# Supplementary Information

Table A. List of primers used for the genotyping and sequencing candidate genes.

| Candidate genes | Primer names | Primer sequences (5′–3′) | PCR fragment size(bp) | Method | References |
|-----------------|--------------|--------------------------|-----------------------|--------|------------|
| VRN-H3          | HvVRNH3CAPS.F | CCGGGATATCTGTACCAAC     | 349                   | Genotyping (CAPS: BclI) | This study |
|                 | HvVRNH3CAPS.R | CTTTCTTTCAATTGTTCCAG     |                       |        | This study |
|                 | VRNH3.F       | TTGCTCCCTCATACCCCTAGC    | 1170                  | Sequencing              | This study |
|                 | VRNH3.R       | CGTTGGAATAATTGACCTTGG    |                       |        | This study |
| VRN-H1          | HvVRNH1.F     | CCCAATATTCTGGCATGACGG    |                       | Genotyping (Indel)      | This study |
|                 | HvVRNH1.R     | CCTCAGGATCCAGACCCAG      |                       |        | This study |
| VRN-H2          | VRNH2.01F     | ATGATGTGCGCCGGTCTT      | 235 (ICB), null (Cheri) | Genotyping       | This study |
|                 | VRNH2.01R     | GCCTGCGTCCACCTC         |                       |        | This study |
|                 | VRNH2.02F     | ACCAGACCAACACAGAAACA    | 334 (ICB), null (Cheri) | Genotyping       | This study |
|                 | VRNH2.02R     | GCCTGCGTCCACCTC         |                       |        | This study |
|                 | VRNH2a.R      | CTTTTGAAATAGTCAAACCAAGA | 1803 (ICB), null (Cheri) | Genotyping and sequencing | This study |
|                 | VRNH2b.R      | CTCCCTCAAATTGCTAAATCA   | 1778 (ICB), null (Cheri) |        | This study |
| PPDH1           | PPDH1.F       | CAACACAAATAACGGGAGCA    | 485                   | Sequencing              | This study |
|                 | PPDH1.R       | CCAGGAGATGAGACGACT      |                       |        | This study |
| PPDH2           | FT3.01F       | GTCTGTAGAGAGCTAGGTTGAC  | 1500                  | Sequencing              | [8]         |
|                 | FT3.02R       | GTGATGCGGACCTACAGC      |                       |        | [8]         |
| ELF3            | ELF3.01F      | TGTCAGAGAAAGGCAATAGAGA  | 170                   | Genotyping and sequencing | This study |
|                 | ELF3.01R      | TGCTTTCCAGAAAATGCTTTGGA |                       |        | This study |
|                 | ELF3.02R      | GCTCAGAACCCTTAGGACCA    | 1020                  |        | [8]         |
| CCA1            | CCA1.F        | ACCAGTTGTCAGCCTTCTCT    | 556                   | Sequencing              | This study |
|                 | CCA1.R        | ATGGCTGTGATTGACAGG      |                       |        | This study |
| LUX1            | LUX1.F        | GCTCGATGGGTGTGTAGAG     | 947                   | Sequencing              | This study |
|                 | LUX1.R        | GACGAGACGCGAGCATCC      |                       |        | This study |
| CEN             | CEN.F         | TCCTCTCATCTCCAGCCATC    | 999                   | Sequencing              | This study |
|                 | CEN.R         | TGACGTACACTGTTCAACA     |                       |        | This study |
Figure A. The alignment of *Vrn-H3* genes between Strider, Triumph, Cheri and ICB181160 with MAFFT version 7. SNPs are with grey background.
**Vrn-H2**

**Table B.** Barley haplotype scoring from five non-synonymous SNPs and one SSR (simple sequence repeat) in the exon 1 and exon 2 of \textit{ZCCT-Ha} gene

| Cultivar/accession | Position of SSR and SNPs in Exon 1 | Exon 2 | Allele | Accession number |
|--------------------|------------------------------------|--------|--------|------------------|
|                    | 127\(^a\) | 151 | 194 | 251 | 291 | 25 |
| Calicuchima-sib    | CAC (7) | A | G | G | C | A | Vrn-H2 | DQ492695 |
| OWB-D              | CAC (5) | G | G | G | C | A | Vrn-H2 | DQ492697 |
| Kompolti korai     | CAC (6) | G | T | A | C | A | Vrn-H2 | DQ492699 |
| Dairokkaku         | CAC (5) | G | G | G | C | G | Vrn-H2 | AY485977 |
| ICB181160          | CAC (7) | G | G | G | G | A | Vrn-H2 | This study |
| Cheri              | - | - | - | - | - | - | vrn-H2 | This study |

OWB-D : Oregon Wolfe Barley Dominant

\(^a\) Number of CAC repeat (coding Histidine)

\(^b\) Detection of presence/absence of the genes \textit{ZCCT-Ha} and \textit{ZCCT-Hb} after sequencing.

**Table C.** Barley haplotype scoring from six nonsynonymous SNPs in the exon 1 and exon 2 of \textit{ZCCT-Hb} gene

| Cultivar/accession | in Exon 1 | in Exon 2 | Allele | Accession number |
|--------------------|-----------|-----------|--------|------------------|
|                    | 221 | 238 | 10 | 29 | 77 | 274 |
| Calicuchima-sib    | G | C | C | A | T | C | Vrn-H2 | DQ492696 |
| OWB-D              | T | C | C | A | C | C | Vrn-H2 | DQ492698 |
| Kompolti korai     | T | C | C | A | C | C | Vrn-H2 | DQ492700 |
| Dairokkaku         | T | C | C | A | C | C | Vrn-H2 | AY485978 |
| Caesarea 26-24     | T | T | A | G | T | G | Vrn-H2 | DQ492701 |
| ICB181160          | T | T | C | G | T | G | Vrn-H2 | This study |
| Cheri              | - | - | - | - | - | - | vrn-H2 | This study |

OWB-D : Oregon Wolfe Barley Dominant

\(^a\) Detection of presence/absence of the genes \textit{ZCCT-Ha} and \textit{ZCCT-Hb} after sequencing.
The genotyping of the *Vrn-H2* was performed with primers which were designed for detecting presence/absence of the *Vrn-H2* gene and furthermore for sequencing of zinc finger-CTT domain transcription factor a (*ZCCT-Ha*) and zinc finger-CTT domain transcription factor b (*ZCCT-Hb*). PCR results and sequencing were showed complete deletion of *Vrn-H2* gene in Cheri. Null allele of *vrn-H2* described as a recessive allele for spring growth habit in barley [1, 2]. Sequencing of *ZCC-Ha* (Table B) and *ZCC-Hb* (Table C) performed for only ICB181160 which has a dominant *Vrn-H2* allele and none of the designed primers amplified from Cheri because deletion of the complete gene.
Genetic interaction of \textit{Vrn-H3} alleles with \textit{Vrn-H1} and \textit{Vrn-H2} alleles for root and shoot traits

\textbf{Figure B.} Interaction plot of \textit{Vrn-H2} and \textit{Vrn-H3} alleles from Cheri and ICB181160 for RDW (root dry weight). All nine possible allele combinations are plotted using R-interaction plot.
Figure C. Interaction plot of *Vrn-H1* and *Vrn-H3* alleles for RV (root volume). All nine possible allele combinations are plotted using R-interaction plot.
Figure D. Interaction plot of *Vrn-H2* and *Vrn-H3* alleles for RV (root volume). All nine possible allele combinations are plotted using R-interaction plot.
Figure E. Interaction plot of *Vrn-H2* and *Vrn-H3* alleles for RL (root length). All nine possible allele combinations are plotted using R-interaction plot.
Figure F. Interaction plot of *Vrn-H2* and *Vrn-H3* alleles for TIL (no. of tillers). All nine possible allele combinations are plotted using R-interaction plot.
**PPDH1**

Table D. Barley haplotype scoring from two described SNPs in the exon8 of *PPDH1* gene

| Cultivar/accession | Position of polymorphisms<sup>a</sup> [4] | Accession number |
|--------------------|------------------------------------------|------------------|
| SNP22 | SNP23 | Allele | |
| Triumph | T | A | *ppdh1* | AY970704 |
| Igri | G | G | *PPDH1* | AY970701 |
| ICB181160 | G | G | *ppdh1* | *This study* |
| Cheri | T | A | *PPDH1* | *This study* |

<sup>a</sup>Detection of the described SNPs in the exon 8 with sequencing.

The dominant *PPDH1* allele is prevalent in wild progenitor *H. vulgare spp. spontaneum* and accelerates flowering under long days in short growing seasons. The recessive *ppdh1* allele causes reduced response to long days and was selected for adaptation to long growing seasons [3]. Previous studies described polymorphisms between dominant and recessive *PPDH1* alleles. In this study, primers were designed spanning from exon 7 to end of exon 8 for detection of previously described mutations(SNP22 and SNP23) [4]. Sequencing of *PPDH1* was showed that wild ICB181160 parent carrying a dominant *PPDH1* allele while Cheri carrying a recessive *ppdh1* allele.
Table E. Barley haplotype scoring from two SNPs in the intron 3 of *PPDH2* gene

| Cultivar/accession | Position of SNP in Intron 3 | Allele | Accession number |
|--------------------|-----------------------------|--------|-----------------|
| Scarlett           | 187 C, 529 A                | PPDH2  | EU331895        |
| Morex              | 187 C, 529 G                | PPDH2  | EU331873        |
| Alexis             | 187 C, 529 A                | PPDH2  | HM133570        |
| OWB-D              | 187 C, 529 A                | PPDH2  | EU331897        |
| ICB181160          | 187 G, 529 G                | PPDH2  | *This study*    |
| Cheri              | 187 C, 529 A                | PPDH2  | *This study*    |

* Detection of the large deletion in the *PPDH2* with sequencing

The dominant *PPDH2* allele is functional and providing early flowering under short days whereas the recessive allele is truncated gene having only part of exon 4 and delay flowering under short days[5]. Previously shown that most of the spring cultivars carrying dominant allele of *PPDH2* gene whereas the majority of winter cultivars possessed recessive null *ppdh2*[6]. Sequencing of the *PPDH2* was carried out in Cheri and ICB181160. PCR results were showed that both of the parents carrying a dominant *PPDH2* allele. Two SNPs were detected in intron 3 which is previously described also by Casao et al. [7].
**HvELF3**

Table F: Barley haplotype scoring from nine SNPs and three indels in the exon 2 and intron 2 of HvELF3 gene

| Cultivar/accession | Exon2  | Intron 2 | Allele | Accession number |
|--------------------|--------|----------|--------|------------------|
|                    | 751\(^a\) | 179\(^b\) | 852 | 235 | 242 | 332 | 334 | 377 | 465 | 573 | 705\(^c\) | 721 | |
| Bonus              | in     | C        | T      | T     | T     | G     | del   | T     | Mat-a | JN180296 |
| Igri               | in     | C        | T      | C     | C     | C     | A      | C     | Mat-a | HQ850272 |
| Mona               | del    | A        | T      | T     | C     | T     | G     | del   | T     | mat-a.8 | HQ850275 |
| ICB181160          | in     | G        | C      | T     | C     | T     | C     | in    | C     | EAM8 | This study |
| Cheri              | in     | G        | del   | C     | T     | C     | C     | A     | C     | in    | C     | EAM8 | This study |

\(^a\) Letters “in” and “del” indicate a 4 bp indel (AAAG).

\(^b\) Letters “in” and “del” indicate a 6 bp indel (TGCATT)

\(^c\) Letters “in” and “del” indicate a 3 bp indel (AAG)

\(^d\) Detection of large deletion and described mutations in exon 2 with sequencing

Transcript based cloning of the EAM8 gene was showed that EAM8 is the orthologue of the Arabidopsis ELF3 and eam8 mutation has a dramatic effect on the expression level of circadian clock and photoperiod pathway genes [8]. First mutant line (mat-a.8) Mari was released commercially in 1961 as an early barley mutant [9]. In field trials under long-day and short-day conditions, it flowered earlier than the mother cultivar Bonus [9]. Primers were designed spanning from exon 2 to exon 3 for detection of previously described mutations [10] and sequencing of HvELF3. PCR results were showed that both of the parents carrying wild EAM8 allele and do not have the detected mutations of mat-a.8 or ert-o.16.
**Text A.** Description of *HvCCA1*, *HvLUX1* and *HvCEN* alleles in cultivar Cheri and ICB181160.

**HvCCA1**

A partially redundant *Arabidopsis thaliana* MYB-transcription factor *AtCCA1* (*CIRCADIAN CLOCK ASSOCIATED*) 1 plays a role in the formation of morning active genes [13]. Previous study experimentally proofed that *HvCCA1* functionality similar to *AtCCA1* [11]. Sequencing of the 556 bp genomic region spanning from exon1 to exon3 of *HvCCA1* sequenced from Cheri and ICB181160. Alignment of the sequences with available *HvCCA1* genes from other cultivars Morex, Triumph and Igri also were not showed any polymorphism. Sequencing results were showed that both of the parents having same allele of the *HvCCA1* gene.

**HvLUX1**

*HvLUX1* is a candidate gene underlying the early maturity 10 (*eam10*) locus in barley. *HvLUX1* characterized as a transcription factor which encodes an MYB domain-containing SHAQKYF-type GARP family protein. The mutation identified in *eam10* changed the first amino acid residue in this conserved motif and this described as *hvlux1* allele[3]. Sequencing of the complete gene was carried out and results were showed that Cheri and ICB181160 both carrying haplotype 1 allele of *HvLUX1* gene.

**HvCEN**

*HvCEN* (*CENTRORADIALIS*) is a barley homologue of *Arabidopsis TFL1* (*TERMINAL FLOWER1*), which regulates inflorescence architecture and flowering time [15]. Three major haplotypes (I, II and III) identified at higher frequencies in domesticated barleys compare to wild progenitors. Major haplotypes I and III (which are later flowering than haplotype II in domesticated barleys) both harbor the mutation encoding the p.Pro135Ala alteration. The haplotype I is most frequently found in wild winter barleys from Fertile Crescent while haplotype III has been selected from wild barley and is completely fixed in northern European spring barley cultivars [15]. In this study *HvCEN* sequences of Cheri and ICB181160 were sequenced and aligned with previously described haplotypes, results were showed that Cheri is carrying a haplotype III allele of *HvCEN* gene while ICB181160 carrying a haplotype I.
REFERENCES

1 Yan L, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, SanMiguel P et al. The wheat VRN2 gene is a flowering repressor down-regulated by vernalization. Science. 2004;303: 1640–1644. doi:10.1126/science.1094305

2 Dubcovsky J, Chen C, Yan L. Molecular characterization of the allelic variation at the VRN-H2 vernalization locus in barley. Molecular Breeding. 2005;15: 395–407. doi:10.1007/s11032-005-0084-6

3 Campoli C, Pankin A, Drosse B, Casao CM, Davis SJ, von Korff M. HvLUX1 is a candidate gene underlying the early maturity 10 locus in barley: phylogeny, diversity, and interactions with the circadian clock and photoperiodic pathways. New Phytologist. 2013;199: 1045–1059. doi:10.1111/nph.12346

4 Turner A, Beales J, Faure S, Dunford RP, Laurie DA. The pseudo-response regulator Ppd-H1 provides adaptation to photoperiod in barley. Science. 2005;310: 1031–1034. doi:10.1126/science.1117619

5 Kikuchi R, Kawahigashi H, Ando T, Tonooka T, Handa H. Molecular and functional characterization of PEBP genes in barley reveal the diversification of their roles in flowering. Plant Physiology. 2009;149: 1341–1353. doi:10.1104/pp.108.132134

6 Casao MC, Karsai I, Igartua E, Gracia MP, Veisz O, Casas AM. Adaptation of barley to mild winters: A role for PPDH2. BMC Plant Biology. 2011;11: 164. doi:10.1186/1471-2229-11-164

7 Casao MC, Igartua E, Karsai I, Lasa JM, Gracia MP, Casas AM. Expression analysis of vernalization and day-length response genes in barley (Hordeum vulgare L.) indicates that VRNH2 is a repressor of PPDH2 (HvFT3) under long days. Journal of Experimental Botany. 2010;62: 1939–1949. doi:10.1093/jxb/erq382

8 Faure S, Turner AS, Gruszka D, Christodoulou V, Davis SJ, Korff MV, et al. Mutation at the circadian clock gene EARLY MATURITY 8 adapts domesticated barley (Hordeum vulgare) to short growing seasons. Proceedings of the National Academy of Sciences. 2012;109: 8328–8333. doi:10.1073/pnas.1120496109

9 Dormling I, Gustafsson Å, Jung HR, von Wettstein D. Phytotron cultivation of Svalöf’s Bonus barley and its mutant Svalöf’s Mari. Hereditas. 1966;56:221–237. 10.1111/j.1601-5223.1966.tb02078.x
10 Zakhrabekova S, Gough SP, Braumann I, Muller AH, Lundqvist J, Ahmann K, et al. Induced mutations in circadian clock regulator Mat-a facilitated short-season adaptation and range extension in cultivated barley. Proceedings of the National Academy of Sciences. 2012;109: 4326–4331. doi:10.1073/pnas.1113009109

11 Kusakina J, Rutterford Z, Cotter S, Martí MC, Laurie DA, Greenland AJ, et al. Barley Hv CIRCADIAN CLOCK ASSOCIATED 1 and Hv PHOTOPERIOD H1 are circadian regulators that can affect circadian rhythms in Arabidopsis. PLoS ONE. 2015;10. doi:10.1371/journal.pone.0127449

12 Hemming MN, Fieg S, Peacock WJ, Dennis ES, Trevaskis B. Regions associated with repression of the barley (Hordeum vulgare) VERNALIZATION1 gene are not required for cold induction. Molecular Genetics and Genomics. 2009;282: 107–117. doi:10.1007/s00438-009-0449-3

13 Locke JCW, Kozma-Bognár L, Gould PD, Fehér B, Kevei É, Nagy F, et al. Experimental validation of a predicted feedback loop in the multi-oscillator clock of Arabidopsis thaliana. Molecular Systems Biology. 2006;2. doi:10.1038/msb4100102

14 Katoh K, Standley DM. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in performance and usability. Molecular Biology and Evolution. 2013;30: 772–780. doi:10.1093/molbev/mst010.

15 Comadran J, Kilian B, Russell J, Ramsay L, Stein N, Ganal M, et al. Natural variation in a homolog of Antirrhinum CENTRORADIALIS contributed to spring growth habit and environmental adaptation in cultivated barley. Nature Genetics. 2012;44: 1388–1392. doi:10.1038/ng.2447