABF-SnRK2 pathway in ABRE-mediated transcription in response to osmotic stress in plants. Physiol. Plant. 147:15–27.

Geisler M, Kleczkowski LA, Karpinski S. 2006. A universal algorithm for genome-wide in silicio identification of biologically significant gene promoter putative cis-regulatory-elements; identification of new elements for reactive oxygen species and sucrose signaling in Arabidopsis. Plant J. 45:384–398.

Goslee SC, Urban DL. 2007. The ecodist package for dissimilarity-based analysis of ecological data. J. Stat. Softw. 22:1–19.

Guillot G, Rousset F. 2013. Dismantling the Mantel tests. Methods Ecol. Evol. 4:336–344.

Hancock AM, Brachi B, Faure N, Horton MW, Jarymowycz LB, Sperone FG, Toomajian C, Roux F, Bergelson J. 2011. Adaptation to Climate Across the Arabidopsis thaliana Genome. Science 334:83 –86.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. 25:1965–1978.

Horton MW, Hancock AM, Huang YS, et al. 2012. Genome-wide patterns of genetic variation in worldwide Arabidopsis thaliana accessions from the RegMap panel. Nat Genet 44:212–216.

Johnson JD, Ferrell WK. 1983. Stomatal response to vapour pressure deficit and the effect of plant water stress. Plant Cell Environ. 6:451–456.

Kalnay E, Kanamitsu M, Kistler R, et al. 1996. The NCEP/NCAR 40-Year Reanalysis Project. Bull. Am. Meteorol. Soc. 77:437–471.

Kang HM, Zaitlen NA, Wade CM, Kirby A, Heckerman D, Daly MJ, Eskin E. 2008. Efficient Control of Population Structure in Model Organism Association Mapping. Genetics 178:1709–1723.

Kenney AM. 2012. Evolutionary genetics and ecology of water use efficiency ([delta] ^{13}C) in Ipomopsis agregata and Arabidopsis thaliana.

Korves TM, Schmid KJ, Caicedo AL, Mays C, Stinchcombe JR, Purugganan MD, Schmitt J. 2007. Fitness Effects Associated with the Major Flowering Time Gene FRIGIDA in Arabidopsis thaliana in the Field. Am. Nat. 169:E141–E157.

Lasky JR, Des Marais DL, McKay JK, Richards JH, Juenger TE, Keitt TH. 2012. Characterizing genomic variation of Arabidopsis thaliana: the roles of geography and climate. Mol. Ecol. 21:5512–5529.

Li Y, Huang Y, Bergelson J, Nordborg M, Borevitz JO. 2010. Association mapping of local climate-sensitive quantitative trait loci in Arabidopsis thaliana. Proc. Natl. Acad. Sci. 107:21199–21204.

Maruyama K, Todaka D, Mizoi J, et al. 2012. Identification of Cis-Acting Promoter Elements in Cold- and Dehydration-Induced Transcriptional Pathways in Arabidopsis, Rice, and Soybean. DNA Res. 19:37–49.

McKay JK, Richards JH, Mitchell-Olds T. 2003. Genetics of drought adaptation in Arabidopsis thaliana: I. Pleiotropy contributes to genetic correlations among ecological traits. Mol. Ecol. 12:1137–1151.

Michaels SD, Amasino RM. 1999. FLOWERING LOCUS C Encodes a Novel MADS Domain Protein That Acts as a Repressor of Flowering. Plant Cell Online 11:949–956.

New M, Lister D, Hulme M, Makin I. 2002. A high-resolution data set of surface climate over global land areas. Clim. Res. 21:1–25.

O’Brien PC. 1984. Procedures for Comparing Samples with Multiple Endpoints. Biometrics 40:1079–1087.

Raufaste N, Rousset F. 2001. Are partial Mantel tests adequate? Evolution 55:1703–1705.

Segrè AV, Groop L, Mootha VK, Daly MJ, Altshuler D, DIAGRAM Consortium, MAGIC investigators. 2010. Common Inherited Variation in Mitochondrial Genes Is Not Enriched for Associations with Type 2 Diabetes or Related Glycemic Traits. PLoS Genet 6:e1001058.

Shindo C, Aranzana MJ, Lister C, Baxter C, Nicholls C, Nordborg M, Dean C. 2005. Role of FRIGIDA and FLOWERING LOCUS C in Determining Variation in Flowering Time of Arabidopsis. Plant Physiol 138:1163–1173.

Stinchcombe JR, Weinig C, Ungerer M, Olsen KM, Mays C, Halldorsdottir SS, Purugganan MD, Schmitt J. 2004. A latitudinal cline in flowering time in Arabidopsis thaliana modulated by the flowering time gene FRIGIDA. Proc. Natl. Acad. Sci. U. S. A. 101:4712 –4717.

Walter H, Lieth H. 1960. Klimadiagramm-Weltatlas. Gustav Fisch. Jena 8000.

Yoder JB, Stanton-Geddes J, Zhou P, Briskine R, Young ND, Tiffin P. 2014. Genomic Signature of Adaptation to Climate in Medicago truncatula. Genetics [Internet]. Available from: http://www.genetics.org/content/early/2014/01/15/genetics.113.159319.abstract

Zhang W, Ruan J, Ho TD, You Y, Yu T, Quatrano RS. 2005. Cis-regulatory element based targeted gene finding: genome-wide identification of abscisic acid- and abiotic stress-responsive genes in Arabidopsis thaliana. Bioinformatics 21:3074–3081.

Zhao K, Aranzana MJ, Kim S, et al. 2007. An Arabidopsis Example of Association Mapping in Structured Samples. PLoS Genet 3:e4.

Tables

Table S1. Results for enrichment tests with selection statistics. Enrichments (frequency of genes in list having SNPs in the 5th percentile of test selection statistics) are calculated as a *z*-score using the distribution from null permutations. Gene lists with significant enrichment of selection statistics are shown in bold. Tests were not stratified by flowering time because selection statistics were calculated on a panel with both types [2].

Selection statistic	*Gene list*	*Abiotic stress*	*Mean (enrichment z-score)*	*Permutation test p*
PHS	eSR	Cold	2.38	**0.0258**
	eSR	Drought	2.57	**0.0198**
	eGEI	Cold	-1.42	0.1526
	eGEI	Drought	-0.21	0.8384
	eSR - eGEI	Cold	2.38	**0.0120**
	eSR - eGEI	Drought	1.13	0.2596
CLR	eSR	Cold	0.86	0.3938
	eSR	Drought	1.14	0.2518
	eGEI	Cold	-0.41	0.6720
	eGEI	Drought	0.31	0.7520
	eSR - eGEI	Cold	0.71	0.4766
	eSR - eGEI	Drought	0.02	0.9914
F_{st}	eSR	Cold	0.54	0.5928
	eSR	Drought	0.29	0.7652
	eGEI	Cold	-1.16	0.2402
	eGEI	Drought	2.46	**0.0142**
	eSR - eGEI	Cold	1.37	0.1692
	eSR - eGEI	Drought	-2.30	**0.0258**
MAF	eSR	Cold	-4.18	**<0.0002**
	eSR	Drought	-2.43	**0.0168**
	eGEI	Cold	1.66	0.1002
	eGEI	Drought	1.96	**0.0434**
	eSR - eGEI	Cold	-3.10	**0.0022**
	eSR - eGEI	Drought	-2.62	**0.0106**

Table S2. Results for enrichment tests with climate associations. The enrichment of candidate gene sets with associations to cold-related climate variables. Enrichments (frequency of genes in list having SNPs in the 5th percentile for *p*-values) are calculated as a *z*-score using the distribution from null permutations. Gene lists with significant enrichment of climate variables are shown in bold.

Accessions	*Climate variable*	*Gene list*	*Abiotic stress*	*Frequency of significant SNPs z-score*	*Permutation test p*
All	Isothermality	eSR	Cold	-0.044	0.9516
All	SD monthly temperature	eSR	Cold	-1.401	0.1650
All	Minimum temperature of coldest month	eSR	Cold	-0.866	0.3882
All	Temperature annual range	eSR	Cold	-0.597	0.5534
All	Mean growing season temperature	eSR	Cold	-2.287	**0.0224**
All	Mean monthly minimum growing season temperature	eSR	Cold	-2.073	**0.0404**
All	CV monthly precipitation	eSR	Drought	1.607	0.1170
All	Mean monthly growing season precipitation	eSR	Drought	-2.061	**0.0412**
All	CV monthly growing season precipitation	eSR	Drought	-0.549	0.5736
All	Mean growing season VPD	eSR	Drought	-0.063	0.9298
All	Inter-annual CV of growing season precipitation	eSR	Drought	-0.107	0.9234
All	Isothermality	eGEI	Cold	-1.412	0.1538
All	SD monthly temperature	eGEI	Cold	2.123	**0.0316**
All	Minimum temperature of coldest month	eGEI	Cold	0.615	0.5372
All	Temperature annual range	eGEI	Cold	1.598	0.1132
All	Mean growing season temperature	eGEI	Cold	0.444	0.6474
All	Mean monthly minimum growing season temperature	eGEI	Cold	-0.641	0.5178
All	CV monthly precipitation	eGEI	Drought	2.968	**0.0036**
All	Mean monthly growing season precipitation	eGEI	Drought	-0.447	0.6494
All	CV monthly growing season precipitation	eGEI	Drought	1.932	0.0534
All	Mean growing season VPD	eGEI	Drought	-0.112	0.9238
All	Inter-annual CV of growing season precipitation	eGEI	Drought	1.275	0.1972
All	Isothermality	eSR - eGEI	Cold	1.389	0.1666
All	SD monthly temperature	eSR - eGEI	Cold	-2.580	**0.0064**
All	Minimum temperature of coldest month	eSR - eGEI	Cold	-0.901	0.3702
All	Temperature annual range	eSR - eGEI	Cold	-1.786	0.0750
All	Mean growing season temperature	eSR - eGEI	Cold	-1.218	0.2200
All	Mean monthly minimum growing season temperature	eSR - eGEI	Cold	-0.071	0.9300
All	CV monthly precipitation	eSR - eGEI	Drought	-2.410	**0.0166**
All	Mean monthly growing season precipitation	eSR - eGEI	Drought	-0.137	0.8940
All	CV monthly growing season precipitation	eSR - eGEI	Drought	-2.042	**0.0406**
All	Mean growing season VPD	eSR - eGEI	Drought	0.091	0.9428
All	Inter-annual CV of growing season precipitation	eSR - eGEI	Drought	-1.271	0.1982
Early-flowering	Isothermality	eSR	Cold	-2.362	**0.0168**
Early-flowering	SD monthly temperature	eSR	Cold	-2.311	**0.0198**

Early-flowering	Minimum temperature of coldest month	eSR	Cold	-2.661	**0.0080**
Early-flowering	Temperature annual range	eSR	Cold	-1.884	0.0622
Early-flowering	Mean growing season temperature	eSR	Cold	-3.176	**0.0010**
Early-flowering	Mean monthly minimum growing season temperature	eSR	Cold	-3.317	**0.0012**
Early-flowering	CV monthly precipitation	eSR	Drought	-1.115	0.2654
Early-flowering	Mean monthly growing season precipitation	eSR	Drought	-1.120	0.2664
Early-flowering	CV monthly growing season precipitation	eSR	Drought	-1.807	0.0760
Early-flowering	Mean growing season VPD	eSR	Drought	-0.289	0.7792
Early-flowering	Inter-annual CV of growing season precipitation	eSR	Drought	-1.760	0.0738
Early-flowering	Isothermality	eGEI	Cold	2.123	**0.0336**
Early-flowering	SD monthly temperature	eGEI	Cold	1.499	0.1296
Early-flowering	Minimum temperature of coldest month	eGEI	Cold	1.629	0.1034
Early-flowering	Temperature annual range	eGEI	Cold	1.878	0.0630
Early-flowering	Mean growing season temperature	eGEI	Cold	1.890	0.0602
Early-flowering	Mean monthly minimum growing season temperature	eGEI	Cold	2.320	**0.0192**
Early-flowering	CV monthly precipitation	eGEI	Drought	1.757	0.0756
Early-flowering	Mean monthly growing season precipitation	eGEI	Drought	-0.639	0.5254
Early-flowering	CV monthly growing season precipitation	eGEI	Drought	2.597	**0.0146**
Early-flowering	Mean growing season VPD	eGEI	Drought	0.371	0.7124
Early-flowering	Inter-annual CV of growing season precipitation	eGEI	Drought	2.283	**0.0208**
Early-flowering	Isothermality	eSR - eGEI	Cold	-2.612	**0.0074**
Early-flowering	SD monthly temperature	eSR - eGEI	Cold	-1.972	**0.0482**
Early-flowering	Minimum temperature of coldest month	eSR - eGEI	Cold	-2.186	**0.0282**
Early-flowering	Temperature annual range	eSR - eGEI	Cold	-2.257	**0.0264**
Early-flowering	Mean growing season temperature	eSR - eGEI	Cold	-2.546	**0.0080**
Early-flowering	Mean monthly minimum growing season temperature	eSR - eGEI	Cold	-3.024	**0.0022**
Early-flowering	CV monthly precipitation	eSR - eGEI	Drought	-2.020	**0.0438**
Early-flowering	Mean monthly growing season precipitation	eSR - eGEI	Drought	0.287	0.7792
Early-flowering	CV monthly growing season precipitation	eSR - eGEI	Drought	-3.062	**0.0024**
Early-flowering	Mean growing season VPD	eSR - eGEI	Drought	-0.443	0.6520
Early-flowering	Inter-annual CV of growing season precipitation	eSR - eGEI	Drought	-2.721	**0.0072**
Late-flowering	Isothermality	eSR	Cold	0.563	0.5738
Late-flowering	SD monthly temperature	eSR	Cold	-0.192	0.8664
Late-flowering	Minimum temperature of coldest month	eSR	Cold	1.362	0.1750
Late-flowering	Temperature annual range	eSR	Cold	-0.463	0.6390
Late-flowering	Mean growing season temperature	eSR	Cold	-2.437	**0.0138**
Late-flowering	Mean monthly minimum growing season temperature	eSR	Cold	-0.692	0.5016
Late-flowering	CV monthly precipitation	eSR	Drought	0.575	0.5682
Late-flowering	Mean monthly growing season precipitation	eSR	Drought	-1.838	0.0668
Late-flowering	CV monthly growing season precipitation	eSR	Drought	0.043	0.9560
Late-flowering	Mean growing season VPD	eSR	Drought	0.506	0.6264
Late-flowering	Inter-annual CV of growing season precipitation	eSR	Drought	-1.466	0.1500
Late-flowering	Isothermality	eGEI	Cold	0.865	0.3902
Late-flowering	SD monthly temperature	eGEI	Cold	1.692	0.1002
Late-flowering	Minimum temperature of coldest month	eGEI	Cold	1.501	0.1444
Late-flowering	Temperature annual range	eGEI	Cold	0.080	0.9276

Late-flowering	Mean growing season temperature	eGEI	Cold	-0.675	0.5098
Late-flowering	Mean monthly minimum growing season temperature	eGEI	Cold	-1.024	0.3058
Late-flowering	CV monthly precipitation	eGEI	Drought	2.328	**0.0182**
Late-flowering	Mean monthly growing season precipitation	eGEI	Drought	1.345	0.1792
Late-flowering	CV monthly growing season precipitation	eGEI	Drought	1.629	0.1070
Late-flowering	Mean growing season VPD	eGEI	Drought	1.750	0.0800
Late-flowering	Inter-annual CV of growing season precipitation	eGEI	Drought	0.638	0.5278
Late-flowering	Isothermality	eSR - eGEI	Cold	-0.693	0.4934
Late-flowering	SD monthly temperature	eSR - eGEI	Cold	-1.762	0.0822
Late-flowering	Minimum temperature of coldest month	eSR - eGEI	Cold	-1.085	0.2754
Late-flowering	Temperature annual range	eSR - eGEI	Cold	-0.225	0.8264
Late-flowering	Mean growing season temperature	eSR - eGEI	Cold	-0.086	0.9318
Late-flowering	Mean monthly minimum growing season temperature	eSR - eGEI	Cold	0.826	0.4122
Late-flowering	CV monthly precipitation	eSR - eGEI	Drought	-2.161	**0.0304**
Late-flowering	Mean monthly growing season precipitation	eSR - eGEI	Drought	-1.777	0.0796
Late-flowering	CV monthly growing season precipitation	eSR - eGEI	Drought	-1.599	0.1118
Late-flowering	Mean growing season VPD	eSR - eGEI	Drought	-1.583	0.1110
Late-flowering	Inter-annual CV of growing season precipitation	eSR - eGEI	Drought	-0.971	0.3320

Table S3. Results for enrichment tests with fitness associations. Enrichments (frequency of genes in list having SNPs in the 5th percentile for *p*-values) are calculated as a *z*-score using the distribution from null permutations. Gene lists with significant enrichment of fitness variables are shown in bold. Tests were not stratified by flowering time because of limited sample size in fitness experiments.

Fitness component	*Location*	*Abiotic stress*	*Gene list*	*Frequency of significant SNPs z-score*	*Permutation test p*
Survival	Finland	eGEI	cold	0.20	0.8270
Survival	Finland	eGEI	drought	0.83	0.4052
Survival	Germany	eGEI	cold	-0.56	0.5888
Survival	Germany	eGEI	drought	0.50	0.6102
Survival	Spain	eGEI	cold	1.07	0.2880
Survival	Spain	eGEI	drought	0.95	0.3414
Survival	UK	eGEI	cold	-1.36	0.1712
Survival	UK	eGEI	drought	-1.03	0.3028
Survival	Finland	eSR	cold	-1.49	0.1382
Survival	Finland	eSR	drought	0.03	0.9692
Survival	Germany	eSR	cold	-1.40	0.1604
Survival	Germany	eSR	drought	-0.06	0.9422
Survival	Spain	eSR	cold	-3.67	**0.0008**
Survival	Spain	eSR	drought	-1.60	0.1066
Survival	UK	eSR	cold	-2.92	**0.0046**
Survival	UK	eSR	drought	-2.08	**0.0360**
Survival	Finland	eSR - eGEI	cold	-0.70	0.4754
Survival	Finland	eSR - eGEI	drought	-0.80	0.4112
Survival	Germany	eSR - eGEI	cold	0.08	0.9326
Survival	Germany	eSR - eGEI	drought	-0.50	0.6026
Survival	Spain	eSR - eGEI	cold	-2.36	**0.0202**
Survival	Spain	eSR - eGEI	drought	-1.39	0.1638
Survival	UK	eSR - eGEI	cold	0.36	0.7152
Survival	UK	eSR - eGEI	drought	0.41	0.6794
Silique N	Finland	eGEI	cold	0.49	0.6178
Silique N	Finland	eGEI	drought	0.60	0.5478
Silique N	Germany	eGEI	cold	1.05	0.2924
Silique N	Germany	eGEI	drought	-0.16	0.8698
Silique N	Spain	eGEI	cold	0.50	0.6226
Silique N	Spain	eGEI	drought	-0.80	0.4258
Silique N	UK	eGEI	cold	0.09	0.9384

Silique N	UK	eGEI	drought	-1.51	0.1282
Silique N	Finland	eSR	cold	-2.19	**0.0338**
Silique N	Finland	eSR	drought	-0.76	0.4478
Silique N	Germany	eSR	cold	-0.12	0.9052
Silique N	Germany	eSR	drought	-0.36	0.7028
Silique N	Spain	eSR	cold	-1.13	0.2590
Silique N	Spain	eSR	drought	-0.74	0.4630
Silique N	UK	eSR	cold	-1.42	0.1556
Silique N	UK	eSR	drought	-1.11	0.2626
Silique N	Finland	eSR - eGEI	cold	-1.24	0.2232
Silique N	Finland	eSR - eGEI	drought	-0.79	0.4336
Silique N	Germany	eSR - eGEI	cold	-1.09	0.2784
Silique N	Germany	eSR - eGEI	drought	0.06	0.9570
Silique N	Spain	eSR - eGEI	cold	-0.87	0.3738
Silique N	Spain	eSR - eGEI	drought	0.58	0.5632
Silique N	UK	eSR - eGEI	cold	-0.57	0.5708
Silique N	UK	eSR - eGEI	drought	1.15	0.2506

Table S4. Results for enrichment tests with selection statistics based on resequencing of 80 accessions. The mean statistic for genes in each gene list, standardized to the null permutations, is shown as “Mean (z-score).” Gene lists with significant enrichment of selection statistics are shown in bold. Tests were conducted without stratifying by flowering time because resequencing data were from too few accessions.

Statistic	*Gene list*	*Abiotic stress*	*Mean (enrichment z-score)*	*Permutation test p*
K_a/K_s	eSR	Drought	-15.76	**<0.0002**
	eSR	Cold	-16.35	**<0.0002**
	eGEI	Drought	-0.74	0.4651
	eGEI	Cold	-4.76	**<0.0002**
	eSR - eGEI	Drought	-3.93	**0.0004**
	eSR - eGEI	Cold	-1.03	0.3013
Promoter θ	eSR	Drought	-3.560	**0.0066**
	eSR	Cold	-3.300	**0.0329**
	eGEI	Drought	2.190	**0.0248**
	eGEI	Cold	1.580	0.1181
	eSR - eGEI	Drought	-3.580	**0.0042**
	eSR - eGEI	Cold	-3.320	**0.0043**

Table S5. [attached .xls file] Table of accessions included in the various data sets analyzed in this study.

Table S6. Enrichment of gene lists for mean rank of motif counts (O'Brien 1984). The mean statistic for genes in each gene list, standardized to the null permutations, is shown as a z-score. For example, a z-score > 0 indicates the motif is more common in the gene list compared to random genes. Gene lists with a significant motif frequency are shown in bold. DRE/CBFs are read both forward and reverse (2d) or only one direction at a time (1d).

Motif	*Treatment*	*Mean eSR Z*	*Mean eSR p*	*Mean eGEI Z*	*Mean eGEI p*	*Mean eSR - Mean eGEI Z*	*Mean eSR - Mean eGEI p*
ABRE	drought	5.86	**<0.0002**	0.48	0.6314	1.36	0.1754
ABRE	cold	1.91	0.0570	12.55	**<0.0002**	-2.89	**0.0044**
DRE/CBF 2d	drought	2.87	**0.0038**	-1.69	0.0812	2.50	**0.0124**
DRE/CBF 2d	cold	1.10	0.2682	3.00	**0.0010**	-0.05	0.9722
DRE/CBF 1d	drought	3.24	**0.0012**	-0.81	0.4278	1.75	0.0748
DRE/CBF 1d	cold	1.28	0.2108	1.24	0.2118	0.77	0.4370

Table S7. The enrichment of specific motifs in the promoters of genes with transcriptional plasticity. The mean statistic for genes in each gene list, standardized to the null permutations, is shown as a z-score. For example, a z-score > 0 indicates the motif is more common in the gene list compared to random genes. Gene lists with a significant motif frequency are shown in bold. Core motifs are shown in bold. Gene lists with a significant motif frequency are shown in bold. DRE/CBFs are read both forward and reverse (2 directions) or only one direction at a time (F-forward or R-reverse).

Motif	*Treatment*	*Mean eSR Z*	*Mean eSR p*	*Mean eGEI Z*	*Mean eGEI p*	*Mean eSR - Mean eGEI Z*	*Mean eSR - Mean eGEI p*
ABREs							
ACGT	drought	4.34	**0.0000**	0.42	0.6826	0.94	0.3602
ACGT	cold	1.43	0.1516	9.53	**0.0000**	-2.25	**0.0236**
ACACGTGG	drought	4.40	**0.0000**	0.49	0.5994	0.86	0.4034
ACACGTGG	cold	1.20	0.2278	1.99	**0.0496**	0.42	0.6768
ACGTG	drought	6.09	**0.0000**	0.32	0.7468	1.56	0.1224
ACGTG	cold	2.21	**0.0318**	12.80	**0.0000**	-2.68	**0.0072**
ACGTGG	drought	4.80	**0.0000**	-0.66	0.5254	2.10	**0.0300**
ACGTGG	cold	1.84	0.0698	8.16	**0.0000**	-1.32	0.1840
ACGTGT	drought	4.42	**0.0000**	0.78	0.4300	0.57	0.5628
ACGTGT	cold	1.46	0.1460	9.18	**0.0000**	-1.93	0.0532
CACGTG Gbox	drought	7.14	**0.0000**	-0.10	0.9252	2.28	**0.0202**
CACGTG Gbox	cold	2.47	**0.0156**	10.70	**0.0000**	-1.54	0.1176
CACGTT TGbox	drought	-0.43	0.6742	-0.52	0.6108	0.38	0.7146
CACGTT TGbox	cold	-1.16	0.2444	0.00	0.9916	-1.12	0.2648
CCACGTGG	drought	3.53	**0.0006**	0.56	0.5578	0.52	0.6110
CCACGTGG	cold	1.09	0.2826	8.89	**0.0000**	-2.27	**0.0184**
DRE/CBFs 2 directions							
GCCGAC CAGCCG	drought	3.24	**0.0012**	-1.88	0.0596	2.80	**0.0032**
GCCGAC CAGCCG	cold	1.55	0.1178	2.97	**0.0024**	0.40	0.6906
AGCCGAC CAGCCGA	drought	2.74	**0.0068**	-0.80	0.4338	1.61	0.0892
AGCCGAC CAGCCGA	cold	0.70	0.4834	2.85	**0.0060**	-0.36	0.7304
GGCCGAC CAGCCGG	drought	-0.42	0.6836	-1.04	0.3010	0.89	0.3782
GGCCGAC CAGCCGG	cold	0.69	0.4754	-0.14	0.8922	0.72	0.4624
DRE/CBFs 1 direction							
GCCGAC F	drought	3.28	**0.0004**	-1.89	0.0526	2.82	**0.0014**
GCCGAC F	cold	1.54	0.1252	2.96	**0.0028**	0.40	0.7006
CAGCCG R	drought	1.66	0.0912	0.68	0.4868	-0.17	0.8594
CAGCCG R	cold	1.10	0.2746	-4.71	**0.0000**	2.79	**0.0066**

AGCCGAC	F	drought	2.75	**0.0070**	-0.82	0.4248	1.61	0.0916
AGCCGAC	F	cold	0.69	0.4924	2.85	**0.0056**	-0.38	0.7252
CAGCCGA	R	drought	1.75	0.0780	-1.61	0.0926	2.09	**0.0262**
CAGCCGA	R	cold	0.48	0.6102	2.51	**0.0130**	-0.45	0.6608
CAGCCGG	R	drought	0.62	0.5320	1.76	0.0908	-1.53	0.1330
CAGCCGG	R	cold	0.20	0.8102	-1.41	0.1578	0.71	0.4726
GGCCGAC	F	drought	-0.42	0.6708	-1.04	0.3016	0.89	0.3832
GGCCGAC	F	cold	0.71	0.4680	-0.15	0.8916	0.73	0.4492

Table S8. Enrichment of gene lists for mean rank of variance in motif frequency among accessions. The mean statistic for genes in each gene list, standardized to the null permutations, is shown as a z-score. For example, a z-score > 0 indicates that genes having the motif have higher variance in motif frequency among accessions than expected. Gene lists with a significant motif frequency are shown in bold. Gene lists with a significant motif frequency are shown in bold. DRE/CBFs are read both forward and reverse (2d) or only one direction at a time (1d).

Motif	*Treatment*	*Var. eSR Z*	*Var. eSR p*	*Var. eGEI Z*	*Var. eGEI p*	*Var. eSR - Var. eGEI Z*	*Var. eSR - Var. eGEI p*
ABRE	drought	-7.08	**<0.0002**	2.16	**0.0336**	-4.48	**<0.0002**
ABRE	cold	-3.38	**0.0006**	-0.64	0.5048	-2.93	**0.0040**
DRE/CBF 2d	drought	-1.13	0.2602	-1.42	0.1604	1.05	0.2920
DRE/CBF 2d	cold	-1.02	0.3154	-2.21	**0.0270**	-0.18	0.8542
DRE/CBF 1d	drought	-1.22	0.2192	-1.63	0.1008	1.22	0.2228
DRE/CBF 1d	cold	-1.21	0.2352	-0.61	0.5260	-0.94	0.3414

Table S9. Variance among accessions in the count of motifs. The mean statistic for genes in each gene list, standardized to the null permutations, is shown as a z-score. For example, a z-score > 0 indicates that genes having the motif have higher variance in motif frequency among accessions than expected. Gene lists with a significant motif frequency are shown in bold. Core motifs are shown in bold. Gene lists with a significant motif frequency are shown in bold. DRE/CBFs are read both forward and reverse (2 directions) or only one direction at a time (F-forward or R-reverse).

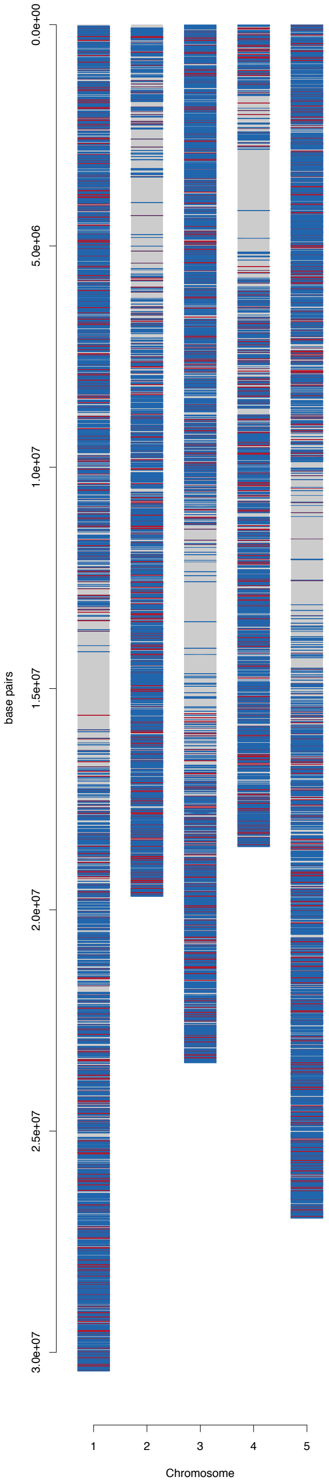
Motif	*Treatment*	*Var. eSR Z*	*Var. eSR p*	*Var. eGEI Z*	*Var. eGEI p*	*Var. eSR - Var. eGEI Z*	*Var. eSR - Var. eGEI p*
ABREs							
ACGT	drought	-5.11	**0.0000**	1.38	0.1800	-2.94	**0.0060**
ACGT	cold	-2.23	**0.0164**	3.18	**0.0012**	-3.39	**0.0002**
ACACGTGG	drought	-0.85	0.3996	2.25	**0.0294**	-2.43	**0.0194**
ACACGTGG	cold	-0.53	0.6140	-2.96	**0.0034**	0.55	0.5662
ACGTG	drought	-5.62	**0.0000**	-0.02	0.9886	-1.73	0.0974
ACGTG	cold	-2.14	**0.0240**	0.38	0.7190	-2.19	**0.0242**
ACGTGG	drought	-2.17	**0.0258**	-0.11	0.9406	-0.56	0.5646
ACGTGG	cold	-1.52	0.1082	-2.37	**0.0126**	-0.57	0.5882
ACGTGT	drought	-5.27	**0.0000**	0.33	0.7224	-1.89	0.0646
ACGTGT	cold	-1.84	0.0552	0.87	0.3668	-2.10	**0.0292**
CACGTG Gbox	drought	-2.92	**0.0032**	-0.24	0.8180	-0.63	0.5030
CACGTG Gbox	cold	-0.90	0.3624	-2.61	**0.0056**	0.10	0.8848
CACGTT TGbox	drought	-3.16	**0.0010**	3.43	**0.0072**	-4.29	**0.0014**
CACGTT TGbox	cold	-1.21	0.1942	-0.63	0.5492	-0.93	0.3494
CCACGTGG	drought	-0.16	0.8782	-0.55	0.6134	0.49	0.6476
CCACGTGG	cold	-0.62	0.5436	-2.64	**0.0062**	0.35	0.7016
DRE/CBFs 2 directions							
GCCGAC CAGCCG	drought	-0.70	0.4802	-1.90	0.0532	1.64	0.0972
GCCGAC CAGCCG	cold	-0.81	0.4248	-1.66	0.0992	-0.17	0.8694
AGCCGAC CAGCCGA	drought	-0.45	0.6556	-1.32	0.1548	1.17	0.2280
AGCCGAC CAGCCGA	cold	-0.26	0.8270	-2.42	**0.0120**	0.62	0.5240
GGCCGAC CAGCCGG	drought	-0.11	0.9070	-0.44	0.6736	0.40	0.7152
GGCCGAC CAGCCGG	cold	-0.47	0.6544	0.34	0.7248	-0.57	0.5762
DRE/CBFs 1 direction							
GCCGAC F	drought	-0.69	0.4912	-1.92	**0.0448**	1.66	0.0888
GCCGAC F	cold	-0.83	0.4154	-1.66	0.0944	-0.18	0.8630

CAGCCG R	drought	-1.64	0.0972	-1.63	0.0770	1.08	0.2600
CAGCCG R	cold	-0.74	0.4748	0.06	0.9544	-0.74	0.4644
AGCCGAC F	drought	-0.43	0.6600	-1.33	0.1566	1.18	0.2296
AGCCGAC F	cold	-0.27	0.8110	-2.42	**0.0144**	0.62	0.5222
CAGCCGA R	drought	-1.81	0.0706	-2.94	**0.0010**	2.35	**0.0094**
CAGCCGA R	cold	-0.26	0.8228	-0.96	0.3280	0.09	0.9092
CAGCCGG R	drought	-1.19	0.2412	-0.79	0.4114	0.44	0.7154
CAGCCGG R	cold	-0.33	0.8080	0.57	0.5834	-0.52	0.6370
GGCCGAC F	drought	-0.12	0.9064	-0.45	0.6656	0.41	0.7034
GGCCGAC F	cold	-0.47	0.6624	0.33	0.7292	-0.57	0.5808

Table S10. Flowering time experiments used to fit a SNP-based model of flowering time category.

Reference	**Photoperiod (hrs)**	**Natural light conditions**	**Temp. (^{o}C)**	*N* accessions	**Notes**
Zhao et al. 2007	16	n/a	18	167	
Zhao et al. 2007	8	n/a	18	162	
Atwell et al. 2010	16	n/a	10	194	
Atwell et al. 2010	16	n/a	16	193	
Atwell et al. 2010	16	n/a	22	193	
Zhao et al. 2007	16	n/a	23	137	
Shindo et al. 2005	n/a	52°37' N, Oct. – March	20-22	153	
Atwell et al. 2010	16	n/a	20	166	
Li et al. 2010	n/a	41°43' N, March.-July	5-27	445	simulated natural day length and temperature
Li et al. 2010	n/a	55°43' N, May – Sep.	5-21	445	simulated natural day length and temperature
Li et al. 2010	n/a	41°43' N, Apr. – Sep.	7-28	445	simulated natural day length and temperature
Li et al. 2010	n/a	55°43' N, June – Nov.	5-21	445	simulated natural day length and temperature
Kenney et al. *In prep.*	16	n/a	18-22	205	drought stress applied to half of individuals

Figure S1. Coding regions of eSR (blue) and eGEI (red) genes from a cold-acclimation experiment (Hannah et al. 2006) mapped across the genome. Genes that are both eSR and eGEI are shown in purple. A small border is added to each gene to improve visibility.



0.0e+00
5.0e+06
1.0e+07
1.5e+07
2.0e+07
2.5e+07
3.0e+07
base pairs
1
2
3
4
5
Chromosome

Figure S2. Coding regions of eSR (blue) and eGEI (red) genes from a drought experiment (Des Marais et al. 2012) mapped across the genome. Genes that are both eSR and eGEI are shown in purple. A small border is added to each gene to improve visibility.

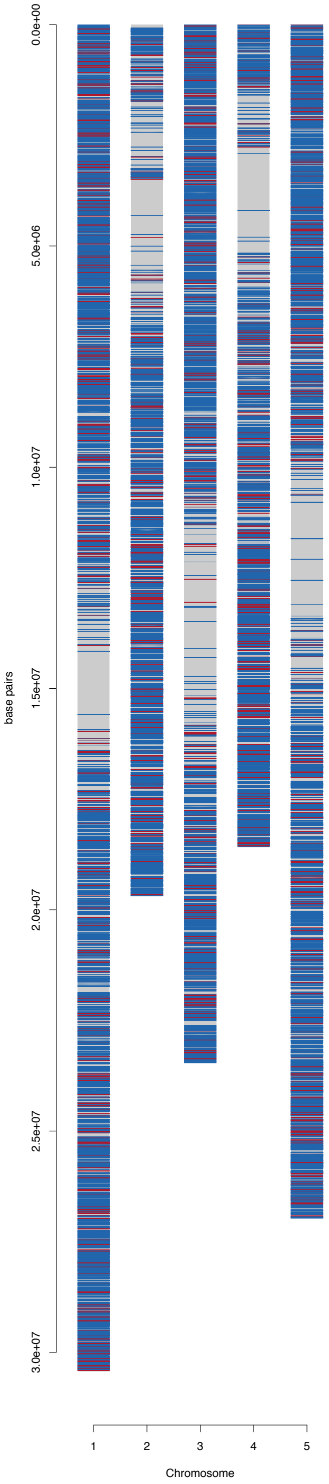

0.0e+00
5.0e+06
1.0e+07
1.5e+07
2.0e+07
2.5e+07
3.0e+07
base pairs
1
2
3
4
5
Chromosome

Figure S3. Schematic of permutation tests for enrichment. Representation of permutation tests for enrichment of eSR and eGEI gene sets with SNP associations to climate, fitness, or selection statistics. 1. GWAS statistics for SNPs (red) within a window where a moving average of correlation *r* among SNP state is > 3 are identified. 2. The observed enrichment test statistic is calculated as the proportion of genes in that set (step 1) that have a SNP with a climate, fitness, or selection statistic in the 0.05 tail of lowest *p*-values. 3. This proportion is then compared to a null distribution generated from randomly permuted gene sets with the same number of genes as the observed set.

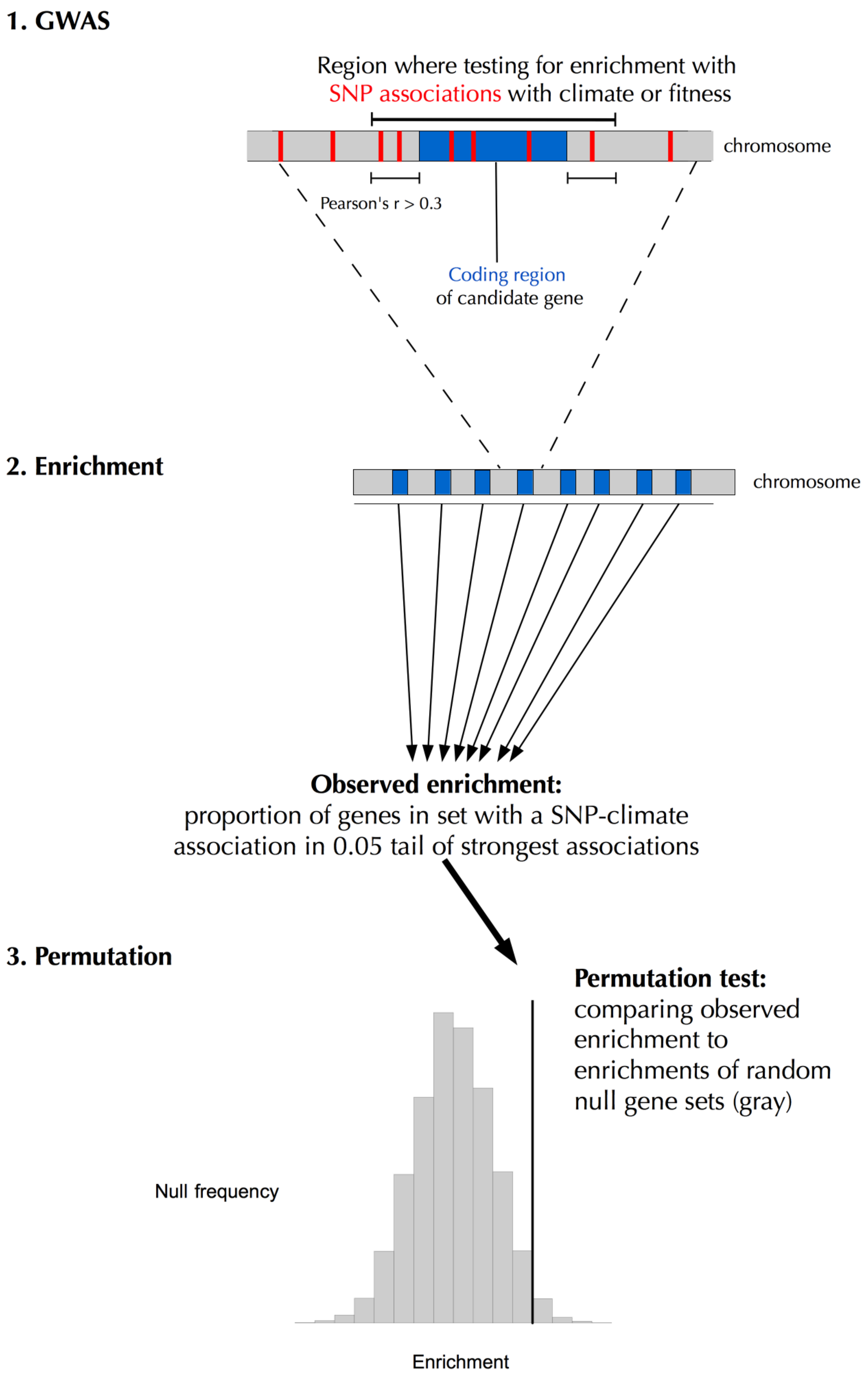

1. GWAS
Region where testing for enrichment with
SNP associations with climate or fitness
chromosome
Pearson's r > 0.3
Coding region
of candidate gene
2. Enrichment
chromosome
Observed enrichment:
proportion of genes in set with a SNP-climate
association in 0.05 tail of strongest associations
3. Permutation
Permutation test:
comparing observed
enrichment to
enrichments of random
null gene sets (gray)
Null frequency
Enrichment

Figure S4. Reaction norm of flowering time across 13 published experiments (Table S10). Lines connect accessions and open circles represent accessions not present in the adjoining experiments in the figure. Green accessions are those classified as late-flowering and blue are early-flowering. Closed black circles represent cluster means used to define early versus late-flowering groups in k-means clustering conducted on accessions present in all experiments.

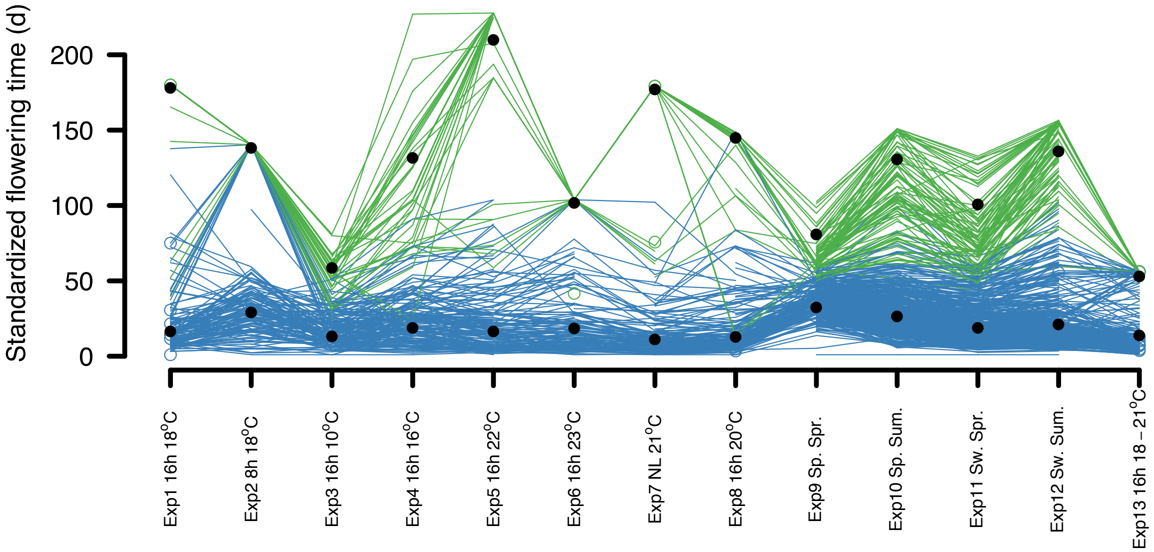


Figure S5. The enrichment of candidate gene sets with associations to fitness variables. Observed enrichments are calculated as a z-score using the distribution from null permutations. Enrichment of eSR genes is shown in blue, while eGEI genes are shown in red. Null permutations are shown as small gray dots ('°' $p < 0.1$, '*' $p < 0.05$, '**' $p < 0.01$, '***' $p < 0.005$).

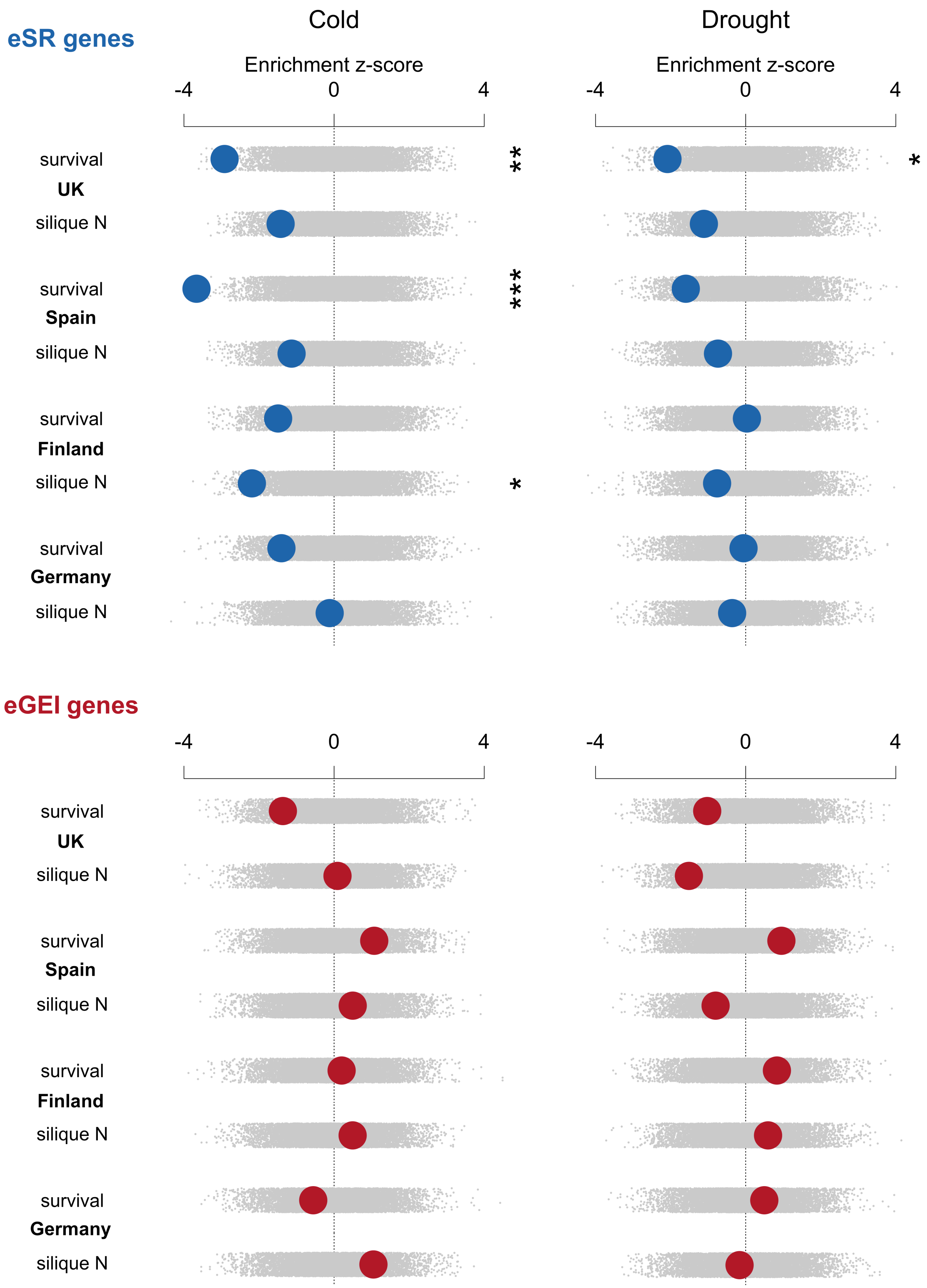

eSR genes
Cold
Drought
Enrichment z-score
Enrichment z-score
-4
0
4
-4
0
4
survival
UK
silique N
survival
Spain
silique N
survival
Finland
silique N
survival
Germany
silique N
**
*
***
*
eGEI genes
-4
0
4
-4
0
4
survival
UK
silique N
survival
Spain
silique N
survival
Finland
silique N
survival
Germany
silique N

Figure S6. Quantile-quantile plots for climate association *t*-statistics for early-flowering accessions. Observed distributions (y-axis) are compared with a theoretical normal distribution (x-axis).

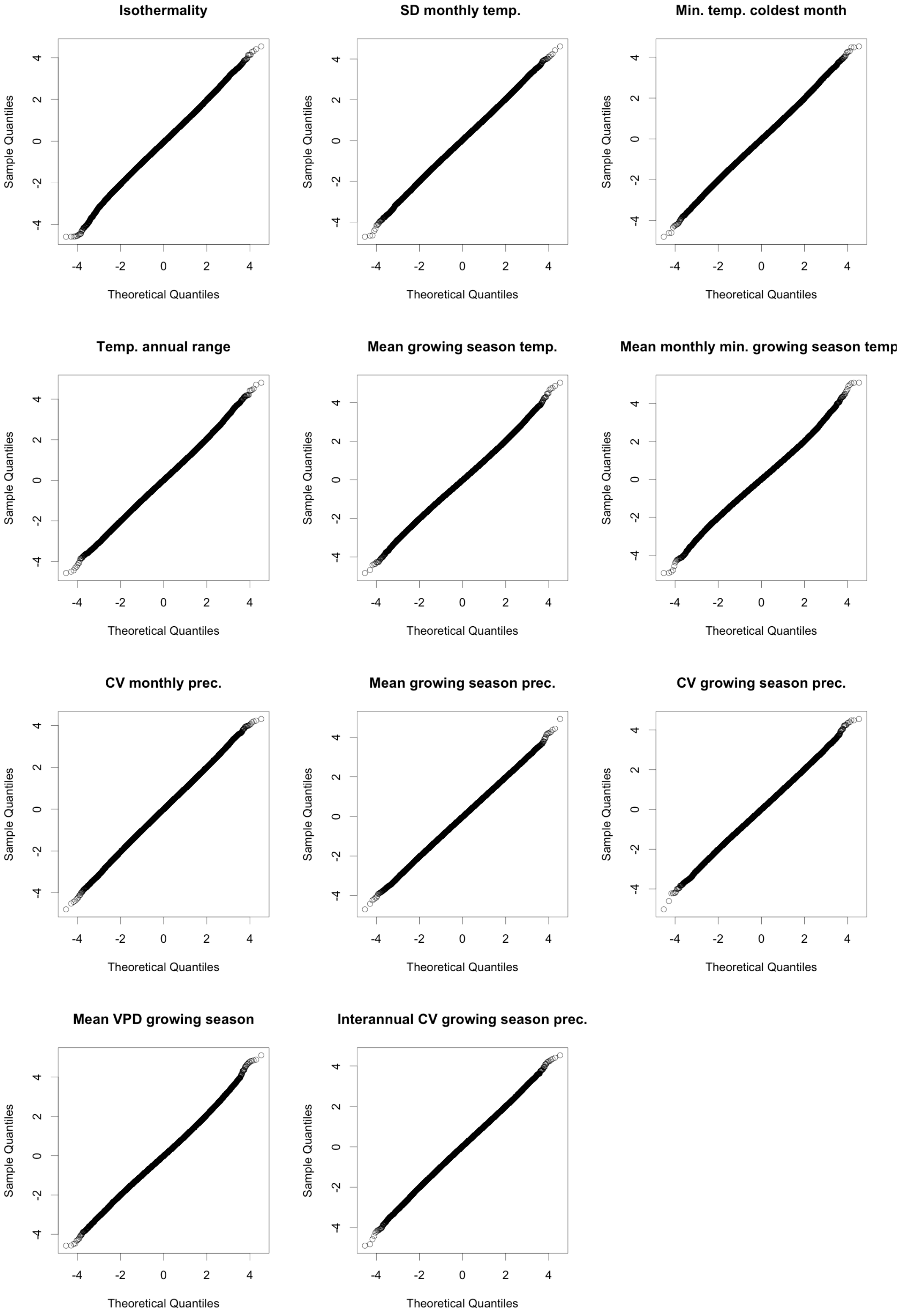

Isothermality
SD monthly temp.
Min. temp. coldest month
Temp. annual range
Mean growing season temp.
Mean monthly min. growing season temp
CV monthly prec.
Mean growing season prec.
CV growing season prec.
Mean VPD growing season
Interannual CV growing season prec.
Sample Quantiles
Theoretical Quantiles
-4
-2
0
2
4

Figure S7. Quantile-quantile plots for climate association *t*-statistics for late-flowering accessions. Observed distributions (y-axis) are compared with a theoretical normal distribution (x-axis).

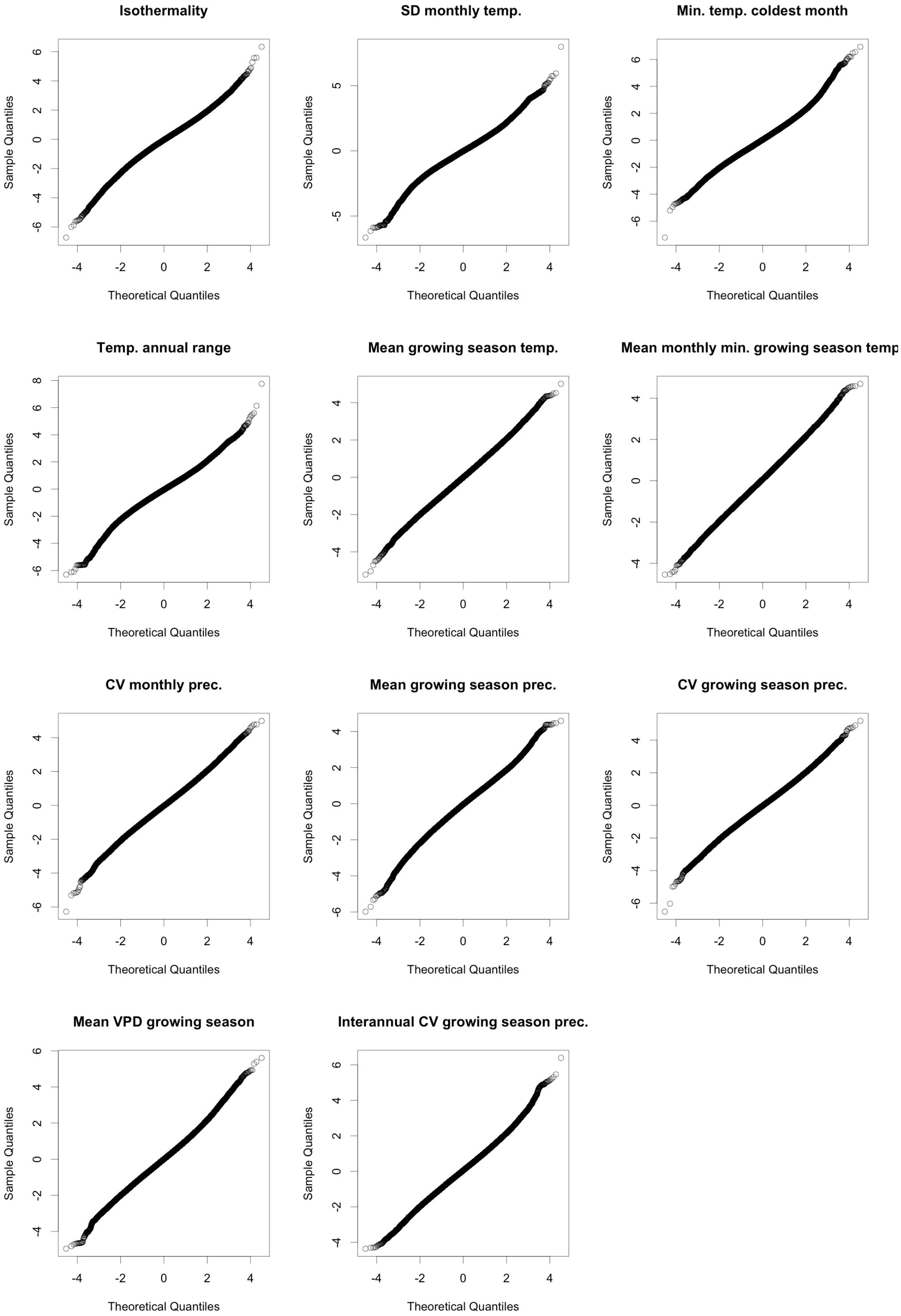

Isothermality
SD monthly temp.
Min. temp. coldest month
Temp. annual range
Mean growing season temp.
Mean monthly min. growing season temp
CV monthly prec.
Mean growing season prec.
CV growing season prec.
Mean VPD growing season
Interannual CV growing season prec.
Sample Quantiles
Theoretical Quantiles
-4
-2
0
2
4
6
8
5
0
-5
-6

Figure S8. Quantile-quantile plots for climate association *t*-statistics for all accessions combined. Observed distributions (y-axis) are compared with a theoretical normal distribution (x-axis).

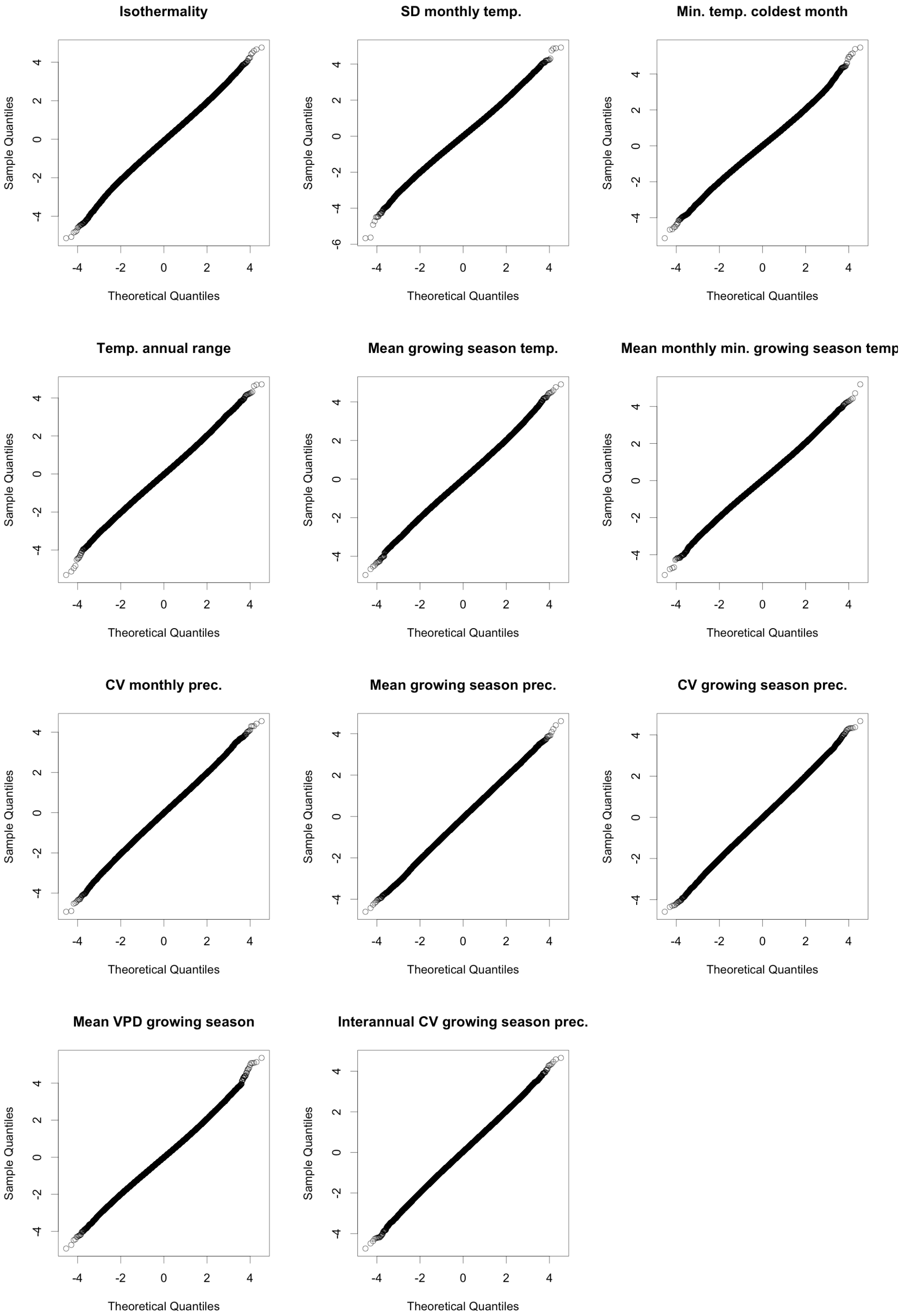
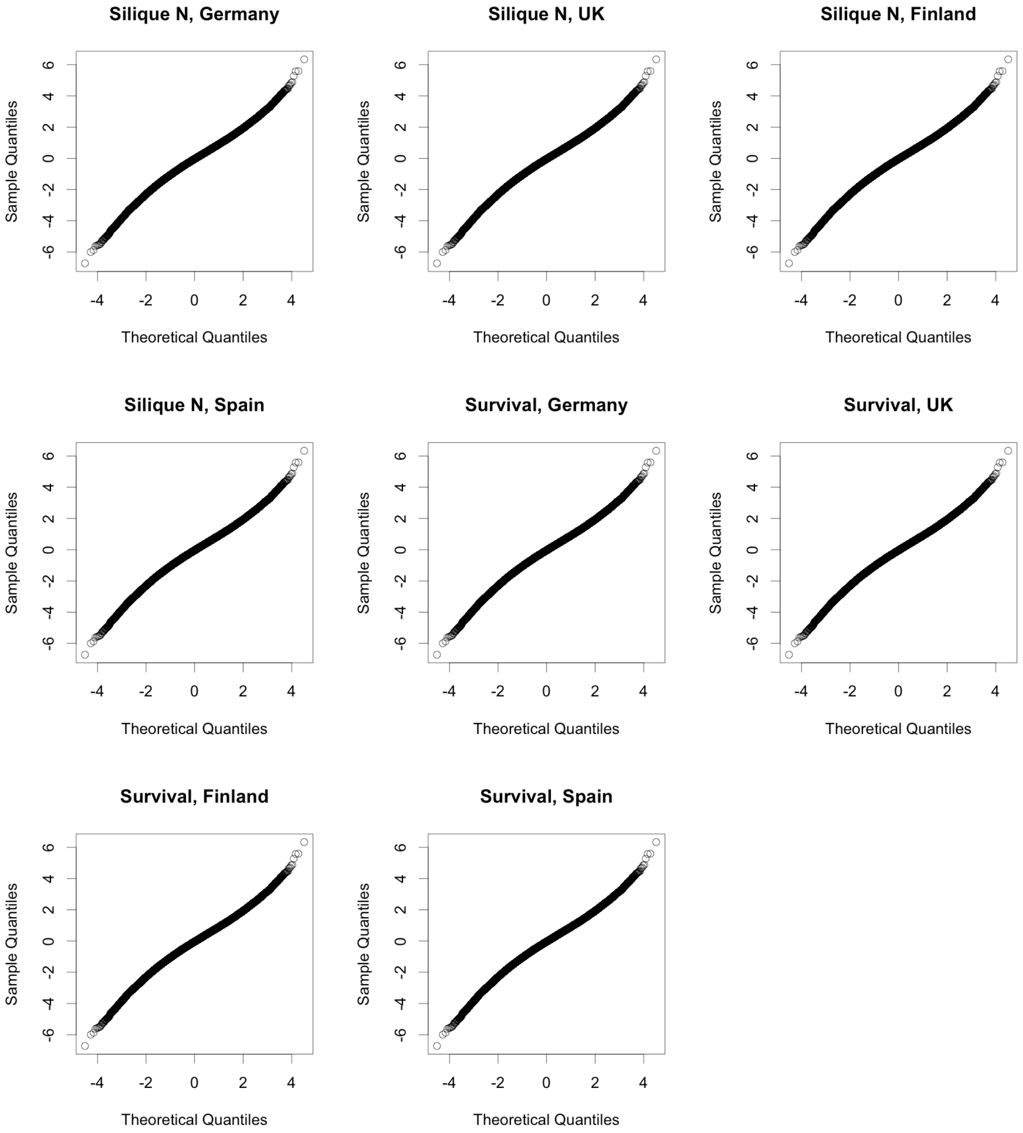

Isothermality
SD monthly temp.
Min. temp. coldest month
Temp. annual range
Mean growing season temp.
Mean monthly min. growing season temp
CV monthly prec.
Mean growing season prec.
CV growing season prec.
Mean VPD growing season
Interannual CV growing season prec.
Sample Quantiles
Theoretical Quantiles
-6
-4
-2
0
2
4

Figure S9. Quantile-quantile plots for fitness association *t*-statistics. Observed distributions (y-axis) are compared with a theoretical normal distribution (x-axis).



Climate outliers removed

Two high-altitude outlier accessions were removed, Kas-2 and Pi-2. The plots below show the distribution of climate data for the remaining accessions. Red lines indicate the boundary between accessions used in association mapping and outliers excluded.

Among all accessions

CV monthly precipitation, accessions removed: Shahdara, Kondara, Sorbo

SD monthly temperature, accessions removed: Kz-1, Kz-9, Per-1, Rubezhnoe-1, Stw-0

Minimum temperature coldest month, accessions removed: Kz-1, Kz-9, Per-1

Temperature annual range, accessions removed: Kz-1, Kz-9

Mean growing season precipitation, accessions removed: Ka-0, Oy-0, Ty-0, Ty-1, UKID115, UKID120

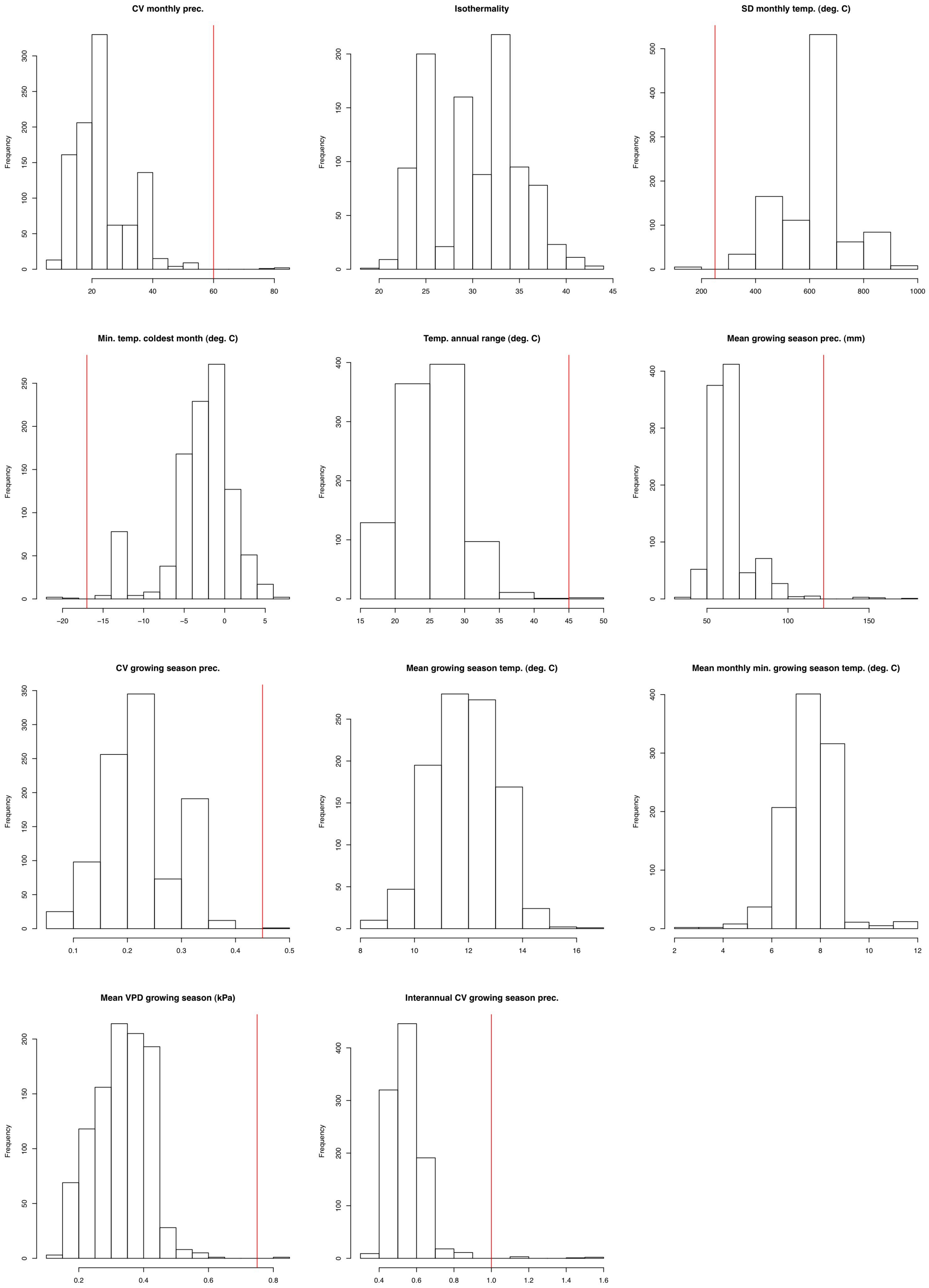
CV growing season precipitation, accessions removed: Kondara

Mean growing season VPD, accessions removed: Lag-1-6

Interannual CV growing season precipitation, accessions removed: Ayu-Dag-3, Kondara, Kz-1, Kz-9, Shahdara, Sorbo

All other climate variables had no outliers

Figure S10. Distributions of climate variables among panels with both early and late-flowering accessions combined. Red lines show outlier thresholds.


CV monthly prec.
Frequency
0
50
100
150
200
250
300
20
40
60
80
Isothermality
Frequency
0
50
100
150
200
20
25
30
35
40
45
SD monthly temp. (deg. C)
Frequency
0
100
200
300
400
500
200
400
600
800
1000
Min. temp. coldest month (deg. C)
Frequency
0
50
100
150
200
250
−20
−15
−10
−5
0
5
Temp. annual range (deg. C)
Frequency
0
100
200
300
400
15
20
25
30
35
40
45
50
Mean growing season prec. (mm)
Frequency
0
100
200
300
400
50
100
150
CV growing season prec.
Frequency
0
50
100
150
200
250
300
350
0.1
0.2
0.3
0.4
0.5
Mean growing season temp. (deg. C)
Frequency
0
50
100
150
200
250
8
10
12
14
16
Mean monthly min. growing season temp. (deg. C)
Frequency
0
100
200
300
400
2
4
6
8
10
12
Mean VPD growing season (kPa)
Frequency
0
50
100
150
200
0.2
0.4
0.6
0.8
Interannual CV growing season prec.
Frequency
0
100
200
300
400
0.4
0.6
0.8
1.0
1.2
1.4
1.6

Among early-flowering accessions

CV monthly precipitation, accessions removed: Shahdara, Kondara, Sorbo

SD monthly temperature, accessions removed: Kz-1, Kz-9, Per-1, Rubezhnoe-1, Stw-0

Minimum temperature coldest month, accessions removed: Kz-1, Kz-9, Per-1

Temperature annual range, accessions removed: Kz-1, Kz-9, Per-1

Mean growing season precipitation, accessions removed: Ka-0, Oy-0, Ty-0

CV growing season precipitation, accessions removed: Kondara

Mean growing season temperature, accessions removed: Lag-1-6

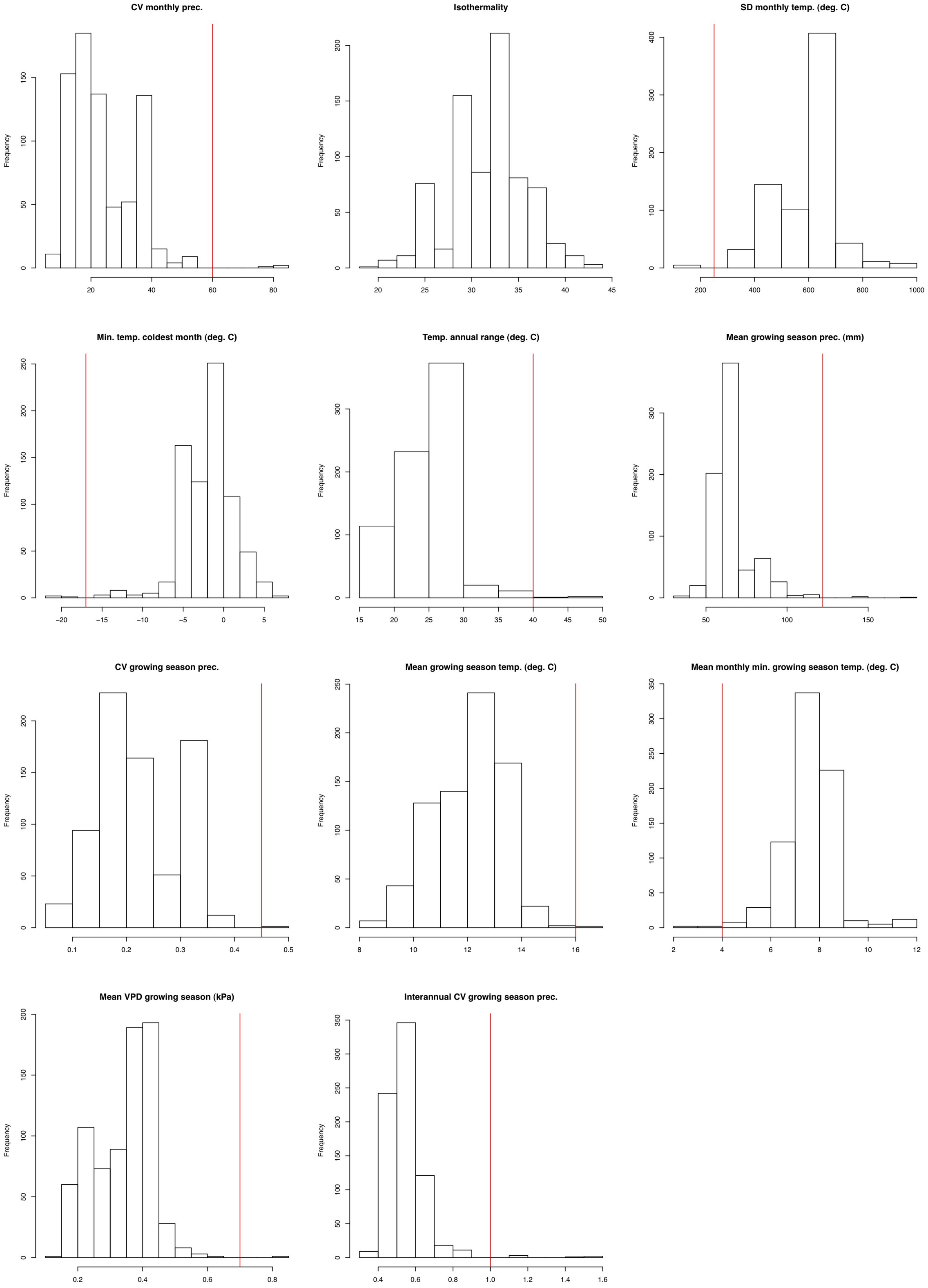
Mean monthly minimum growing season temperature, accessions removed: Alc-0, Ka-0, Kz-1, Kz-9

Mean growing season VPD, accessions removed: Lag-1-6

Interannual CV growing season precipitation, accessions removed: Ayu-Dag-3, Kondara, Kz-1, Kz-9, Shahdara, Sorbo

All other climate variables had no outliers

Figure S11. Distributions of climate variables among panels with early-flowering accessions. Red lines show outlier thresholds.


CV monthly prec.
Frequency
0
50
100
150
20
40
60
80
Isothermality
Frequency
0
50
100
150
200
20
25
30
35
40
45
SD monthly temp. (deg. C)
Frequency
0
100
200
300
400
200
400
600
800
1000
Min. temp. coldest month (deg. C)
Frequency
0
50
100
150
200
250
−20
−15
−10
−5
0
5
Temp. annual range (deg. C)
Frequency
0
100
200
300
15
20
25
30
35
40
45
50
Mean growing season prec. (mm)
Frequency
0
100
200
300
50
100
150
CV growing season prec.
Frequency
0
50
100
150
200
0.1
0.2
0.3
0.4
0.5
Mean growing season temp. (deg. C)
Frequency
0
50
100
150
200
250
8
10
12
14
16
Mean monthly min. growing season temp. (deg. C)
Frequency
0
50
100
150
200
250
300
350
2
4
6
8
10
12
Mean VPD growing season (kPa)
Frequency
0
50
100
150
200
0.2
0.4
0.6
0.8
Interannual CV growing season prec.
Frequency
0
50
100
150
200
250
300
350
0.4
0.6
0.8
1.0
1.2
1.4
1.6

Among late-flowering accessions

Mean growing season precipitation, accessions removed: Ty-1, UKID115, UKID120

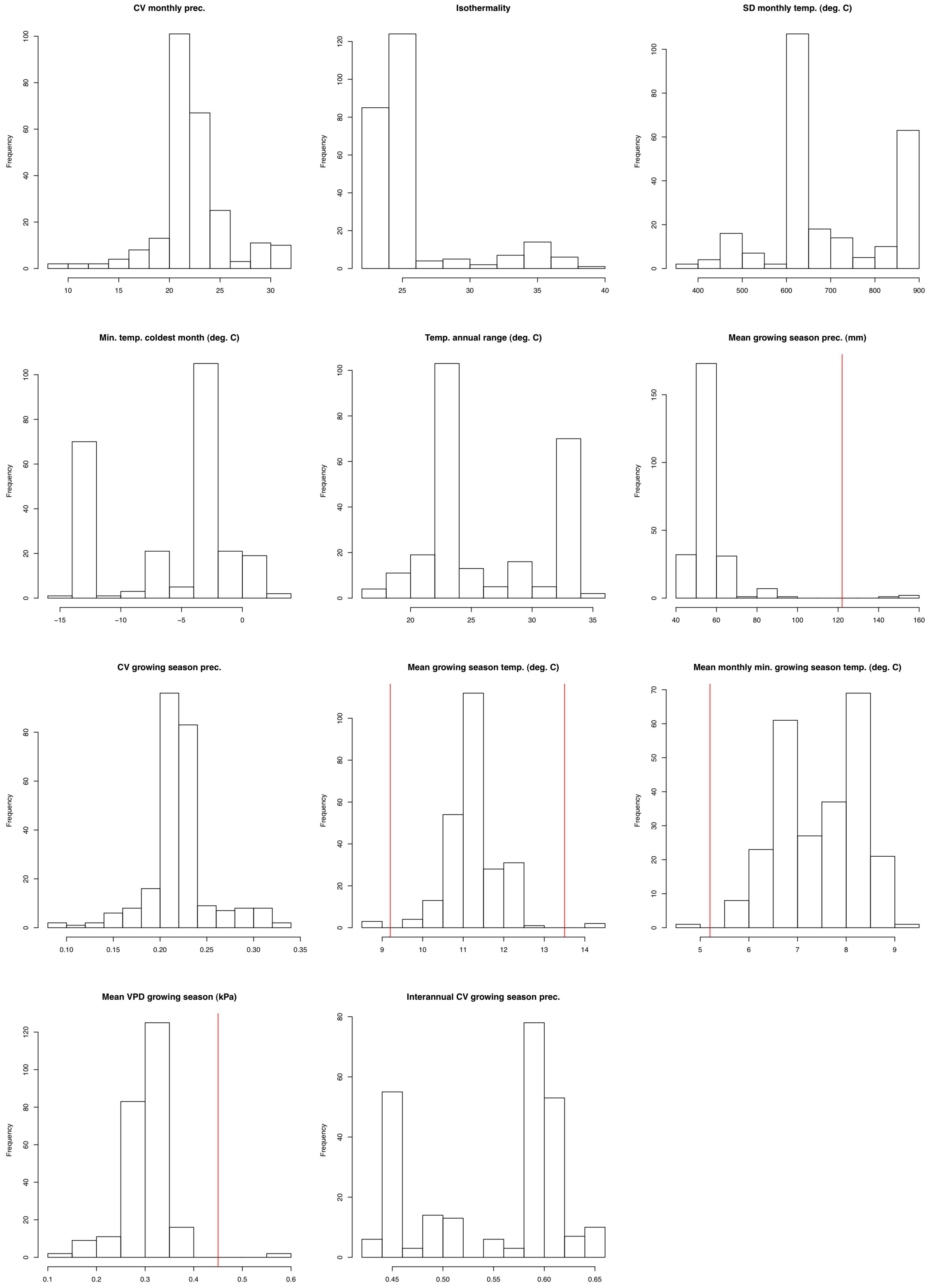
Mean growing season temperature, accessions removed: Blh-1, Blh-2, Mc-0, UKID115, UKID120

Mean monthly minimum growing season temperature, accessions removed: Mc-0

Mean growing season VPD, accessions removed: Blh-1 Blh-2

All other climate variables had no outliers

Figure S12. Distributions of climate variables among panels with late-flowering accessions. Red lines show outlier thresholds.



CV monthly prec.

Isothermality

SD monthly temp. (deg. C)

Min. temp. coldest month (deg. C)

Temp. annual range (deg. C)

Mean growing season prec. (mm)

CV growing season prec.

Mean growing season temp. (deg. C)

Mean monthly min. growing season temp. (deg. C)

Mean VPD growing season (kPa)

Interannual CV growing season prec.