A regulator of early flowering in barley (*Hordeum vulgare* L.)

Ahmed Ibrahim1,2, Matthew Harrison1, Holger Meinke1, Yun Fan1, Peter Johnson1, Meixue Zhou1*

1 Tasmanian Institute of Agriculture, University of Tasmania, Tasmania, Australia, 2 Department of Plant Science, Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria

* Meixue.Zhou@utas.edu.au

Abstract

Heading date (HD) of cereals is an important trait for adaptation to diverse environments and is critical for determining yield and quality and the number of genes and gene combinations that confer earliness in barley under short days is limited. In our study, a QTL for early flowering was identified from the cross between an Australian malting barley cultivar and a Chinese landrace. Four sets of near isogenic lines (NILs) were developed with a QTL located on chromosome 5H at the interval of 122.0–129.0 cM. Further experiments were conducted to investigate how this gene was regulated by photoperiod using the NILs with three sowing dates from autumn to summer. The NILs carrying the earliness allele were significantly earlier than the late genotype at all sowing dates. This gene was different from previously reported vernalisation genes that are located at a similar position as no vernalisation was required for all the NILs. The difference between this gene and *Eam5* (*HvPHYC*) locus which also located between two co-segregated markers (3398516S5, 122.5 cM, and 4014046D5, 126.1 cM), is that with the existence of *Ppd-H1* (*Eam1*), *Eam5* has no effect on ear emergence under long days while the gene from TX9425 still reduced the time to ear emergency. The locus showed no pleiotropic effects on grain pasting properties and agronomic traits except for spike length and number of spikelets per spike, and thus can be effectively used in breeding programs. The array of early heading dates caused by interactions of *Eam5* gene with other maturity genes provides an opportunity to better fine tune heading dates with production environments, which can be critical factor in barley breeding.

Introduction

Barley is an important cereal crop grown worldwide under a wide range of environments [1]. The broad adaptation of this crop to varying climatic and regional conditions is in part caused by the diversity in flowering time (anthesis, Zadoks GS61) or heading date (HD, Zadoks GS51) or tipping (awn emergence, Zadoks GS 49) [2–4]. These terms are often used interchangeably by many scientist [4]. The main factors affecting HD are photoperiod, vernalization, temperature and management [5–10]. These factors provide the physiological and genetic basis for variations in the duration of developmental stages, such as double ridge (DR), terminal spikelet...
(TS), heading, anthesis and grain filling [11, 12]. Genotypes vary in their photoperiodic response [13, 14], with temperature being very important to plant physiological processes [15] especially variations in duration to spikelet initiation [16], heading and flowering (anthesis) in cereals [14, 17]. It follows that a linear association generally describes the relationship between cumulative temperatures over the growing season and heading/anthesis in barley [16, 18, 19] and in many other cereals. To initiate flowering, winter barley requires vernalisation, i.e. exposure to prolonged temperatures below 10˚C for a period between 4 to 6 weeks [20, 21, 22].

Early maturity of cool-season cereals like barley under short-day environments is vital in many grain producing regions of the world. Barley grown in Eastern Asia has evolved unique earliness mechanisms that stimulate early ear emergence under short days even when vernalization is not necessary. These mechanisms may involve several maturity genes interacting together in either additive, epistatic or pleiotropic way in order to regulate earliness. Apart from these allelic and no-allelic interactions, intra-locus mutations at Vrn loci, Eps and HvPHYC loci are responsible for earliness in both barley and wheat [23, 24] with a rich allelic variation at Vrn-H1 or/and HvPhyC [25]. Our understanding of these interactions is gradually improving as we learn more about their mechanisms of expression and that some of these genes are functional under long days. Four major genes are reported to be responsible for vernalization. These include Vrn-H1 (Sgh2), Vrn-H2 (Sgh1), Vrn-H3(Sgh3) and Vrn4 on chromosomes 5H, 4H, 7H and 5H, respectively [21, 26–31]. These four loci interact in an epistatic fashion to determine vernalization sensitivity in barley and wheat [32]. For example, the winter barley cultivars which are responsive to vernalization have vrn-H1_vrn-H2_vrn-H3 haplotype [29, 32, 33] and more recently were found to have the recessive mutant vrn4 [34]. A model of heading-time regulation in both photoperiod groups (Ppd-H1; ppd-H1) under long day condition was proposed by Alqudah et al [25].

Heading date in barley is regulated by photoperiod response genes; the first identified being Ppd-H1 (Eam1), which is a pseudo-response regulator gene (HvPRR37) that is effective under long days and is located on chromosome 2H [25, 35–37]. The second photoperiod gene, Ppd-H2 (HvFT3), is located on chromosome 1H and regulates flowering time under short days [38, 39].

Earliness in intrinsic or per se genes determine the time and duration of reproductive phases [40, 41]. These QTL manifest their expression after all sources of variation in basic vegetative period (BVP) or maturity-related traits (such as vernalization and photoperiod) have been met [42–46]. Thus, Eps QTL are important in fine-tuning HD and anthesis in barley [41, 47, 48]. Eps QTL have significant effects on the time and duration of reproductive phase and spikelet number [41] which directly affect the grain yield [48]. Eps QTL also have significant effects on grain protein [49] which is inversely related to the starch [50, 51]. The EARLY FLOWERING3 (ELF3) locus regulates flowering under the influence of photoperiod [52]. The recessive allele (elf3, eam8, mat-a) of this gene in barley causes early flowering in both short days (SDs) and long days (LDs) [52] and an insensitivity of barley to photoperiod [35, 52].

Apart from the Eps QTL, an early maturity factor in chromosome 5H was reported by Wexelsen [53]. The gene belongs to the family of photoreceptors, phytochromes, which helps plants to perceive, interpret, and translate light signals that modulate and synchronize their growth and development in any given environments [54, 55]. These phytochromes are involved in plant metabolism including flowering, shade avoidance, dormancy, and germination [56]. Close linkage to the rough awn trait demonstrates that Eam5 is likely the locus reported by Laurie et al. [28] and later identified at the HvPHYC locus after which Szucs et al. [57] mapped to a similar position as that of Vrn-H1. HvPHYTOCHROME C (HvPHYC) is reported to be a candidate gene to Eam5 [58]. Nishida et al. [59] found that a mutation at HvPHYC, due
amino-acid substitution in the GAF domain, influenced HD under LDs. It was later found to affect ear emergence under long and non-inductive short days [58, 60]. Thus, Eam5 likely interacts with many photoperiod response loci under an array of photoperiods. In addition, a casein kinase alpha (HvCK2α-5H) gene is also reported to be closely linked to the Vrn-H1 [59]. This gene encodes the α subunit of CK2 protein and regulates flowering, which is found in cereals including rice, wheat and barley under both short and long days [59].

Beside the photoperiod and vernalization genes which have been cloned, there is little information on the genetics, physiological and biochemical functions of other genes regulating early ear emergence in barley. For instance, HvPHYC gene has been recently fine mapped but there is discrepancy surrounding the expression and interaction of this gene with the environment. Further work is required to determine their quantitative effects [61]. This manuscript provides some details about interactions of one component of the circadian clock with various photoperiod regimes in essentially fixed genetic background in barley.

From thousands of F10 recombinant inbred lines from the cross of TX9425 and Franklin, we have identified another early flowering allele that may be distinct from other QTL on chromosomes 2H, 3H and 6H which were reported earlier [39]. Four pairs of near isogenic lines (NILs) were developed to investigate: 1) the location of this early flowering gene; and 2) the effect of this gene on agronomic traits, yield components, and grain quality.

Materials and methods

Development of near-isogenic lines

NILs were selected from over 1,000 F9 derived F10 recombinant inbred lines (RILs) of the cross of TX9425 and Franklin. Franklin (Shannon/Triumph) is a late maturing malting variety from Australia [62] with the seeds being sourced from the University of Tasmania and the Australian Grains Genebank. TX9425 (Taixing 9425) is a Chinese landrace which has semidwarf and early maturity genes, most likely the dominant alleles of the Ppd-H1, the spring Vrn-H1 allele and the recessive allele of the Eps5HL [39]. From the RIL population four lines segregating on heading date were further selfed to produce homozygous early and late lines, leading to four pairs of the NILs. They are Eps5HL-116 (-E/-L), Eps5HL-317-1(-E/-L), Eps5HL-317-2 (-E/-L), Eps5HL-322 (-E/-L) with–E carrying earliness allele. All NILs have both the spring Vrn-H1 allele as vernalization is not needed to all lines and Ppd-H1 since the segment containing this gene is from TX9425 (Fig 1).

Genotyping the NILs

Genomic DNA of NILs was extracted from the leaf tissue of four-week old seedlings, according to the plant DNA extraction protocol for DArT analysis (https://www.diversityarrays.com/files/DArT_DNA_isolation.pdf). The two parental cultivars and four pairs of NILs were genotyped with DArTseq (http://www.diversityarrays.com/dart-application-dartseq). Around 10,000 polymorphism molecular markers with known positions were chosen for comparing the differences between NILs and the relationships to their parents.

Field experimentation

Field experiments were conducted at Mt Pleasant Laboratories, at the Tasmanian Institute of Agriculture (TIA) in Launceston, Tasmania (Latitude: -41.4702 Longitude: 147.1392), where the day length ranges from 9 to 15 hours (S1 Fig). The four pairs of NILs and both parents were sown in tanks (1.50 x 3.0 x 1.0 m) with a spacing of 20.0 cm x 7.0 cm (S2 Fig). Tanks were filled with sandy loam soils and fitted with irrigation facilities to avoid any water stress. The
NILs/parents were grown in a randomized complete block design with three replications. Three different sowing dates, 14/January/2015 (SD1, summer), 13/March/2015 (SD2, summer/autumn) and 15/May/2015 (SD3, autumn), were selected to represent the photoperiods/temperatures (S1 Fig). Agronomic practices such as fertilizer rate and regular weeding were similar to local practices. Traits measured shown in Table 1 include: heading date (HD) (when the first spikelet emerged in one of the tillers of 50% of the plants) in calendar time, growing degree days to heading date (GDD), plant height (PH), number of nodes on the main plant, peduncle length, spike length, awn length and number of fertile spikelets per spike. Harvested grain was tested for pasting properties. Climatic data were taken from the nearest meteorological station, and GDD accumulation was calculated following the classical method:

$$GDD = \sum_{i=1}^{n} \left( \frac{T_{\text{min}} + T_{\text{max}}}{2} - T_{b} \right)$$

Where \( n \) = number of days taken for a particular growth stage to be accumulated, and \( T_{\text{min}} \), \( T_{\text{max}} \) = minimum and maximum daily air temperatures in °C, respectively, and \( T_{b} \) = base temperature threshold for barley, which is 0 °C [63]

**Analysis of pasting properties**

The determination of pasting properties was conducted according to the methods described by [64, 65]. After harvesting and threshing, the samples in each NIL pair were air dried. 10.0 g of grains were sampled from each of the NIL pairs in each replication and ground in a
Cylotech 1903 Mill. 4.0 g of the flour was slurried into 25.0 g of 0.1M of silver nitrate (AgNO₃) solution in an aluminium canister. The slurry was then thoroughly mixed by moving the paddle both vertically and stirring in the canister before placing it into a Rapid Visco-Analyser (RVA-4D, Newport Scientific, Australia). The RVA instrument was used to determine the pasting properties. The RVA instrument was used for 10 s at 960 rpm then reduced to 160 rpm for the remainder of the test run. The initial temperature was 50 °C, held for 1.0 minute, then heated to 95 °C for 3.7 minutes and was maintained at 95 °C for 2.5 minutes before cooling to 50 °C over 3.8 min, and finally maintained at 50 °C for 2.0 min. The measured parameters for pasting properties include: peak viscosity (PV), highest viscosity during heating; time to peak viscosity (TTPV); trough (T), lowest viscosity after cooling started; breakdown (BD), PV minus T; final viscosity (FV), highest viscosity after the temperature had returned to 50 °C; setback (SB), FV minus T; pasting temperature (PT), temperature at which the trace left the baseline [65].

### Statistical analysis

SAS version 9.4 was used to conduct ANOVA to estimate the significances of the differences between each of the pairs, whilst the mean of each trait within genotypes was ranked used Tukey’s test [66].

### Results

#### Mapping early flowering QTL

The genotyping of the four pairs of NILs was conducted using DArTseq with over 30,000 markers. Fig 1 shows that for the Eps5HL-317-1 pair, except for the 122–129 cM region of chromosome 5H, genetic background was identical for the early (Eps5HL-317-1-E) and late (Eps5HL-317-1-L) lines. Similar regions were located in the other three pairs of NILs except that the region with different background was much greater (122–140 cM) (Fig 2) for the

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**Table 1. Traits scored and their abbreviations.**

| Traits                           | Symbol | Unit   | Description                                                                 |
|----------------------------------|--------|--------|-----------------------------------------------------------------------------|
| Heading date                     | HD     | d      | Number of days from sowing to when fifty percent of the spike appears       |
| Thermal time (growing degree days)| TT/GDD | °Cd    | Accumulated thermal time from day 0 of the sowing day to current growth stage |
| Plant height                     | PH     | cm     | Measured from collar to the peak of the awns                              |
| Spike length                     | SpkL   | cm     | Measured from the base of the spike to the tip                             |
| Spikelet number                  | SpkN   | Spk/spike | Number of spikelets in a spike                                           |
| Peduncle length                  | PedL   | cm     | Measured from the last node to the base of the spike                       |
| Rapid Visco-analyser Unit        | RVU    | RVU    | An RVU is approximately equal to 10 cP.                                    |
| Peak Viscosity                   | PV     | RVU    | Highest viscosity during cooking                                           |
| Time To Peak Viscosity           | TTPV   | min    | Time taken to reach the peak                                               |
| Trough                           | TR     | RVU    | Lowest viscosity after cooling started                                     |
| Breakdown                        | BD     | RVU    | Peak viscosity minus trough (PV-TR)                                        |
| Final Viscosity                  | FV     | RVU    | Maximum viscosity after the temperature had returned to 50 °C             |
| Setback                          | SB     | RVU    | Final viscosity minus trough (FV-TR)                                       |
| Pasting Temperature              | PT     | °C     | Temperature when the rate of increase in viscosity reaches 11.5 RVU in 0.2 min |

https://doi.org/10.1371/journal.pone.0200722.t001
Eps5HL-322 pair. The earliness allele was from TX9425. More than 100 SNP/DArT markers co-segregated with the trait. As reported previously [39] two major QTL for early maturity were found in a DH population originating from the cross of TX9425 and Franklin. One QTL is located on chromosome 3H that is closely linked to \textit{sdw1} and \textit{uzu1} genes. This early maturity allele is absent in all these NILs. The other early maturity QTL is located on chromosome 2H (most likely \textit{Eam1}) which exists in all the NILs (data not shown).

Effects of sowing time on heading dates (HD) and GDD

Sowing dates resulted in significant differences in HD and GDD (Table 2, S3 Fig). All four NILs and their parents (TX9425 and Franklin) had the fewest days to heading in SD1, followed by SD2 and SD3 (Table 2). Consistent differences between lines with early and late alleles existed in all the sowing dates. Heading days (HDs) for TX9425 were 41, 105 and 125 d for SD1, SD2 and SD3, respectively. HDs for Franklin were 55, 151 and 162 for SD1, SD2 and SD3, respectively. All NILs were earlier than Franklin but later than TX9425 except SD1 with
The early genotypes of the NILs and TX9425 flowering at same time. HDs of the NILs with the early allele were 41, 131 and 136 for SD1, SD2 and SD3, respectively, while those with the late allele were 45, 149 and 155 for SD1, SD2 and SD3, respectively (Table 2). The NILs carrying the early allele were approximately four days earlier than those with the late alleles in SD1. The differences between the two alleles were much greater with 18 and 20 days in SD2 and SD3, respectively (Table 2). The relative HDs for each sowing date were similar for all pairs across sowing dates, with less difference in SD1 (Fig 3). When considering the effects of accumulated temperature for the HD to be expressed, similar trend was observed in the GDD with consistent differences among the SDs and among the genotypes except in Franklin (S2 Fig)

Table 2. Means of heading dates and other different traits of two parent varieties and four pairs of NILs under different sowing dates*.

| Genotype       | HD    | GDD  | PH    | SpkL | SpkN | PedL | InterL |
|----------------|-------|------|-------|------|------|------|--------|
|                | SD1   |      | SD2   |      |      |      | SD3    |        |
| TX9425         | 41.0±2.2 | 756.3±38.2b | 36.0±0.7b | 4.9±0.1b | 20.7±1.5b | 38.3±4.6a | 16.6±0.8a |
| Franklin       | 55.0±4.4 | 980.1±25.7a | 47.1±2.6a | 9.3±0.0a | 24.3±1.2a | 29.0±2.2b | 16.8±2.5a |
| Eps5HL-116-E   | 42.3±1b  | 774.1±37b | 87.9±0.5a | 7.5±0.2b | 22.0±1.2b | 33.3±1.8a | 17.6±0.8a |
| Eps5HL-116-L   | 46.7±1.3a| 846.4±32a | 87.7±0.8a | 9.1±0.1a | 25.0±1.0a | 35.3±5.5a | 15.0±1a  |
| Eps5HL-317-1-E | 41.1±1b  | 756.6±37b | 83.9±0.2b | 7.8±0.1b | 21.3±1.5b | 28.7±0.3a | 19.3±1.7a |
| Eps5HL-317-1-L | 46.0±1.7a| 840.5±26.5a | 95.9±4a | 9.2±0.2a | 25.3±1.5a | 30.1±0.8a | 17.5±0.7a |
| Eps5HL-317-2-E | 41.2±2b  | 756.8±38b | 84.2±1b | 7.6±0.1b | 22.4±1.5b | 37.0±2.6a | 19.0±1.3a |
| Eps5HL-317-2-L | 45.3±1.6a| 824±21.4a | 89.7±2.5a | 8.9±0.1a | 25.0±1a | 33.0±2.3b | 16.2±1.7a |
| Eps5HL-322-E   | 41.0±1b  | 756.3±27b | 70.0±1.1b | 7.5±0.1b | 21.2±2b | 29.2±2a | 20.2±2a |
| Eps5HL-322-L   | 45.7±1.1a| 839.6±21a | 76.5±1.3a | 9.0±0.0a | 25.0±1.7a | 31.7±1.2a | 15.6±2.5b |
|                | SD2   |      | SD3   |      |      |      |        |        |
| TX9425         | 105.0±5.5b | 1065.2±42b | 94.5±2.1a | 6.0±0.3b | 28.0±1.5b | 25.7±2.2a | 12.1±2.3b |
| Franklin       | 152.0±2.1a | 1378.9±61a | 84.7±7.1b | 11.8±0.2a | 29.0±1.1a | 24.0±1.4a | 14.2±2.1a |
| Eps5HL-116-E   | 131.0±2b  | 1238.1±23b | 89.8±4.1a | 10.0±0b | 24.3±1.5b | 30.4±2.3a | 18.2±a   |
| Eps5HL-116-L   | 148.9±3a  | 1351.0±14a | 90.0±4a | 11.8±0.2a | 28.7±1.5a | 33.4±2.3a | 20.0±2.5a |
| Eps5HL-317-1-E | 132.2±2b  | 1246.9±22b | 97.1±8.6a | 9.8±0.2b | 25.3±0.5b | 25.8±5a | 14.2±2.2b |
| Eps5HL-317-1-L | 149.0±3.1a| 1351.6±27a | 98.2±6.5a | 12.0±0.2a | 28.7±1.1a | 24.1±9a | 20.0±2.5a |
| Eps5HL-317-2-E | 131.3±3b  | 1238.7±21b | 82.1±2.8b | 10.0±0b | 24.3±0.6b | 29.3±2a | 20.0±2.2a |
| Eps5HL-317-2-L | 149.0±2a  | 1351.6±25a | 94.2±3.1a | 11.8±0.3a | 28.0±1.7a | 29.1±2a | 15.2±2.4a |
| Eps5HL-322-E   | 131.0±2b  | 1238.1±17b | 89.4±3.3b | 10.0±0b | 24.3±0.6b | 28.0±1.8a | 18.0±1.4a |
| Eps5HL-322-L   | 149.4±2.6a| 1352.0±18a | 92.3±1.6a | 11.8±0.3a | 28.0±2a | 26.8±1.3a | 16.0±0.8a |

*Means in same column for each NIL pair followed by same letter are not significantly different (P = 0.05); Means ±Standard deviation; Abbreviations are defined in Table 1.

https://doi.org/10.1371/journal.pone.0200722.t002
Effects of early heading on agronomic traits and yield components

Different heading dates were associated with some of the agronomic traits and yield components. The average spike length of genotypes carrying the late allele was 1.3 cm longer than the spike length of those carrying the early allele (Table 2). Similar results were obtained for the number of fertile spikelets per spike with SD3 having the most spikelets per spike. Genotypes carrying the late allele had more spikelets per spike that those carrying the early allele (Table 2).

No significant differences between early and late lines were observed for peduncle length and internode length for most of the NILs (Table 2 and S1 Table). Franklin had significantly shorter peduncle length (PedL) than NILs and TX9425 in sowing dates 1 and 3, although 317-2-L under sowing date 1 and 322-L under sowing date 3 also had shorter PedL. However, significant differences in plant height between early and late lines were found in three of the four pairs, with genotypes having the late allele being slightly taller than those having the early allele (Table 2).

Sowing dates had significant effects on overall performance of all the traits. Spike length was shorter in SD1 (7.5–9.2 cm) than that in SD2 (9.8–12.0 cm) and SD3 (11.1–14.3 cm). Similar results were found for other traits with those from SD1 being shorter and having fewer spikelets per spike (Table 2). The differences between the early and late alleles were consistent for different SDs even though significant interactions were observed between the alleles and spike length and spikelets number per spike.
Effects of heading date on pasting properties

Fig 4 and Table 3 show that Franklin had generally higher PV, T, FV but lower BD, SB and PT values than TX9425. Both parents showed similar TTPV values. Differences were found between NIL pairs but no significant differences between early and late alleles were observed for all pasting properties (S2 Table). The Eps5HL-116, Eps5HL-317-1 and Eps5HL-317-2 pairs were very close to Franklin in most of the parameters while the Eps5HL-322 pair was close to TX9425 (Table 3). The Eps5HL-116 pair showed longer TTPV than both parents and other NIL pairs.

Discussion

A heading date locus was identified in the long arm of chromosome 5H

TX9425, a Chinese landrace, has been reported to have different stress tolerances [67–73], semidwarf and early maturity genes [39]. In this study, a different gene/QTL was identified for
heading date with the earliness allele derived from this cultivar. This locus was mapped to a position of 122–129 cM region of chromosome 5H using four pairs of near isogenic lines. The heading dates of the lines with the earliness allele occurred about 20 d earlier than those with the lateness allele in the normal growing season (autumn sowing) in Tasmania. This QTL was not identified in the DH population originating from the cross of the same parents (TX9425 and Franklin), most likely due to the relatively smaller effects which are often masked by the effects of two other major QTL [39] thus making difficult for comparative analysis with other major genes. By comparing the position in physical maps [74], the locus is situated at a similar position to \textit{Vrn-H1}, a vernalisation gene [2] and two other flowering regulators, \textit{HvPHYC} and \textit{HvCK2\alpha-5H} [58, 59].

\textit{Vrn-H1} is expressed when plants are exposed to prolonged cold temperatures (vernalization) usually in winter cultivars to switch to reproductive phase [75]. Since TX9425 originated from East Asia and shows a spring growth habit, it likely has the spring \textit{Vrn-H1} allele at this locus. Pankin et al. [58] reported that \textit{Vrn-H1} locus is tightly linked to \textit{HvPHYC (Eam5)} with no recombinants being detected between \textit{Vrn-H1} and \textit{HvPHYC} from a large BC1\textsubscript{F}\textsubscript{2}:3 population. Most of East Asian accessions have \textit{HvPHYC} haplotypes 1, 3 and 4. These haplotypes existed in both winter and spring types even though the growth habit of most of these accessions were not defined [58]. Thus the \textit{HvPHYC} haplotype in TX9425 needs to be further investigated.

The first report of an early maturity factor in chromosome 5H was by Wexelsen (1934) [53]. Close linkage to the rough awn trait demonstrates that this likely the locus reported by Laurie et al. (1995) [28] and later identified at the \textit{HvPHYC} locus (\textit{Eam5}). \textit{Eam5} was mapped to the similar position of \textit{Vrn-H1} [57] and \textit{HvPHYC} is reported as the candidate gene [58–60]. This gene is well adapted to the environments of China and Japan and is found in ICARDA/CIMMYT genetic stocks (CMB85533, Higuerrilla’2/Gobernadora) [58]. The amino-acid substitution in the GAF domain is the major influence of heading date under LDs [59]. However, \textit{HvPHYC} may interact with other genes such as \textit{Vrn-H1}, \textit{sdw1} and \textit{Ppd-H1} to induce early flowering under long and non-inductive short days [58, 60]. The QTL identified in this study behaved more like \textit{HvPHYC} reported by Pankin et al. (2014) [58] who observed 23 and 3 days differences in flowering between the Bowman (late genotype) and Bowman(\textit{eam5}) (early genotypes) under both short and long days, respectively. However, with the existence of \textit{Ppd-H1}, \textit{eam5} showed no significant effect on maturity, i.e. no differences between Bowman (\textit{Ppd-H1})

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**Table 3. Pasting properties of two parent varieties and four pairs of NILs**.

| Variety/line | PV  | T   | BD  | FV  | SB  | TTPV | PT   |
|--------------|-----|-----|-----|-----|-----|------|------|
| TX9425       | 412b| 310b| 102a| 699b| 389a| 6.38a| 84.2a|
| Franklin     | 451a| 357a| 94b | 715a| 358b| 6.38a| 82.1a|
| EpsSHL-116-E | 450a| 350a| 100a| 731a| 381a| 6.51a| 81.0a|
| EpsSHL-116-L | 448a| 354a| 93a | 720a| 365a| 6.56a| 79.3a|
| EpsSHL-317-1-E | 45a  | 345a| 110a| 748a| 403a| 6.28a| 79.7a|
| EpsSHL-317-1-L | 435a | 320a| 115a| 695a| 375a| 6.24a| 80.8a|
| EpsSHL-317-2-E | 444a| 321a| 123a| 727a| 406a| 6.18a| 80.7a|
| EpsSHL-317-2-L | 467a| 346a| 122a| 761a| 415a| 6.27a| 78.4a|
| EpsSHL-322-E | 417a| 309a| 109a| 701a| 392a| 6.27a| 82.2a|
| EpsSHL-322-L | 427a| 315a| 111a| 677a| 362a| 6.42a| 83.9a|

*Values in same column followed by same letter are not significantly different (P = 0.05). Comparisons are made within each pairs. Abbreviations are defined in Table 1.*

https://doi.org/10.1371/journal.pone.0200722.t003
and Bowman (Ppd-H1 + cam5) while the QTL identified in this study still showed earliness, indicating a possible new allele for early flowering.

**Early heading affects some agronomic traits but not flour pasting properties**

Previously reported eps QTL have been found to have direct influence on spike morphology, including length of the spike, spike density and thousand kernel weights in cereals [44, 76–78] since the duration of vegetative period is positively correlated to spike length and spikelet number per spike [79]. NILs carrying the late alleles including the late parent Franklin were found to have longer spike length and higher grain number than those carrying the early alleles across all sowing dates (Table 2), due to prolonged duration of the spike developmental stages [41, 76, 78].

Ppd-H1 was reported to have pleiotropic effects on plant height [80] and a QTL regulating heading date was found to be closely linked to the sdw1 (denso) gene in barley [81]. Ppd-H1 also seems to be one of the key genetic determinants for plant height and tiller number with Ppd-H1 reducing the number of tillers per plant [82]. QTL regulating plant height in the DH population of TX9425 and Franklin are located on 2H and 3H [39], likely usu1 and sdw1 (denso), respectively. However, none of these are in similar positions to early heading gene or are likely present in the NILs. Among four NIL pairs, 116 pair showed no significant difference in plant height, indicating no pleiotropic effect between the 5HL segment and plant height. The difference in the height between the alleles in the 317–1, 317–2 and 322 NIL pairs could be due to heading date or a different QTL responsible for plant height. Further studies are needed to confirm this.

Flour pasting properties are important quality traits and have close relationship with malting quality [83, 64] and food processing quality [65]. Pasting properties have been found to be influenced by genotype [83, 84] and environment [65, 84–86]. Earliness per se can also influence grain protein content [49] thus pasting properties [87]. Several QTL have been identified for pasting properties in barley [86]. These QTL for pasting properties are located on chromosomes 1H, 2H, 3H, 4H, 6H and 7H [86] with no QTL on 5H, indicating that may be unlikely that this chromosome segment would affect pasting properties. Indeed, this was confirmed in the current experiment, with no significant differences measured between NILs with the early and the late alleles.

In conclusion, a QTL on chromosome 5HL that causes variations in heading/flowering date and growing degree-days to heading was identified from the cross between TX9425 and Franklin. Using different pairs of NILs, the gene was mapped to 122–129 cM with a large number of co-segregating markers. This locus was found to have less sensitivity to temperature and photoperiod compared with other maturity/vernatisation genes at similar positions, indicating a possible new allele for early flowering. The chromosome region results in significant effects on some agronomic traits such as the length of spike and the number of spikelets per spike, but has less effect on flour pasting properties. Since the maturity effects of Eam5 are highly variable, closely linked molecular markers could be useful in facilitating the utilization of this gene. These markers are much closer than previously reported Raw1 locus, which is about 5 cm away from this earliness locus.

**Supporting information**

S1 Fig. Mean monthly temperatures and day length hours per month in 2015 for Tasmania. Arrows are three different sowing dates. (TIF)
S2 Fig. Morphological differences in ear emergence, maturity and spike length of four pairs of NILs carrying Eps5-317-1-E and Eps5HL-317-1-L alleles.
(TIF)

S3 Fig. Growing degree-day to heading date of different pairs of NILs, TX9425 and Franklin from different sowing dates.
(TIF)

S1 Table. Mean square values from the analysis of variance for all the traits studied for each pair of the NILs (early and late) and the parents (TX9425 and Franklin).
(DOCX)

S2 Table. Mean square values from the analysis of variance for all the pasting properties studied for each pair of the NILs (early and late) and the parents (TX9425 and Franklin).
(DOCX)

Acknowledgments
This work was supported by Grains Research & Development Corporation (GRDC) of Australia.

Author Contributions
Conceptualization: Meixue Zhou.
Data curation: Ahmed Ibrahim, Peter Johnson.
Formal analysis: Yun Fan, Meixue Zhou.
Funding acquisition: Meixue Zhou.
Supervision: Meixue Zhou.
Writing – original draft: Ahmed Ibrahim.
Writing – review & editing: Matthew Harrison, Holger Meinke, Yun Fan, Peter Johnson, Meixue Zhou.

References
1. Cockram J, Jones H, Leigh F, O’Sullivan D, Powell W, Laurie D, et al. Control of flowering time in temperate cereals: genes, domestication, and sustainable productivity. Journal of Experimental Botany. 2007; 58(6):1231–44. https://doi.org/10.1093/jxb/erm042 PMID: 17420173
2. Cuesta-Marcos A, Igartua E, Codesal P, Russell JR, Molina-Cano JL, Moralejo M, et al. Heading date QTL in a spring × winter barley cross evaluated in Mediterranean environments. Molecular Breeding. 2008; 21(4):455–71.
3. Ren X, Li C, Boyd W, Westcott S, Grime C, Sun D, et al. QTLs and their interaction determining different heading dates of barley in Australia and China. Crop and Pasture Science. 2010; 61(2):145–52.
4. Alqudah AM, Schnurbusch T. Heading date is not flowering time in spring barley. Frontiers in Plant Science. 2017; 8:896. https://doi.org/10.3389/fpls.2017.00896 PMID: 28611811
5. McMaster G, Wilhelm W. Phenological responses of wheat and barley to water and temperature: improving simulation models. The Journal of Agricultural Science. 2003; 141(02):129–47.
6. Hossain A, Teixeira da Silva J, Lozovskaya M, Zvolinsky V, Mukhortov V. High temperature combined with drought affect rainfed spring wheat and barley in south-eastern Russia: yield, relative performance and heat susceptibility index. Journal of Plant Breeding and Crop Science. 2012; 4(11):184–96.
7. González FG, Slafer GA, Miralles DJ. Floret development and spike growth as affected by photoperiod during stem elongation in wheat. Field Crops Research. 2003; 81(1):29–38.
8. Snape J, Butterworth K, Whitechurch E, Worland A. Waiting for fine times: genetics of flowering time in wheat. Euphytica. 2001; 119(1–2):185–90.

9. Meinke H, Hammer GL, Chapman SC. A sunflower simulation model: II. Simulating production risks in a variable sub-tropical environment. Agronomy Journal. 1993; 85(3):735–42.

10. Harrison MT, Tardieu F, Dong Z, Messina CD, Hammer GL. Characterizing drought stress and trait influence on maize yield under current and future conditions. Global Change Biology. 2014; 20(3):867–78. https://doi.org/10.1111/gcb.12381 PMID: 24038882

11. Boyd WJR, Li CD, Grime C. Genetic control of heading date in spring barley. In: 13th Australian Barley Technical Symposium. Fremantle, Western Australia, 26–30 August 2007.

12. Juskiw P, Jame Y, Kryzanowski L. Phenological development of spring barley in a short season growing area. Agronomy Journal. 2001; 93:370–9.

13. Boyd WJR, Li C, Grime C, Cakir M, Potipibool S, Kaveeta L, et al. Conventional and molecular genetic analysis of factors contributing to variation in the timing of heading among spring barley (Hordeum vulgare L.) genotypes grown over a mild winter growing season. Crop and Pasture Science. 2003; 54(12):1277–301.

14. Roberts E, Summerfield R, Cooper J, Ellis R. Environmental control of flowering in barley (Hordeum vulgare L.). I. Photoperiod limits to long-day responses, photoperiod-insensitive phases and effects of low-temperature and short-day vernalization. Annals of Botany. 1988; 62(2):127–44.

15. Went F. The effect of temperature on plant growth. Annual Review of Plant Physiology. 1953; 4(1):347–62.

16. Hay R, Ellis R. The control of flowering in wheat and barley: what recent advances in molecular genetics can reveal. Annals of Botany. 1998; 82(5):541–54.

17. Yin X, Kroplf MJ, Horie T, Nakagawa H, Centeno HG, Zhu D, et al. A model for photothermal responses of flowering in rice. I. Model description and parameterization. Field Crops Research. 1997; 51(3):189–200.

18. Hay R, Kirby E. Convergence and synchrony—a review of the coordination of development in wheat. Crop and Pasture Science. 1991; 42(5):661–700.

19. Ellis R, Roberts E, Summerfield R, Cooper J. Environmental control of flowering in barley (Hordeum vulgare L.). II. Rate of development as a function of temperature and photoperiod and its modification by low-temperature vernalization. Annals of Botany. 1988; 62:145–58.

20. MASWHEAT. Abiotic Stress and Agronomic Traits: Vernalization requirement. http://maswheat.ucdavis.edu/protocols/Vrn/. 2015.

21. Sasani S, Hemming MN, Oliver SN, Greenup A, Tavakol-Afshari R, Mahfoozi S, et al. The influence of vernalization and daylength on expression of flowering-time genes in the shoot apex and leaves of barley (Hordeum vulgare). Journal of Experimental Botany. 2009; 60(7):2169–78. https://doi.org/10.1093/jxb/erp098 PMID: 19357429

22. von Zitzewitz J, Szűcs P, Dubcovsky J, Yan L, Francia E, Pecchioni N, et al. Molecular and structural characterization of barley vernalization genes. Plant Molecular Biology. 2005; 59(3):449–67. https://doi.org/10.1007/s11103-005-0351-2 PMID: 19357429

23. Mizuno N, Kinoshita S, Kinoshita N, Hijida H, Fujita M, Kato K, et al. Loss-of-function mutations in three homoeologous PHYTOCLOCK1 genes in common wheat are associated with the extra-early flowering phenotype. PLoS ONE. 2016; 11(10). e0165618. https://doi.org/10.1371/journal.pone.0165618 PMID: 27788250

24. Pourkheirandish M, Komatsuda T. The importance of barley genetics and domestication in a global perspective. Annals of Botany. 2007; 100(5):999–1008. https://doi.org/10.1093/aob/mcm139 PMID: 17761690

25. Alqudah AM, Sharma R, Pasam RK, Graner A, Kilian B, Schnurbusch T. Genetic dissection of photoperiod response based on gwas of pre-anthesis phase duration in spring barley. PloS ONE. 2014; 9(11):e113120. https://doi.org/10.1371/journal.pone.0113120 PMID: 25420105

26. Trevaskis B, Bagnall DJ, Ellis MH, Peacock WJ, Dennis ES. MADS box genes control vernalization-induced flowering in cereals. Proceedings of the National Academy of Sciences. 2003; 100(22):13099–104.

27. Distelfeld A, Li C, Dubcovsky J. Regulation of flowering in temperate cereals. Current Opinion in Plant Biology. 2009; 12(2):178–84. https://doi.org/10.1016/j.pbi.2008.12.010 PMID: 19195924

28. Laurie DA, Pratchett N, Snape J, Bezant J. RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter × spring barley (Hordeum vulgare L.) cross. Genome. 1995; 38(3):575–85. PMID: 18470191

29. Karsai I, Szűcs P, Mészáros K, Filichkina T, Hayes P, Skinner J, et al. The Vrn-H2 locus is a major determinant of flowering time in a facultative winter growth habit barley (Hordeum vulgare L.) mapping
population. Theoretical and Applied Genetics. 2005; 110(8):1458–66. https://doi.org/10.1007/s00122-005-1979-7 PMID: 15834697

30. Kippes N, Debernardi JM, Vasquez-Gross HA, Akpinar BA, Budak H, Kato K, et al. 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. 2015; 112(39):E5401–E5410.

31. Kato K, Yamashita M, Ishimoto K, Yoshino H, Fujita M. Genetic analysis of two genes for vernalization response, the former Vrn2 and Vrn4, by using PCR based molecular makers. In: Pogna NE, Romano M, Pogna E, Galterio G (eds), Proceedings of 10th international wheat genetic symposium, Italy. 2003:971–3.

32. Cuesta-Marcos A, Szűcs P, Close TJ, Filichkin T, Muehlbauer GJ, Smith KP, et al. Genome-wide SNPs and re-sequencing of growth habit and inflorescence genes in barley: implications for association mapping in germplasm arrays varying in size and structure. BMC Genomics. 2010; 11(1):707.

33. Takahashi R, Yasuda S. Genetics of earliness and growth habit in barley. In: Nilan RA (ed.), Proceedings of the 2nd International Barley Genetic Symposium, 1971. Press: Pullman, (WA). Pp388-408.

34. Kippes N, Chen A, Zhang X, Lukaszewski AJ, Dubcovsky J. Development and characterization of a spring hexaploid wheat line with no functional VRN2 genes. Theoretical and Applied Genetics. 2016; 129:1417–28. https://doi.org/10.1007/s00122-016-2713-3 PMID: 27112150

35. Faure S, Turner A, Gruszka D, Christodoulou V, Davis S, von Korff M, 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(21):8328–33.

36. Wang J, Yang J, Jia Q, Zhu J, Shang Y, Hua W, et al. A new QTL for plant height in barley (Hordeum vulgare L.) showing no negative effects on grain yield. PloS ONE. 2014; 9(2):e90144. https://doi.org/10.1371/journal.pone.0090144 PMID: 24587247

37. Ren X, Li C, Cakir M, Zhang W, Grime C, Zhang X, et al. A quantitative trait locus for long photoperiod response mapped on chromosome 4H in barley. Molecular Breeding. 2012; 30(2):1121–30.

38. Börner A, Buck-Sorlin G, Hayes P, Malyshew S, Korzun V. Molecular mapping of major genes and quantitative trait loci determining flowering time in response to photoperiod in barley. Plant Breeding. 2002; 121(2):129–32.

39. Wang JM, Yang JM, McNeil DL, Zhou MX. Identification and molecular mapping of a dwarfing gene in barley (Hordeum vulgare L.) showing no negative effects on grain yield. PloS ONE. 2014; 9(2):e90144. https://doi.org/10.1371/journal.pone.0090144 PMID: 24587247

40. Bullrich L, Appendino M, Tranquilli G, Lewis S, Dubcovsky J. Mapping of a thermo-sensitive earliness per se gene on Triticum monococcum chromosome 1A. Theoretical and Applied Genetics. 2002; 105(4):585–93. https://doi.org/10.1007/s00122-002-0982-5 PMID: 12582508

41. Lewis S, Faricelli ME, Appendino ML, Valárík M, Dubcovsky J. The chromosome region including the earliness per se locus Eps-A m 1 affects the duration of early developmental phases and spikelet number in diploid wheat. Journal of Experimental Botany. 2008; 59(13):3595–607. https://doi.org/10.1093/jxb/em209 PMID: 18836186

42. Appendino M, Slafer GA. Earliness per se and its dependence upon temperature in diploid wheat lines differing in the major gene Eps-A1 alleles. The Journal of Agricultural Science. 2003; 141(02):149–54.

43. Kuchel H, Hollamby G, Langridge P, Williams K, Jefferies S. Identification of genetic loci associated with ear-emergence in bread wheat. Theoretical and Applied Genetics. 2006; 113(6):1103–12. https://doi.org/10.1007/s00122-006-0370-7 PMID: 16896709

44. Faricelli EM, Valárík M, Dubcovsky J. Control of flowering time and spike development in cereals: the earliness per se Eps-1 region in wheat, rice, and Brachypodium. Functional & Integrative Genomics. 2010; 10(2):293–306.

45. Valárík M, Linkiewicz A, Dubcovsky J. A microcolinearity study at the earliness per se gene Eps-A m 1 region reveals an ancient duplication that preceded the wheat–rice divergence. Theoretical and Applied Genetics. 2006; 112(5):945–57. https://doi.org/10.1007/s00122-005-0198-6 PMID: 16432738

46. Zikhali M, Leverington-Waite M, Fish L, Simmonds J, Orford S, Wingen LU, et al. Validation of a 1DL earliness per se (eps) flowering QTL in bread wheat (Triticum aestivum). Molecular Breeding. 2014; 34(3):1023–33. https://doi.org/10.1007/s11032-014-0094-3 PMID: 25242885

47. Slafer G. Genetic basis of yield as viewed from a crop physiologist’s perspective. Annals of Applied Biology. 2003; 142(2):117–28.

48. Griffiths S, Simmonds J, Leverington M, Wang Y, Fish L, Sayers L, et al. Meta-QTL analysis of the genetic control of ear emergence in elite European winter wheat germplasm. Theoretical and Applied Genetics. 2009; 119(3):383–95. https://doi.org/10.1007/s00122-009-1046-x PMID: 19403758

49. Hermel M, White J, Graeff S, Claupein W. The impact of Vernalization requirement, photoperiod sensitive and earliness per se on grain protein content of bread wheat. Euphytica. 2008; 163(2):309–20.
50. Fox G, Kelly A, Bowman J, Inkerman A, Poulsen D, Henry R. Is malting barley better feed for cattle than feed barley? Journal of the Institute of Brewing. 2009; 115(2):95–104.

51. Gupta M, Abu-Ghannam N, Gallagh M. Barley for brewing: characteristic changes during malting, brewing and applications of its by-products. Comprehensive Reviews in Food Science and Food Safety. 2010; 9(3):318–28.

52. Boden SA, Weiss D, Ross JJ, Davies N, Trevaskis B, Chandler PM, et al. EARLY FLOWERING3 regulates flowering in spring barley by mediating gibberellin production and FLOWERING LOCUS T expression. The Plant Cell. 2014; 26(4):1557–69. https://doi.org/10.1105/tpc.114.123794 PMID: 24781117

53. Wexelsen H. Quantitative inheritance and linkage in barley. Hereditas. 1934; 18(3):307–48.

54. Mathews S. Evolutionary studies illuminate the structural-functional model of plant phytochromes. The Plant Cell. 2010; 22(1):4–16. https://doi.org/10.1105/tpc.109.072280 PMID: 20118225

55. Biyashev R, Ragab R, Maughan J, Maroof S. Molecular mapping, chromosomal assignment, and genetic diversity analysis of phytochrome loci in barley (Hordeum vulgare). Journal of Heredity. 1997; 88(1):21–6.

56. Franklin KA, Quail PH. Phytochrome functions in Arabidopsis development. Journal of Experimental Botany. 2010; 61(1):4–16. https://doi.org/10.1105/tpc.109.072280 PMID: 20118225

57. Szeicz P, Karsai I, Von Zitzewitz J, Meszaros k, Cooper L. Positional relationships between photoperiod response QTL and photoreceptor and vernalization genes in barley. Theoretical and Applied Genetics. 2006; 112:1277–85. https://doi.org/10.1007/s00122-006-0229-y PMID: 16489429

58. Pankin A, Campoli C, Dong X, Kilian B, Sharma R, Himmelbach A, et al. Mapping-by-sequencing identifies HvPHYTOCHROME C as a candidate gene for the early maturity 5 locus modulating the circadian clock and photoperiodic flowering in barley. Genetics. 2014; 198(1):383–96. https://doi.org/10.1534/genetics.114.165613 PMID: 24996910

59. Nishida H, Ishihara D, Ishii M, Kaneko T, Kawahigashi H, Akashi Y et al. Phytochrome C is a key factor controlling long day-flowering in barley. Plant Physiology. 2013; 163:804–14. https://doi.org/10.1104/pp.113.222570 PMID: 24014575

60. Hill CB, Li C. Genetic architecture of flowering phenology in cereals and opportunities for crop improvement. Frontiers in Plant Science. 2016; 7:1906. https://doi.org/10.10110/tpc.109.072280 PMID: 20118225

61. 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(12):1388–92. https://doi.org/10.1038/ng.2447 PMID: 23160098

62. DPIT. 'Franklin Barley'. Department of Primary Industry Tasmania Plant Varieties Journal Application No: 1989/018 Received: 04-Apr-1989, Accepted: 06-Apr-1989, and Granted: 19-Jan-1990 http://www.austliie.edu.au/journals/MurUEJL/2003/40.html

63. Nuttonson MY. A comparative study of lower and upper limits of temperature in measuring the variability of day-degree summations of wheat, barley, and rye. American Institute of Crop Ecology. 1956. 18:1–42.

64. Zhou MX, Mendham NJ. Predicting barley malt extract with a Rapid Viscoanalyzer. Journal of Cereal Science. 2005; 41:31–6.

65. Zhou MX, Robards K, Glennie-Holmes M, Helliwell S. Structure and pasting properties of oat starch. Cereal Chemistry. 1998; 75:273–81

66. Stell R, Torrie J, Dickey D. Principles and procedures of statistics: a biometrical approach. New York: MacGraw-Hill. 1980; 2nd edition.

67. Li H. A statistical framework for SNP calling, mutation discovery, association mapping and population genetic parameter estimation from sequencing data. Bioinformatics. 2011; 27(21):2987–93. https://doi.org/10.1093/bioinformatics/btr509 PMID: 21903627

68. Li HB, Kilian A, Zhou MX, Wenzl P, Huttner E, Mendham N, et al. Construction of a high-density composite map and comparative mapping of segregation distortion regions in barley. Molecular Genetics and Genomics. 2010; 284(5):319–31. https://doi.org/10.1007/s00438-010-0570-3 PMID: 20830217

69. Li HB, Vaillancourt R, Mendham N, Zhou MX. Comparative mapping of quantitative trait loci associated with waterlogging tolerance in barley (Hordeum vulgare L.). BMC Genomics. 2008; 9(1):401.

70. Fan Y, Shabala S, Ma Y, Xu R, Zhou MX. Using QTL mapping to investigate the relationships between abiotic stress tolerance (drought and salinity) and agronomic and physiological traits. BMC Genomics. 2015; 16(1):43.
71. Xu R, Wang J, Li C, Johnson P, Lu C, Zhou MX. A single locus is responsible for salinity tolerance in a Chinese landrace barley (*Hordeum vulgare* L.). PloS ONE. 2012; 7(8):e43079. https://doi.org/10.1371/journal.pone.0043079 PMID: 22916210

72. Pang J, Zhou MX, Mendham N, Shabala S. Growth and physiological responses of six barley genotypes to waterlogging and subsequent recovery. Crop and Pasture Science. 2004; 55(8):895–906.

73. Li H, Durbin R. Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics. 2009; 25(14):1754–60. https://doi.org/10.1093/bioinformatics/btp324 PMID: 19451168

74. Mayer KFX, Waugh R, Brown JWS, Schulman A, Langridge P, Platzer M, et al. A physical, genetic and functional sequence assembly of the barley genome. Nature. 2012; 491(7426):711–6. https://doi.org/10.1038/nature11543 PMID: 23075845

75. 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(2):107–17. https://doi.org/10.1007/s00438-009-0449-3 PMID: 19404679

76. Święcka S, Berdzik M, Myśkó B. Genetic mapping of the *ScHd1* gene in rye and an assessment of its relationship with earliness per se and plant morphology. Journal of Applied Genetics. 2014; 55(4):469–73. https://doi.org/10.1007/s13353-014-0223-z PMID: 24840745

77. Gawroński P, Schnurbusch T. High-density mapping of the earliness per se-3A* (Eps-3A*) locus in diploid einkorn wheat and its relation to the syntenic regions in rice and *Brachypodium distachyon*. Molecular Breeding. 2012; 30(2):1097–108.

78. Zikhali M, Griffiths S. The effect of Earliness per se (Eps) genes on flowering time in bread wheat. In: Oghihara Y, Takumi S, Handa H (eds.), Advances in Wheat Genetics: From Genome to Field. Proceeding of the 12th International Wheat Genetic Symposium. 2015.

79. Kiss T, Balla K, Bányai J, Veisz O, Karsai I. Effect of different sowing times on the plant developmental parameters of wheat (*Triticum aestivum* L.). Cereal Research Communications. 2014; 42(2):239–51.

80. Laurie AD, Pratchett N, Beazant JH, Snape JW. Genetic analysis of a photoperiod response gene on the short arm of chromosome 2 (2H) of *Hordeum vulgare* (barley). Heredity-London-. 1994; 72:619–27.

81. Barua U, Chalmers KJ, Thomas WTB, Hackett CA, Lea V, Jack P et al. Molecular mapping of genes determining height, time to heading and growth habit in barley (*Hordeum vulgare*). Genome. 1993; 36:1080–7. PMID: 18470049

82. Alqudah AM, Koppolu R, Wolde G, Graner A, Kilian B, Schnurbusch T. The genetic architecture of barley plant stature. Frontiers in Genetics. 2016; 7:117. https://doi.org/10.3389/fgene.2016.00117 PMID: 27446200

83. Zhou MX, Li HB, Chen ZH, Mendham NJ. Combining ability of barley flour pasting properties Journal of Cereal Science. 2008; 48:789–93.

84. Zhou MX, Glennie-Holmes M, Roberts G, Robards K, Helliwell S. The effect of growing sites on grain quality of oats and pasting properties of oatmeals. Crop and Pasture Science. 1999; 50(8):1409–16.

85. Singh S, Gupta AK, Gupta SK, Kaur N. Effect of sowing time on protein quality and starch pasting characteristics in wheat (*Triticum aestivum* L.) genotypes grown under irrigated and rain-fed conditions. Food Chemistry. 2010; 122(3):559–65.

86. Wang J, Yang J, McNeil D, Zhou MX. Mapping of quantitative trait loci controlling barley flour pasting properties. Genetica. 2010; 138:1191–200. https://doi.org/10.1007/s10709-010-9511-7 PMID: 21063749

87. Zhou MX, Robards K, Glennie-Holmes M, Helliwell S. Effects of enzyme treatment and processing on pasting and thermal properties of oats. Journal of the Science of Food and Agriculture. 2000; 80 (10):1486–94.