Latitude and epistatic effects uncover novel stable regulators of flowering time on chromosomes 5A and 3A in winter wheat

Salma Benaouda[1], Said Dadshani[1], Patrice Koua[1], Jens Léon[1], Agim Ballvora[1]*

[1]Institute for Crop Science and Resource Conservation, Chair of Plant Breeding, University of Bonn, Bonn, Germany

Correspondence:

✉Agim Ballvora

Katzenburgweg 5, 53115 Bonn

ballvora@uni-bonn.de

Tel. +49 228 737400

INRES Plant Breeding Rheinische Friedrich-Wilhelms-University Bonn 53115 Germany Tel.: 12 +49228737400 Fax: +49-(0)228-73-2045 Email: ballvora@uni-bonn.de

Funding: German Research Foundation (DFG), priority program 1530: "Flowering time control: From natural variation to crop improvement". BRIWECS project funded by the German Federal Ministry of Education and Research (BMBF) grant 031A354.

Abstract

Modern bread wheat has a huge genetic potential to adjust its heading date with favorable conditions that has remained largely unexplored so far. In this study, we used an association panel of in Germany adapted cultivars that was tested in multi-location field trials across Germany over three years. The genotypic response to climatic parameters variation depending on location and year uncovered the implication of photoperiod in promoting transition to flowering in higher latitudes, while spring temperature accelerates flowering in lower ones. Spring temperature overdominates other factors in decreasing the days to heading whereas the higher amount of solar radiation is delaying it. Genome wide scan detected a so far unknown stable locus TaHd14 on chromosome 5A. Including non-adapted cultivars, the exotic allele TaHd119 on chromosome 3A could be identified. The later explains up to 33% of the genetic variance and accelerates heading date by 5.63 days. The response to the competition of latitude dependent climatic variables detected fine tuning QTL responding to temperature and photoperiod in lower and higher latitudes, respectively. A novel locus TaHd12 on chromosome 5A showed significant epistatic interactions with 15 known operators of HD regulation when exotic cultivars were included in the analysis.

Keywords: Wheat, heading time, environment, latitude, GWAS, stable QTL, fine tuning, epistasis

1 INTRODUCTION

Heading date (HD), representing the initiation of flowering time, is one of the most targeted and extensively studied traits for breeding programs that have as ultimate goal to breed performing cultivars that fit to different climatic conditions while maintaining a high and stable yield production over years. Plants capable to adapt to changing climatic conditions are able to avoid inappropriate stress factors such as frost, heat and drought by adjusting its flowering time to seasonal changing conditions in order to protect the floral organs (Fjellheim, Boden & Trevaskis 2014). Such adaptive mechanisms of controlling the timing of starting the transition from vegetative to reproductive phase can be a tool for selecting cultivars that match to different climates and geographical regions and even to adapt regional cultivars to coming climate changes (Guedira *et al.* 2016).

Wheat (*Triticum aestivum* L.) is the leading food grain crop and is a staple source of nutrients for around 40% of the world's population (FAO 2019). The adaptability of wheat to a wide climatic conditions derived from large natural variations, which has been favored by allelic diversity in genes regulating growth and developmental stages especially flowering time pathway (Worland, 2001). Three distinct pathways interact to control flowering time in wheat: vernalization, photoperiod and earliness *per se* (Distelfeld et al., 2009; Herndl et al., 2008; Kamran et al., 2014; Snape et al., 2001). The group of four vernalization (*VRN)* genes regulates the molecular mechanisms for the requirement of vernalization and exposure to cold in wheat (Allard et al., 2012; Distelfeld et al., 2009; Trevaskis et al., 2007). *VRN1* and its paralog *VRN-D4* encode a MADS-box gene with high similarity to *Arabidopsis (Arabidopsis thaliana)* meristem identity protein APETALA*1* (AP1) (Kippes et al., 2015; Yan et al., 2003). *VRN2* locus includes two tandemly duplicated genes *ZCCT1* and *ZCCT2* (Yan et al., 2004). These genes encode proteins carrying a putative zinc finger and a *CCT* domain referred to CONSTANS (CO),

CONSTANS-like (COL) and TIMING OF CAB1 (TOC1) (Putterill, Robson, Lee, Simon & Coupland 1995; Strayer *et al.* 2000; Robson *et al.* 2001). *VRN3* is a homolog of the *Arabidopsis* photoperiod gene *FLOWERING LOCUS T* (Yan et al., 2006). Natural allelic variation in one or many of *VRN* genes leads to differentiate between winter and spring growth habit. The allele combination *vrn1/Vrn2/vrn3* confers the strict winter growth habit due to the dominance of *VRN2* and recessiveness of *VRN1* and *VRN3* (Takahashi, 1970; Yan et al., 2006). Wheat is a photoperiod senstive crop and flowering after accumulation of a critical day length has been satisfied. The day length responsive gene, *Ppd-D1*, is an ortholog of pseudo-response regulator (PRR) of *Arabidopsis* in wheat (Turner, Beales, Faure, Dunford & Laurie 2005; Beales, Turner, Griffiths, Snape & Laurie 2007). The semi-dominant deletion of 2,089 bp upstream from the coding region in the allele *Ppd-D1a* caused insensitivity to photoperiod and accelerate flowering time (Beales *et al.* 2007; Shaw, Turner & Laurie 2012). Earliness *per se (Eps)* is referred to the remaining earliness inducing variation in flowering time when vernalization requirements and photoperiodic sensitivity are fulfilled (Worland, 1996; Yasuda & Shimoyama, 1965).

Latitude as complex environmental determinant plays a pivotal role in temperature regimes, photoperiod and solar radiation fluctuations, which influence the growth and reproduction of plants (Li, Suzuki & Hara 1998; Craufurd & Wheeler 2009). As temperature affects all growth phases, success has been gained in using temperature-based variables for estimating dates for key development stages (Slafer & Rawson 1995; Atkinson & Porter 1996). Thermal time or growing degree day (GDD) estimated by different statistical models is the variable mostly used for predicting the timing in days for transition from one phenological stage to the next (Eagles *et al.* 2010; Rousset *et al.* 2011; Allard *et al.* 2012; Cane *et al.* 2013).

Numerous strategies have been adopted to decipher the genetic control of flowering time in wheat such as candidate gene approach (Eagles, Cane & Vallance 2009; Eagles *et al.* 2010; Rousset *et al.* 2011;

Bentley *et al.* 2013), and the meta-QTL analysis, which includes individual and separte QTL studies. The leter was used firstly in maize and was conducted in wheat as well using either biparental populations or collections of association panels (Hanocq, Laperche, Jaminon, Lainé & Le Gouis 2007; Griffiths *et al.* 2009; Reif *et al.* 2011; Bentley *et al.* 2013; Kamran *et al.* 2014). On the other hand, facilities gained via high-throughput genotyping and sequencing technologies beside the developement of powerful statistical tools based on linkage disequilibrium (LD) could be exploited in genome wide scans (Jander *et al.* 2002; Pletcher *et al.* 2004; Flint-Garcia *et al.* 2005; Frazer *et al.* 2007; Kang *et al.* 2008). Identification of causal genetic-interactions through epistasis analysis can decipher in better way the genetic regulation of complex traits (Phillips 2008). The epistasis is referring to an interaction between a pair of loci in dependent manner making that the resulting phenotype of one locus is conditioned by the genotype at the second locus (Carlborg & Haley 2004). Therefore, many genome wide scan studies used epistatic analysis as a complementary approach to discover more genomic regions associated with intricate traits in different crops including maize, wheat and rapeseed (Buckler et al., 2009; Liu et al., 2012; Steinhoff et al., 2012; Würschum et al., 2013). Given this background, the aim of this study was to dissect the genetic regulation of flowering time and detection of novel QTL and epistatic interactions underlying HD in winter wheat under different environments across Germany in respect of latitude gradient. The particular goals of the current study were (1) to assess, with high accuracy, the interaction of flowering time with the environmental stimuli in a geographical context, (2) to provide insights into stable and fine tuning genetic factors controlling HD, which can be exploited in wheat breeding programs, (3) and to evaluate the contribution of epistasis in the genetic architecture of flowering time.

2 MATERIAL AND METHODS

2.1 Plant material

We used a collection set made of 213 elite bread wheat (*Triticum aestivum* L.) cultivars released between 1966 and 2016 (Voss-Fels et al., 2019). The set was containing 162 cultivars from Germany (winter type), 34 from other Western European countries and 17 exotic cultivars from Mexico, India, USA, Australia, Moldava and Chile (winter and facultative types). We used two subsets for genome wide association studies (GWAS). Subset1 referring to the 162 wheat cultivars developed and adapted in Germany. Subset2 is grouping all the 213 wheat cultivars.

2.2 Experimental set-up

The experiments were conducted in three consecutive years from 2015 to 2017 at six locations across Germany following a gradient latitude: Moosburg an der Isar 48°28′ N/11°56′E (Loc1), Klein-Altendorf 50°37'N /6°59′E (Loc2), Rauischholzhausen 50°46'N/8°53'E (Loc3), Quedlinburg 51°47'N/11°09'E (Loc4), Hannover 52°22'N/9°44'E (Loc5) and Kiel 54°19'N/10°08'E (Loc6). In total 17 environments were included in the study (Loc3 was analysed only in 2015 and 2016).

2.3 Scoring of heading date and measurements of environmental factors

HD was recorded according to two reference dates: the first (HD_winter), as number of days from January 1st until the day when 75% of the ears of an observation plot are visible according to stage BBCH58 (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie) (Meier 1997). The second (HD_spring) was recorded from the day GDD (Growing degree days) kept being positive for at least five consecutive days until the day of reaching BBCH58 stage (day/date of heading) in each environment. The accumulated GDD is calculated using Peterson equation (Peterson 1965): GDD $= \sum_{i=1}^{n} \left\{ \left(\frac{Tmax+Tmin}{2} \right) - Tb \right\}$, where n = the number of days taken for the completion of a particular

134 growth phase. The basic threshold temperature used for wheat is (Tb) = 4.0°C (Cao & Moss 1989).
135 Thus, HD_winter and HD_spring refer to HD scoring starting from winter and spring, respectively. The
136 measurements of environmental stimuli were recorded beginning from both reference dates until the day
137 of heading. The daily measurements of temperatures, global solar radiation and precipitations were
138 obtained from local weather stations placed directly at the experimental field in each location (A
139 summary of these data is shown in Table S1). For temperature, the maximal (Tmax) and minimal (Tmin)
140 values were calculated from reference date until the day of heading for a given cultivar. For the other
141 factors, the accumulated values of daily measurements starting from the reference date until the day of
142 heading were used. Daylength, including civil twilight (h), was computed daily following Forsythe et
143 al. (1995).
144 Field trials were conducted in plots of size between 4.5 and 12 m^2. The experimental sites had diverse
145 soil characteristics and sowing density was 330 viable seeds per m^2 in 2 replicates (See supplemetary
146 Table 3 in Voss-Fels et al., 2019).

147 **2.4 Allelic variation analysis of flowering time known genes:**

148 All cultivars were screened for known vernalization (*VNR1, VRN2, and VRN3*) and photoperiod genes
149 (*Ppd1*). The genotyping included the recessive and dominant alleles of *VRN-A1*(*vrn-A1*, *Vrn-A1a, Vrn-*
150 *A1b, Vrn-A1c)* (Yan et al., 2004), *VRN-B1 (vrn-B1, Vrn-B1)* (Chu *et al.* 2011), *VRN-D1 (vrn-D1*,*Vrn-*
151 *D1a, Vrn-D1b)* (Fu *et al.* 2005), null allele *ZCCT-A1, ZCCT-B1* and *ZCCT-D1* (Zhu, Tan, Cao & Yan
152 2011) and functional alleles *ZCCT-A2, ZCCT-B2* and *ZCCT-D2* of *VRN2* (Distelfeld et al., 2009; Kippes
153 et al., 2016), *VRN3 (vrn-B3, Vrn-B3a, Vrn-B3b, Vrn-B3c)* (Chen et al., 2013), photoperiod-insensitive
154 alleles *Ppd-A1a, Ppd-B1a, Ppd-D1a and* sensitive alleles *Ppd-A1b, Ppd-B1b* and *Ppd-D1b* of *Ppd1*
155 (Beales *et al.* 2007; Nishida *et al.* 2013). The primers and the protocols used to amplify the target
156 fragments are summarized in table S2. DNA extraction was conducted following the protocol of DNeasy

Plant Mini Kit (Qiagen, Hilden, Germany). The PCR amplification reactions were performed in a 25 µL reaction volume containing 100 ng of genomic DNA, 5×Taq DNA polymerase reaction buffer, 10 µM of forward and reverse primers, 100 µM of dNTP, and 0.5 unit of Taq DNA polymerase (NEB, Frankfurt, Germany). The PCR were conducted in thermocycler Flex cycler (Analytik GmbH, Jena, Germany). PCR profiles were visualized by electrophoresis on a 1 to 2% agarose gel stained with ethidium bromide.

2.5 Phenotypic data analysis

Analysis of variance (ANOVA) was performed adopting general linear-model (Gilmour, Thompson & Cullis 1995) in Proc Mixed procedure in SAS 9.4 (SAS Institute, 2015). Variance components of genotypes (G), locations (L), years (Y) as well as their interactions (G*Y), (G*L*) and (G*L*Y) were determined by the restricted maximum likelihood (REML) method assuming a random model in SAS 9.4.

Broad-sense heritability (H^2) estimates were calculating following the method described by Holland et al. (2003): $H^2 = \frac{V_G}{V_G + \frac{V_{G*E}}{E} + \frac{V_E}{E}}$ where V_G: genetic variance, V_{G*E}: variance of genotype × environment, E: environment, V_E: variance of error term. Hierarchical clustering analysis was performed in R using "hclust" function, the distance (dissimilarity) between clusters is calculated with the "complete linkage" method, and "pvclust" package was used to calculate the *p*-values for hierarchical clustering. Principal component analysis (PCA) was run using the built-in R function prcomp.

2.6 QTL mapping

The diversity panel was genotyped using the map of 24,216 informative SNP markers based on Infinium iSelect 15K chip and the 135K Axiom Exome Capture Arrays (Dadshani, Mathew, Ballvora, Mason & Léon 2021). Principal component analysis was performed by using the prcomp core function in R (Team

2013). Marker-based identical-by-state (IBS) kinship matrix was calculated with the A.mat function of the R package rrBLUP (Endelman 2011) and the Pair-wise measures of linkage disequilibrium (LD) between two SNP with the package PLINK version 1.9 (Chang *et al.* 2015). For QTL mapping a multiple QTL model using the PROC MIXED procedure in SAS 9.4 was utilized. Iteratively, the forward selection and backward elimination approach described in (Bauer *et al.* 2009) was used to reduce the number of false-positives and endorsing the true QTL. Threshold of *P*-value ≤0.001 and false discovery rate (FDR) was set at 5% for the iterative multi-locus approach in the QTL model (Kilpikari & Sillanpää 2003). Further increase of accuracy for detection of true QTL was achieved by implementation of 10-fold cross-validation procedure with 20% leave-out. QTL analysis was conducted following the linear model: $Y_{ik} = \mu + M_i + E_{k+} M_i * E_k + \varepsilon_{ijk}$, where Y_{ik} is the vector of phenotypic values, μ: general mean, M_i: the fixed effect of i-th marker; E_k: the fixed effect of k-th environment (location-by-year), $M_i * E_k$: the fixed interaction effect of i-th marker with the k-th environment, and ε_{ijk}: the residual. The genetic variance explained by a single SNP marker (P^G) was calculated as following: $P^G = SQ_M/SQ_g$, where SQ_M is the sum of squares of $_{i\_th}$ marker and SQ_g was calculated as the type I sum of squares (Type I SS) of the genotype in the ANOVA model. The total proportion of the genotypic variance P_G for each marker was calculated by including all markers with QTL effect in the ANOVA model. While the individual proportion of P^G of a specific marker is calculated by excluding other markers from the ANOVA model.

2.7 Epistatic interactions

In PROC MIXED procedure in SAS 9.4, the two-way multilocus approach was used for epistatic interactions involving the environment factor in the following model: $Y_{ijk} = \mu + M_{1i} + M_{2j} + M_{1i} \times M_{2j} + E_k + M_{1i} \times M_{2j} \times E_k + \varepsilon_{ijk}$, where Y_{ijk}: the vector of phenotypic values; μ: general mean; M_{1i}: the fixed effect of $_{i\text{-}th}$ marker1, M_{2j}: the fixed effect of $_{j\text{-}th}$ marker2, $M_{1i} \times M_{2j}$: the fixed

interaction effect of $_{i\text{-}th}$ marker1 with $_{j\text{-}th}$ marker2, E_k : fixed effect of $_{k\text{-}th}$ environment (location by year), $M_{1i} \times M_{2j} \times E_k$:fixed interaction of the $_{i\text{-}th}$ marker1 with the $_{j\text{-}th}$ marker2 genotype and $_{k\text{-}th}$ environment; ε_{ijk}: the residual. Threshold of *P*-value ≤0.001 and FDR < 5% were implemented in the model for more accuracy in detecting true epistatic interactions. The proportion of the genotypic variance explained by each single epistatic interaction was estimated in the same way as genetic variance for single SNP marker.

2.8 *In silico* analysis

The known vernalization *VRN* and photoperiod *Ppd* genes were mapped physically on the wheat genome sequence using the following approach: the core sequence information of markers was blasted against the genome sequence draft (Table S3). Further, the genes included in the flanking regions were downloaded und their annotations were checked using the last updated version of the gene annotation from the International Wheat Genome Sequencing Consortium and EnsemblPlants platforms. The start position of each gene was extracted from blasting outputs and were exploited later in the QTL and epistatic analyses. For some reported SSR markers only the primer sequences were available in GrainGenes database (wheat.pw.usda.gov). In this case, the sequence of the primers was blasted to find the corresponding physical positions and the same steps were followed for blasting using IWGSC RefSeq v1.1 gene annotation platform.

3 RESULTS

3.1 Phenotypic assessment of heading date-by-environment

To characterize the phenotypic performance, the genotypes of subset1 and subset2 were tested at six different locations for three years. The mean HD_winter across all environments ranged from 147.93 and 142.07 to 158.32 for subset1 and subset2, respectively (Table 1). The variance components of genotype and of interactions genotype-by-year, genotype-by-location and genotype-by-location-by-year are highly significant for subset2 compared with subset1. Student's *t*-test showed a very high significant difference ($p \leq 0.01$) between HD scorings of subset1 and subset2 (Table S4). The heritability estimation was high by 0.89 for adapted cultivars and 0.96 by including the exotic ones. The exotic cultivars originating from Australia, Mexico, Serbia, Moldova and USA were found in early flowering group [on the left side of the distribution graphic (Figure 1)]. Cultivars from France are the earliest flowering ones in the European germplasm. All latest flowering varieties originate from Germany with HD range of 10.39 days.

To better estimate the effect of environment, HD was evaluated using two reference dates for scoring. HD_winter revealed less distinctness among environments due to overlapping of the scorings in all locations over the three years. Exception is Loc6 (North), where HD was delayed by 14.5 days in 2015 compared to 2016 and 2017. Loc1 (South) recorded an advanced HD by 12.6 days in 2016 compared with the other years (Figure 2a). According to HD_spring, an overlapping of HD scorings over years was noticed exclusively in Loc6, while in other locations, two to three clearly distinguishable clusters could be differentiated. In 2016, we observed a reduction of days to heading in Loc1, Loc2, Loc3 and Loc5 by 54, 59, 68 and 72 days, respectively, except in Loc6 (Figure 2b). PC analysis was conducted to identify the combination of variables that better explained the environmental variation in Germany. The

first two axis of the PCA accounted for ca 71% (Figure 2c). Daylength, Tmax of spring, Tmin of winter and global radiation of spring contribute the most by 13.7%, 13.5%, 12.6% and 11.6%, respectively in the total environment variability (Figure 2d). The genotype effect on HD variation in interaction with environmental factors, selected by PCA, was checked via ANOVA. The location influenced the HD variation due to the genotypic response to Tmax, daylength and global radiation by 53%, 34% and 13%, respectively. The genetic response to "year-to-year" fluctuations of Tmax (Figure S1) explained 70% of HD variation, while genotypic interactions with daylength and global radiation seem to be stable from year to year and lead to very week HD alterations (Table S5). Significant hierarchical clustering (*p*-value <0.05) uncovers how similar is the flowering behavior between 17 environments based on the genetic response to the fluctuation of the most important climatic factors (based on PCA and ANOVA). Tmax of spring lead to the most similar clustering to HD pattern given in Figure 2a, compared with other parameters, showing high closeness between low and middle latitude in 2016 (Loc1 and Loc2, r>0.9), as well as in high ones (Loc5 and Loc6, r>0.9). The global radiation of spring revealed a strong cluster grouping over all the years in loc6 as well. HD variation based on winter reference date (Figure 2b) narrow tightly the grouping based on day length, which revealed the dissimilarity of loc1-2016 and loc6-2015 to the other environments (Figure S2).

3.2 Effect of latitude-associated genetic response on HD variation

In order to identify the latitude-associated effect of climatic parameters on HD variation, correlation analysis in each location was performed. For spring measurements, Tmax effect showed a high matching with latitude gradient and reduced strongly the days to heading from the South (99% in Loc1) to the North (26% in Loc6). The impact of Tmin is following the same trend, with 98% in Loc1, 79 % in Loc2, 81% in Loc3 and continues decreasing to 0.04% in Loc6. The global radiation showed a moderate

correlation with HD in the North but strongly positive in other locations. Using the winter reference date, only Tmin effect on HD showed a tendency linked to latitude gradient, with less consistent relationship to HD. The correlation between HD and the precipitations goes from strongly positive to strongly negative for both reference dates. The rainfall in spring correlates more negatively with HD in Loc3, Loc5, Loc6, while the positive correlation is not changing in the other locations when comparing winter and spring counting (Figure S3). Focusing on spring records, ANOVA revealed that the genotype response to Tmax fluctuation explained HD variation by 98.4% in the South and 10.7% in the North, showing strong reliance to latitude gradient across locations. The response to daylength is highly depending on latitude as well, but following the opposite trend than Tmax. The interaction genotype-by-daylength altered very weekly HD in in the South and central regions. From Loc4, the effect of the response to daylength increased to 89% in the North. No significant HD change could be explained by the genotype-by-radiation interaction in all locations (Table 2).

3.3 Genotyping the population for major flowering time regulatory genes

In order to identify the growth habit of the two subsets, the genotypes were screened at the known flowering time loci. For subset1, the analysis based on allele specific primers using PCR revealed the presence of three recessive alleles *vrnA1, vrn-B1, vrn-D1*, at locus *VRN1*. The screening showed the presence of null alleles *ZCCT-A1* and *ZCCT-D1* as well the functional alleles *ZCCT-B2* and *ZCCT-D1* at *VRN2*, which lead to conclude that the German cultivars carry a dominant *Vrn-2*. The spring allele *Vrn-3B*, photoperiod insensitive allele *Ppd-D1a* and sensitive allele *Ppd-D1b* could be detected too. In total, 95% of the adapted germplasm carries the allelic combination *vrn-1/Vrn-2/Vrn-3Bc/Ppd-D1b* (Figure 3). Except *Vrn-3Bc* (Figure S4), which is a spring allele, *vrn-1/Vrn-2/Ppd-D1b* is responsible for the strict winter growth habit of the majority of the German cultivars. Only a minority (5%) harbors

289 the insensitive allele *Ppd-D1a* beside the same *VRN* alleles. For subset2, *VRN-D1/ Ppd-D1* appears to
290 be the allelic pair mostly associated with growth habit for the European cultivars. Referring to the origin
291 of selected cultivars, 88% of those from central Europe follow a winter growth attitude. The facultative
292 behavior related to *Vrn-D1a/ Ppd-D1a* was detected in 9 % of the south-European cultivars (France and
293 Serbia), while 3% of cultivars harbor *Vrn-D1a/ Ppd-D1a* (France). Different *VRN/Ppd* -1 allelic
294 associations identified in the non-European wheat collection were mostly spring alleles (*Vrn-A1, Vrn-*
295 *B1 and Ppd-B1a).*

296 **3.4 Identification of stable and fine tuning QTL for heading date**

297 We aimed to identify stable genetic regions controlling HD independently of environmental factors. For
298 subset1, GWA analysis resulted in 27 QTL (Figure 4a, Table S6) above the threshold of $P < 0.0001$.
299 Among them four loci mapped on chromosomes 5A and 5B were selected as highly associated to HD.
300 The strongest QTL TaHd14 located on 5A is peaked by marker GENE_3500_336 at 117,4Mbp and
301 explains 13.18% and 23.78% of the total and individual proportions of the genotypic variance,
302 respectively (Table 3, Table S4). By including the exotic cultivars (subset2) into GWAS, we selected
303 six QTL distributed on chromosomes 2B, 3A, 4A, 4B, 5B and 7B above the significance threshold of (-
304 log10 =15) (Figure 4b, Table S7). The strongest effect was detected by the peak marker AX-111134276
305 at 556,6Mbp of the QTL TaHd119, which explained 33% and 46% of the total and individual
306 proportions of the genetic variance, respectively. The allelic variation at TaHd119 located on
307 chromosome 3A is altering HD by 5.63 days (Table 3). Loci close to *VRN-A1, VRN-A2, VRN-B* and
308 *VRN-D3* genes were detected by QTL TaHd218, TaHd69, TaHd71 and TaHd91, respectively (Table
309 S8). We identified nine QTL shared between both subsets. Altogether, they showed an increased effect
310 by 2 to 2.6 folds in subset2 compared to subset1 (Table S9). Looking at the allelic level, the adapted

311 cultivars revealed a very high monomorphism at the six QTL identified in subset2. The ratio of the
312 exotic alleles present in the adapted background was less than 1.85% per each QTL.

313 Further, to better understand the genetic modulation or fine tuners of the transition to reproductive phase
314 in response to particular environmental parameters, we performed the genome-wide scan per each
315 environment. In total, 84 SNPs distributed across 17 environments were identified (Table S10). We
316 detected some shared QTL among the specific location-by-year combinations (Table S11). In 2015,
317 three possibly homeoelog QTL were detected at the very distal end of chromosomes 2A, 2B and 2D.
318 This region was shared by locations at lower latitude until middle part of Germany (Loc1 to Loc3),
319 whereas northern regions (Loc5 and Loc6) had a common QTL on the short arm of chromosome 5A.
320 Another QTL on the proximal of the short arm of chromosome 2B showed up in Loc4 and Loc5. The
321 year 2016 was the warmest among the three years of the experiment in the southern and central locations
322 where three loci at the distal end of chromosome 4B were shared, as well as the homeoelog locus on
323 chromosome 4D. The loci detected in 2017 followed no trend with latitude gradient. The overall effect
324 of revealed fine-tuning QTL spans from inducing early flowering time by 2.6 days (Loc5-2016) to
325 delaying it by 4.45 days (Loc2-2015) (Table S10).

326 Since all genotypes were tested in 17 environments, we were able to calculate the flowering time
327 response to various meteorological parameters for each genotype separately after vernalization. We
328 calculated the Pearson correlation coefficients between HD and the mean records of climate variables
329 in February, March and April and used these as new traits in GWAS. This new approach leads to the
330 detection of a few significant QTL. We only counted the annotated genes associated with the detected
331 loci and identified four QTL for temperature, seven for day length and five for radiation (Table S12).

332

3.5 Identification of epistatic interactions involved in heading date control in winter wheat

To evaluate how the interaction among genetic loci affects flowering time, genome-wide epistatic interaction analysis was performed. Using the subset1, 32 significant epistatic interactions were detected, and explained up to 3.8% of the genetic variance (Table S13). One locus on chromosome 5A (marker AX-158565287) at 698,1 Mbp was involved in 14 epistatic interactions with loci located on chromosomes 1B, 2B, 3B, 4A, 4B, 5A, 5B and 5D including the strongest QTL, TaHd14 identified in the same subset (Table 5a). This locus located upstream (37 Mb) in close vicinity of ZCCT2, the core protein of *VRN2* gene. We detected 30 significant epistatic interactions using the subset2, which explained up to 7.8% of the genetic variance (Table S14). Two loci mapped on chromosomes 5A and 1B at 158.2Mbp and 654.7Mbp, respectively, showed the strongest epistatic interaction in the subset2, explaining 7.8% of the genetic variance. The combination of minor alleles of both regions induced HD by 4.64 days earlier compared with that of major alleles. The locus TaHd12 was implicated in 15 digenic interactions for subset2 (Table 5b)

4. DISCUSSION

4.1 Response of heading date to local and seasonal interplays of environmental factors

Heading time variation is occurring between individuals across very small temporal and spatial scales, where local climatic conditions caused part of within-population variation (Dahlgren, von Zeipel & Ehrlén 2007). This explains the heading interval of 10.39 days among the adapted cultivars within latitude range of 6°. The reduced genotypic variance of HD in subset1 compared to subset2 is attributed to the local adaptation impact of the German cultivars. The genetic response of HD is more depending on location than on year. This indicates the importance of multi-locations trials with broad distribution

for the genetic estimation of a highly heritable trait such as HD (Holland, Nyquist & Cervantes-Martínez 2003). On the other hand, the high variance of genotype-by-location-by-year interaction for both sets shows that all cultivars respond very differently to the 17 environments and that the European winter wheat contain an immense genetic potential appropriate to study complex traits like flowering time.

The interplay of climatic factors is influencing all phenological events of plants including flowering time in barley (Jones & Thornton 2003), rice (Mall & Aggarwal 2002; Prasad, Boote, Allen Jr, Sheehy & Thomas 2006) and wheat (Manderscheid et al., 2003; Kouchaki & Nasiri, 2008). Using an environment specific date for counting days to heading based on GDD revealed that Tmax of spring is the climatic parameter mostly responsible for the determination of similarity between environments showing closer HD. This is due to high genotypic response to fluctuations of Tmax, which depends on location and year. Furthermore, we found that Tmax and Tmin of spring dominate strongly other factors in reducing days to heading from the lowest latitude to the middle ones. This ascertainment matches with other studies (Menzel *et al.* 2006; Miller-Rushing *et al.* 2007; Record 2009; Moore & Lauenroth 2017). The elevated solar radiation accumulation was highly associated with delayed HD, except in the highest latitude. The high UV-B radiation plays a crucial regulatory role in plant growth and morphology (Bornman *et al.* 2015), however, many reports confirm the delay of flowering time as response to high natural UV-B radiation in different plant species, such as maize (Saile-Mark, Tevini & Mark 1996), roses (Terfa, Roro, Olsen & Torre 2014) and pea (Roro *et al.* 2016). Although other factors such as soil conditions like moisture, and temperature could affect HD, the PCA showed that 71% of the environmental variation was explained by the variables considered in the study, which provides reliability and robustness to the results obtained.

4.2 Substituted effect of latitude dependent temperature and daylength on heading date

With all the measurements performed in this study, we did not see a relationship between latitude and HD. Nevertheless, the genotypic response to daylength in dependence on Tmax is the key factor that should be considered to understand the HD variation in respect of latitude. Dramatic acceleration of flowering with increasing light amount as response to daylength was observed in several annual plant species (Tsegay *et al.* 2005; Opseth, Holefors, Rosnes, Lee & Olsen 2016; Chiang *et al.* 2018). However, daylength and increasing light amount may have no or less effect when flowering is induced by milder temperatures between 15°C and 22°C (King, Pate & Johnston 1996; Sønsteby & Heide 2008). Indeed, higher Tmax and lower Tmin in spring are recorded in the lower latitude compared with higher one and the year-to-year thermal change is decreasing when latitude increased. By contrast, daylength is prolonged faster during spring season in the higher latitude than in the lower one (Figure 6). This leads to conclude that the impact of high seasonal fluctuations of temperature in the low latitude on HD seems to be substituted by the immense daylength seasonal variation occurring in the high latitude when moving from winter to spring. Consequently, plants are adapted to use temperature as sensor of favorable conditions for starting HD in lower latitudes. Because of the stable year-to-year thermal change in the higher latitudes, plants use photoperiod as more reliable indicator of the changing seasons than temperature. This assessment is strongly supported by the genotypic response to Tmax of spring, which is the opposite of genotypic response to daylength. Finally, as the seasonal alteration of daylength is the only environmental input that is constant from year to year, this might explain the similar HD behavior in the North over three years and the increased HD variation as we headed further South.

4.3 The roles of *VRN* and *PPD* genes in flowering time control

The allele combination *vrn1/Vrn2/Ppd-D1b* is responsible for strict winter growth habit in the adapted germplasm. Our results are in line with Langer et al. (2014), who reported that 82% of the European winter wheat cultivars harbor daylength sensitive allele *Ppd-D1b* with 100% dominance of winter allele *vrn-1*. Since the majority (95%) of the adapted cultivars carry the same allelic variation at *VRN* genes, neither the HD range of 10.39 days nor the genetic variance showed by the German cultivars can be convincingly explained by the allelic variation at *Ppd-D1* locus, as only 5% of the cultivars harbor the insensitive allele *Ppd-D1a*. The candidate gene approach disclosed the presence of *VRN* and *PPD* alleles established as result of long-term adaptation to winter conditions. Nevertheless, the HD variation due to genetic variance and interaction with environment is very likely involving more genetic regulators responsible for HD variation after fulfillment of vernalization and photoperiod requirements. On the other hand, spring alleles at *VRN* and *PPD* were more frequent in the exotic cultivars. The insensitive alleles at *Ppd-B* reported by Nishida et al. (2013) have an equal HD inducing effect as *Ppd-D1*.

4.4 Novel stable QTL alleles regulating the time of heading

Studying the genetic control of HD in multi-environment trials is of great significance for detection of QTL stably expressed in different environments. The overall effect of the four detected significant stable QTL is with 20.63% higher compared with that of six QTL (9.5%) reported by Langer et al. (2014) that tested more European winter wheat cultivars but in very close locations for one single year. Granted that the size of population is a determinant factor in GWAS, the incorporation of QTL × environment interaction, which maintains the genetic variance, may improve the power of GWAS to find relevant and broadly adapted QTL (Cantor, Lange & Sinsheimer 2010; Thomas 2010). TaHd14 is a novel locus regulating HD in the European germplasm and located distantly from the SSR marker Xgwm293 in the

small arm of chromosome 5A and involved in genetic control of height in wheat (Griffiths *et al.* 2009) (Figure S5). Showing high proportion of explaining the genetic variance, TaHd14 is a promising candidate for further fine mapping approaches.

Increasing the phenotypic variance is highly required for high resolution mapping and allele mining (Ersoz, Yu, Buckler, Varshney & Tuberosa 2007; Uchiyama *et al.* 2013). The strongest QTL TaHd119, harboring exotic allele, is flanked by two previously reported SSR markers Xbarc45 (Griffiths *et al.* 2009) and WMC264 (Zanke *et al.* 2014). The known Earliness *per se* gene *Eps-3A* mapped genetically to the distal part of chromosome 3A (Gawroński & Schnurbusch, 2012) was found far from the flanking region of TaHd119 (Figure S5). The identification of the QTL close to *VRN* genes in subset2 is most probably due to different vernalization requirements, caused by the exotic alleles, which could carry natural variations that lead to need of shorter exposure to cold (Yan et al., 2004; Fu et al., 2005; Kippes et al., 2015). Nevertheless, the proportions of *VRN* genes in explaining the genetic variance are very low compared to the locus TaHd119. Despite of the expected differences in daylength and circadian clock adaptations of exotic cultivars, no QTL related to *Ppd-D1* could be detected. This is probably due to LD decay in chromosome 2D and the selective sweep around the *Ppd-D1* (Bentley *et al.* 2013).

4.5 Fine tuning QTL undergo the competition of latitude dependent climatic variables

The fine tuning QTL of specific microenvironments are matching with latitudinal competition of environmental cues affecting HD. We found that spring temperature is a dominant regulator of HD in lowest and middle latitudes. Consequently, the shared homeoelogs QTL in the distal region of chromosomes 2 and 4 detected in lowest and middle latitudes are likely temperature sensitive loci. Indeed, one of the three homeoelogs on chromosome 2D were located close to FT-interacting protein 1-like (FTIP) involved in flowering time locus T (FT) protein transport, in response to ambient temperature

(Liu et al., 2012). Despite their small effect, thermo-sensitive genes play an essential role for adaptation to specific climatic conditions (Snape *et al.* 2001; Lewis, Faricelli, Appendino, Valárik & Dubcovsky 2008). Another locus on chromosome 5A, found to be a member of Auxin/B3 appeared exclusively in the high latitudes, where we showed that photoperiod acts as reliable proxy for initiating the floral transition. Auxin is promoting floral timing (Ueda *et al.* 2008), while transcriptional and growth responses to auxin are modulated by circadian clock (Covington & Harmer 2007). Finally, many fine-tuning QTL known by their role in flowering time were confirmed by exploring the correlation coefficients between HD and each of the environmental parameters in GWAS.

4.6 Epistatic interactions

We identified one locus located in the *VRN2* gene region that is implicated in 14 genetic interactions. This strongly suggests that *VRN-A2* plays a central role in the regulatory network controlling heading time in the German germplasm. The epistatic effect of *VRN* loci in genetic control of flowering time in European winter wheat was proposed by Reif et al., (2011) who reported the likely involvement of *VRN-A1* in four epistatic interactions.. The identification of genes of the intervals interacting with *VRN2* revealed a significant interaction between this locus and Apetala2/Ethylene (AP2/ERF) on chromosome 5A explaining 2.14% of the genetic variance. This class of AP2/ERF genes is well described in flowering pathway in *Arabidopsis* for regulating the correct timing of the transition of the spikelet meristem to the floral meristem in maize (Chuck, Meeley & Hake 1998). Similarly, we found that the other chromosomal regions interacting with the *VRN2* harbor protein families such as MATH-BTB, bHLH, WD40, Agamous/MADSbox, DsPTP1, and PLC-C2, known to contribute in flowering time regulation in many plant species (Hazebroek & Metzger 1990; Yanofsky *et al.* 1990; Sheldon *et al.* 1999; Georges *et al.* 2009; Ito *et al.* 2012; Chen, Bernhardt, Lee & Hellmann 2015; Jiang, Chen, Luo & Peck 2018).

Interestingly, the novel locus TaHd12 that has a small QTL effect in adapted germplasm, showed strong epistatic effect when adding the exotic cultivars to the analysis. Some of the 15 interacting loci were mapped very close to key regulatory elements of flowering time, like FYPP (Kim *et al.* 2002), *TaFT3*, *Alpha-Beta hydrolase (ABH)* (Sun & Ni, 2011), *tRNA methyltransferase (Trm1)* (Chen, Jäger & Zheng 2010; Guo *et al.* 2019), *Eps-3A*, *VRN-B1*, and *Vrn-3/FT* genes on chromosomes 1A,1B, 2B, 3A, 5B, 7A, respectively. Numerous other epistatic interactions found in this study were significant but showed small effects in explaining the genetic variance.

5 CONCLUSION

In this study, we elucidated the latitude associated competitive effect of environmental factors on flowering time regulation. In the light of year-to-year differential fluctuations of temperature and seasonal change of daylength, the genetic response to climatic stimuli selects thermo-sensitive loci in low latitudes and photoperiod susceptible loci in high ones for starting the transition to the reproductive phase. The allele combinations of known *VRN* and *PPD* genes responsible for the winter and facultative growth habits of adapted and exotic cultivars were determined. We were able to enrich the flowering time pathway in Germany adapted wheat with potential QTL attributing stable effect across different environments and exotic alleles that induce greater HD alteration. A novel locus TaHd12, detected on chromosome 5A, gained more epistatic implications for controlling flowering time in non-adapted winter wheat. Further, we propose a pivotal epistatic role of *VRN2* based on its genetic interactions with key regulatory elements in the adapted germplasm. Our findings can be exploited in wheat breeding process for developing cultivars adapted to different environments, and offer new insights in understanding the mechanisms of the genetic architecture underlying flowering time in wheat.

ACKNOWLEDGMENT

We thank the German Research Foundation (DFG) for funding this research under the priority program 1530, “Flowering time control: From natural variation to crop improvement”. We address our acknowledgment also to the project partners of BRIWECS project funded by the German Federal Ministry of Education and Research (BMBF) grant 031A354 and SECOBRA Saatzucht GmbH for the phenotypic data. We thank as well Lea Hördemann for her help in the laboratory work.

CONFLICT OF INTEREST

The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be envisaged and/or construed as a conflict of interest.

AUTHORS CONTRIBUTIONS

Competing interests

The authors declare no competing interests.

SUPPLEMENTARY DATA

Excel Tables

Table Sxl1 Summary of Heading date scoring and daily measurements of environmental factors per location and year

Table Sxl2 Primer used for the analysis of allelic variation at *VRN* and *PPD* genes

Table Sxl3 Physical mapping of VRN and PPD genes based on reported flanking Marker in cM

Table Sxl4 ttest results of significant Heading date difference between subset1 and subset2

Table Sxl5 ANOVA of climatic variable and heading date depending on location and year

Table Sxl6 Summary of QTL ^{X} Environment GWAS subset 1

Table Sxl7 Summary of QTL ^{X} Environment GWAS subset 2

Table Sxl8 Genotypic variance of QTL TaHd119 compared to VRN genes

Table Sxl9 Significant QTL for flowering time shared between adapted cultivars (Sb1) and non-adapted ones (Sb2)

Table Sxl10 Fine tuning QTL in the German germplasm

Table Sxl11 Shared fine-tuning QTL ^{X} Environment per location in the same year in the subset 1

Table Sxl12 GWAS of coefficients between HD and the mean records of climate variables in February, March and April

Table Sxl13 Epistatic interactions detected in subset1

Table Sxl14 Epistatic interactions detected in subset2

Figures

Figure S1 Measurements of climatic factors per environment according to winter and spring reference dates

Figure S2 Hierarchical clustering of the interaction HD*environmental factors including six locations and three year

Figure S3 Geographical heatmap summarizing the correlation between the climatic factors

Figure S4 PCR pattern screening of adapted cultivars (162) at *Vrn-3Bc*, visualized in 2% electrophoresis gel

Figure S5 Physical mapping of strongest detected QTL for heading date trait using the German germplasm (marker in red color) and exotic cultivars (marked in green color) in chromosomes 5A and 3A, respectively

REFERENCES

Allard V., Veisz O., Kõszegi B., Rousset M., Le Gouis J. & Martre P. (2012) The quantitative response of wheat vernalization to environmental variables indicates that vernalization is not a response to cold temperature. *Journal of Experimental Botany* **63**, 847–857.

Atkinson D. & Porter J.R. (1996) Temperature, plant development and crop yields. *Trends in Plant Science* **1**, 119–124.

Bauer A.M., Hoti F., Von Korff M., Pillen K., Léon J. & Sillanpää M.J. (2009) Advanced backcross-QTL analysis in spring barley (H. vulgare ssp. spontaneum) comparing a REML versus a Bayesian model in multi-environmental field trials. *Theoretical and applied genetics* **119**, 105–123.

Beales J., Turner A., Griffiths S., Snape J.W. & Laurie D.A. (2007) A pseudo-response regulator is misexpressed in the photoperiod insensitive Ppd-D1a mutant of wheat (Triticum aestivum L.). *Theoretical and Applied Genetics* **115**, 721–733.

Bentley A.R., Horsnell R., Werner C.P., Turner A.S., Rose G.A., Bedard C., … Howells R.M. (2013) Short, natural, and extended photoperiod response in BC2F4 lines of bread wheat with different Photoperiod-1 (Ppd-1) alleles. *Journal of experimental botany* **64**, 1783–1793.

Bornman J.F., Barnes P.W., Robinson S.A., Ballare C.L., Flint S.D. & Caldwell M.M. (2015) Solar ultraviolet radiation and ozone depletion-driven climate change: effects on terrestrial ecosystems. *Photochemical & Photobiological Sciences* **14**, 88–107.

Buckler E.S., Holland J.B., Bradbury P.J., Acharya C.B., Brown P.J., Browne C., … Glaubitz J.C. (2009) The genetic architecture of maize flowering time. *Science* **325**, 714–718.

Cane K., Eagles H.A., Laurie D.A., Trevaskis B., Vallance N., Eastwood R.F., … Martin P.J. (2013) Ppd-B1 and Ppd-D1 and their effects in southern Australian wheat. *Crop and Pasture Science* **64**, 100–114.

Cantor R.M., Lange K. & Sinsheimer J.S. (2010) Prioritizing GWAS results: a review of statistical methods and recommendations for their application. *The American Journal of Human Genetics* **86**, 6–22.

Cao W. & Moss D.N. (1989) Temperature Effect on Leaf Emergence and Phyllochron in Wheat and Barley. *Crop Science* **29**, cropsci1989.0011183X002900040038x.

Carlborg Ö. & Haley C.S. (2004) Epistasis: too often neglected in complex trait studies? *Nature Reviews Genetics* **5**, 618–625.

Chang C.C., Chow C.C., Tellier L.C.A.M., Vattikuti S., Purcell S.M. & Lee J.J. (2015) Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience* **4**, s13742-015.

562 Chen F., Gao M., Zhang J., Zuo A., Shang X. & Cui D. (2013) Molecular characterization of
563 vernalization and response genes in bread wheat from the Yellow and Huai Valley of China. *BMC*
564 *plant biology* **13**, 199.

565 Chen L., Bernhardt A., Lee J. & Hellmann H. (2015) Identification of Arabidopsis MYB56 as a novel
566 substrate for CRL3BPM E3 ligases. *Molecular plant* **8**, 242–250.

567 Chen P., Jäger G. & Zheng B. (2010) Transfer RNA modifications and genes for modifying enzymes in
568 Arabidopsis thaliana. *BMC plant biology* **10**, 201.

569 Chiang C., Aas O.T., Jetmundsen M.R., Lee Y., Torre S., Fløistad I.S. & Olsen J.E. (2018) Day
570 extension with far-red light enhances growth of subalpine fir (Abies lasiocarpa (hooker) Nuttall)
571 seedlings. *Forests* **9**, 175.

572 Chu C.G., Tan C.T., Yu G.T., Zhong S., Xu S.S. & Yan L. (2011) A novel retrotransposon inserted in
573 the dominant Vrn-B1 allele confers spring growth habit in tetraploid wheat (Triticum turgidum
574 L.). *G3: Genes| Genomes| Genetics* **1**, 637–645.

575 Chuck G., Meeley R.B. & Hake S. (1998) The control of maize spikelet meristem fate by
576 theAPETALA2-like gene indeterminate spikelet1. *Genes & Development* **12**, 1145–1154.

577 Clapham D.H., Ekberg I., Eriksson G., Norell L. & Vince-Prue D. (2002) Requirement for far-red light
578 to maintain secondary needle extension growth in northern but not southern populations of Pinus
579 sylvestris (Scots pine). *Physiologia Plantarum* **114**, 207–212.

580 Cooper M. & DeLacy I.H. (1994) Relationships among analytical methods used to study genotypic
581 variation and genotype-by-environment interaction in plant breeding multi-environment
582 experiments. *Theoretical and Applied Genetics* **88**, 561–572.

583 Covington M.F. & Harmer S.L. (2007) The circadian clock regulates auxin signaling and responses in
584 Arabidopsis. *PLoS Biol* **5**, e222.

585 Craufurd P.Q. & Wheeler T.R. (2009) Climate change and the flowering time of annual crops. *Journal*
586 *of Experimental botany* **60**, 2529–2539.

587 Dadshani S., Mathew B., Ballvora A., Mason A.S. & Léon J. (2021) Detection of breeding signatures
588 in wheat using a linkage disequilibrium-corrected mapping approach. *Scientific reports* **11**, 1–12.

589 Dahlgren J.P., von Zeipel H. & Ehrlén J. (2007) Variation in vegetative and flowering phenology in a
590 forest herb caused by environmental heterogeneity. *American Journal of Botany* **94**, 1570–1576.

591 Distelfeld A., Li C. & Dubcovsky J. (2009a) Regulation of flowering in temperate cereals. *Current*
592 *opinion in plant biology* **12**, 178–184.

593 Distelfeld A., Tranquilli G., Li C., Yan L. & Dubcovsky J. (2009b) Genetic and molecular
594 characterization of the VRN2 loci in tetraploid wheat. *Plant physiology* **149**, 245–257.

595 Eagles H.A., Cane K., Kuchel H., Hollamby G.J., Vallance N., Eastwood R.F., … Martin P.J. (2010)
596 Photoperiod and vernalization gene effects in southern Australian wheat. *Crop and Pasture*
597 *Science* **61**, 721–730.

598 Eagles H.A., Cane K. & Vallance N. (2009) The flow of alleles of important photoperiod and
599 vernalisation genes through Australian wheat. *Crop and Pasture Science* **60**, 646–657.

600 Endelman J.B. (2011) Ridge regression and other kernels for genomic selection with R package rrBLUP.
601 *The Plant Genome* **4**, 250–255.

602 Ersoz E.S., Yu J., Buckler E.S., Varshney R.K. & Tuberosa R. (2007) Genomics-assisted Crop
603 Improvement, Vol. 1: Genomics Approaches and Platforms.

604 FAO (2019) No Title. *FAOSTAT statistics database: Crops, available at*
605 *www.fao.org/faostat/en/#data/QC*.

Fjellheim S., Boden S. & Trevaskis B. (2014) The role of seasonal flowering responses in adaptation of grasses to temperate climates. *Frontiers in plant science* **5**, 431.

Flint-Garcia S.A., Thuillet A., Yu J., Pressoir G., Romero S.M., Mitchell S.E., … Buckler E.S. (2005) Maize association population: a high-resolution platform for quantitative trait locus dissection. *The Plant Journal* **44**, 1054–1064.

Frazer K.A., Eskin E., Kang H.M., Bogue M.A., Hinds D.A., Beilharz E.J., … Nilsen G.B. (2007) A sequence-based variation map of 8.27 million SNPs in inbred mouse strains. *Nature* **448**, 1050–1053.

Fu D., Szűcs P., Yan L., Helguera M., Skinner J.S., Von Zitzewitz J., … Dubcovsky J. (2005) Large deletions within the first intron in VRN-1 are associated with spring growth habit in barley and wheat. *Molecular genetics and genomics* **273**, 54–65.

Gawroński P. & Schnurbusch T. (2012) High-density mapping of the earliness per se-3Am (Eps-3A m) locus in diploid einkorn wheat and its relation to the syntenic regions in rice and Brachypodium distachyon L. *Molecular breeding : new strategies in plant improvement* **30**, 1097—1108.

Georges F., Das S., Ray H., Bock C., Nokhrina K., Kolla V.A. & Keller W. (2009) Over-expression of Brassica napus phosphatidylinositol-phospholipase C2 in canola induces significant changes in gene expression and phytohormone distribution patterns, enhances drought tolerance and promotes early flowering and maturation. *Plant, cell & environment* **32**, 1664–1681.

Gilmour A.R., Thompson R. & Cullis B.R. (1995) Average information REML: an efficient algorithm for variance parameter estimation in linear mixed models. *Biometrics*, 1440–1450.

Goulart M.F., Lemos Filho J.P. & Lovato M.B. (2005) Phenological variation within and among populations of Plathymenia reticulata in Brazilian Cerrado, the Atlantic Forest and transitional sites. *Annals of Botany* **96**, 445–455.

Griffiths S., Simmonds J., Leverington M., Wang Y., Fish L., Sayers L., … Snape J. (2009) Meta-QTL analysis of the genetic control of ear emergence in elite European winter wheat germplasm. *Theoretical and Applied Genetics* **119**, 383–395.

Guedira M., Xiong M., Hao Y.F., Johnson J., Harrison S., Marshall D. & Brown-Guedira G. (2016) Heading date QTL in winter wheat (Triticum aestivum L.) coincide with major developmental genes VERNALIZATION1 and PHOTOPERIOD1. *PloS one* **11**.

Guo Q., Ng P.Q., Shi S., Fan D., Li J., Zhao J., … Do T. (2019) Arabidopsis TRM5 encodes a nuclear-localised bifunctional tRNA guanine and inosine-N1-methyltransferase that is important for growth. *PloS one* **14**, e0225064.

Hanocq E., Laperche A., Jaminon O., Lainé A.-L. & Le Gouis J. (2007) Most significant genome regions involved in the control of earliness traits in bread wheat, as revealed by QTL meta-analysis. *Theoretical and Applied Genetics* **114**, 569–584.

Hazebroek J.P. & Metzger J.D. (1990) Thermoinductive regulation of gibberellin metabolism in Thlaspi arvense L.: I. Metabolism of [2H]-ent-Kaurenoic Acid and [14C] Gibberellin A12-Aldehyde. *Plant Physiology* **94**, 157–165.

Herndl M., White J.W., Graeff S. & Claupein W. (2008) The impact of vernalization requirement, photoperiod sensitivity and earliness per se on grain protein content of bread wheat (Triticum aestivum L.). *Euphytica* **163**, 309–320.

Holland J.B., Nyquist W.E. & Cervantes-Martínez C.T. (2003) Estimating and interpreting heritability for plant breeding: an update. *Plant breeding reviews* **22**.

Inouye D.W., Saavedra F. & Lee-Yang W. (2003) Environmental influences on the phenology and abundance of flowering by Androsace septentrionalis (Primulaceae). *American Journal of Botany* **90**, 905–910.

Ito S., Song Y.H., Josephson-Day A.R., Miller R.J., Breton G., Olmstead R.G. & Imaizumi T. (2012) FLOWERING BHLH transcriptional activators control expression of the photoperiodic flowering regulator CONSTANS in Arabidopsis. *Proceedings of the National Academy of Sciences* **109**, 3582–3587.

Jackson M.T. (1966) Effects of microclimate on spring flowering phenology. *Ecology* **47**, 407–415.

Jacob Y., Mongkolsiriwatana C., Veley K.M., Kim S.Y. & Michaels S.D. (2007) The nuclear pore protein AtTPR is required for RNA homeostasis, flowering time, and auxin signaling. *Plant Physiology* **144**, 1383–1390.

Jander G., Norris S.R., Rounsley S.D., Bush D.F., Levin I.M. & Last R.L. (2002) Arabidopsis map-based cloning in the post-genome era. *Plant physiology* **129**, 440–450.

Jiang L., Chen Y., Luo L. & Peck S.C. (2018) Central roles and regulatory mechanisms of dual-specificity MAPK phosphatases in developmental and stress signaling. *Frontiers in Plant Science* **9**, 1697.

Jones P.G. & Thornton P.K. (2003) The potential impacts of climate change on maize production in Africa and Latin America in 2055. *Global environmental change* **13**, 51–59.

Kamran A., Iqbal M. & Spaner D. (2014) Flowering time in wheat (Triticum aestivum L.): a key factor for global adaptability. *Euphytica* **197**, 1–26.

Kang H.M., Zaitlen N.A., Wade C.M., Kirby A., Heckerman D., Daly M.J. & Eskin E. (2008) Efficient Control of Population Structure in Model Organism Association Mapping. *Genetics* **178**, 1709 LP – 1723.

Kilpikari R. & Sillanpää M.J. (2003) Bayesian analysis of multilocus association in quantitative and qualitative traits. *Genetic Epidemiology: The Official Publication of the International Genetic Epidemiology Society* **25**, 122–135.

Kim D.-H., Kang J.-G., Yang S.-S., Chung K.-S., Song P.-S. & Park C.-M. (2002) A phytochrome-associated protein phosphatase 2A modulates light signals in flowering time control in Arabidopsis. *The Plant Cell* **14**, 3043–3056.

King R.W., Pate J.S. & Johnston J. (1996) Ecotyptic Differences in the Flowering of Pimelea ferruginea (Thymelaeaceae) in Response to Cool Temperatures. *Australian Journal of Botany* **44**, 47–55.

Kippes N., Chen A., Zhang X., Lukaszewski A.J. & Dubcovsky J. (2016) Development and characterization of a spring hexaploid wheat line with no functional VRN2 genes. *Theoretical and Applied Genetics* **129**, 1417–1428.

Kippes N., Debernardi J.M., Vasquez-Gross H.A., Akpinar B.A., Budak H., Kato K., … Dubcovsky J. (2015) Identification of the VERNALIZATION 4 gene reveals the origin of spring growth habit in ancient wheats from South Asia. *Proceedings of the National Academy of Sciences* **112**, E5401–E5410.

KOUCHAKI A.L.I.R. & NASIRI M.M. (2008) Impacts of climate change and CO2 concentration on wheat yield in Iran and adaptation strategies.

Langer S.M., Longin C.F.H. & Würschum T. (2014) Flowering time control in European winter wheat. *Frontiers in Plant Science* **5**, 1–11.

Lawlor D.W. & Mitchell R.A.C. (2000) Crop ecosystem responses to climatic change: wheat. *Climate change and global crop productivity*, 57–80.

Lewis S., Faricelli M.E., Appendino M.L., Valárik M. & Dubcovsky J. (2008) The chromosome region including the earliness per se locus Eps-Am1 affects the duration of early developmental phases and spikelet number in diploid wheat. *Journal of experimental botany* **59**, 3595–3607.

Li B., Suzuki J.-I. & Hara T. (1998) Latitudinal variation in plant size and relative growth rate in

Arabidopsis thaliana. *Oecologia* **115**, 293–301.

Liu L., Liu C., Hou X., Xi W., Shen L., Tao Z., … Yu H. (2012a) FTIP1 is an essential regulator required for florigen transport. *PLoS Biol* **10**, e1001313.

Liu Y., He Z., Appels R. & Xia X. (2012b) Functional markers in wheat: current status and future prospects. *Theoretical and Applied Genetics* **125**, 1–10.

Mall R.K. & Aggarwal P.K. (2002) Climate change and rice yields in diverse agro-environments of India. I. Evaluation of impact assessment models. *Climatic Change* **52**, 315–330.

Manderscheid R., Burkart S., Bramm A. & Weigel H.-J. (2003) Effect of CO2 enrichment on growth and daily radiation use efficiency of wheat in relation to temperature and growth stage. *European Journal of Agronomy* **19**, 411–425.

Marquis R.J. (1988) Phenological variation in the neotropical understory shrub Piper arielanum: causes and consequences. *Ecology* **69**, 1552–1565.

Meier U. (1997) *Growth stages of mono-and dicotyledonous plants*. Blackwell Wissenschafts-Verlag.

Menzel A., Sparks T.H., Estrella N., Koch E., Aaasa A., Ahas R., … Zust A. (2006) European phenological response to climate change matches the warming pattern. *Global Change Biology* **12**, 1969–1976.

Miller-Rushing A.J., Katsuki T., Primack R.B., Ishii Y., Sang D.L. & Higuchi H. (2007) Impact of global warming on a group of related species and their hybrids: Cherry tree (Rosaceae) flowering at Mt. Takao, Japan. *American Journal of Botany* **94**, 1470–1478.

Moore L.M. & Lauenroth W.K. (2017) Differential effects of temperature and precipitation on early- vs. Late-flowering species. *Ecosphere* **8**.

Nishida H., Yoshida T., Kawakami K., Fujita M., Long B., Akashi Y., … Kato K. (2013) Structural variation in the 5′ upstream region of photoperiod-insensitive alleles Ppd-A1a and Ppd-B1a identified in hexaploid wheat (Triticum aestivum L.), and their effect on heading time. *Molecular breeding* **31**, 27–37.

Opseth L., Holefors A., Rosnes A.K.R., Lee Y. & Olsen J.E. (2016) FTL2 expression preceding bud set corresponds with timing of bud set in Norway spruce under different light quality treatments. *Environmental and Experimental Botany* **121**, 121–131.

Peterson R.F. (1965) Wheat crop series, Ed. Polunin, N. *Inter Science Publication Inc. New York* **422**.

Phillips P.C. (2008) Epistasis--the essential role of gene interactions in the structure and evolution of genetic systems. *Nature reviews. Genetics* **9**, 855–867.

Pletcher M.T., McClurg P., Batalov S., Su A.I., Barnes S.W., Lagler E., … Bogue M.A. (2004) Use of a dense single nucleotide polymorphism map for in silico mapping in the mouse. *PLoS biology* **2**.

Prasad P.V. V, Boote K.J., Allen Jr L.H., Sheehy J.E. & Thomas J.M.G. (2006) Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field crops research* **95**, 398–411.

Putterill J., Robson F., Lee K., Simon R. & Coupland G. (1995) The CONSTANS gene of Arabidopsis promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. *Cell* **80**, 847–857.

Record P. (2009) The Responses of Species to Climate Over Two Centuries : An Analysis of the Marsham Author (s): T . H . Sparks and P . D . Carey Published by : British Ecological Society Stable URL : http://www.jstor.org/stable/2261570. *Society* **83**, 321–329.

Reif J.C., Maurer H.P., Korzun V., Ebmeyer E., Miedaner T. & Würschum T. (2011) Mapping QTLs with main and epistatic effects underlying grain yield and heading time in soft winter wheat.

741 *Theoretical and Applied Genetics* **123**, 283–292.

742 Robson F., Costa M.M.R., Hepworth S.R., Vizir I., Pin˜ eiro M., Reeves P.H., … Coupland G. (2001)
743 Functional importance of conserved domains in the flowering-time gene CONSTANS
744 demonstrated by analysis of mutant alleles and transgenic plants. *The Plant Journal* **28**, 619–631.

745 Roro A.G., Terfa M.T., Solhaug K.A., Tsegaye A., Olsen J.E. & Torre S. (2016) The impact of UV
746 radiation at high altitudes close to the equator on morphology and productivity of pea (Pisum
747 sativum) in different seasons. *South African Journal of Botany* **106**, 119–128.

748 Rousset M., Bonnin I., Remoué C., Falque M., Rhoné B., Veyrieras J.-B., … Le Gouis J. (2011)
749 Deciphering the genetics of flowering time by an association study on candidate genes in bread
750 wheat (Triticum aestivum L.). *Theoretical and Applied Genetics* **123**, 907.

751 Saile-Mark M., Tevini M. & Mark U. (1996) Effects of solar UVB radiation on growth, flowering and
752 yield of central and southern European maize cultivars (Zea mays L.). *Photochemistry and*
753 *Photobiology* **64**, 457–463.

754 Shaw L.M., Turner A.S. & Laurie D.A. (2012) The impact of photoperiod insensitive Ppd-1a mutations
755 on the photoperiod pathway across the three genomes of hexaploid wheat (Triticum aestivum). *The*
756 *Plant Journal* **71**, 71–84.

757 Sheldon C.C., Burn J.E., Perez P.P., Metzger J., Edwards J.A., Peacock W.J. & Dennis E.S. (1999) The
758 FLF MADS box gene: a repressor of flowering in Arabidopsis regulated by vernalization and
759 methylation. *The Plant Cell* **11**, 445–458.

760 Slafer G.A. & Rawson H.M. (1995) Base and optimum temperatures vary with genotype and stage of
761 development in wheat. *Plant, Cell & Environment* **18**, 671–679.

762 Snape J.W., Butterworth K., Whitechurch E. & Worland A.J. (2001) Waiting for fine times: genetics of
763 flowering time in wheat. In *Wheat in a global environment*. pp. 67–74. Springer.

764 Sønsteby A. & Heide O.M. (2008) Environmental control of growth and flowering of Rubus idaeus L.
765 cv. Glen Ample. *Scientia Horticulturae* **117**, 249–256.

766 Steinhoff J., Liu W., Reif J.C., Della Porta G., Ranc N. & Würschum T. (2012) Detection of QTL for
767 flowering time in multiple families of elite maize. *Theoretical and Applied Genetics* **125**, 1539–
768 1551.

769 Strayer C., Oyama T., Schultz T.F., Raman R., Somers D.E., Más P., … Kay S.A. (2000) Cloning of the
770 Arabidopsis clock gene TOC1, an autoregulatory response regulator homolog. *Science* **289**, 768–
771 771.

772 Sun X.-D. & Ni M. (2011) HYPOSENSITIVE TO LIGHT, an alpha/beta fold protein, acts downstream
773 of ELONGATED HYPOCOTYL 5 to regulate seedling de-etiolation. *Molecular Plant* **4**, 116–
774 126.

775 Takahashi R. (1970) Genetics of earliness and growth habit in barley. *Barley genetics* **2**, 388–408.

776 Team R.C. (2013) R: A language and environment for statistical computing.

777 Terfa M.T., Roro A.G., Olsen J.E. & Torre S. (2014) Effects of UV radiation on growth and postharvest
778 characteristics of three pot rose cultivars grown at different altitudes. *Scientia Horticulturae* **178**,
779 184–191.

780 Thomas D. (2010) Gene–environment-wide association studies: emerging approaches. *Nature Reviews*
781 *Genetics* **11**, 259–272.

782 Trevaskis B., Hemming M.N., Dennis E.S. & Peacock W.J. (2007) The molecular basis of vernalization-
783 induced flowering in cereals. *Trends in plant science* **12**, 352–357.

784 Tsegay B.A., Lund L., Nilsen J., Olsen J.E., Molmann J.M., Ernsten A. & Juntttila O. (2005) Growth

responses of Betula pendula ecotypes to red and far-red light. *Electronic Journal of Biotechnology* **8**, 17–23.

Turner A., Beales J., Faure S., Dunford R.P. & Laurie D.A. (2005) The pseudo-response regulator Ppd-H1 provides adaptation to photoperiod in barley. *Science* **310**, 1031–1034.

Uchiyama K., Iwata H., Moriguchi Y., Ujino-Ihara T., Ueno S., Taguchi Y., … Watanabe A. (2013) Demonstration of genome-wide association studies for identifying markers for wood property and male strobili traits in Cryptomeria japonica. *PloS one* **8**, e79866.

Ueda A., Li P., Feng Y., Vikram M., Kim S., Kang C.H., … Fukuhara T. (2008) The Arabidopsis thaliana carboxyl-terminal domain phosphatase-like 2 regulates plant growth, stress and auxin responses. *Plant molecular biology* **67**, 683.

Voss-Fels K.P., Stahl A., Wittkop B., Lichthardt C., Nagler S., Rose T., … Baig M.M. (2019) Breeding improves wheat productivity under contrasting agrochemical input levels. *Nature plants* **5**, 706–714.

Williams I.H. (1960) Effects of environment on Rubus idaeus LV Dormancy and flowering of the mature shoot. *Journal of Horticultural Science* **35**, 214–220.

Worland A.J. (1996) The influence of flowering time genes on environmental adaptability in European wheats. *Euphytica* **89**, 49–57.

Worland T. (2001) Genetic basis of worldwide wheat varietal improvement. *The world wheat book: a history of wheat breedingWorland, T. (2001). Genetic basis of worldwide wheat varietal improvement. The World Wheat Book: A History of Wheat Breeding, 59–100.*, 59–100.

Würschum T., Maurer H.P., Dreyer F. & Reif J.C. (2013) Effect of inter-and intragenic epistasis on the heritability of oil content in rapeseed (Brassica napus L.). *Theoretical and applied genetics* **126**, 435–441.

Yan L., Fu D., Li C., Blechl A., Tranquilli G., Bonafede M., … Dubcovsky J. (2006) The wheat and barley vernalization gene VRN3 is an orthologue of FT. *Proceedings of the National Academy of Sciences* **103**, 19581–19586.

Yan L., Helguera M., Kato K., Fukuyama S., Sherman J. & Dubcovsky J. (2004) Allelic variation at the VRN-1 promoter region in polyploid wheat. *Theoretical and applied genetics* **109**, 1677–1686.

Yan L., Loukoianov A., Tranquilli G., Helguera M., Fahima T. & Dubcovsky J. (2003) Positional cloning of the wheat vernalization gene VRN1. *Proceedings of the National Academy of Sciences* **100**, 6263–6268.

Yanofsky M.F., Ma H., Bowman J.L., Drews G.N., Feldmann K.A. & Meyerowitz E.M. (1990) The protein encoded by the Arabidopsis homeotic gene agamous resembles transcription factors. *Nature* **346**, 35–39.

Yasuda S. & Shimoyama H. (1965) Analysis of internal factors influencing the heading time of wheat varieties. *Berichte des Ohara Instituts für landwirtschaftliche Biologie, Okayama Universität* **13**, 23–38.

Zanke C., Ling J., Plieske J., Kollers S., Ebmeyer E., Korzun V., … Röder M.S. (2014) Genetic architecture of main effect QTL for heading date in European winter wheat. *Frontiers in Plant Science* **5**, 1–12.

Zheng J., Ge Q., Hao Z. & Wang W.C. (2006) Spring phenophases in recent decades over eastern china and its possible link to climate changes. *Climatic Change* **77**, 449–462.

Zhu X., Tan C., Cao S. & Yan L. (2011) Molecular differentiation of null alleles at ZCCT-1 genes on the A, B, and D genomes of hexaploid wheat. *Molecular breeding* **27**, 501–510.

TABLES

Table 1: Summary statistics for heading date for subset1 and 2.

	Subset1	Subset2
Max	158.32	158.32
Min	147.93	142.07
Mean	153.24	152.30
SD	6.03	6.36
CV	3.93	4.18
σ^2_G	1.13***	2.54***
$\sigma^2_{G\,x\,Y}$	2.14***	3.04***
$\sigma^2_{G\,x\,L}$	4.99***	6.87***
$\sigma^2_{G\,x\,L\,x\,Y}$	11.94***	14.37***
σ^2_{error}	2.52	2.51
H^2	0.89	0.96

Abbreviations: Standard deviation SD. Coefficient of variation CV (in percentage). Variance components for genotypic variance (σ^2_G), genotype-by-year variance ($\sigma^2_{G\,x\,Y}$), genotype-by-location variance ($\sigma^2_{G\,x\,L}$) genotype-by-location-by-year variance ($\sigma^2_{G\,x\,L\,x\,Y}$). *** Significance at <0.001 probability level. Heritability H^2

852 **Table 2**: Percentage of the mean of squares extracted from ANOVA for the genotype interaction with

853 environmental variables and heading date in subset1 (adapted germplasm) including six locations

854 following latitude gradient.

		Loc1 (South)		**Loc2**		**Loc3**		**Loc4**		**Loc5**		**Loc6 (North)**	
Source of variance	DF	MQ	**%**	MQ	**%**	MQ	**%**	MQ	**%**	MQ	**%**	MQ	**%**
Genotype*Tmax_Spr	161	788.43	**98.4****	696.98	**85.4****	184.67	**0.96****	546.45	**0.77****	40.35	**0.11****	12.98	**0.11****
Genotype*Daylength	161	9.41	**1.2****	49.41	**6.1****	6.89	**0.04****	159.33	**0.23****	293.34	**0.76****	107.61	**0.89****
Genotype*G.Rad_Spr	161	3.65	**0.5****	69.31	**8.5****	0.00	**0.00**	1.06	**0.00**	50.54	**0.13****	0.23	**0.00**
Error		0.12		0.13		0.02		0.10		0.33		0.17	

855

856 Abbreviations: Degree of freedom DF. Mean squares MQ. ** Significance at the 0.01 probability level. Loc:

857 Location. Maximal temperature of spring Tmax_Spr. Global radiation of springG.Rad_Spr.

858

859

860

861

862

863

864

865

866

867

868

869

870

871

872

873

874

875

876

Table 3: Significant QTL for flowering time detected in the winter wheat association panels of subset1 und subset2

	QTL	Marker[a]	Chr[b]	Position[c]	Flanking region[d]	MAF[e]	F_Value[f]	*P*[g]	-Log$_{10}$(*P*)	FDR[h]	*PG*[i]	SNP effect[j]
Subset 1	TaHd12	Ra_c69221_1167	5A	41,427,451	41,427,419 - 41,458,586	0.37	26.06	9.40E-07	6.03	9.00E-04	2.78	0.97
	TaHd14	GENE_3500_336	5A	117,495,484	110,667,048 - 117,495,484	0.47	49.3	6.14E-11	10.21	4.25E-07	13.18	-1.2
	TaHd20	BS00022191_51	5A	476,402,782	466,013,993 - 477,546,011	0.35	28.54	3.14E-07	6.5	4.72E-04	2.46	1.05
	TaHd23	BS00024829_51	5B	693,611,551	693,611,551 - 693,679,909	0.26	28.11	3.75E-07	6.43	4.72E-04	2.21	-1.19
Subset 2	TaHd109	AX-158603420	2B	720,796,133	720,796,133 - 730,190,623	0.11	120.4	2.45E-17	16.61	5.54E-19	1.54	5.09
	TaHd119	AX-111134276	3A	556,662,059	556,548,610 - 564,943,896	0.10	159.69	4.25E-19	18.37	1.97E-23	33.01	5.63
	TaHd150	AX-158581720	4A	593,486,064	581,869,248 - 596,506,881	0.12	113.35	3.86E-16	15.41	3.45E-18	1.77	6.27
	TaHd175	Jagger_c3991_101	5B	488,820,722	478,130,002 - 490,769,429	0.08	126.61	1.14E-17	16.94	8.06E-20	1.82	6.01
	TaHd214	AX-158601566	7B	2,944,225	2,944,225	0.09	155.01	2.68E-18	17.57	4.31E-23	7.09	5.83
	TaHd216	AX-158567788	7B	416,768,820	411,058,927 - 426,859,548	0.13	111.72	1.23E-15	14.91	4.89E-18	2.77	5.25

[a] The peak marker of QTL for flowering time showing the highest -Log10(P)

[b] The chromosome harboring the peak marker.

[c] The physical position in bp of the peak marker

[d] The physical interval of the most significant QTL harboring the peak marker

[e] The minor allele frequency set to >5%

[f] F-test statistic value

[g] *P* value threshold set to $p \leq 0.001$

[h] False discovery rate (FDR) set to ≤ 0.05

[i] Proportion of the genotypic variance explained by the QTL in %

[j] Effect in days of the allele substitution on flowering time

FIGURE LEGENDS

Figure 1 Phenotypic distribution of HD_winter in mean value per country of origin of 213 cultivars of the diversity wheat panel (subset2). The mean is based on data collected from six locations across Germany and over three years 2015, 2016 and 2017

Figure 2 Comparison of HD variation based on winter and spring reference dates of scoring. a-for HD-winter and b, for HD_Spring. Locations are denoted in x-axis, HD scorings are denoted in y-axis. The colors refer to years. c:Visualization of Principal Component Analysis of the variability among the environmental factors. The contributions of each variable to the principal components Dim1 and Dim2 is indicated by percentage and colors. d) Summary of the contribution of each variable by combining Dim1 and Dim2. The red dashed line indicates the expected average contribution. The environmental factors that are below the red threshold of the expected average contribution are considered less important.

Figure 3 Frequency in percentage of allele combinations of *VRN* and *PpdD1* genes detected in different wheat germplasm according to the country of origin. For vernalization genes, dominant and recessive alleles are designed with capital and small letters, respectively. For photoperiod genes, letter "a" designs the insensitive allele and letter "b" indicates the sensitive one.

Figure 4 GWAS for heading date using adapted (subset1) and adapted plus non-adapted (subset2) winter wheat cultivars. a and b Manhattan plot showing the identified QTL in the subset1 and subset2, respectively. The y-axes refers to the –log10 (P) values of the SNP markers. The chromosomes are denoted on the x axes. The red dots refer to the significant SNP markers above the cut-off red line. The SNP markers density per chromosome for each subset is shown above the x-axis. The number of SNP

markers within 10 Mbp window size is indicated in categories and colors on the right side of Manhattan plot.

Figure 5 Epistatic interactions detected in subset1 (a) and in subset2 (b). From outside to inside, the layers indicate: the length of chromosomes in Mb, then the organization of chromosomes per subgenome A, B and D, then the mapping of SNP markers used for GWAS, then the QTLs presented according to their –log10 P values extracted from GWAS. The last inner curved lines indicate significant interactions between SNP markers highlighted in colors. The known flowering time genes are indicated with green arrow. The detected genes are highlighted in red. The blue color designs the QTL with epistatic effect.

Figure 6 Seasonal change of Tmax (a) and daylength (b) including three years in loc1 (Moosburg) and in loc6 (Kiel). The mean of Tmax per month is indicated in numbers. Daylength, including civil twilight (h), was computed daily following Forsythe et al. (1995)