

# Genetic loci mediating circadian clock output plasticity and crop productivity under barley domestication

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## Summary

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Received: 5 November 2020  
Accepted: 6 February 2021

New Phytologist (2021) **230**: 1787–1801  
doi: 10.1111/nph.17284

**Key words:** barley, circadian clock, domestication, grain yield, plasticity, selective sweep, temperature compensation.

- Circadian clock rhythms are shown to be intertwined with crop adaptation. To realize the adaptive value of changes in these rhythms under crop domestication and improvement, there is a need to compare the genetics of clock and yield traits.
- We compared circadian clock rhythmicity based on Chl leaf fluorescence and transcriptomics among wild ancestors, landraces, and breeding lines of barley under optimal and high temperatures. We conducted a genome scan to identify pleiotropic loci regulating the clock and field phenotypes. We also compared the allelic diversity in wild and cultivated barley to test for selective sweeps.
- We found significant loss of thermal plasticity in circadian rhythms under domestication. However, transcriptome analysis indicated that this loss was only for output genes and that temperature compensation in the core clock machinery was maintained. Drivers of the circadian clock (DOC) loci were identified via genome-wide association study. Notably, these loci also modified growth and reproductive outputs in the field. Diversity analysis indicated selective sweep in these pleiotropic DOC loci.
- These results indicate a selection against thermal clock plasticity under barley domestication and improvement and highlight the importance of identifying genes underlying for understanding the biochemical basis of crop adaptation to changing environments.

## Introduction

Growth and metabolism follow rhythms that allow resonance between environmental dynamics (e.g., day and night) and molecular pathways that regulate these biological activities (McClung, 2006). The core circadian clock drives these rhythms, as reflected in the cyclic patterns of different layers or outputs, such as photosynthesis, cell division, and metabolism (e.g. starch synthesis and degradation). Circadian output and rhythmicity are often used as approximations of the core clock activities (e.g. leaf movement and CO<sub>2</sub> fixation; Tindall *et al.*, 2015). Since quantitative reverse transcription PCR analysis of circadian clock genes is destructive and time-consuming, and luciferase assay requires costly apparatus and reagents (Somers *et al.*, 1998), nondestructive methods have been developed to allow species-wide non-transgenic and high-temporal-resolution analysis (Tindall *et al.*, 2015). Some of these remote methods include following leaf

movement (Edwards & Millar, 2007) or photosynthetic activity by capturing prompt fluorescence (F) and delayed fluorescence (DF) of Chl (Gould *et al.*, 2009; Dakhiya *et al.*, 2017; Bdolach *et al.*, 2019) or dynamics of gas exchange (Bohn *et al.*, 2001) or dynamics of gas exchange (Bohn *et al.*, 2001). Advances in throughput measurement of circadian rhythms in plants have paved the way for large-scale comparisons between naturally adapted and interspecific populations (Anwer & Davis, 2013).

Notably, some of the critical phenotypes under crop domestication have been linked with mutations in the circadian clock's genetic network, with a connection found between clock variation and life-history traits that is relevant for adaptation to the agricultural setup (Bendix *et al.*, 2015). For example, selection of a rare beet (*Beta vulgaris*) plant with partial loss of function of the pseudo-response regulator (PRR) gene *BOLTING TIME CONTROL 1* led to reduced sensitivity to photoperiod that was restored by vernalization, thus conferring the typical biennial cultivation mode (Pin *et al.*, 2012). In wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), and sorghum, such PRR-mediated

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insensitivity to long days was crucial for the transition to the Northern Hemisphere, mostly in the regulation of photoperiod flowering (Turner *et al.*, 2005; Murphy *et al.*, 2011). Selection for other clock gene alleles – for example, *eam8* mutated at the barley *EARLY FLOWERING3* (*HvELF3*), which disrupts the dependence of flowering on time-of-day light inputs – allowed cultivation further north from the Fertile Crescent, in Europe's shorter growing season (Faure *et al.*, 2012). Very recently, Müller *et al.* (2015), based on rhythms of leaf movement under continuous light, showed that the circadian clock in tomato has decelerated under domestication and that the clock phase has shifted. They explained the light-conditioned effects of the *EID1* and *LNK2* mutations (i.e. changes in the light input to the core clock) as a way of adapting to the changed day–night cycles at higher latitudes of modern vs wild tomatoes (Müller *et al.*, 2018).

Despite these examples of circadian clock changes under domestication, and their assumed adaptive value, it is not yet clear whether selection under domestication and breeding worked for or against the clock's plasticity; that is, changes in the core genes or outputs under changing environments, or what characteristic of the rhythms is affected (period or amplitude, or both). One ongoing question in the study of the circadian clock is the extent of the clock's robustness to environmental changes, and if plasticity, rather than robustness, or temperature compensation was selected for during crop evolution. In other systems, studies on temperature compensation and its underlying mechanism often focus on temperature-dependent changes in core clock components and how these assist to maintain a *c.* 24 h cycle. For example, in *Neurospora*, the clock component casein kinase 1 has two distinct temperature-dependent phosphorylation sites. The different effects of phosphorylation of each site and relationship between increased temperature and kinase activity match earlier models in which temperature compensation could be achieved if two temperature-dependent reactions oppose one another (Hastings & Sweeney, 1957; Narasimamurthy & Virshup, 2017). Another way to define and examine temperature-compensation regulators in plants is to compare the permissive temperature range for rhythmicity; for example, by monitoring leaf movement in null mutant and wild-type *GIGANTEA* genotypes of *Arabidopsis* grown at 17°C vs 27°C (Gould *et al.*, 2006).

In this study, we utilized our SensyPAM high-throughput F-based circadian rhythm measurement platform to estimate and compare the relative plasticity of the clock under optimal and high temperatures. We performed this phenotypic analysis on three barley panels that represent the evolution from wild relatives via landraces to modern cultivars. In addition, we performed a genome scan of a multiparent interspecific population between wild and cultivated barley to identify specific drivers of clock (DOC) loci underlying the changes in the circadian clock characteristics between thermal environments (their plasticity). We hypothesized that these DOC loci may also control major agricultural traits, such as grain yield (GY); therefore, we conducted field trials in two different environments for the same population. Finally, we were interested in knowing if there had been selection for these DOC loci that regulate clock plasticity during barley domestication or improvement.

## Materials and Methods

### Plant material

To compare circadian clock thermal plasticity under domestication, we used three barley panels: 316 wild barley accessions (*Hordeum vulgare* ssp. *spontaneum*) collected from 51 sites across most of Israel (Barley 1K (B1K) collection; Supporting Information Table S1; Hubner *et al.*, 2009); the US Spring Two-Row Multi-Environment Trial panel consisting of 232 elite varieties from five diverse US breeding programs (Neyhart *et al.*, 2019); and the Barley Core Collection (BCC), which includes 142 cultivated lines and 42 landraces collected from across the globe (Table S1; Haseneyer *et al.*, 2010). For the genome-wide association study (GWAS), we used 338 lines originated from the interspecific multiparent barley population Halle Exotic Barley (HEB)-25, which were developed by the introduction of 25 wild barley accessions into a common cultivated (cv Barke) genetic background (Maurer *et al.*, 2015).

### Clock phenotype under optimal and high temperature

Plants were grown in 50 ml pots with planting mixture 'Green' (Even Ari Green, Bet Elazari, Israel), using an irradiance of  $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ , and irrigated daily. Plants were grown in long-day (LD) conditions (14 h : 10 h, light : dark; 05:00 h was Zeitgeber time 0 (ZT0) and 19:00 h was ZT14) at a constant temperature of 20–22°C for *c.* 4 wk until the emergence of the fourth leaf. For the short-day (SD) experiment, plants were grown for 4 wk in conditions (10 h : 14 h, light : dark) in which 07:00 h was ZT0 and 17:00 h was ZT10. Following this entrainment, the plants were transferred to the high-throughput SensyPAM (SensyTIV, Aviel, Israel), which was custom designed to allow F measurements in up to 240 plants for each experiment following the methodology in Bdolach *et al.* (2019). For the clock measurement, F was measured every 2.5 h for 3 d in continuous light. We measured the same plant: once under optimal temperature (OT; 22°C) and a second time under high temperature (HT; 32°C), with four to five plants per genotype. Between the OT and HT run the day length was returned to 14 h : 10 h, light : dark, for two nights. For the clock analysis, the nonphotochemical quenching (NPQ) steady-state values under actinic light ( $\text{NPQ}_{\text{ss}} = (F_m - F_{m \text{ ss}})/F_{m \text{ ss}}$ ) was calculated and normalized to the mean of each experimental cycle as shown previously (Bdolach *et al.*, 2019). The circadian clock's free-running period, amplitude, and amplitude error were extracted using the BioDare2 website (<https://biodare2.ed.ac.uk>). The input data were set to 'cubic dtr', and 'MFourFit' was used as the analytical method (Zielinski *et al.*, 2014).

### Field phenotype of the Halle Exotic Barley population

Field phenotype were carried out during 2015–2016 in Gilat (31.3339°N and 34.6661°E) and during 2019–2020 in Yotveta (29.8939°N, 35.0643°E). Both field sites were in the southern part of Israel with no photoperiod difference between the sites. A

total of 1320 lines of HEB-25 were grown in Gilat with a plot setup under drought and control regimes. For every line, 36 plants were shown in square plots of 1 m × 1 m in both treatments. In total, the drought treatment received 275 mm water and the control received 380 mm water (Fig. S1; Table S2).

In Yotveta, 338 HEB-25 lines were grown in two net houses under two environments: ambient temperature (AT) and HT. Initially, the seeds were germinated in a protected net house. After 20 d, seedlings were transplanted in the net house. Each experimental setup has a dimension of 18 m × 6 m, with three different blocks of 1600 cm × 80 cm. Eight plants per each line were transplanted in a plot. At 4 d posttransplantation, HT was applied by covering the HT net house roof top partially with polythene to allow release of humidity as well. We were able to maintain an average temperature difference of 4°C and 2°C between the HT and AT net house during the day and night period, respectively (Fig. S2; Table S3).

### Statistical analysis

JMP v.14.0 statistical package (SAS Institute, Cary, NC, USA) was used for statistical analyses and to generate reaction norms for the means and SE of the different traits and for principal component analysis. Student's *t*-tests between treatments were conducted per panel using the 'Fit Y by X' function. A factorial model was employed for the ANOVA, using the 'Fit model', with temperature treatment and panel as fixed effects.

### Transcriptome comparison between gene pools

A transcriptome comparison was undertaken between the gene pools of barley (i.e. wild: seven lines; landrace: 10 lines; cultivated: 10 lines). Plants were grown in a growth room under LD conditions (14 h : 10 h, light : dark) at 20–22°C until the two-leaf stage (21 d). Plants were grown in a 308-holes tray, 35 cm × 70 cm, size with 'Green' planting mixture. The plants were then shifted to continuous light for 24 h, in either OT (22°C) or HT (32°C). Leaves from each of the wild, landrace, and cultivated plants were sampled in pool every 4 h starting at ZT29 (10:00 h, 14:00 h, 18:00 h, 22:00 h, 02:00 h and 06:00 h), in three replicates. Total RNA was isolated using TRI Reagent®, and after quality checking and a denaturing gel run according to Tiwari & Grover (2019), the RNA samples were sent for massively parallel single-cell RNA sequencing analysis at the Nancy and Stephen Grand Israel National Center for Personalized Medicine, Weizmann Institute of Science (<https://g-incpm.weizmann.ac.il/>).

### Quantitative expression-analysis methods

Illumina adapters were removed from the demultiplexed fastq files using TRIMMOMATIC (v.0.36; Bolger *et al.*, 2014). Poly-A tails that were at least three bases in length were then removed using an in-house script, keeping reads that were at least 25 bases in length after trimming. The trimmed reads were then aligned to the *Hordeum* genome assembly (Ensemble database;

*Hordeum\_vulgare*.IBSC\_v2.dna.toplevel.fa) using STAR (Dobin *et al.*, 2013). The STAR indexing step (runModegenomeGenerate) used the gff file *Hordeum\_vulgare*.IBSC\_v2.48.gff3. The output SAM files were converted to BAM files using SAMTOOLS (v.1.6; H. Li *et al.*, 2009), and the number of reads overlapping each gene in the gff3 file were counted using the ESAT v.1 toolkit. Normalized counts (trimmed mean of *M*-values) were obtained with the R package EDGER (Robinson *et al.*, 2009), and the statistical tests for differential expression were performed with R package DESEQ2 (v.1.18.1; Love *et al.*, 2014).

### Genome-wide association study

A GWAS was carried out to identify trait variations under OT and HT, and to assess digenic interactions (two-dimensional (2D) scan). Since the methods (genetic model and statistics) chosen for the genome scan have a major effect on the loci identified, we compared several options to determine the most reliable signals that we could support by more than one method. We therefore performed the genome scan using three different analyses: extended Bayesian lasso (EBL), linear mixed model (LMM), and TASSEL pipeline. The details of each model are given in Methods S1.

### Selective sweeps

To detect genomic regions under selection during the domestication and improvement of barley, we used genome-wide genotyping-by-sequencing data for barley germplasm collections of the German Federal *ex situ* Genebank (Milner *et al.*, 2019). The collections include 2714 wild, 12 585 landrace, and 5145 cultivar germplasm lines. For computational feasibility and to avoid a bias due to genetic nonrandomness of the sampled lines, we selected 500 representative lines from each of the three groups, using hierarchical clustering based on the Ward method with Euclidean distance using the R function 'hclust'. The R function 'cutree' was used to cluster the lines from each group into clusters of 500, and one line was selected from each cluster. The selective sweeps were detected using a likelihood-based method implemented in SWEEP v.3.2.1 (Pavlidis *et al.*, 2013). The composite likelihood ratio (CLR) was calculated for Genebank landraces and Genebank cultivars using a grid of 1000.

In addition to CLR values, we calculated reduction in nucleotide diversity (ROD) values. The nucleotide diversity  $\pi$  was calculated for each group (i.e. wild, landrace, and cultivar) with TASSEL v.5.0 (Bradbury *et al.*, 2007) using a 1000 kb window and a step size of 100 kb.  $\pi_{\text{wild}}/\pi_{\text{landrace}}$  and  $\pi_{\text{landrace}}/\pi_{\text{cultivar}}$  were calculated as ROD values of domestication and improvement, respectively. From both the CLR and ROD, the top 5% of the windows or regions with highest values were defined as selective sweeps.

Because the HEB population and the German Federal *ex situ* Genebank germplasm collections were genotyped using different single-nucleotide polymorphisms (SNPs), it was difficult to directly compare the genomic positions of significant signals from the GWAS using the HEB population and selective sweep signals

from the germplasm collections. To enable this comparison, we estimated candidate regions of the significant GWAS signals based on linkage disequilibrium blocks. The linkage disequilibrium between SNPs was evaluated using the Pearson correlation coefficient squared ( $r^2$ ). We estimated the candidate regions with  $r^2 > 0.6$  (Yano *et al.*, 2016).

## Results

### Differential circadian rhythmicity of nonphotochemical quenching and its thermal responses among wild, landrace, and breeding lines of barley

To determine the thermal plasticity of the circadian clock period and amplitude, we phenotyped wild *H. vulgare* ssp. *spontaneum* (from the B1K collection), *H. vulgare* landraces, and more recent breeding material from the USA and world-wide. We used the SensyPAM platform for measuring NPQ<sub>ISS</sub> under OT (22°C) and HT (32°C) and in continuous light for 3 d and determined rhythmicity (period and amplitude). On average, the period of the B1K accessions was shortened under HT compared with OT by 1.7 h (Fig. 1a,b). The heat responses varied between relatively robust accessions, such as B1K-21-15 with a delta of period ( $\Delta$ Period) of 0.03 h, to highly plastic ones, such as B1K-18-05 with a  $\Delta$ Period of  $-8.38$  h (Fig. 1b; Tables S4, S5). Changes in clock amplitude in B1K were even more dramatic than those for period, with average values being doubled under HT compared with OT (Fig. 1c,d; Tables S4, S5). The plasticity in amplitude also varied among accessions, ranging between a decrease of 35.5% (B1K-05-15) to a  $>316.6\%$  increase (B1K-15-05; Tables S4, S5). Moreover, there was no overlap in mean  $\pm$  SE amplitude levels between the two environments (Fig. 1c).

Like that of wild B1K, the cultivated panel also showed a varied level of responses for both period and amplitude plasticity (Fig. 1b,d). This variation may be brought about by the diversity existing in each germplasm set (Fig. S3), which, surprisingly, was not significantly different between panels (Fig. S3a,b). Comparing clock rhythms of the wild material and the cultivated panel showed a slight trend of clock deceleration in barley under domestication (Fig. 1a), although the amplitude was significantly higher (112%) in cultivated plants than in the wild-type in OT (Fig. 1c; Table S4). Moreover, there was a clear difference in clock plasticity between the wild and breeding plant material. Unlike the clear plasticity observed in the fully wild plants (B1K; Student's *t*-test;  $P < 0.0001$  for  $\Delta$ Period different than zero), the mean plasticity of the *H. vulgare* breeding lines was not significantly different than zero for period and amplitude (Student's *t*-test;  $P = 0.5$ ,  $P = 0.09$ , respectively).

Finally, the subset 'landraces/traditional cultivars' from the BCC collection (Stracke *et al.*, 2009) was analyzed. Thermal plasticity of the clock period was similar in direction to that found in the B1K and in the opposite direction for amplitude. The mean  $\Delta$ Period of the landrace was not significantly different from that of the wild barley panel, and significantly different from the cultivar (Tukey–Kramer,  $P < 0.05$ ); that is, a mean  $\Delta$ Period of  $-1.16 \pm 2.62$  h (Fig. 1a,b; Tables S4, S5). Whereas clock

amplitude and plasticity were significantly different between the wild B1K and landrace accessions, with the latter being more similar to the cultivars in thermal amplitude response (Tukey–Kramer,  $P < 0.05$ ), the  $\Delta$ Amplitude in B1K averaged  $+127.7\%$ ; that in the landraces mean was  $+4.56\%$  (Fig. 1c,d).

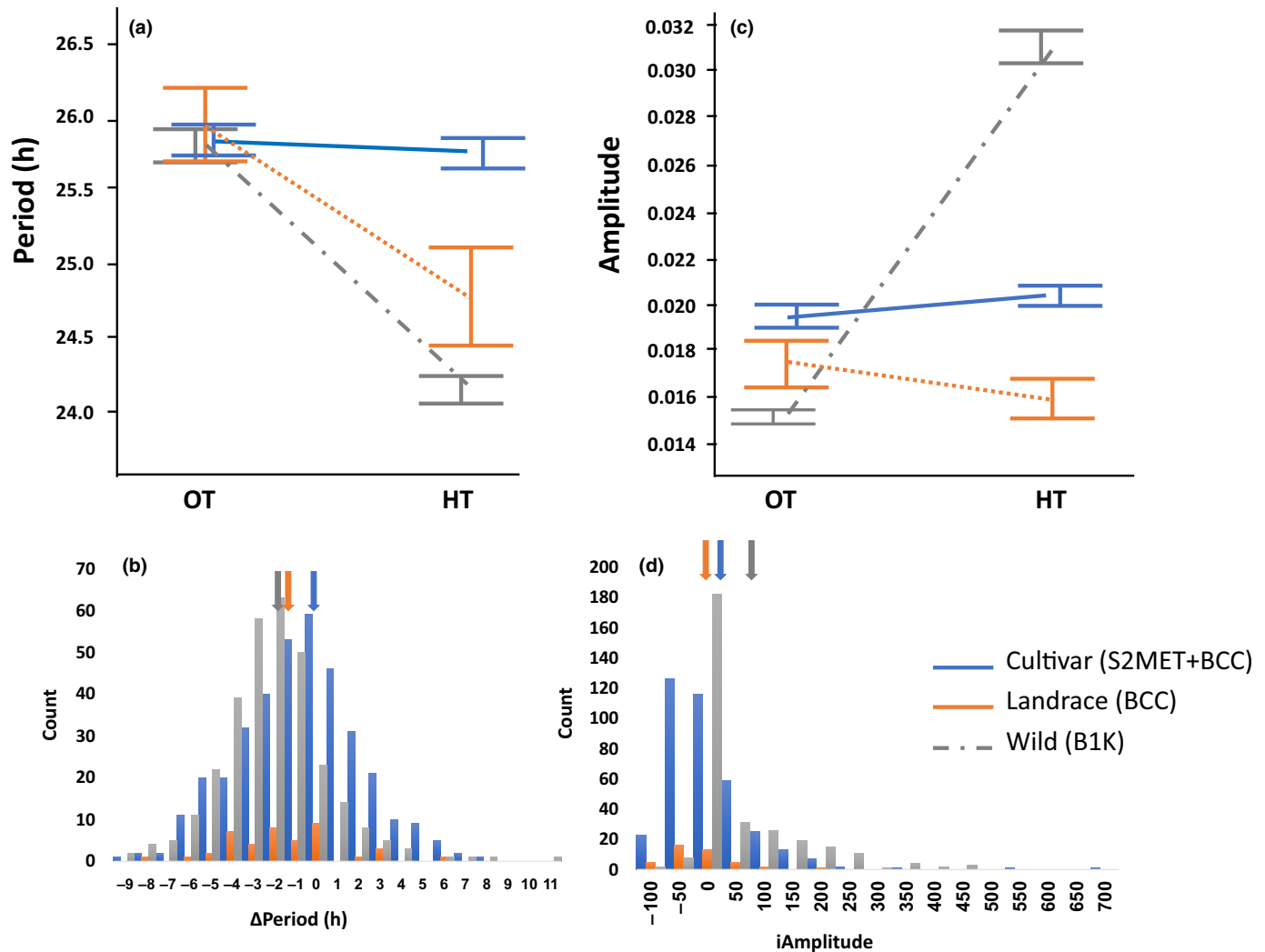
We compared lines from the BCC panel (Haseneyer *et al.*, 2010), which contains both landraces and cultivars collected across the globe with different origin. The thermal plasticity of the period ( $\Delta$ Period) was significantly higher in landraces ( $-1.40 \pm 2.31$  h) than in the cultivars ( $0.26 \pm 2.89$ ). This is compared with lack of significant differences for the thermal plasticity of the amplitude ( $\Delta$ Amplitude; Table 1). This is similar to the results with the cultivated panel (Fig. 1b,d). We found no significant difference between the landrace and the cultivar from Europe (EU) and West Asia and North Africa (WANA). This is compared with a significantly higher  $\Delta$ Period for those accessions coming from America (AM) and East Asia (EA) (Table 1).

### Comparison of gene-expression plasticity of clock core and output genes in wild and cultivated barley gene pool

Transcriptome analysis for the core clock genes displayed the expected rhythmicity, but no significant difference in either the wild or cultivated lines resulting from the temperature shift were detected (Figs 2a, S4). To examine what seems to be a temperature-compensation mechanism (Gould *et al.*, 2006), which maintains these rhythms regardless of the temperature shift, the expression rhythmicity (period and amplitude) of these genes was examined using the BioDare platform. Indeed, overall, the period of these genes did not change significantly between temperatures, with means of 23.48 h (cultivated) and 23.80 h (wild) in OT (one-way ANOVA,  $P < 0.8704$ ) and 22.90 h (cultivated) and 24.26 h (wild) in HT ( $P < 0.5075$ ) (Fig. 2b). Comparison of the amplitude value for the core genes in cultivated (mean 1.022) and wild (mean 0.692) lines at OT and the cultivated (mean 0.8562) and wild (mean 0.7312) lines at HT revealed no significant differences ( $P < 0.2959$  and  $P < 0.65$ , respectively; Fig. 2c).

Next, the expression patterns of selected photosynthesis and NPQ-related output genes were calculated under OT and HT conditions in wild, landrace, and cultivated lines. The light-harvesting Chl-binding (Lhcb) proteins Lhcb1, Lhcb2 and Lhcb3 are components of light-harvesting complex II (LHCII) trimers, Lhcb4 (CP29) and Lhcb5 (CP26) are minor, monomeric antenna proteins of photosystem II (PSII; Z. Li *et al.*, 2009). Photosystem I (PSI) subunit F (PsaF) is a PSI reaction center protein (Z. Li *et al.*, 2009). Overall, the expression of output genes in HT condition were lower than in OT conditions. The output genes *PsaF*, *CP26*, and *Lhcb2* showed differential expression in wild and cultivated lines during the night under the OT condition (Fig. 2d). In the cultivated lines, these genes showed four-fold lower expression (under HT vs OT conditions) during the night (22:00 h) resulting from the temperature shift, whereas in the wild lines their levels were comparable at night in OT and HT (Fig. 2d). *CP29* showed the highest expression difference in wild and cultivated lines as a result of the temperature shift from OT to HT, especially during nighttime (22:00 h; Fig. S5). The





**Fig. 1** Differential circadian rhythms and thermal plasticity among the wild, landrace, and cultivated panels of barley. (a) Circadian rhythm reaction norm for mean and SE clock period under optimal temperature (OT; 22°C) and high temperature (HT; 32°C). (b) The histogram of period plasticity ( $\Delta\text{Period} = \text{Period}^{\text{HT}} - \text{Period}^{\text{OT}}$ ) for all the three panels. (c) Reaction norm for mean and SE clock amplitude under OT and HT of all three panels. (d) Histogram of the amplitude plasticity (index of amplitude,  $i\text{Amplitude} = [(\text{Amplitude}^{\text{HT}}/\text{Amplitude}^{\text{OT}}) - 1] \times 100$ ). The gray bar depicts the wild *Hordeum spontaneum* panel ( $n = 311$ ), the blue bar is the advanced cultivated *Hordeum vulgare* breeding material ( $n = 374$ ), and the orange bar is the landraces ( $n = 42$ ). B1K, Barley 1K; BCC, Barley Core Collection; S2MET, Spring Two-Row Multi-Environment Trial. The clock circadian rhythm is calculated from the nonphotochemical quenching steady-state values under actinic light of an average of four replicates per line using the BioDare2 website (<https://biodare2.ed.ac.uk/login>).

NPQ gene *ZEAXANTHIN EPOXIDASE* displayed a rhythmic expression pattern in all lines under OT and HT conditions, whereas *VIOLAXANTHIN DEEPOXIDASE*, which is part of the carotenoid-biosynthesis pathway and also participates in NPQ (Li Z *et al.*, 2009), displayed differential expression in wild and cultivated lines during the night in OT (Fig. S5). BioDare analysis was performed for 20 output genes to examine differences in expression rhythmicity. In OT, the period of cultivated (mean 23.56 h) and wild (mean 22.36 h) cultivars were comparable ( $P > 0.2963$ ). On average, based on expression of these output genes over the time course, the period of cultivated lines remained similar under both conditions (mean 24.65 h at HT), whereas the period of wild lines was significantly shortened by the transition to HT (mean 20.82 h;  $P > 0.0028$ ). By contrast, the amplitude of the output genes was not significantly different

in either cultivated (mean 0.4372) or wild (mean 0.5345) under OT conditions ( $P > 0.5075$ ) or in cultivated (mean 0.6281) or wild (mean 0.5018) cultivars under HT conditions ( $P > 0.5238$ ) (Fig. 2f). The difference in OT and HT for *PsaF*, *Lhcb2*, and *CP26* remained constant and showed a similar expression pattern (Fig. 2d). *PsbS* also showed a differential expression in cultivated and wild under OT, especially during the evening and at night (Fig. S5).

#### Genome-wide association study of the interspecific population identifies a genetic network among DOC loci

Overall, the thermal plasticity of the clock period in the HEB population was similar in direction to that found in the wild cultivars (i.e. a mean  $\Delta\text{Period}$  of  $-1.42 \pm 2.55$  h; Tables S4, S5).

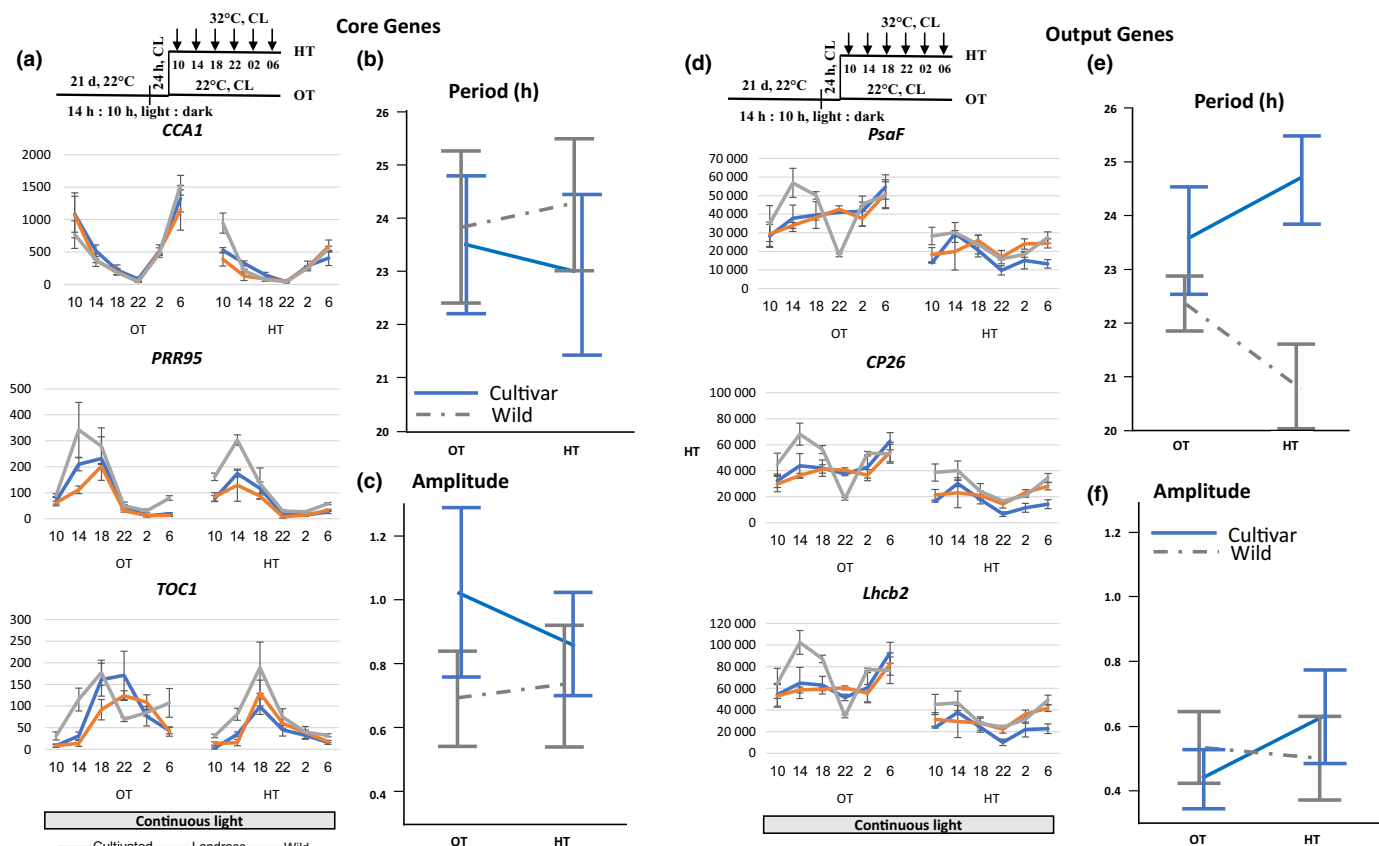
**Table 1** Mean and SD of thermal plasticity ( $\Delta$ Period and  $\Delta$ Amplitude) in the Barley Core Collection with four different origins.

Biological status	Region of origin	No. lines	Mean $\pm$ SD	
			$\Delta$ Period (h)	$\Delta$ Amplitude
Cultivar	AM	26	2.22 $\pm$ 2.54 a	0.00141 $\pm$ 0.0063 a
Cultivar	EA	26	1.21 $\pm$ 2.22 a	-0.00487 $\pm$ 0.0085 a
Cultivar	EU	83	-0.73 $\pm$ 2.77 b	-0.00066 $\pm$ 0.0097 a
Cultivar	WANA	7	0.73 $\pm$ 3.14 b	-0.00089 $\pm$ 0.0141 a
Landrace	EU	13	-1.86 $\pm$ 2.79 b	-0.00212 $\pm$ 0.01 a
Landrace	WANA	26	-1.18 $\pm$ 2.04 b	-0.00096 $\pm$ 0.0086 a
Cultivar	Average	142	0.26 $\pm$ 2.89 A	-0.001061 $\pm$ 0.009 A
Landrace	Average	39*	-1.40 $\pm$ 2.31 B	-0.001346 $\pm$ 0.009 A

AM, America; EA, East Asia; EU, Europe; WANA, West Asia and North Africa.  
Numbers with different letters are significantly different. Tukey–Kramer (lowercase letters) between biological status and region of origin and *t*-test between biological status and region of origin and *t*-test between biological status (uppercase letters).  
\*Three lines excluded from EA.

However, we observed a lesser, albeit significant, difference in the changes of the clock amplitude between the B1K and HEB collections; whereas the  $\Delta$ Amplitude in B1K averaged +128.4%, the  $\Delta$ Amplitude in the HEB lines was half that (+65.6%). Since the HEB population is composed of 25 subpopulations, these comparisons were run on individual families. This analysis showed that, as between the B1K sites, there was a spectrum of responses between the families with different HID (*H. spontaneum* accession) donors (Figs S6, S7). For example, the  $\Delta$ Amplitude in the HEB-02 population (wild donor HID004; Maurer *et al.*, 2015) was on average +180%, whereas that of lines derived from HEB-14 (HID144) was almost unchanged ( $\Delta$ Amplitude = -4.2%; Fig. S8).

A genome scan for the loci affecting the trait per se (under OT or HT), or their plasticity (delta of trait), was conducted with 3013 markers (Table S6). By using a significant threshold determined by Bonferroni correction with false-detection rate of 0.1, we identified four significant quantitative trait loci (QTLs), two for each period and amplitude. Each of these four QTLs was



**Fig. 2** Temporal expression and rhythms of the core clock and output genes under optimal temperature (OT, 22°C) and high temperature (HT, 32°C) in barley gene pools. (a) Line graph expression of representative core circadian clock *CCA1*, *PRR95*, and *TOC1* genes in wild (gray), landrace (orange), and cultivated (blue) barley lines under OT (left panel) and HT (right panel) under continuous light. Reaction norms for the means and SE of core clock genes' (b) period and (c) amplitude under OT and HT conditions. (d) Line graph expression of representative output genes in wild (gray), landrace (orange), and cultivated (blue) barley lines under OT (left panel) and HT (right panel) under continuous light. Reaction norms for the means and SE of output clock genes' (e) period and (f) amplitude under OT and HT conditions. Samples were harvested at 4 h interval starting from 10:00 h to 06:00 h (Zeitgeber; ZT29, ZT33, ZT37, ZT41, ZT45 and ZT49). Values are means of three biological replicates  $\pm$  SE. Top: schematic diagram showing the experimental condition. CL, continuous light; L, light; D, dark. The y-axes show trimmed mean of *M*-values values in all graphs. Period and amplitude were calculated with BioDARE2 software.

detected in all three genome scan pipelines (Fig. S9a–c). These QTLs were termed DOC, and their location, size based on linkage disequilibrium values, effect, and phenotypic contribution are summarized in Table S7 and S8. One locus, *DOC1.1* on chromosome 1 (position 548 354 572–556 777 555), was associated with period in HT (Fig. 3a; Table S8). The second QTL, termed *DOC5.1*, resides on chromosome 5 (position 605 805 151–609 517 829), and it was associated with variation of the period under OT and delta period (Fig. 3a; Table S8). In both *DOC1.1* and *DOC5.1*, the wild allele accelerated the period for all the significant markers in these loci (Fig. 3b,c). *DOC3.1*, which resides on chromosome 3 (position 29 085 440–36 987 723), was associated with variation of the amplitude under HT and delta amplitude (Fig. 3d; Table S8). We identified *DOC3.2*, which was also associated with variation of the amplitude (position 51 509 488; logarithm of the odds: 4.63), near *DOC3.1* (Fig. 3d; Table S8). Notably, the increase in amplitude in HT for both *DOC3.1* and *3.2* loci were attributed to wild alleles for all markers in these loci (Fig. 3e,f; Table S8). It is interesting to note that, based on the linkage disequilibrium pattern (Table S7), the region of *DOC3.2* spans the barley domestication gene *BTR* and circadian clock gene *GIGANTEA* (Dunford *et al.*, 2005). Apart from these four DOC loci, which we identified by all three methods used (Fig. S8), we also detected more QTLs for the trait per se and their plasticity (Fig. S9a–d; Table S8).

### Two-dimensional genome-wide association study for clock diversity highlights the contribution of epistasis to circadian clock diversity

From both a statistical and a biological point of view, epistatic interactions can alter the efficacy of detecting causal QTLs due to mutually conditional effects, as we previously showed for MKS1 and MKS2 in tomato (Ben-Israel *et al.*, 2009). The interactions between the loci appearing with high additive values were mostly heat conditioned and acted on clock amplitude (Fig. S10b; Table S9). Simultaneously, conditioning was also found for period (Fig. S11; Table S9), where each member of an interacting pair conditioned a different trait for the other. Epistatic interactions that included DOC loci were not limited to the specific traits for which they were detected in the one-dimensional (1D) analysis. For example, *DOC1.1* was an epistatic locus for both HT period and HT amplitude (Table S9), whereas in the 1D analysis it was only detected for period. All the interactions including the DOC loci (Fig. 4), and other QTLs identified by 2D analysis, are summarized in Table S9. *DOC1.1* was found to accelerate the period under HT when homozygous wild loci of *DOC1.1* interacted with heterozygous loci on chromosome 5 (Fig. 4a). For HT amplitude, we found interaction of *DOC3.1* and *DOC3.2* with chromosomes 5 and 3, respectively, which favored amplitude increase under HT. When the heterozygous allele of *DOC3.1* interacts with homozygous wild allele of chromosome 5 (Fig. 4b) and also when homozygous wild allele of *DOC3.2* interacts with cultivated homozygous allele of chromosome 3, an increase in amplitude under HT was noticed (Fig. 4b, c). *DOC5.1* interacted with different markers on chromosome 7

for OT period and resulted with a period shortening when homozygous wild allele of *DOC5.1* interacted with the heterozygous wild alleles in chromosome 7 (Fig. 4d). Other significant interactions are summarized in Table S9.

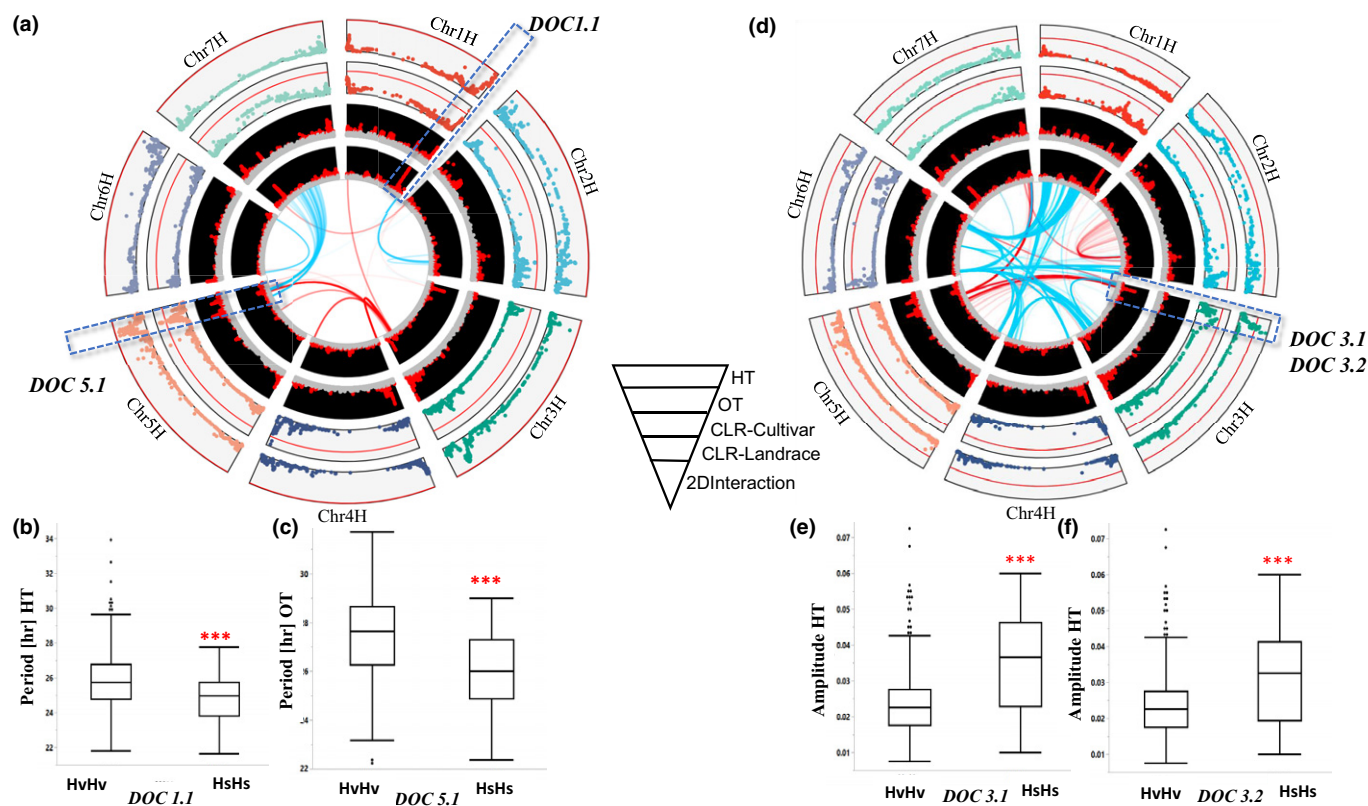
### DOC loci are significantly associated with temperature-dependent effects on heading and grain yield

To test for pleiotropic effects of the DOC loci, we performed a GWAS with phenotypes we collected from the same HEB population in four experiments in the south of Israel: two experiments in Gilat, under control and drought conditions, and two experiments in Yotveta, under AT and HT. We also compared genome scans with QTL results that had been published for the same population by other groups: at Kühnfeld Experimental Station of Martin Luther University Halle-Wittenberg (51°29′46.47″N; 11°59′41.81″E; Maurer *et al.*, 2015); the International Center for Biosaline Agriculture, Dubai, United Arab Emirates (25°5.847′N; 55°23.464′E; Saade *et al.*, 2016); and Dundee, Scotland (Herzig *et al.*, 2018).

In the Gilat experiment, the wild allele at *DOC3.1* and *DOC3.2*, which we linked with higher amplitude for the wild allele in HT (Fig. 3e,f), was associated with a significant reduction in GY and GY per plant, by 50% and 25%, respectively, in drought vs control environments (Fig. 5a,b; Table S10). The wild allele doubled total dry matter and almost doubled vegetative DW under high temperature at Yotveta (Fig. 5c,d). In experiments conducted previously at Halle, this QTL reduced 1000-grain weight and increased plant height (Maurer *et al.*, 2015). An association was also elucidated by a general linear model analysis between the wild allele of *DOC1.1*, which was linked to period shortening for the wild allele in HT, and a 26% and 12% increased GY under drought and control conditions (in Gilat), respectively (Fig. S12a,b; Table S10). The wild allele also increased GY per plant under the two treatments in Gilat (Fig. S12c). We also found that the same QTLs shorten plant development by 2–3 d in a comparison between carriers of the wild and cultivated alleles in other studies (Maurer *et al.*, 2015; Herzig *et al.*, 2018). In those studies, these effects on flowering time were associated with *HvELF3*, which is located near *DOC1.1*. Similarly, Saade *et al.* (2016) showed that the wild allele in this QTL causes earlier flowering and maturity under both control and saline conditions and increases the harvest index. We also found a significant epistasis between *DOC1.1* and *DOC3.1* for GY in Yotveta under drought. The combination of wild allele of *DOC1.1* with the cultivated allele of *DOC3.1* had a higher GY than the other alleles' combinations (Fig. S12d).

### DOC loci overlap with footprints of selection under domestication

Our phenotypic and genotypic results indicated that there was a change in the circadian clock output during barley domestication and improvement and that DOC loci underlie some of those changes. Furthermore, since some of these DOC loci mediate GY, the question arose whether these loci were under selection



**Fig. 3** Genome scan identifies drivers of the clock (DOC) loci. Circos plots depicting the genome-wide association study (GWAS) results in the HEB-25 multiparent population for (a) period and (d) amplitude and their plasticity. Barley chromosomes in the plot are depicted in different colors. The outermost layer and the one in from that represent Manhattan plots with  $-\log_{10}(P)$  of one-dimensional GWAS for high temperature (HT; 32°C) and optimal temperature (OT; 22°C), respectively. Red lines in the Manhattan plots indicate significant threshold ( $P = 0.01$ , established by 1000 permutations). The innermost layer and the layer next to it represent the composite likelihood ratio values from SWEED for landraces and cultivars, respectively, for Asia. Blue and red lines within the centers of the circles indicate significant digenic interactions detected by two-dimensional two-locus GWAS under OT and HT, respectively ( $P = 0.05$ , established by 1000 permutations). (b–f) Box plots for DOC markers that show differences in the allele effect for wild and cultivated markers: (b) SCRI\_RS\_156009 of *DOC1.1* for period at HT, (c) SCRI\_RS\_196175 of *DOC5.1* for period at OT, (e) BOPA1\_4844\_1737 of *DOC3.1* and (f) BOPA2\_12\_31475 of *DOC3.2* for amplitude at HT. \*\*\*,  $P < 0.001$ . In the box plots, the horizontal lines and the vertical boxes represent the median, and the ends of the boxes are the upper and lower quartiles.

during domestication. There is ample evidence now that barley domestication was not a single or double event, but that domesticated barley has a complex reticulate genealogy (Hass *et al.*, 2019; Poets *et al.* 2015; Pankin *et al.* 2018). For example, it was found that the domestication gene *BTR1* dominated in European landraces whereas *BTR2* dominated in Asian landraces (Pourkheirandish *et al.*, 2015). Therefore, we performed an analysis of the genetic sweeps between common wild barley and domesticated barley separately for the Asian and European groups (Fig. 6).

Among the top 5% value from the CLR and ROD data (Tables S11, S12), there was a clear significant sweep in all chromosomes. The number of selective sweeps detected by ROD was higher for landrace vs cultivar than for wild vs landrace in both the Asian and European groups, which indicated selection of more improvement genes in the more recent past. Our analysis also detected the location of the known barley domestication locus *Btr1/2* and *Intermedium-c* within the selection sweep. *BTR* is responsible for the nonbrittleness of the spike, whereas the *Intermedium-c* is a modifier of lateral spikelet fertility in barley

and the orthologue of which is also the domestication gene in maize (Pourkheirandish *et al.*, 2015).

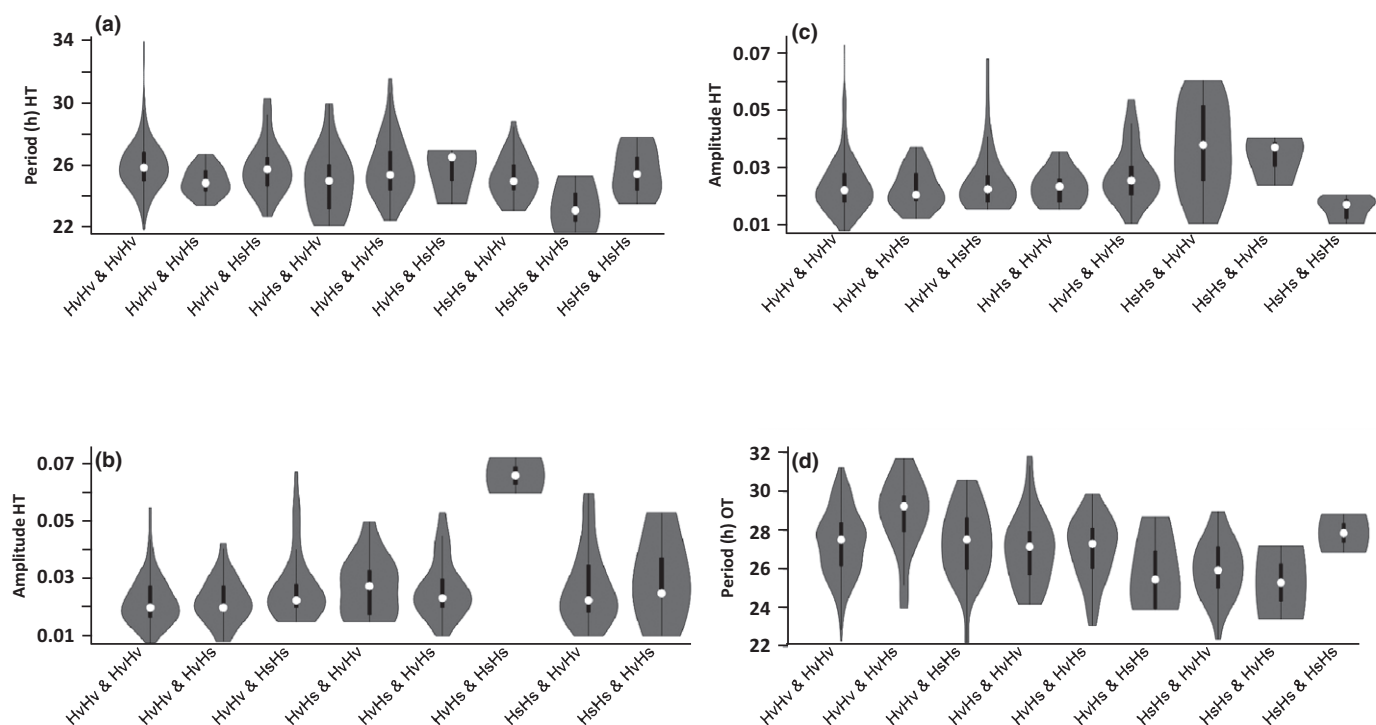
Notably, both SWEED CLR and sigma pi values identified *DOC1.1*, *DOC3.1*, and *DOC5.1* in the selection sweep during the domestication and improvement period of barley evolution in both Asia (Fig. 6a–d) and Europe (Fig. 6e–h). In addition to the DOC loci (Fig. 4), we identified some of the loci involved in the significant epistatic interactions with the imprint of the sweep (Table S1).

## Discussion

### Measuring circadian clock changes using high-throughput platforms

A clear difference between the wild and modern breeding materials was that the wild accessions accelerated their NPQ rhythmicity and increased their amplitude oscillation under hot conditions, whereas the cultivated genotypes are much less responsive and, overall, maintain similar peripheral (output)





**Fig. 4** Epistatic interactions involving the drivers of the clock (DOC) loci. (a) *DOC1.1* (represented by SCRI\_RS\_175218) interaction with SCRI\_RS\_3429 on chromosome 5 for period under high temperature (HT; 32°C). (b) *DOC3.1* (represented by BOPA2\_30431) interaction with SCRI\_RS\_1481120 on chromosome 5 for amplitude under HT. (c) *DOC3.2* (represented by SCRI\_RS\_199987) interaction with SCRI\_RS\_180027 on chromosome 3 for amplitude under HT. (d) *DOC5.1* (represented by SCRI\_RS\_210928) interaction with SCRI\_RS\_175879 on chromosome 5 for period under optimal temperature (OT; 22°C). In the violin images, the white dots represent the median and the thick black vertical lines represent the interquartile range. Wider sections of the violin plot represent a higher probability that members of the population will take on the given value, whereas the thinner sections represent a lower probability.

rhythms (Fig. 1). We used a high-throughput readout of NPQ to obtain measures of clock rhythmicity (period and amplitude) by analyzing oscillator and output genes. In a previous study, we showed that the wild barley *frp2.2* locus accelerates the F-based clock under hot conditions, yet examination of *CCA1* and *TOC1* temporal expression did not reveal any significant effect on these core clock genes (Bdolah *et al.*, 2019). In the current study, we expanded this analysis by performing whole-genome transcriptomic analysis and compared the plasticity of more core clock genes, as well as that of output genes related to NPQ. We further expanded this analysis to include three groups of genotypes that represent the evolution of barley from domestication to improvement. In all three gene pools, the core clock genes maintained their rhythmicity under both OT and HT environments (Fig. 2a). Moreover, none of the core genes, including input genes, showed any significant difference in expression pattern in OT and HT conditions between the gene pools, as reflected by the expression of core genes and by BioDare analysis (Fig. 2b). Therefore, these observations for the core clock genes support the existence of temperature-compensation mechanisms (Gould *et al.*, 2006; Sorek & Levy, 2012; Ford *et al.*, 2016).

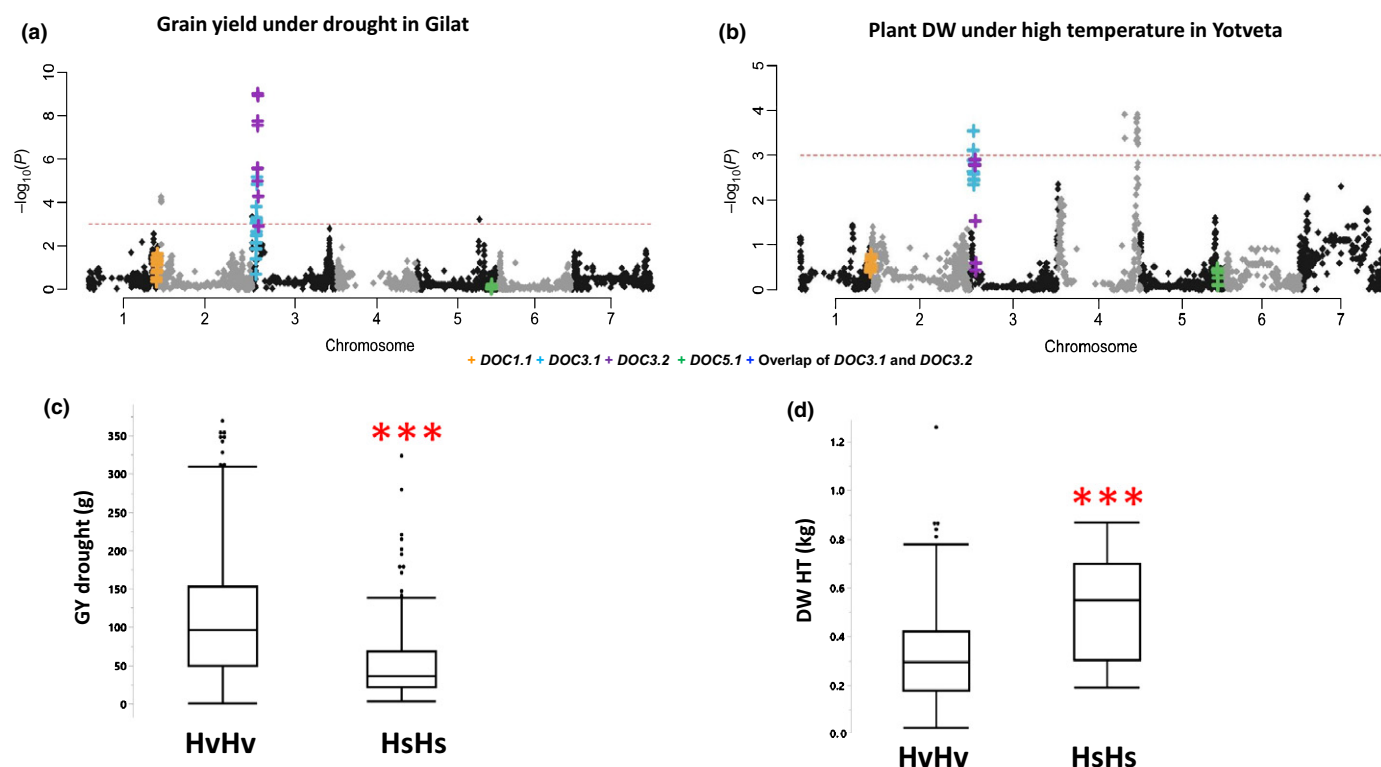
The fact that expression of the output genes did not show such robust rhythms (Fig. 2e), but rather followed the trend observed with the SensyPAM platform (Fig. 1), highlights three important points. First, the output or peripheral activities of the clock do not necessarily follow the same principles of temperature

compensation as the core clock network. Second, from a phenomics point of view, high-throughput phenotypes, such as F or DF (Gould *et al.*, 2009), are proxies for the core circadian clock rhythms and care should be taken to distinguish between the two (output and core). Third, the plasticity of the output activities and the hitherto unknown genes underlying these peripheral activities (outside the core clock) is likely to have important implications for understanding the evolution of the clock while considering new biological functions in crop adaptation.

### Nature of the genes underlying circadian clock output plasticity

In plants, NPQ overcomes excess absorbed light by switching LHCII of PSII to a heat-dissipation function (Goss & Lepetit, 2015; Ruban, 2016). The energy quenching, which is a major component of NPQ, is induced by high trans-thylakoid  $\Delta pH$  in excess light. This induction depends on the xanthophyll cycle, where de-epoxidization of violaxanthin and antheraxanthin forms zeaxanthin (Z. Li *et al.*, 2009).

Here, we focused on the circadian clock output to calculate the expression of PSII and NPQ-related genes. In-depth analysis of output genes specifically related to PSII showed that the temporal gene expression of *Lhcb1* was comparable in wild and cultivated lines under OT, whereas it differed between the two under HT. Other *Lhcb* genes, such as *Lhcb2*, *Lhcb3*, *CP29/Lhcb4*, *CP26/*



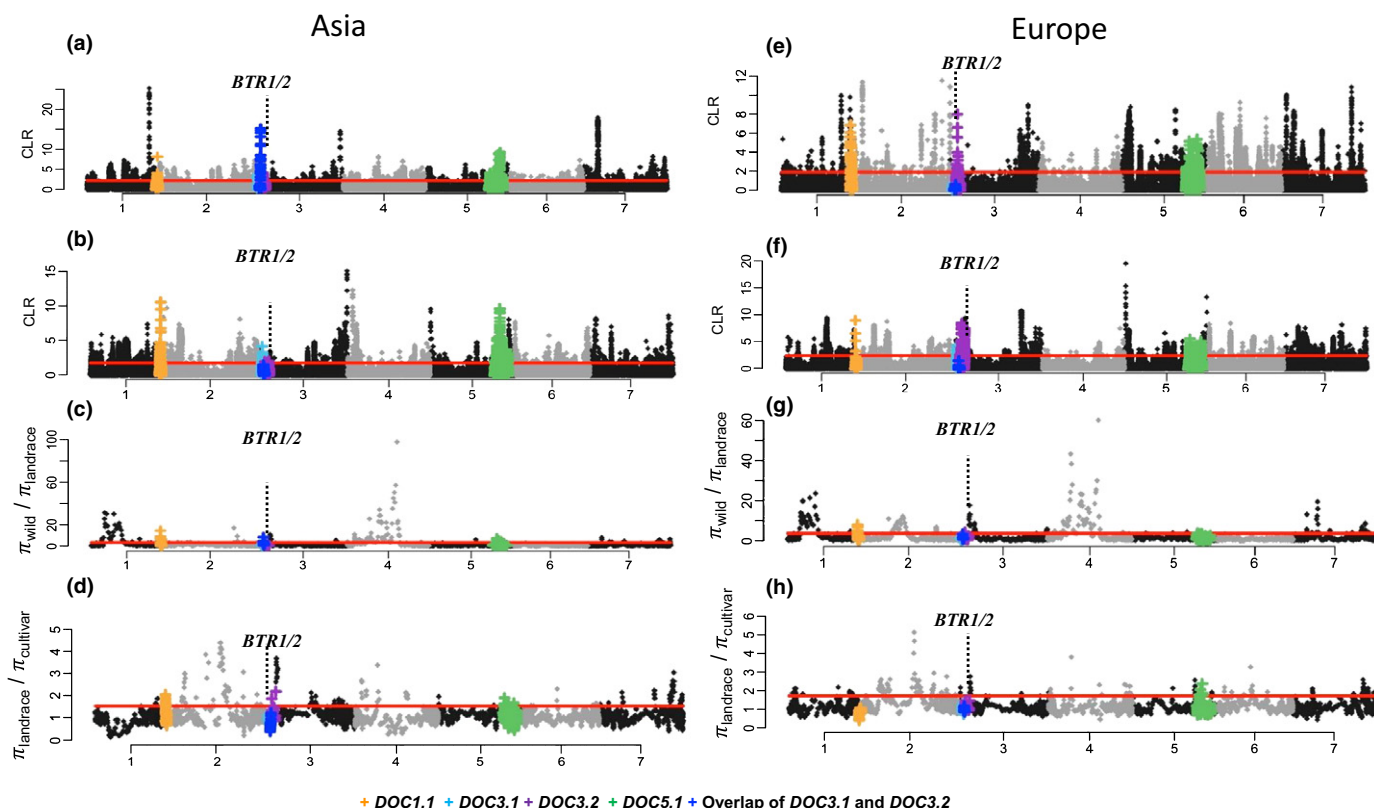
**Fig. 5** Pleiotropy of the drivers of the clock (DOC) loci for plant growth and grain yield (GY) in field experiments at Gilat and Yotveta. (a, b) Manhattan plots with  $-\log_{10}(P)$  of one-dimensional genome-wide association study for (a) GY under drought conditions in Gilat and (b) plant DW in Yotveta. The different DOCs are color coded with crosses: yellow, DOC1.1; blue, DOC3.1; violet, DOC3.2; green, DOC5.1. (c, d) Box plots for DOC3.1 with the wild genotype (HsHs) showing (c) significant decreasing effect ( $P < 0.0001$ ) on GY and (d) significant increasing effect ( $P < 0.0001$ ) on DW compared with carriers of the cultivated alleles (HvHv). HT, high temperature ( $32^{\circ}\text{C}$ ). Error bars in this plot depict the 95% confidence interval. The bottoms and tops of the boxes depict the 25<sup>th</sup> and 75<sup>th</sup> percentiles, and the lines inside represent the 50<sup>th</sup> percentile (median). Outliers are shown as open circles. \*\*\*,  $P < 0.001$ .

*Lhcb5* and *PsaF*, also showed a differential expression pattern in the time course analysis in OT and HT conditions. The excess radiation on the plants in HT may impose significant stress and damage to PSII (Björkman & Demmig-Adams, 1995), and such a transduction of heat responses would allow systemic resilience. Recently, Yarkhunova *et al.* (2016, 2018) suggested that the circadian clock might contribute to regulation of thermal dissipation of excess energy and through connections between clock period and both PSII efficiency and NPQ. Our observation that the timing of the differential plasticity for NPQ activity was primarily during the night may suggest that those physiological pathways play an additional role in some process other than photochemistry. Detailed physiological and molecular analyses of near-isogenic lines for the DOC loci identified in this study, including the differential transcriptome and metabolome changes, should unravel the main pathways that would explain these temporal differences and their systemic consequences.

### Candidate genes underlying the DOC loci

Our genetic scan identified four main DOC loci, and the comparative transcriptome analysis indicated that the differential plasticity of genes related to NPQ, our proxy for the clock

rhythms, occurs mainly during the subjective night under continuous light. One obvious way to find candidate genes is to look at the list of barley genes in the DOC region and link them to the clock function based on previous circadian clock studies in plants. One candidate gene is *Elf3*, which resides near *DOC1.1*. The circadian clock gene *EAM8* (orthologue of the *Arabidopsis thaliana* circadian clock regulator *ELF3*), which accelerates the transition from vegetative to reproductive growth and inflorescence development, also resides in this part of the chromosomal arm (Faure *et al.*, 2012). Moreover, very recently, Jung *et al.* (2020) showed that *A. thaliana* ELF3 prion domain forms condensates *in vitro* in a temperature-dependent manner, thereby modulating circadian rhythms. Moreover, Silva *et al.* (2020) studied *Elf3* as part of the evening complex (EC) and showed that *in vitro* EC is able to act as a thermosensor by lowering of the DNA binding at higher temperature in *Arabidopsis* and thereby affecting the suppression of downstream genes. This may suggest that natural variation in the protein or at the *Elf3* or in its expression timing in barley could directly coordinate the expression of hundreds of key regulators of photosynthesis, the circadian clock, phytohormones, signaling, growth, and response to the environment (Ezer *et al.*, 2017).



**Fig. 6** The drivers of the clock (DOC) loci reside within genomic regions that were under selection during domestication (landrace) and improvement (cultivar). The y-axes represent composite likelihood ratio (CLR) and diversity reduction index ( $\pi_a/\pi_b$ ), and the x-axes represent chromosome location. The horizontal red lines indicate the top 5% CLR and reduction index values that suggest the existence of a selection signature for signals that exceed that line for (a) CLR of Asia\_landrace, (b) CLR of Asia\_cultivar, (c) distribution of nucleotide diversity reduction index during domestication ( $\pi_{wild}/\pi_{landrace}$ ) Asia, (d) improvement ( $\pi_{landrace}/\pi_{cultivar}$ ) Asia, (e) CLR of Europe\_landrace, (f) CLR of Europe\_cultivar, (g) distribution of nucleotide diversity reduction index during domestication ( $\pi_{wild}/\pi_{landrace}$ ) Europe, and (h) improvement ( $\pi_{landrace}/\pi_{cultivar}$ ) Europe. Dotted lines indicate location of known domestication gene *BTR1/2*. The different DOCs are color coded with crosses: yellow, DOC1.1; blue, DOC3.1; violet, DOC3.2; green, DOC5.1.

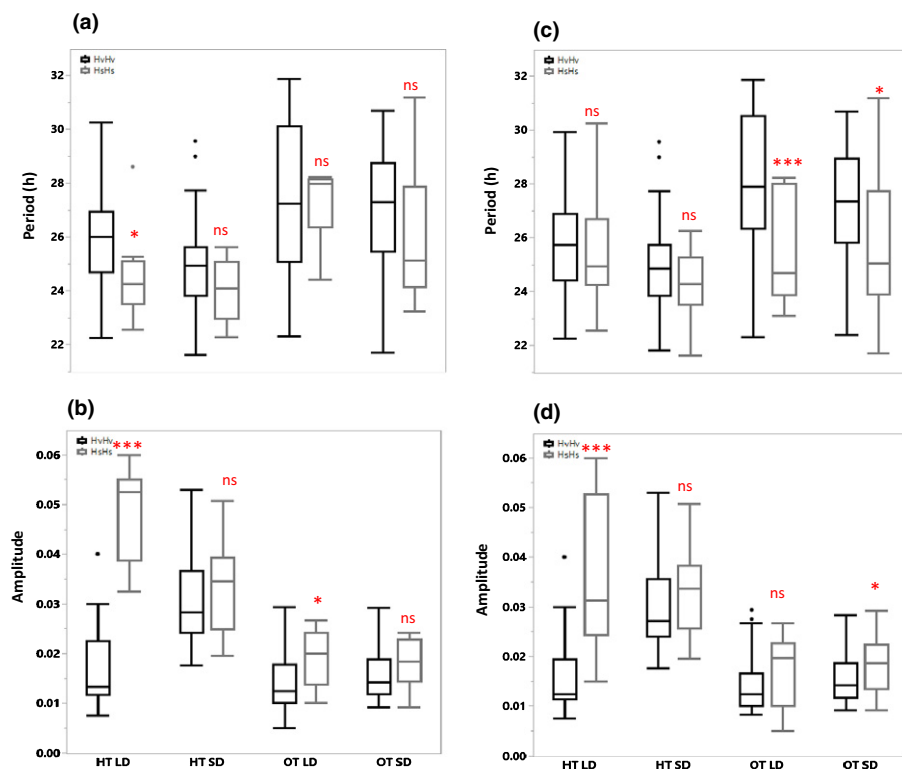
### The adaptive value of plasticity vs robustness and its genetic basis

The type of genetic material investigated in this study, as well as other comparisons between genotypic and phenotypic diversity under domestication (Wright *et al.*, 2005; Caicedo *et al.*, 2007; Olsen & Wendel, 2013), considers nuclear genome diversity that had obviously undergone a bottleneck (Tanksley & McCouch, 1997; Prusty *et al.*, 2018). Moreover, recent studies are attempting to identify signatures of selection by identifying genetic sweeps across these nuclear genomes between wild and cultivated panels (Bourguiba *et al.*, 2012; Shi & Lai, 2015). In our study, the DOC loci were found to be in the top range of the selection sweep and, therefore, could possibly be part of a subsection of loci that have both a role in circadian clock and have undergone changes due to domestication and improvement. How do these genetic changes between cultivated and wild alleles for the DOC loci that we identified in the current study correspond to the known history of barley domestication and improvement?

Barley cultivation from ancestral *H. vulgare* ssp. *spontaneum* to derived *H. vulgare* landraces and cultivars involved a transition from growth under SDs (winter growth in the Southern Levant) to longer days (spring season in the Northern Hemisphere). We

further tested 59 HEB genotypes segregating for *DOC3.1* and *DOC5.1* under SD or LD entrainment (10 h : 14 h, light : dark). The analysis under shorter days suggested that these differences in the output rhythms, and their differential plasticity, are dependent on day length and may coincide with the transition of the wild ancestors to longer-day cultivation (Fig. 7). Notably, the relatively decelerated rhythm associated with the cultivated vs wild genotypes (HvHv vs HsHs) at the *DOC5.1* QTL (identified under OT; Fig. 2) is much more emphasized under LD (28.17 h vs 25.66 h, respectively) than SD (27.17 h vs 25.73 h, respectively) (Fig. 7a). Moreover, the significant increasing effects of the wild *DOC3.1* allele on the amplitude under HT (0.0048 vs 0.0017) were lost when we tested these changes under SDs (Fig. 7b).

Moreover, the analysis of the differences in plasticity within the BCC collection (Table 1) indicate that the amplitude of thermal plasticity diverged from the wild at an early point during domestication; by contrast, the period thermal plasticity occurred later, during improvement or adaptation to new areas. It seems that, according to this comparison (Table 1), changes in the period were still ongoing during the transition of the cultivar to different niches, such as America and East Asia, and may have been related to crop improvement in these regions (local



**Fig. 7** Effect of the drivers of the clock (DOC) loci are day-length dependent. The quantile plots represent means of clock output period and amplitude for HEB lines carrying the wild or cultivated alleles (HsHs and HvHs, respectively) in *DOC3.1* or *DOC5.1*. Plants were measured for clock rhythmicity under two thermal environments, optimal temperature (OT; 22°C) and high temperature (HT; 32°C) and short day (SD; 10 h : 14 h, light : dark) and long day (LD; 14 h : 10 h, light : dark) entrainment before the clock measuring in the SensyPAM. The means of circadian period (a, c) and amplitude (b, d) for carriers of Hs vs Hv alleles for *DOC3.1* and *DOC5.1* were compared by Student's *t*-test. These comparisons were conducted for values obtained under SD or LD entrainment, and between plants grown under OT or HT. Error bars in this plot depict the 95% confidence interval. The bottoms and tops of the boxes are the 25<sup>th</sup> and 75<sup>th</sup> percentiles, and the lines inside represent the 50<sup>th</sup> percentile (median). Outliers are shown as open circles. \*, *P* < 0.05; \*\*\*, *P* < 0.001; ns, not significant.

adaptation). Beyond sorting the timing of the selection events between domestication and improvement, identifying the genetic agents of selection against thermal clock plasticity under barley domestication could help us to maintain, or improve, crop yield despite global warming. In our study, *DOC1.1* is one example of how a wild allele was selected out during domestication but seems to have a benefit for crop productivity in current and future abiotic stress scenarios (drought and heat, which is characteristic of Gilat; Fig. S1). Realizing which alleles have stress-conditioned effects (i.e. plasticity) is a key in incorporating these gene alleles in future adapted varieties.

## Acknowledgements

This work was supported by the Israeli Science Foundation (ISF) program (1270/17), the Chief Scientist Grant (20-01-0080) from the Ministry of Agriculture, and the CAPITALISE/Horizon2020 (AMD-862201-2) grants to EF. We thank Booky Katz and his team in the Yotveta Experimental Unit. Also, to Jamal Atrash and Saker El-Atrash for their valuable work in the field trials in Gilat. The technical assistance of laboratory members Avital Beery, Orit Amir-Segev, and the editing of the manuscript by Ayelet Kurtz-Sohn are highly appreciated.

## Author contributions

MRP, EB, RS, DB and EF designed the field experiments and collected, analyzed, and interpreted the data. LDT, EB, ADF, and EF designed the transcriptome experiments and collected, analyzed, and interpreted the data. JLN, KP and KPS provided

the cultivated and genotyped interspecific population for the field and SensyPAM analysis. EY, MRP and ADF analyzed the genomic data and performed GWAS and selective sweep analysis. MRP, EB, EY, LDT, KK and EF were involved in discussion and the writing of the manuscript. MRP, EB and EY contributed equally to this work.

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## Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

**Fig. S1** Irrigation treatments throughout the growing season in Gilat.

**Fig. S2** Temperature and relative humidity profile in Yotveta during the experimental period.

**Fig. S3** Genome-wide diversity between wild B1K wild population and the US breeding panel.

**Fig. S4** Temporal expression and rhythms of the core clock genes under optimal and high temperature (OT and HT, respectively) in barley gene pools.

**Fig. S5** Temporal expression and rhythms of the clock output genes under optimal and high temperature (OT and HT, respectively) in barley gene pools.

**Fig. S6** HEB-25 family with differential period thermal plasticity.

**Fig. S7** HEB-25 family with differential amplitude thermal plasticity.

**Fig. S8** Manhattan plots for drivers of the clock (DOC) loci for high-temperature (HT) amplitude, optimal-temperature (OT) period and HT period detected from GLM, EBL and LMM models of genome scan.

**Fig. S9** QTL allele effect detected from single genome scan.

**Fig. S10** Additive interaction heat map for clock amplitude.

**Fig. S11** Additive interaction heat map for clock period.

**Fig. S12** General linear model (GLM) analysis shows pleiotropic effect of the *DOC1.1* locus for plant yield under drought in the field experiments at Gilat.

**Methods S1** Genome-wide association study (GWAS).

**Table S1** Description of the Barley1K, BCC collection sites and their biological status.

**Table S2** Details of the GILAT Meteorological data.

**Table S3** Details of the Yotveta temperature and humidity profile during the experimental period.

**Table S4** Circadian clock phenotypes of the different accessions in the wild barley (B1K), interspecific (Halle Exotic Barley; HEB) and cultivated (S2MET) panels under optimal and high temperatures (OT and HT).

**Table S5** The interactions between the different gene pools and the environments.

**Table S6** SNP genotypes of different accessions of the HEB panel.

**Table S7** Linkage disequilibrium values of the HEB-25 population.

**Table S8** Significant QTL loci from single marker GWAS of HEB population.

**Table S9** Significant epistatic interactions in the HEB population.

**Table S10** Pleiotropic effect of the DOC loci.

**Table S11** SNP-Pi values of barley domestication in Asia and Europe.

**Table S12** CLR values of SweeD for barley domestication in Asia and Europe.

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