HEINRICH HEINE
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PHOTOPERIOD 1 (*Ppd-H1*) and *VERNALIZATION 1* (*HvVRN1*) Interact to Control Reproductive Development under High Ambient Temperatures in Barley (*Hordeum vulgare* L.)

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Mahwish Ejaz

aus Jhelum-Pakistan

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der Heinrich-Heine-Universität Düsseldorf

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I. Zusammenfassung

Ein Anstieg der globalen Durchschnittstemperaturen und das Auftreten extremer Wetterereignisse bedrohen die Getreideerträge weltweit. Hohe Temperaturen während der reproduktiven Entwicklung beeinträchtigen die Ährenentwicklung und die Fruchtbarkeit der Blüte und sind daher eine der Hauptursachen für Ertragsverluste in den Getreidearten Gerste und Weizen. Derzeit sind Strategien zur Züchtung von Getreidesorten mit verbessertem Ertrag unter hohen Temperaturen begrenzt, aufgrund fehlender Kenntnisse über die genetischen Faktoren, die die Spross-und Ährenenentwicklung unter hohen Temperaturbedingungen steuern. In dieser Studie habe ich gezeigt, dass Gene der zirkadianen Uhr, der Photoperiode- und Vernalisationsstoffwechselwege die reproduktive Entwicklung und die Fruchtbarkeit der Blüten unter hohen Umgebungstemperaturen in Gerste kontrollieren. Eine natürliche Mutation im *PHOTOPERIOD RESPONSE 1* Gen (*Ppd-H1*), die vor allem in Sommergerste vorkommt, verzögert die reproduktive Entwicklung und verringert die Zahl der Blüten und Körner pro Ähre unter hoher Temperatur. Im Gegensatz dazu führte das Wildtyp-Allel von *Ppd-H1* oder ein nicht funktionsfähiges Allel von *EARLY FLOWERING 3 (HvELF3)*, ein Repressor von *Ppd-H1*, zu einer beschleunigten reproduktiven Entwicklung und einer erhöhten Anzahl Körner pro Ähre unter hohen Temperaturen. Außerdem verzögerte hohe Temperatur die Phase und verringerte die Amplitude von Uhrgenen. *Ppd-H1* und *HvELF3* abhängige Unterschiede in der reproduktiven Entwicklung unter hohen Temperaturen korrelierten mit der Expression der Mads-Box-Gene *HvVRN1*, *HvBM3* und *HvBM8* in den Blättern. Ausserdem hatte regulatorische Variation im ersten Intron des wichtigsten Vernalisationsgens *HvVRN1* einen Einfluss auf die reproduktive Entwicklung unter hohen Temperaturen. Hohe Temperaturen verzögerten die Ährchenbildung durch die Herunterregulierung des Winteralleles, nicht aber des Sommerallels von *HvVRN1*, auch nach der Vernalisierung. Darüber hinaus waren die Expressionslevels des *FLOWERING LOCUS T1* Gens, welches maßgeblich an der Blütenbildung beteiligt ist, unter hoher Umgebungstemperatur unabhängig vom genetischen Hintergrund reduziert. Schliesslich zeige ich, dass *Ppd-H1* genetisch mit *HvVRN1* interagiert, wobei *Ppd-H1* die Entwicklung nur im Hintergrund eines Sommer- *HvVRN1*, nicht aber eines Winter *Hvvrn1* Allels beschleunigt. Meine Arbeit demonstriert deshalb, dass die Photoperiode- und Vernalisationsgene Signale der Umgebungstemperatur zur Feinabstimmung der reproduktiven Entwicklung in Gerste integrieren.

II. Summary

An increase in global average temperature and the occurrence of extreme temperature events threaten crop productivity worldwide. High temperature during reproductive development impairs developmental timing, spike development and floret fertility and is therefore a major cause of yield losses in the temperate cereals barley and wheat. At present, strategies to breed cereal varieties with improved yield under high temperatures are limited due to a lack of knowledge on the genetic factors that control shoot and spike development under high temperature conditions. In this study, I have demonstrated that genes from the circadian clock, photoperiod and vernalization pathways control reproductive development and floral fertility in response to high ambient temperatures in barley. I have established that a natural mutation in the major photoperiod response gene, *Ppd-H1,* prevalent in spring barley slows down reproductive development and decreases the number of florets and seeds per spike under high ambient temperature. In contrast, the wild-type *Ppd-H1* or a non-functional allele of *EARLY FLOWERING 3 (HvELF3),* which is an upstream regulator of *Ppd-H1,* accelerated the reproductive development and maintained high seed numbers per spike under high temperatures. Furthermore, high temperature delayed the phase and reduced the amplitude of clock genes. *Ppd-H1* and *HvELF3* dependent differences in reproductive development under high temperatures correlated with the expression levels of the *BARLEY MADS*-box genes *HvVRN1, HvBM3* and *HvBM8* in the leaves. Moreover, regulatory variation in the first intron of the major vernalization gene *HvVRN1* also affected developmental timing under high temperatures. High temperatures strongly delayed spikelet initiation by downregulating the expression of the full-length winter allele *Hvvrn1*, but not the spring allele *HvVRN1*, even after vernalization. In addition, the transcript levels of *FLOWERING LOCUS T1* (*HvFT1*), the major floral integrator gene, were reduced unter high ambient temperature independently of genetic variation at *Ppd-H1* and *HvVRN1*. Lastly, I show that *Ppd-H1* genetically interacts with regulatory variation at *HvVRN1* where *Ppd-H1* accelerates shoot apex development in the background of a spring *HvVRN1* but not a winter *Hvvrn1* allele. My work thus demonstrated that the photoperiodic and vernalization pathways integrate high temperature signals to fine-tune reproductive development in barley.

III. Scientific Aims

High temperature during reproductive development impairs spike development and floret fertility and is therefore a major cause of yield losses in the temperate cereal barley (*Hordeum vulgare* L.). However, genetic components controlling reproductive development in response to high ambient temperatures have not yet identified. The barley genepool comprises exotic germplasm, which is characterized by high genetic diversity and adaptation to stressful climatic conditions. These resources represent valuable genetic material to dissect the genetic and molecular control of temperature responses and identify alleles that improve reproductive development under high ambient temperature in barley.

1) The physiological genetic control of developmental timing in response to high ambient temperatures has been characterized in the model eudicot species *Arabidopsis thaliana*. This knowledge provides a basis to explore the genetic control of developmental responses to high ambient temperatures in the monocot barley. I give an overview over the current state of research on the genetic basis of reproductive development under high ambient temperature in *Arabidopsis thaliana.* I discuss similarities and differences in developmental phenotypes as controlled by high ambient temperature between Arabidopsis and cereal crops. In addition, I propose how known flowering pathways, such as the photoperiod and vernalization pathway may integrate different environmental cues to fine-tune shoot and spike development. Finally, I dissect the reported effects of high ambient temperature on different developmental stages of the shoot apex, spike morphology and floret fertility in cereal crops.
2) High ambient temperature alters flowering time in barley, however, the effects of high ambient temperature on shoot apex development and its genetic control are not known. I, therefore, aimed to characterize the microscopic and macroscopic development of the shoot apex in response to high ambient temperature in barley. Further, I investigated the effects of the flowering time gene *PHOTOPERIOD RESPONSE 1* (*Ppd-H1*), the circadian clock gene *EARLY FLOWERING 3* (*HvELF3*) and the vernalization gene *VERNALIZATION 1* (*HvVRN1*) on developmental timing, spike development and fertility in barley. For this purpose, I scored shoot development in introgression lines carrying natural variants at the candidate genes introgressed from wild and landrace barley. Finally, my objective was to identify molecular changes in the leaf that correlate with developmental timing and spike development under high temperature in different introgression lines

IV. Chapter 1-- Phenotypic Plasticity under High Ambient Temperature: Flowering and Reproduction

1.1 Introduction

Flowering at the right time is an important decision particularly for cereal crops to ensure their reproductive success and thereby is of both adaptive and economic significance. Plants, being sessile, can adapt to changing environmental cues such as light and temperature. In contrast to animals, plants can produce new organs throughout their lifespan from their pluripotent stem cells present at the tip of shoot apical meristem (SAM), from which above ground parts such as stems, leaves, and flowers are developed. This characteristic of plants is under endogenous (genetic) and environmental control which enables them to adapt and respond to changing environmental condition.

Major environmental cues are photoperiod, circadian clock, and temperature, which are integrated by plants to time shoot development. Genetic variation in developmental timing in response to environmental cues was important for successful adaptation of crop plants to different eco-geographic cultivation areas. The major crop plants, rice, wheat, maize and barley are monocot grasses and are either tropical grasses (maize, rice) adapted to warmer climates or temperate grasses (barley and wheat) adapted to cooler environments. Climate models predict that an increase in global average temperature will have large impacts on crop yield. High temperature during reproductive development impairs spike development and floret fertility and is therefore a major cause of yield losses in the temperate cereals barley and wheat. Understanding the genetic and molecular basis for ambient temperature-induced changes in reproductive development will play a crucial role to ensure future yield stability of these cereals.

In this chapter, I review barley phenology and the genetic control of inflorescence development in response to environmental cues. Since current knowledge of reproductive development under high ambient temperature is limited in grasses such as barley, I have summarized how thermosensory pathways modulate flowering time in the model eudicot Arabidopsis (*Arabidopsis thaliana*). This knowledge from the model plant can provide insights into the genetic control of reproductive development under high temperature in barley. The last section of this chapter reports on previous studies about ambient temperature

effects on flowering and spike fertility in grass species such as barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), rye (*Secale cereale*), rice (*Oryza sativa*), Brachypodium *(Brachypodium distachyon)*, and sorghum (*Sorghum bicolor*). Thus, this chapter provides the current state of knowledge about reproductive development under ambient temperatures in Arabidopsis and grass species.

1.1.1 Inflorescence Morphology and Spike Development in Barley

Barley (*Hordeum vulgare*), a member of the grass family *Poaceae*, is a temperate cereal crop. Like many other grass species such as wheat (*Triticum aestivum*), oats (*Avena sativa*), rice (*Oryza sativa*), maize (*Zea mays*), it is a source of food and feed. Therefore, inflorescence and spike development, which directly control grain yield, are essential traits to study. The activity of shoot apical meristems (SAM) has a significant impact on grain yield. The SAM develops during embryogenesis, which gives rise to all the aboveground plant organs, i.e., stem, leaves, and flowers during postembryonic development after germination (Barton, 2010). In Arabidopsis, during the vegetative phase, the shoot apex form leaves in a spiral arrangement with very short internodes forming a rosette. After the vegetative phase, the shoot apex undergoes the transition to bolting and becomes an inflorescence meristem (IM), which still produces small cauline leaves, separated by long internodes. After this transient phase, the IM produces floral meristems (FMs), and each of them gives rise to one individual pedicellate flower resulting in a raceme inflorescence (E. S. Coen, 1991; Weberling, 1992; Benlloch et al., 2007).

The shoot and inflorescence architecture of grasses, recapitulates the basic structures described in the model plant, but also clearly differs in morphology. In barley and wheat, the SAM gives rise to auxiliary meristems (AMs) in the leaf axils of the basal nodes, which later develop into secondary shoots or tillers (Tanaka et al., 2013). The inflorescence meristem generates spikelet meristems (SM) and floral meristems (FM). A spikelet produces of one to several flowers or florets enclosed by two glumes (a modified bract) (von Bothmer and Komatsuda, 2011). These spikelets are arranged on both sides of rachis in an alternate fashion forming either a two or a six-row spike. Barley spikes produce a triple spikelet meristem (TSM) constituting one central and two lateral spikelet meristems. In two-rowed spike, only the central spike produces seeds whereas in six-rowed spike forms all the three meristems are fertile (Zohary and Hopf, 1993; Forster et al., 2007; von Bothmer and Komatsuda, 2011; Sakuma et al., 2011). Barley meristem does not produce a terminal spikelet, thereby has an indeterminate inflorescence. Barley flowers (florets) are bisexual and contain one pistil and three stamens, and two flap-like structures in place of petals, the lodicules. At the time of

anthesis, the lodicules become turgid and help in floret opening. Florets are covered by bract-like structure, the palea (inner) and lemma (outer) and further subtend by two reduced bracts, the glumes (Figure 1) (Kellogg, 2007).

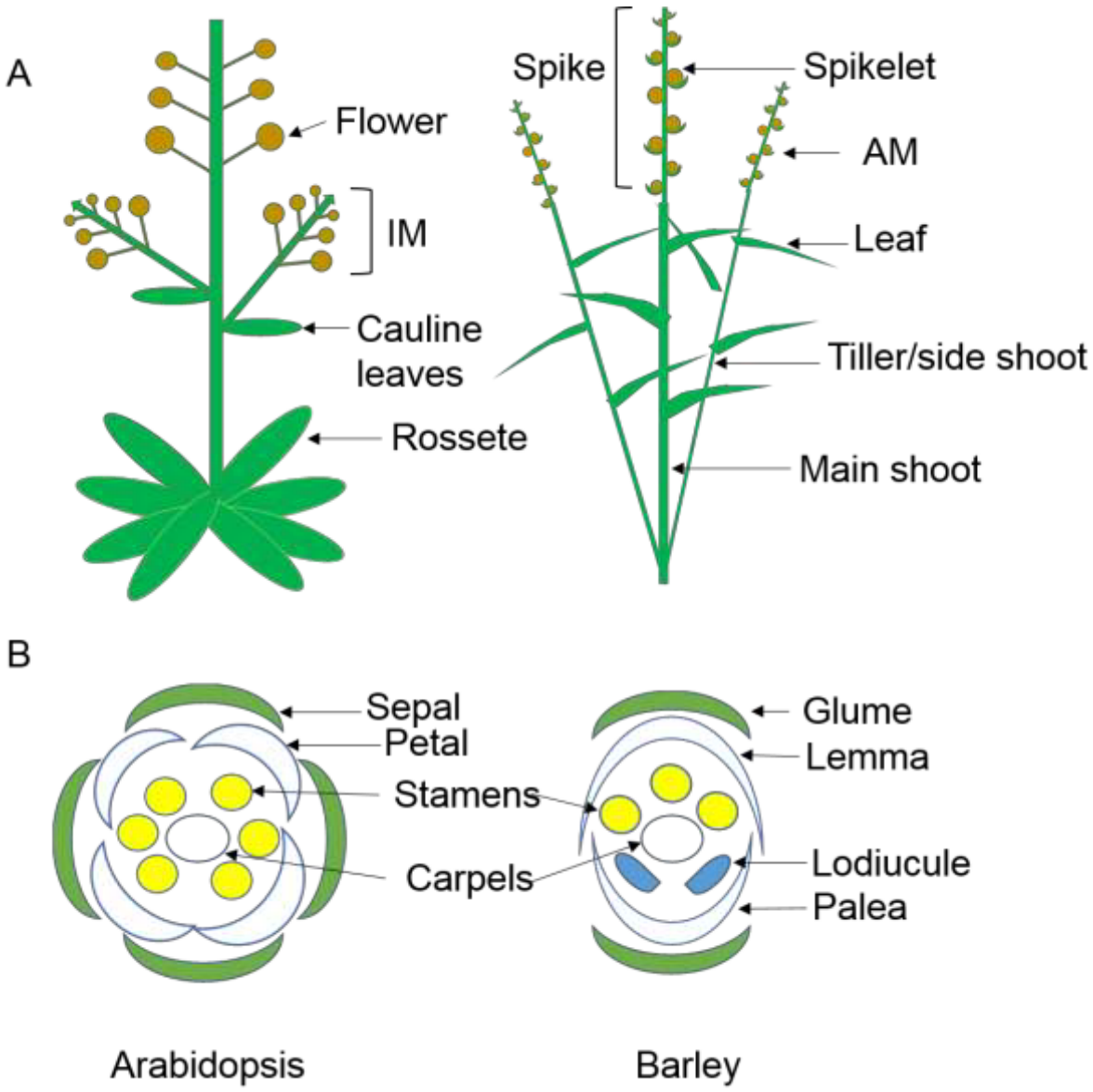

Figure 1. Inflorescence patterning and flower/spikelet structures of Arabidopsis and barley. **A)** Schematic diagram of the inflorescence architecture of Arabidopsis (Raceme) and barley (Spike). Both Arabidopsis and barley show indeterminate growth of the inflorescence compared to wheat and rice (not shown here) which form a terminal spikelet **B)** Schematic representation of Arabidopsis flower and barley spikelet. Abbreviations: IM, Inflorescence meristem, AM, Auxiliary meristem. (Adapted from E. S. Coen, 1991; Tanaka et al., 2013)

Pre-anthesis development in barley consists of three distinctive growth phases that are broadly classified as; 1) vegetative phase, 2) early reproductive phase and 3) a late reproductive phase (Slafer and Rawson, 1994; González et al., 2002). After germination, the vegetative shoot apex forms single ridges that develop into leaf primordia as semicircular whorls, at the basal-apical growing points, growing upwards within the tube-like structures of already present leaf sheaths (Briggs, 1978). At the point, when the shoot apex stops producing leaf primordia, floral primordia are initiated. This stage is also referred to as transition from vegetative to reproductive development. During this phase, the shoot apex elongates and develops into the double ridge stage; each of upper ridges develops faster compared to the lower ridge and forms spikelet primordia, while the development of lower ridge is repressed resulting into rachis internodes of the spikes later (Briggs, 1978). In barley, each spikelet produces one floret primordium, which develops into a flower. Floral development starts from the center of the inflorescence and later spread towards distal and proximal parts. At the onset of floral development, early reproductive phase ends (Slafer and Rawson, 1994). Unlike Arabidopsis, the time between spikelet initiation and stem elongation is prolonged in grasses during which the shoot apex remains at the base of the stem (Briggs, 1978; Kemp and Culvenor, 1994). This trait is evolved explicitly in grasses to protect the shoot apex from grazing damage (Briggs, 1978).

The late reproductive phase of shoot apex development in barley is relatively long and includes differentiation of spikelet meristems, floral organs, and then spike growth during stem elongation. Stem elongation involves internode elongation and growth of the shoot apex upwards. This phase starts with the initiation of three stamen primordia followed by one-carpel primordia in the center of each floret. At the carpel primordium stage, a maximum number of floral primordia are already initiated, and awns start developing, thus also known as awn primordium stage. In the following stages, development of ovary and anthers takes place, and anthers develop into a four-locule structure. To ensure timely pollination, the developmental processes of male and female organogenesis are synchronized (Briggs, 1978). After the development of floral organs, they start growing in size and change in morphology; as the style elongates, stigmatic branches start appearing and the anthers change in color from transparent white to green. This phase also corresponds to overall spike growth and can visually be assessed by a fully expanded flag leaf, also called booting phase in cereals (Zadoks et al., 1974). As soon as all floral organs have been formed, they start maturing; anthers become yellow and stigmatic branches fall apart. At this stage, about one-centimeter-long awns are visible out of the flag leaf sheath, which is scored as heading date or flowering time in barley and other cereals (Large, 1954; Zadoks et al., 1974). Pollination takes place

right after heading stage. Various scales were developed to score reproductive development in barley (Bonnett, 1935; Sharman, 1947; Large, 1954). These are based on scoring macroscopic changes of the main shoot or on evaluating microscopic changes of the shoot apical meristem. Waddington et al. (1983) developed a quantitative scale from germination to anthesis, to score the morphogenesis of the shoot apex and carpels of the inflorescence. This scale scores the carpel development in the most advanced floral primordia at the shoot apex at a scale of 1-10 (Figure 2). This scale is the best choice to score development when morphological growth and shoot apex development are not synchronized, for example under changing environmental conditions such as high temperature or drought (Waddington et al., 1983).

The duration of different developmental phases of SAM affects flowering time and are associated with different yield components (Rawson, 1970; Rawson, 1971; Slafer and Rawson, 1994) (Figure 2). The length of vegetative and early reproductive phase determines the number of spikelet primordia that are initiated on the shoot apex while the late reproductive phase determines the number of fertile florets established (Alqudah and Schnurbusch, 2014; Digel et al., 2016). The duration of the stem elongation phase before anthesis and spike dry weight is associated with floret survival and potential seed set (Slafer and Rawson, 1996; Miralles and Richards, 2000). A prolonged duration of the stem elongation phase favors spike growth and increases spike dry weight, which is due to the competition between stem and spike for dry matter accumulation (Brooking and Kirby, 1981; Miralles et al., 1998). Environmental factors and genetic variation also affect the duration of the developmental phases of the shoot apex and thereby affect flowering time and yield related traits (Fischer, 1985; Digel et al., 2015; Ejaz and von Korff, 2017). Genetic control of reproductive development and interaction with different environmental cues in barley is described in the following section.

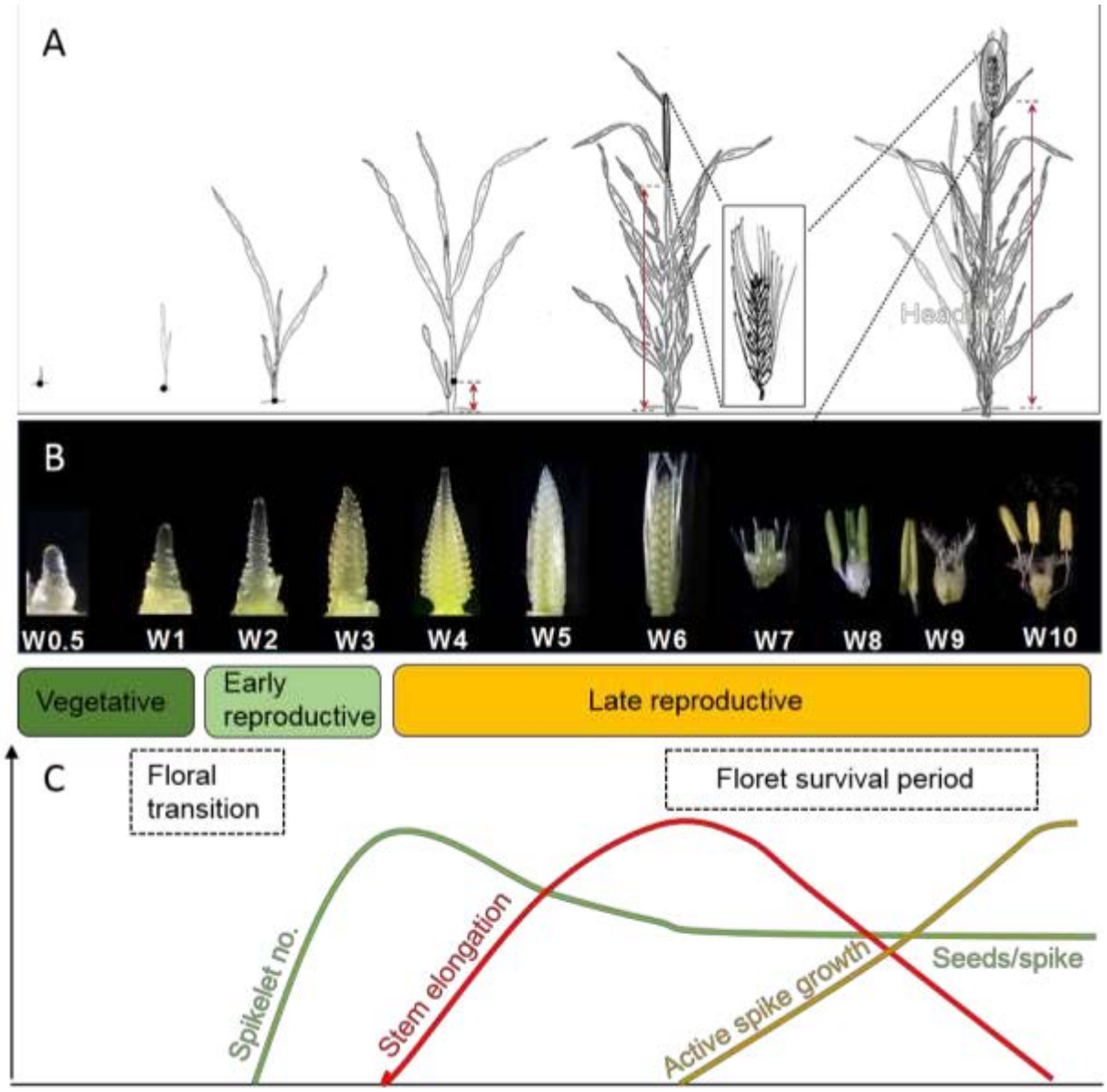

Figure 2 Phases of pre-anthesis development in barley. A) Illustration of barley shoot morphogenesis and spike growth. Black dots indicate the location of the shoot apical meristem (SAM) inside the leaf sheath. The SAM moves upwards during stem elongation represented by red arrows (Briggs, 1978; del Moral et al., 2002; Digel et al., 2015). B) Pre-anthesis development of the SAM represented at a scale of 0.5-10 (modified from Waddington et al. 1983). C) Spikelet initiation occurs between W1 and W2 when the shoot apex transforms from single ridge to double ridge. The early reproductive phase ends at the stamen primordium stage (W3-3.5) when the shoot apex starts growing upwards in the stem. The late reproductive phase (LRP) starts at carpel primordium stage (W4), when the maximum number of floral primordia are formed and anthesis occurs at W10. The number of primordia decreases in the early stages of LRP and final seed number is determined in the late stages of LRP (Miralles and Richards, 2000; González et al., 2003; Slafer, 2003; Sreenivasulu and Schnurbusch, 2012).

1.1.2 Genetic Control of Reproductive Development by Environmental Cues in Barley

Flower development is regulated by various environmental signals such as photoperiod, vernalization and ambient temperature and endogenous cues such as the circadian clock, hormone homeostasis and carbon availability (Simpson et al., 1999; Reeves and Coupland, 2000; Ausín et al., 2005; McClung and Davis, 2010). These environmental cues interact with genetic components that have resulted in the adaptation of barley to different growing seasons. Barley is a facultative long day (LD) plant and flowers early under LD conditions, whereas short days (SD) delay flowering (Turner et al., 2005). *PHOTOPERIOD 1* (*Ppd-H1*), a homolog of *PSEUDO-RESPONSE REGULATOR (PRR)* genes in Arabidopsis, is a major photoperiod response gene in barley (Turner et al., 2005). The wild-type *Ppd-H1* allele is responsible for early flowering in wild and winter barley under LDs (Turner et al., 2005). In contrast, a natural recessive mutation in the CCT domain of *Ppd-H1* causes reduced sensitivity to photoperiod resulting in late flowering under LDs. This delay in flowering correlates with a reduced expression of *HvFT1*, a homolog of the Arabidopsis *FLOWERING LOCUS T (FT)* in barley (Turner et al., 2005; Campoli et al., 2012). Thus, barley genotypes carrying the dominant *Ppd-H1* allele show increased expression levels of *HvFT1* (Turner et al., 2005). Likewise, increased expression of *Ppd1* in wheat up-regulates the expression of *FT* homolog *TaFT1* (Shaw et al., 2012). FT moves from leaves to the shoot apical meristem at the time of vegetative to floral transition to induce flowering in Arabidopsis (Corbesier et al., 2007).

In addition, *EARLY FLOWERING3 (ELF3)*, a repressor of light signals to the circadian clock, acts as an upstream regulator of the photoperiod pathway genes and contributes to photoperiodic development in Arabidopsis (Zagotta et al., 1996; Suárez-López et al., 2001). *ELF3* forms together with *EARLY FLOWERING4 (ELF4)* and *LUX ARRHYTHMO (LUX)*, the so-called “evening complex” (EC) that functions as a nighttime repressor of gene expression in the circadian clock of Arabidopsis (Nusinow et al., 2011; Herrero and Davis, 2012). The circadian clock is an autonomous oscillator that produces endogenous biological rhythms with a period of about 24 h is represented by at three interconnected feedback loops (Ronald and Davis, 2017). The central loop includes two partially redundant *MYB*-like transcription factors *CIRCADIAN CLOCK ASSOCIATED1 (CCA1)* and *LATE ELONGATED HYPOCOTYL (LHY),* and a member of *PSEUDO RESPONSE REGULATOR (PRR)* gene family the *TIMING OF CAB EXPRESSION1 (TOC1).* In the morning, *CCA1* and *LHY* proteins

accumulate to repress the transcription of evening expressed gene *TOC1* (Pokhilko et al., 2012). The morning-phased loop includes *PRR* genes; *PRR7* and *PRR9* that are structurally related to *TOC1,* inhibit the transcriptional activation of *CCA1/LHY* during the day (Farré et al., 2005; Nakamichi et al., 2012). *PRR7* and *PRR9* are repressed by the evening complex (EC) genes in the evening to activate *CCA1/LHY*. The *CCA1* inhibits the evening complex (EC) genes late at night which connects the EC to the central oscillator (Lu et al., 2012; Kamioka et al., 2016). The evening phased loop consist of auto-regulated feedback of *TOC1* with *GI (GIGANTEA)* (Kim et al., 2007). The transcription factors (TFs) regulating the feedback loops of the oscillator also directs the output pathways of the circadian clock (Kamioka et al., 2016).

Several studies on Arabidopsis reveals that the circadian clock influences plant performance and adaptation to abiotic stresses (Dodd et al., 2005; Legnaioli et al., 2009). Orthologues of Arabidopsis clock genes have been identified in barley and termed *as HvCCA1, HvGI, HvPRR1, HvPRR37 (Ppd-H1), HvPRR73, HvPRR59,* and *HvPRR95*, thus demonstrating that the circadian clock genes are structurally conserved in barley (Campoli et al., 2012). Unlike in Arabidopsis, barley clock mutants, however, do not show substantial effects on plant morphology, growth, and metabolism (Habte et al., 2014). However, they play a significant role in flowering time that has been demonstrated in the identification of clock mutants in barley. *early maturity 8 (eam8)* mutation produces a photoperiod insensitive phenotype, which flowers rapidly independent of the photoperiod in barley (Börner et al., 2002). Faure et al. (2012) have shown that *eam8 is* an orthologue of Arabidopsis *EARLY FLOWERING 3,* and an essential component of the evening complex in the circadian clock. *eam8* mutation has been employed in commercial spring barley varieties with the *ppd-H1* mutation for cultivation in short growing seasons (Zakhrabekova et al., 2012). Despite the presence of the *ppd-H1* mutation, *eam8* (*Hvelf3*) mutants show misexpression of *ppd-H1* in the night and increased expression of *HvFT1* (Faure et al., 2012). Campoli et al. (2013) identified the circadian clock gene *HvLUX1* underlying the *early maturity 10* (*eam10*) locus in barley which is an orthologue of the Arabidopsis clock gene *LUX ARRHYTHMO*. *HvLUX1* interacts with *ppd-H1* to promote flowering under LDs and SDs (Campoli et al., 2013). In addition, *Phytochrome C*, an upstream regulator of the circadian clock, (Nishida et al., 2013; Chen et al., 2014; Pankin et al., 2014) has been shown to disrupt the expression of clock genes and to interact with *ppd-H1* to promote early flowering in SDs (Pankin et al., 2014). Apart from photoperiod and the circadian clock, temperature changes also affect timing of flowering in barley.

Barley is characterized by winter and spring growth habits. Winter barley needs a period of cold (vernalization) for flowering, whereas spring barley does not respond to vernalization and flowers in the absence of a cold period (Gasser et al., 1998; Hemming et al., 2009). Two major vernalization response genes *VERNALIZATION 1* (*HvVRN1*) *and VERNALIZATION 2 (HvVRN2)* regulate flowering time in winter barley. Hv*VRN2* is highly expressed before cold exposure under long photoperiods (Yan et al., 2004). *HvVRN2* is a member of *ZCCT* (zinc finger and CCT domain) gene family. *HvVRN2* does not have a clear orthologue in Arabidopsis, however, its function is analogous to *FLOWERING LOCUS C (FLC)* in Arabidopsis (Yan et al., 2004). In barley and wheat, *VERNALIZATION 1 (VRN1* in wheat*,* Hv*VRN1* in barley *)*, a *MADS-box* transcription factor, is upregulated during vernalization and represses *HvVRN2* (Trevaskis et al., 2007; Hemming et al., 2008). *HvVRN1* is a homologue of the Arabidopsis meristem identity gene *APETALA1 (AP1)* that initiates floral transition (Murai et al., 2003; Shitsukawa et al., 2007). In barley, *HvVRN1* is characterized by a series of different deletions and insertions in the first regulatory intron, which has been linked to differences in vernalization response and flowering behavior (Hemming et al., 2009). Furthermore, Hemming et al., (2009) identified the regions in the first intron of *HvVRN1* that are related to the reduced expression of *HvVRN1* before vernalization. These regions, however, are not associated with cold induction of this gene. Expression of *HvVRN1* is also epigenetically regulated, and high expression levels of *HvVRN1* are maintained after vernalization (Oliver et al., 2009). Before vernalization, *HvVRN1* transcripts are repressed and this repression correlates with the presence of *H3K27me3* histone marks at the chromatin of *HvVRN1*. Cold exposure changes the chromatin state by decreasing the *H3K27me3* histone and increasing the *H3K4me3* levels at the *HvVRN1* locus, which is associated with high *HvVRN1* expression (Oliver et al., 2009). Increase in Hv*VRN1* downregulates *HvVRN2* and upregulates *HvFT1* expression in the presence of dominant *Ppd-H1* under LD conditions (Hemming et al., 2008). In contrast, spring barley does not have a vernalization requirement to flower because of the mutation within the CCT domain *HvVRN2* or a complete deletion of the gene (Yan et al., 2004; Dubcovsky et al., 2005). Moreover, deletions in the first intron of *HvVRN1* in the spring barley control the expression of *HvVRN1* independent of vernalization and a concomitant downregulation of *HvVRN2* (Tranquilli and Dubcovsky, 2000). While low-temperature signaling (vernalization) is well studied in barley, much less is known about the control of reproductive development in response to variation in ambient growth temperatures.

1.2 High Ambient Temperature Mediated Plant Responses

1.2.1 High Ambient versus Stress Temperatures

In this section, I focus on plant responses to high ambient temperatures, which usually ranges between 20-30°C. High ambient temperatures responses are different from heat stress responses which are studied at temperatures >35°C (Balasubramanian et al., 2006; Saidi et al., 2011). A slight increase in temperature or high ambient temperature can have significant effects on flowering time of plants (Fitter and Fitter, 2002; Wigge, 2013). Temperature responses are species/genotype specific and depend on developmental phase and organs of the plant (Porter and Gawith, 1999). Genotypes from thermally variable habitats have a higher ability to maintain their performance under extreme conditions (Berry and Bjorkman, 1980).

1.2.2 Effects of Ambient Temperature on Photosynthesis

The photosynthetic apparatus of plants is sensitive to changes in temperature, and its sensitivity varies among genotypes and species because of their inherent features and complex interactions with the prevailing environment (Berry and Bjorkman, 1980; Quinn, P. and Williams, 1985). Various studies have suggested that inhibition of net photosynthesis (Pn) at temperatures <40°C is a rapidly reversible process compared to its inhibition at the lethal temperatures (>40°C) (Weis, 1981; Sharkey et al., 2001; Salvucci et al., 2004). Irreversible photosynthesis inhibition at temperatures >40°C is the results of damage in thylakoid membranes and subsequent damage to photosystem II (PSII) (Berry and Bjorkman, 1980; Seemann et al., 1984; Bilger et al., 1987). In reversible photosynthesis inhibition response, limiting factors include photochemistry, enzymes of carbon assimilation, photorespiration, and utilization of photosynthates (Berry and Bjorkman, 1980; Kobza and Edwards, 1987). In wheat, a study on the impact of different leaf-temperatures (15-45°C) on photosynthetic carbon metabolism suggested that the photosynthesis rate under high temperatures in C3 plants depends on the activity of Rubisco (Kobza and Edwards, 1987). Various studies in Arabidopsis also confirmed that a decrease in net photosynthesis (Pn) is linked to the inactivation of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) due to the inhibition of Rubisco activase A (Kim and Portis, 2005). Rubisco activase (RCA) regulates the activity of Rubisco in the light reaction of photosynthesis in plants, which is a temperature sensitive process (Salvucci et al., 1985). In many plants, RCA is composed of two isoforms of different sizes (short, RCAβ and long RCAα) which are produced via alternative splicing of pre mRNA (Salvucci et al., 1985; Werneke et al., 1989; To et al., 1999; Zhang et al., 2002).

The long form, RCAα in cotton, maize, and wheat (*Triticum aestivum*) has significant thermostable properties that can work better under heat shock conditions (Law and Crafts-Brandner, 2001; Salvucci et al., 2003; Ayala-Ochoa et al., 2004). In the Syrian barley cultivars, high temperature (35°C) caused a reduction in the quantum efficiency of photosystem II (ΦPSII) and the net photosynthesis (Pn) (Rollins et al. 2013). Leaf proteomic analysis also revealed that heat treatment increased the expression of Rubisco activase B while downregulated the Rubisco activase A (Rollins et al., 2013). These studies suggest that RCA is one of the major factors that control the photosynthesis under high ambient temperatures and is one of the potential targets for crop improvement (Kurek et al., 2007). Most of the photosynthesis studies in wheat, barley, and other crop species accounted for moderate and stress temperatures. Recently, high ambient temperatures have received much focus of research compared to the stress temperatures due to the gradual increase in global temperature. In Arabidopsis, high temperature (27°C) reduces Pn under SDs (10/14h, day/night) in wild-type plants, whereas *rca* mutants, having a thermostable chimeric Rubisco activase (from tobacco), show an increase in photosynthesis rate at high temperature (Kumar et al., 2009). However, another study shows that net photosynthesis (Pn) remains constant at temperatures ranging from 25-30°C in 12/12h (day/night) photoperiod conditions in Arabidopsis (Kim and Portis, 2005). It has been suggested that the temperature dependence of photosynthesis is strongly influenced by light which either has a direct effect on photosynthetic properties or the indirect impact of photorespiration by affecting stomatal conductance (Berry and Bjorkman, 1980). These differential temperature responses to photosynthesis in the above-mentioned studies might be due to the different photoperiod conditions used. While thermostable RCA slightly improved photosynthesis at high ambient temperature, the effects on seed set were substantial compared to the photosynthesis effect (Kumar et al., 2009). The authors suggest that it could be that dealing with heat sensitive activase, plant delays other aspects of development. In barley, optimum photosynthesis temperature ranges between 15 and 20°C and above which photosynthesis decreases up to 35% at 30°C (Todd, 1982; Rollins et al., 2013). Increase in ambient growth temperature has shown an accelerated movement of C^{14} labeled photosynthates from the leaves to the spike in wheat (Wardlaw et al., 1980). The authors suggest that the increased carbon demand for reproductive organs is due to the carbon loss through respiration at high temperature. Despite the increased carbon supply, carbon loss due to the excessive respiration resulted in 25% reduction in dry seed weight (Wardlaw et al., 1980).

Dodd et al. (2005) have demonstrated that Arabidopsis plants with no defects in the circadian clock component fix more carbon, have high stomatal conductance and chlorophyll contents

and thereby, obtain an advantage over the circadian clock mutants. In barley, high ambient temperatures (28°C) reduced net photosynthesis in SDs and increased photosynthesis rates in LDs under controlled conditions (Ejaz, unpublished data, not shown). Moreover, Pn was strongly induced when plants were unexpectedly shifted to the high temperature for a short period in LDs (Ejaz, unpublished data, not shown). Interestingly, these results also demonstrated that Pn remained unaltered in the barley clock mutants BW290 (Hv*elf3*) and BW284 (Hv*lux1*) in SD and LD which indicates that Hv*ELF3* and Hv*LUX1* affect net photosynthesis (Pn) at high temperature in barley (Ejaz, unpublished data, not shown). An increase in the net photosynthesis (Pn), under long days and under unexpected temperature shifts could be due to an increased enzymatic activity under high ambient temperature. The above results suggest that photosynthesis remained unaffected at high temperature in barley circadian clock mutants BW290 (Hv*elf3*) and BW284 (Hv*lux1*) under SD and LD conditions. The insensitivity to high temperature suggests that these barley clock mutants might have a higher photosynthetic acclimation potential. Photosynthetic acclimation potential is defined as the genetic ability of a genotype to improve its performance under new growth regime which can be acquired by environmentally induced changes in the photosynthetic characteristics of a plant (Berry and Bjorkman, 1980).

1.2.3 Plant Architectural Changes in Response to High Temperatures

Plant survival and productivity are also influenced by the shoot architecture (Lee et al., 2014). Therefore, understanding architectural changes in response to high ambient temperatures is crucial. The phenotypic adaptations to high-temperature help plants to protect their photosynthetic tissues from the stress (Casal, 2012). Recently, studies in Arabidopsis have demonstrated that exposure of plants to high ambient temperature results in dramatic changes in growth and development. Phenotypic responses to escape from high temperature include a rapid extension of the plant axes, leaf hyponasty and early flowering (Koini et al., 2009). In Arabidopsis, high temperature accelerates flowering in SD independent of the photoperiod pathway (Balasubramanian et al., 2006). High temperature accelerates stem elongation and promotes leaf elevation from the soil surface, which facilitates the cooling of leaves (Crawford et al., 2012). These authors also demonstrate that Arabidopsis plants, which display elongated stems and leaves, increase the transpiration in well-watered conditions. The authors suggested that these architectural changes result in diffusion of water vapors from stomata, thus aid in the cooling process. The capacity of leaf cooling has been positively associated with fruiting efficiency and plant fitness (Radin et al., 1994). However, under water shortage, there is a trade-off between leaf cooling and the possibly harmful effects of

excessive water loss. In addition to exhibiting elongated stems, Arabidopsis plants grown at high temperature produced a reduced number of leaves compared to the plants grown at normal temperatures. Also under well-watered conditions, the stomatal size and densities were reduced at high temperatures suggesting that adaptations to high temperature may promote water conservation (Crawford et al., 2012). In Arabidopsis, high temperature mediated architectural modifications suggest that leaf cooling over water conservation has been selected during evolution (Crawford et al., 2012). Since ambient temperature and water availability are strongly interconnected, understanding shoot architecture and water use is very crucial in barley and other cereals to maximize yield.

1.2.4 High Ambient Temperature Directed Molecular Changes

Molecular aspect of adaptation involves changes in gene expression in response to fluctuating growth condition, the phenomenon that plants also demonstrate for different stress conditions. Transcription factors (TFs) are the critical nodes between signal transduction and activation of specific proteins against a particular environmental stimulus, during a particular developmental phase or tissues of the plant (Riechmann et al., 2000). TF are sequence-specific DNA binding proteins that bind at specific sites of the promoter regions (cis-acting) or regulate the expression of downstream target genes (trans-acting). TFs from the circadian clock and other flowering pathways have been implicated in ambient temperature response. Multiple molecular mechanisms, such as stability of regulatory proteins and temperature dependent alternative splicing of transcription factors involved in reproductive development have been suggested to regulate the flowering response to high ambient temperature in Arabidopsis (reviewed in Capovilla et al., 2014). In the following section, I discuss two newly identified major molecular pathways involved in ambient temperature flowering response in Arabidopsis.

a) Circadian Clock: A Regulator of Ambient Temperature Control of Flowering

The circadian clock plays a vital role to regulate physiological processes in plants facing abiotic stresses and regulates seasonal flowering and fruit set (Sanchez et al., 2011; Grundy et al., 2015). Daily changes in photic and thermal response play a significant role in the clock entrainment (Boikoglou et al., 2011). Clock controlled pathways direct many developmental processes in response to changes in light and temperature. In Arabidopsis, *EARLY FLOWERING 3 (ELF3)* which is a repressor of light signals to the circadian clock also functions as a fundamental element of ambient temperature response (Thines and Harmon,

2010). In diurnal light/dark cycles, high ambient temperatures impede the transcription of *ELF3*, thereby relieving the expression of the bHLB transcription factor, *PHYTOCHROME INTERACTING FACTOR 4 (PIF4)*, at night (Box et al., 2014). PIF4, a protein involved in light and temperature signaling, activates *FT*, a major floral integrator, by direct binding at the promoter regions of the gene (Kumar et al., 2012). The *FT* protein is an essential regulator of the flowering pathway that activates downstream floral meristem identity genes and thereby accelerates flowering (Turck et al., 2008). High temperature increases PIF4 binding, at the target promoters, to five folds compared to normal temperatures (Kumar et al., 2012). The increased binding ability of PIFs at high temperature works at the level of chromatin accessibility at the promoters of target genes. H2A.Z nucleosome occupancy at PIF binding sites is high at cool temperatures compared to high temperature suggesting that H2A.Z nucleosomes mediate temperature signals in Arabidopsis (Kumar and Wigge, 2010). Collectively, clock controlled floral induction at high temperatures is mediated by an *ELF3/PIF4* pathway and the transcriptional activation of the genes in this pathway is, at least in part, mediated by H2A.Z nucleosome thermosensing in Arabidopsis.

b) The Role of MADS-box Transcription Factors in the Thermosensory Flowering Pathway

The function of *MADS*-box TFs in the control of flower and fruit development has been extensively studied in plants. *MADS*-box genes regulate a variety of functions in plants such as flowering time, meristem and organ identity and also fruit and leaf development (Theißen and Saedler, 2001; Becker and Theißen, 2003; Theißen et al., 2016). Among a plethora of *MADS*-box genes involved in reproductive development, most of them function as floral inducers. However, *FLOWERING LOCUS C (FLC)*, a *MADS* domain transcription factor, is a major flowering repressor in Arabidopsis (Choi et al., 2011). *FLC* expression is induced by *FRIGIDA (FRI)*, and at low temperatures (vernalization). *FLC* expression is epigenetically silenced by a set of genes, which belong to the autonomous pathway, e.g., *FCA*, *FPA* (RNA binding proteins) and *FLD (FLOWERING LOCUS D)* (Michaels and Amasino, 2001). However, when FLC is expressed, its protein interacts with SHORT VEGETATIVE PHASE (SVP) and a flowering promoter SUPPRESSOR OF CONSTANS 1 (SOC1) (Searle et al. 2006). The *MADS*-box transcription factor *SVP* is also a negative regulator of flowering and expressed during vegetative development in Arabidopsis (Hartmann et al., 2000). SVP represses *SOC1* expression in the shoot apex and partly modulates *FT* expression in leaves (Li et al., 2008). *FLC* has five homologs in Arabidopsis including *FLM* (*FLOWERING LOCUS M*) and *MAF* 2-5 (*MADS AFFECTING FLOWERING 2-5). FLC* and *FLM* both act as

flowering repressors (Scortecci et al., 2001; Ratcliffe et al., 2003). Among these, *FLM* plays a significant role in ambient temperature regulation of flowering and is characterized by temperature dependent alternative splicing (Balasubramanian et al., 2006). Similar to *FLC*, *FLM* interacts with SVP to repress the expression of *FT* under high ambient temperatures (Lee et al., 2007; Lee et al., 2013). In Arabidopsis, *FLM* transcribes two splice forms *FLMβ* and *FLMδ* (Posé et al., 2013). These transcripts integrate exons at the second or third position, respectively, which translate into a *MICK* domain involved in protein-protein interaction (Jiao and Meyerowitz, 2010; Posé et al., 2013). The *FLMβ* splice form is present at low temperatures whereas *FLMδ* is more predominant at high temperature (Lee et al., 2013; Posé et al., 2013). These *FLM* splice variants form a complex with SVP that bind to DNA as a repressor in a temperature-dependent manner. The *FLMβ*-SVP complex acts as a floral repressor at low temperature. In contrast, at high temperature, splice variant *FLMδ* competes with *FLMβ* to form a complex with SVP and thus acts as a dominant-negative regulator of flowering (Lee et al., 2013; Posé et al., 2013). Moreover, the vernalization gene *FLC* also modulates ambient temperature responses in Arabidopsis. *flc-3* mutants show temperature insensitivity in the range of 23-27°C (Lee et al., 2013). However, expression of *FLC* is downregulated at the transcriptional level at 23°C as compared to 16°C (Blázquez et al., 2003). Downregulation of *FLC* expression results in the upregulation of floral integrators *FT, SOC1, TSF* (Lee et al., 2013). Also, *FLC* directly interacts with SVP, *FT*, and *SOC1* suggesting the role of *FLC* in flowering at high temperatures. Although these studies demonstrate the role of vernalization pathway genes *FLM* and *FLC* in control of flowering under high temperature, a major thermosensory pathway through which these genes are recruited is yet to be identified.

1.3 Barley is A Model Plant in Grasses to Understand Plant Developmental Responses under Changing Climate Scenarios

Barley is cultivated in diverse eco-geographic areas, ranging from North Europe to semi-arid areas (von Bothmer, 1995). Barley demonstrates higher yields under non-optimal growth conditions and low-input farming, compared to wheat (Visioni et al., 2013; Gürel et al., 2016; Shen et al., 2016). In many crop-growing areas, several abiotic factors such as temperature, drought and soil nutrition affect crop yield (Dolferus et al., 2011). Barley has good adaptability to adverse climatic conditions indicative of its large deposit of genes for adaptation and tolerance to such climates (Cattivelli et al., 2011). Moreover, barley is a self-pollinated and a diploid species, which makes genetic studies easy to perform compared to hexaploid wheat and the cross-pollinated species maize. Besides, diverse genetic stocks are available to study natural genetic variation in barley (Franckowiak and Lundqvist, 2012). Furthermore, extended collinearity and conserved gene contents with other members of Triticeae also make it an excellent model for grass species (Hayes, P.M., A. Castro, L. Marquez-Cedillo, A. Corey, C. Henson, B.L. Jones, J. Kling, D. Mather, I. Matus, C. Rossi, 2003). However, genetic studies regarding high ambient temperature are insufficient in barley. In the next section, I have summarized a few quantitative trait loci (QTL) case studies in wheat and barley that reflects the value of relating precise and appropriate phenotyping with genetic studies can help breeding crops resilient to high ambient temperatures.

1.4 Targeted Breeding for High Ambient Temperatures Adaptation

Crop performance is a consequence of interactions between a number of genes and environment and the management practices (Collins and Tuberosa, 2008). The conventional breeding approach involves recombining alleles from within elite genotypes and selecting among a large number of plants for expression of the desired trait that have resulted in gradual yield gains (Braun et al., 2010). However, these breeding approaches alone seem to be insufficient in the present climate change scenarios, which mounts pressure to improve cop performance at a rapid pace. Subsequently, genetic dissection of qualitative traits controlling adaptive responses of crops to environments is a prerequisite to enhance crop performance under global warming (Collins and Tuberosa, 2008). Breeding efforts for high ambient

temperature adaptation have long been hindered in crops because the underlying molecular mechanisms are largely unknown. Therefore, the principal breeding strategy involves systematic deployment of yield specific genes, which is possible in crops meeting the following criteria; availability of fully sequenced genomes to identify functional polymorphism and the knowledge of how genes interact to determine the expression of complex traits in a targeted environment (Reynolds et al., 2012). Robertson, (1985) postulated that quantitative trait loci (QTLs) or quantitative alleles contain polymorphism of minor nature at the genic level, which results in gene products of different efficiencies while qualitative alleles possess major genetic rearrangements or changes in the regions that affects the normal functioning of the gene. Another aspect to consider for breeding for targeted environment is the identification of adaptive QTLs where the expression of QTL is dependent on the temperature intensity (Vargas et al., 2006).

Wild barley provides a valuable genetic source for improving quantitative tolerance to high temperatures. A collection of 326 barley genotypes comprising of 320 wild barley accessions and 6 local cultivars from 74 countries were evaluated for genetic diversity in various phenotypic traits under high ambient temperature in barley (Abou-Elwafa and Amein, 2016). Based on phenotypic traits such as; days to flowering, number of tillers per plant, plant height, chlorophyll content, spike length, thousand kernel weight, and single plant yield, clustering analysis divided the genotypes into two distinct groups; genotypes adapted to cold environments and high temperature tolerant genotypes demonstrating varying degree of tolerance to high temperatures within the group. The authors have suggested the importance of these findings for further association mapping study that would reveal QTLs related to high ambient temperature in barley (Abou-Elwafa and Amein, 2016).

In wheat, unique QTLs have been mapped on chromosome 2A that controlled grain yield and its components such as grain weight/spike, grain number/spike, thousand grain weight, grain filling rate and duration under heat stress (Bhusal et al., 2017). This QTL study was performed based on the field data from two years and the maximum increase in temperature around flowering was 4.2°C and 0.9°C respectively (Bhusal et al., 2017). The identified QTLs in this study needs to be validated under controlled conditions (Bhusal et al., 2017).

In rice, Shanmugavadivel et al. (2017) identified QTLs for grain yield and spike fertility under high ambient temperature. A mapping population previously developed from a cross between heat susceptible and tolerant cultivars was used in identifying QTLs under high temperatures. Five QTLs were identified on chromosomes 3, 5, 9 and 12 for stress tolerance and the susceptibility index of spikelet sterility and yield. The major QTLs identified for yield and spikelet susceptibly explained 21% and 16% of phenotypic variation, respectively. These

results provide high-density SNP (single nucleotide polymorphism) map on a relatively large RIL (Recombinant Inbred Line) population in controlled conditions that can be further fine mapped to identify the candidate genes conferring tolerance under high temperature in rice (Shanmugavadivel et al., 2017).

High temperature often causes premature foliar senescence during and after the reproductive development in cereals that results in poor grain quality and yield losses (Vijayalakshmi et al., 2010; Lobell and Gourdji, 2012). Grain yield in cereals that are high-carbon crops, deciphers the source-sink association with canopy carbon capture and nitrogen remobilization (Thomas and Ougham, 2014). Senescence in plants is triggered by chlorophyll catabolism resulting the diversion of nutrients from leaves to the reproductive organs (Molisch, 1928). Under abiotic stresses, stay green, a delayed foliar senescence, shows a positive correlation with yield in many plant species (Guo and Gan, 2012; Thomas and Ougham, 2014). Genetic studies have demonstrated that QTLs for temperature and drought responses coincides with the loci identified for leaf senescence (Ougham et al., 2007; Vijayalakshmi et al., 2010; Jordan et al., 2012; Emebiri, 2013). Moreover, the stay green trait has been selected as a linked phenotype with plant development and plant height in the modern wheat cultivars.

For instance, a stay green QTL maps on chromosome 2D, which is close to the major photoperiod response locus *PPD-D1* and the stature gene *Rht8 (Reduced height 8)* (Pestsova and Röder, 2002; Verma et al., 2004). In barley, 334 doubled haploid lines were exposed to terminal heat stress and used to correlate near infrared (NIR) spectral data to physiological and biochemical responses associated with the stay-green phenotype (Gous et al., 2015). In wheat, a QTL on the short arm of chromosome 3B which affects the stability of grain weight is also linked with the stability of chlorophyll ('stay-green') and shoot weight when plants were exposed to a brief heat shock (37°C/27°C day/night) in early grain filling stage (Shirdelmoghanloo et al., 2016). These results suggest that accelerated or a delayed maturation of the reproductive organs under high temperatures might correlate to the genetic control of senescence responses in the two organs. Similar to studies described above, barley plants were grown under high ambient temperature (28°C) also senesced early due to the chlorophyll loss during the treatment (personal observation). This observation needs to be confirmed which only requires two non-destructive chlorophyll measurements before and after exposure to high ambient temperature.

Overall, QTLs that affect flowering time often influence yield under abiotic stresses because of the duration and the intensity of the stresses that affect the timing and duration of the crop life cycle (Reynolds et al., 2008). So far, not much is known about QTL/genes linked to pre-anthesis development in response to high ambient temperature in barley.

1.5 Reproductive Development under High Ambient Temperatures and the Underlying Molecular Control in Grasses

1.5.1 Previous Physiological Studies Supporting the Effects of High Ambient Temperature on Reproductive Development

Climate models have predicted shifts in flowering time of different wheat genotypes due to an increase in seasonal ambient temperatures (Zheng et al., 2016). Effects of high temperature on flowering time have already been recorded about half a century ago in barley and wheat (Walster, 1920; Faris and Guitard, 1969; Tingle et al., 1970; see also Gol et al., 2017). The concept of photothermal time was developed to characterize the plant development under fluctuating environment especially photoperiod and temperature (Ellis et al., 1988; Roberts et al., 1988; Ellis et al., 1989). Photothermal time describes a genotype dependent linear relationship of the reciprocal of rate of development to the photoperiod and mean diurnal temperature (Ellis et al., 1988; Roberts et al., 1988; Ellis et al., 1989). This concept of photothermal time employs only between base and maximal temperature as well as between ceiling and critical photoperiod. Depending on the genotype, the ceiling and the critical photoperiod for barley as a long day plant were ≤10hd-1 and ≥13hd-1, respectively (Roberts et al., 1988). In addition, the critical photoperiod varies with temperature, suggesting a close interaction between photoperiod and temperature (Ellis et al., 1988). Optimal temperatures vary between species and genotypes and range, for example in wheat, between 17-23°C while temperatures beyond this range impair growth and development (Porter and Gawith, 1999). In barley, it was shown that high temperature (28°C) delayed reproductive development compared to cooler temperatures (20°C) (Ellis et al., 1988). The authors speculated that the delay in flowering at high temperature could be a de-vernalization response. Several studies have established the de-vernalization response in different crops species (Gregory, 1945; Gregory and Purvis, 1948; Purvis and Gregory, 1952; S. Tzay-Fa, 2000). The effects of vernalization are reversed when plants are immediately shifted to high ambient temperatures (usually 30°C) after a period of cold (Bernier et al., 1981; Michaels and Amasino, 2000). However, high temperatures also delayed flowering time in spring barley genotypes without vernalization treatment, which indicates that the delay in reproductive development is not a sole effect of de-vernalization as has been proposed by Ellis et al., (1988).

Rawson and Richards, (1993) investigated the effects of different temperatures (33.3/20 °C and 20/12 °C, day/night) on six isogenic lines of a wheat cultivar varying at the major photoperiod gene *PPD1* and vernalization genes *VRN*1, 2, 3 and 4. The authors showed that high temperature, in short days (9h light), delayed the floral transition but accelerated the late reproductive phase until flowering. Rawson and Zajac, (1993) examined the effects of high temperature (25/15°C and 17/7°C, day night), on two spring wheat varieties; early season Hartog and mid-season Late Hartog, under the photoperiods of 9h, 11h, 13h and 15 h, and seed vernalization of 0, 2 and 4 weeks. High temperature accelerated ear emergence in all the photoperiod and vernalization treatments, and the temperature effects were increased two folds in the early season cultivar. Unexpectedly, high temperature delayed time to ear emergence under short days and 4 weeks of seed vernalization treatment in Late Hartog by 2 weeks. Data presented in the above studies comes from experiments performed under controlled conditions. To examine the effects of temperature on flowering time in field conditions, Ruwali et al., (1988) grew seven wheat genotypes at three hot locations in India and scored days to anthesis. Three mean daily temperatures 21.5°C, 22.8°C and 25°C and 11.3h, 11.6h and 12h photoperiod conditions were set by choosing different sowing dates. An increase of 5-6°C temperature delayed the time to anthesis by about 10 days at 12h photoperiod. However, results of this study are not comparable because the variation in photoperiods is small and high temperature effects have not been analyzed under short days (Ruwali et al., 1988). Overall, these studies show that ambient temperature interact with photoperiod and affect flowering time in wheat.

1.5.2 Effects of Ambient Temperature on Developmental Timing

A renewed interest in the genetic and physiological basis of reproductive development in barley and wheat followed the recent findings that mild changes in the ambient temperatures delays or accelerates reproductive development and flowering. In an Australian winter barley cultivar, an increase in temperature from 15°C to 25°C accelerated flowering time in long days and delayed the early phase of reproductive development in short days (Hemming et al., 2012). Karsai et al., (2013) have examined the effects of different ambient temperatures (13°C, 16.5°C, 18°C and 23°C) on the duration of different phases of development in 168 barley genotypes of diverse origins and growth habits under controlled conditions. The authors identified six different developmental responses that were dependent on the growth habit. One cluster of winter barley genotypes accelerated the development whereas the development of one spring barley cluster was delayed at 23°C (Karsai et al., 2013).

Ejaz and von Korff, (2017) examined the effect of high ambient temperature on shoot apex development and flowering time under control (20°C/16°C) and high temperatures (28°C/ 24°C) in LDs (16 h light/ 8 h dark), in spring barleys and the derived introgression lines with winter and spring alleles at *Ppd-H1, HvVRN1* and a functional and non-functional hv*elf3* allele. High ambient temperatures accelerated or delayed reproductive development depending on the photoperiod response gene *Ppd-H1* and its upstream regulator *HvELF3* (Ejaz and von Korff, 2017). A natural mutation in *Ppd-H1* prevalent in spring barley delayed the late reproductive phase of shoot apex development and flowering time, while the wild-type *Ppd-H1* or a mutant *Hvelf3* allele accelerated late reproductive phase of development and flowering time (Ejaz and von Korff, 2017). High temperature also delayed shoot apex development in spring barley under SDs while the development of the introgression lines carrying wild-type *Ppd-H1* or a mutant *Hvelf3* allele was not affected by high temperatures under SDs (Ejaz, unpublished data). Furthermore, the authors showed that the effect of *Ppd-H1* on early reproductive development under high temperatures was dependent on vernalization response gene *HvVRN1*. Only in the background of a spring *HvVRN1* allele or after up-regulation of *Hvvrn1* by vernalization, the wild-type *Ppd-H1* allele accelerated early reproductive development under high ambient temperatures (Ejaz and von Korff, 2017). Our study demonstrates that *Ppd-H1* and *HvVrn1* interacted to control inflorescence development under high ambient temperatures and this interaction is mediated by *Ppd- H1*, which is functional under LDs, but not under SDs (Digel et al., 2015). To identify the interaction between high ambient temperature and vernalization responses (previously described as de-vernalization), shoot apex development in spring barley Scarlett and the derived introgression line carrying winter *Hvvrn1 (IL-Hvvrn1)* was scored at control and high temperatures after vernalizing plants for 4 weeks. As without vernalization, high temperature delayed shoot apex development in both genotypes, however, the high temperature mediated delay in development was not strong in *IL-Hvvrn1* compared to non-vernalized conditions. Shoot apex development was postponed by 4 days in the spring barley Scarlett and 7 days in *IL-Hvvrn1* under high compared to controlled temperature. These results indicated that even after vernalization variation at *Hvvrn1* affected development in response high temperature, suggesting that high temperature direct the developmental responses through the vernalization pathway (Ejaz., Unpublished data). Temperature response in wheat was also recently shown to be controlled by *VRN1* and *PPD1* (Kiss et al., 2017). The authors investigated the effects of high temperature on 19 wheat cultivars at two photoperiods (16h and 12h light) and three temperatures (11°C, 18°C and 25 °C). Phenotypic responses under these six environments placed the genotypes into two major groups based on allelic variation at *PPD1*. In genotypes

carrying the photoperiod insensitive allele, high temperatures (25°C) delayed the development under both LDs and SDs, though effects of temperature and day length were small. In photoperiod sensitive genotypes, however, high temperature strongly delayed development under SDs and to a lesser extent under LDs. Temperature effects were even more pronounced in *PPD1* sensitive genotypes that required vernalization. Consequently, temperature effects were much stronger in genotypes with a weak *PPD1* allele and a strong vernalization requirement (Kiss et al., 2017). Overall, these studies demonstrate that high temperature responses are dependent on day length and vernalization requirement in wheat and barley, which are essentially determined by photoperiod sensitivity and vernalization genes (Karsai et al., 2013; Ejaz and von Korff, 2017; Kiss et al., 2017). Moreover, high temperature primarily delays the onset of the stem elongation phase independent of vernalization requirement in barley and wheat (Ejaz and von Korff, 2017; Kiss et al., 2017).

1.5.3 Early Stage of Development – Induction of Spikelet Primordia

Seed or grain number per spike is an essential trait in cereals that contributes to final grain yield (Dolferus et al., 2011). Different studies in wheat have demonstrated that grain number can be affected by increases in ambient temperature throughout the floral transition to anthesis (Slafer and Rawson, 1994). Aspinall, (1969) have investigated the effects of high temperature (30°C) on induction of spikelet primordia and showed that high-temperature delays spikelet initiation in barley, which is influenced by day length and light intensity (also reviewed in Gol et al., 2017) . In wheat, high temperatures of >19°C decrease the number of spikelet primordia and delay the initiation of terminal spikelet (Slafer and Rawson, 1994). High temperature (33.3/20 °C and 20/12 °C, day/night) accelerated the double ridge formation in long days and decreased the initiation of spikelet primordia in six wheat cultivars varying for photoperiod and vernalization genes (Rawson and Richards, 1993). Similarly, high temperature reduced the spikelet primordia in two early and late season spring wheats and temperature effects were increased by short photoperiods (9h) and a seed vernalization treatment (4 weeks) (Rawson and Zajac, 1993). The authors suggest that high temperature decreased the overall rate of development and the final number of organs (spikelets). However, in general, a delayed spikelet initiation at control temperature accelerates the rate of induction of spikelet primordia which depends on photoperiod and vernalization (Evans, 1960; Rawson, 1970; Rawson, 1971). Therefore, Rawson and Zajac, (1993) suggested that carbon availability strongly influences that number of primordia that needs to be considered at high temperature. This follows the hypothesis that during the vegetative phase the shoot apex accumulates a stockpile of leaf primordia, which after spikelet initiation develops into spikelet

primordia (Evans, 1960; Rawson, 1970; Rawson, 1971). However, as the duration for leaf primordia to become a fully expanded leaf increases, the rate of stacking of primordia declines (Evans, 1960; Rawson, 1970; Rawson, 1971). Therefore, an increase in the duration of the vegetative phase under high temperature would not favor the induction of spikelet primordia but could reduce the number of queued primordia because of carbon shortage (Rawson and Zajac, 1993). Thus, the effects of high temperature on induction of spikelet primordia are not only influenced by the developmental timing, but also the nutrient status at the shoot apex. In contrast, high temperature (28/24°C) did not affect the spikelet initiations and the number of spikelets produced at the end of early reproductive phase in the spring barley genotypes Bowman and Scarlett under LDs (Ejaz, unpublished data). However, spikelet initiation did not occur under high ambient temperatures of 28/24°C in a spring genotype with an introgression of a winter *Hvvrn1* allele (Ejaz and von Korff, 2017). Altogether, the effects of high ambient temperature on spikelet induction and grain number and interactions with photoperiod and vernalization needs to be further investigated.

1.5.4 Fertility, Seed Set-Microspore, Carpel Development

Grain number is determined by the number of spikelets established on a spike and the number of grains developed inside the florets. The late reproductive phase of pre-anthesis is the most sensitive phase that affects floral organ development inside the florets (Satake and Yoshida, 1978; Miralles and Richards, 2000; González et al., 2003; Slafer, 2003; Reynolds et al., 2009; Sreenivasulu and Schnurbusch, 2012). In addition to flowering response, high temperature reduced floret and seed number per spike in spring barley genotypes (Ejaz and von Korff, 2017). High ambient temperatures caused a larger reduction in seed number (34-74%) in spring barleys than in the ILs carrying a nonfunctional *Hvelf3* and a dominant *Ppd-H1*. Quantitative variation in the reduction of seed number under high ambient temperatures was dependent on *HvELF3* and *Ppd-H1* (Ejaz and von Korff, 2017). High ambient temperatures affected floral fertility and seed set by damaging pistils and stamens in cereals such as barley, wheat, rice, sorghum (Saini and Aspinall, 1982; Saini et al., 1983; Abiko et al., 2005; Jagadish et al., 2007; Jain et al., 2007). In rice, high ambient temperature affects the patterns of flowering and the number of spikelets that reach anthesis. Jagadish et al., (2007) examined the effects of episodes of high temperature on spikelet fertility at a high temperatures of >33.7 °C during anthesis and demonstrated that high-temperature episodes of <1h are sufficient to cause sterility in rice. Increases in temperature negatively affected panicle extrusion, flowering time and pollen viability in rice (Das et al., 2014). Effects of high temperatures on pollen viability were much stronger in low land compared to upland rice cultivars suggesting

that there is genetic variation for fertility under high ambient temperatures in rice (Rang et al., 2011; Das et al., 2014). In wheat, high temperatures (30°C) caused, for example, an abnormal embryo sac development or its complete absence as compared to the control temperature (20°C). In addition, the high temperature applied at the onset of meiosis in the anthers affected anther development and dehiscence (Saini and Aspinall, 1982). The early phase of anther development is sensitive to changes in temperature. In wheat, at the onset of meiosis, a 10°C increase in temperature for three days resulted in abnormal microsporogenesis resulting in a mixture of pollen of different abnormalities (Saini et al., 1984). During meiosis, high temperature causes premature tapetal degeneration. Pollen mother cells (PMC) could complete meiosis, but microspores failed to undergo pollen grain mitosis 1 (PMG1) phase. Therefore, abnormal microspores developed exine but lack cytoplasm (Saini et al., 1984). Besides, some microspores complete the PGM1 phase and develop into normal pollen but could not complete pollen grain mitosis 2 (PGM2) and did not accumulate starch (Saini et al., 1984). In barley, high-temperature causes an advancement of meiosis of pollen mother cells (PMC) and inhibits cell proliferation of anther walls cells (Sakata et al., 2000; Abiko et al., 2005; Oshino et al., 2011). Therefore, anther length at high temperature is reduced two folds compared to the plants grown under control temperature (Sakata et al., 2010).

In controlled conditions, barley plants demonstrate three developmental responses at high temperature which reduce overall fertility in barley (Sakata et al., 2000). Exposure to high temperature at the primary microsporogenesis stage (which starts at an early stage of panicle differentiation) results in empty pollen grains without cytoplasm that is similar to the first phenotype described by Saini et al. (1984) in wheat. The high temperature at meiosis of PMC results in a complete lack of pollen grains resulting in empty anthers. The effect of high temperature on mitosis result in abnormal and immature microspores that is similar to the second phenotypes described above in wheat (Abiko et al., 2005). Among all the developmental stages, microsporogenesis and meiosis were the most sensitive stages and were severely affected by high temperatures in rice (Satake and Yoshida, 1978). In addition, the decrease in viable pollen grains has been suggested as the major cause of high temperature-induced sterility in rice while pistils remain receptive to be fertilized up to a temperature of 41°C (Das et al., 2014). High temperature affects pollen viability by reducing the duration of meiotic stages and pollen maturation time in rye and wheat (Bennett et al., 1972). However, the underlying molecular and physiological mechanisms triggering high ambient temperature induced sterility have not been thoroughly investigated.

1.5.5 Phytohormones

High ambient temperature causes premature pollen abortion and affects the expression levels of genes involved in cell developmental processes in developing anthers in barley. It has been shown that high-temperature induced pollen abortion is regulated by the auxin pathway in both Arabidopsis and barley (Sakata et al., 2000; Oshino et al., 2007; Sakata et al., 2010). High temperatures reduced the endogenous auxin levels in developing anthers, which correlated with reduced tissue-specific expression of auxin biosynthesis YUCCA genes in barley and Arabidopsis. High-temperature induced male sterility was reversed by exogenous auxin applications (Sakata et al., 2010; Oshino et al., 2011). These results suggest that auxin biosynthesis genes direct high temperature responses that results in the decrease in auxin biosynthesis, which is the limiting factor for pollen viability in barley. Growth promoting phytohormone gibberellins (GA) are essential for the pollen development in many crops species (Blázquez et al., 1998). Low ambient temperatures (8°C decrease from control temperatures) altered the endogenous bioactive GA levels and the expression of GA biosynthetic genes such as *GAox3* (*GA oxidase 3*) and *GA3ox1* (*GA3 oxidase 1*) in the leaves in rice (Sakata et al., 2014). Moreover, GA reversed the male sterility induced by low ambient temperatures in rice (Sakata et al., 2014). Altogether, these findings suggest that high temperatures control fertility by altering hormone homeostasis in grasses, while the underlying molecular mechanisms need further studies.

1.5.6 Chromatin and Transcriptional Changes

It has been shown that chromatin accessibility is altered under high temperatures in *Brachypodium distachyon* (Boden et al., 2013). High temperature decreased H2A.Z nucleosome occupancy at the grain filling stage and this correlated with reduced grain size and yield. In contrast to Arabidopsis, high temperature does not affect nucleosome occupancy during the vegetative phase in Brachypodium (Boden et al., 2013). The authors argue that the differences between developmental stages could be specific to Brachypodium. However, nucleosome sensitivity to increased temperature depends on the developmental stage of the plant (Abiko et al., 2005). One explanation for these stage-specific distinct responses is the adaptation to low temperatures during early development and high temperature during the reproductive phase (Angus et al., 1981; Slafer and Savin, 1991). To unravel the connections between the H2A.Z nucleosome and the reproductive development in response to high temperature, it is essential to identify the genes controlling the binding of the nucleosome to their promoters (Boden et al., 2013). An important question that remained unanswered in this study is to identify the mechanism underlying the variable response of H2A.Z-nucleosomes to

high ambient temperatures at the vegetative and reproductive stages. H2A.Z is present in the proximity of transcription start sites (TSS) of both active and inactive promoters of the genes. However, acetylated H2A.Z is only present at the TSS of active genes in humans (Valdés-Mora et al., 2012). Acetylated H2A.Z correlates with gene activity at genome level in yeast (Millar et al., 2006) and plays a role in nucleosome destabilization and an open conformation of chromatin (Ishibashi et al., 2009). Therefore, Boden et al., (2013) argue that the degree of acetylation of H2A.Z might have a role in tissue-specific temperature responses. Moreover, these authors also observed high-temperature dependent induction of gene expression in vegetative seedlings where the high temperature does not affect H2A.Z-nucleosomes occupancy. These findings are similar to Arabidopsis that H2A.Z-nucleosomes do not explain all the transcriptional responses to high ambient temperature (Kumar and Wigge, 2010). The identification of comparable temperature responsive pathways in other grass species will be of interest.

High temperature mediated transcriptional inhibition in barley affects anther development and pollen viability (Abiko et al., 2005). They performed serial analysis of gene expression (SAGE) in barley anthers at different ambient temperatures (control 20°C /15°C and high 30°C/25°C). At control temperatures, various genes including histone H3, H4 and glycine-rich RNA-binding protein genes were upregulated before the development and differentiation of anther cell wall but not at high temperatures. Moreover, transcriptional reactivation of these genes was also inhibited when plants were shifted from high to control temperature after four days. However, high temperature induced the hyper-phosphorylation of ser-5 residue of the C-terminal domain (CTD) of the largest subunit of RNA polymerase II (RPB1) (Abiko et al., 2005). The hyper-phosphorylation response indicates the activation or suppression of CDK kinases and phosphatases that might lead to a general transcriptional inhibition and activation of stress-related genes in Arabidopsis (Koiwa et al., 2002; Koiwa et al., 2004). Moreover, phosphorylation levels of the C-terminal domain might play a role to determine the temperature sensitivity of specific tissues (Koiwa et al., 2002; Koiwa et al., 2004). Overall, these results demonstrate that early development and differentiation of barley anthers are susceptible to high ambient temperature and changes gene expression.

1.5.7 Sugar and Lipid Metabolism

In sorghum, a 6°C increase in ambient temperature affected the transcriptional activity of genes involved in sugar to starch metabolism and changed sucrose uptake in the microspores tested at different stages from pre-meiotic to the late mitotic phase (Jain et al., 2007). Microspores from high-temperature treatment accumulated less starch and exhibited poor in-

vitro germination. There was no sucrose detected under high temperature compared to control temperature, and starch levels were also reduced up to 50% under high temperature in sorghum microspores both at early and late stages of development (Jain et al., 2007). High temperature altered expression of the genes controlling sugar breakdown and use (*SbIncw1, SbIvr2, Sh1*, and *Sus1*), transport (*Mha*1 and *MST*1) and starch biosynthesis (*Bt2, SU1, GBSS1*, and *UGPase*) that was revealed in northern blot experiments. High temperature resulted in a substantial decrease in the steady-state transcript abundance of *SbIncw*1 gene and CELL WALL INVERTASE (CWI) proteins both in microspores and in sporophytic tissues of sorghum florets (Jain et al., 2007). Together, these data propose that high ambient temperature injuries impair the CWI-mediated sucrose hydrolysis and resulting in the lack of sucrose biosynthesis. The authors suggested that CWI-mediated sugar catalysis might be the upstream molecular dysfunction resulting in altered carbohydrate metabolism and starch deficits at high temperature. Expression CWI genes in the vegetative and the reproductive organs of maize have been reported (Kim et al., 2000). Also in this study, high-temperature induced expression of these genes in the style and stigma demands a focus in this direction in other grass species. Therefore, future high temperature studies must consider pollen receptivity by the stigma of the female reproductive tissues. However, caution should be taken that sorghum has acquired an efficient carbon assimilation system (C^4 plants) under high ambient temperatures as compared to the wheat or barley (C^3 plants) (Kresowich et al., 2005).

To maintain membrane integrity and fluidity, plants alter lipid composition of the membranes in response to changes in ambient temperatures (Larkindale and Huang, 2004; Chen et al., 2006; Zheng et al., 2011). These alterations include lipid peroxidation, increases in reactive oxygen species (ROS), and increases in Ox-lipids (oxidized acyl chains) that act as signaling molecules to transmit environmental perturbations (Andersson et al., 2006). Narayanan et al. (2016) analyzed the lipid profiles of the two different wheat genotypes based on the differences in the physiological responses to high temperature. High temperature altered lipid profiles in the leaves by lipid remodeling and reducing the unsaturation level of lipids suggestive of an adaptation response to temperature changes (Narayanan et al., 2016). (Zhang et al., 2016) have shown that a mitochondrial Lipase *EXTRA GLUME1 (EG1)*, a lipid metabolism gene coordinate floral robustness by transcriptional regulation under high temperature in rice. However, connections between metabolism and transcriptional regulation of flower development genes are mostly unknown in grasses.

1.5.8 Genetic Pathways Controlling Ambient Temperature Responses in Cereals

Photoperiod and vernalization response pathways have been implicated in mediating stress dependent signals to control development. However, little is known about the genetic components involved in temperature sensing and how ambient temperatures impact the genes involved in photoperiod and vernalization pathways in temperate cereals (Bullrich et al., 2002; Appendino and Slafer, 2003; Slafer, 2003; Lewis et al., 2008; Borràs-Gelonch et al., 2012; Hemming et al., 2012). In barley, for example, Hemming et al., (2012) have shown that an increase of temperature from 15 °C to 25 °C accelerates reproductive development in long days but does not affect the expression of genes involved in photoperiod pathway. They show that high temperature changed the expression of the genes previously identified in vernalization pathway. The expression of *HvODDSOC2*, a *MADS*-box repressor of elongation and flowering, was 8-fold increased at high temperature in short days in vernalized barley plants (Hemming et al., 2012). The expression of *MADS*-box genes is responsive to a broad range of temperatures (Hemming and Trevaskis, 2011). Similarly, expression levels of *HvODDSOC2* varied in the temperature range of 12-22°C in non-vernalized barley plants that suggest the effect of temperature on *HvODDSOC2* expression is independent of reproductive development (Hemming et al., 2012). However, the authors could not identify any effect of temperature changes on the expression levels of major vernalization response genes (Hv*VRN1, HvVRN2, HvVRN3 (HvFT1)*) and the photoperiod gene (*Ppd-H1*) in their experimental conditions. Although interactions between temperature and photoperiods affect reproductive development in wheat (Slafer and Rawson, 1996; Borràs-Gelonch et al., 2012), the genetic control of high-temperature responses have not been explored. Kiss et al. (2017) have analyzed gene expression of *PPD1* and *VRN1*, *VRN2* and *VRN3 (FT1)* under three ambient temperatures (11°C, 18°C and 25°C) and in both long and short days across the 11 wheat genotypes. Temperature variation altered the expression levels of *PPD1*, *VRN1* and *VRN2*, whereas *VRN3 (FT1)* expression correlated with the development (Kiss et al., 2017). Moreover, high temperature antagonistically influenced the expression levels of *VRN1* and *VRN2*. High temperature strongly downregulated the expression of *VRN1* and transiently increased the *VRN2* expression (Kiss et al., 2017). These authors propose a potential role for *VRN2* that affects reproductive development not only under vernalization but also under different ambient temperatures in wheat.

In barley, high ambient temperature delayed the expression phase and reduced the amplitude of clock genes and repressed the floral integrator gene *FLOWERING LOCUS T1* (Ejaz and von Korff, 2017). *Ppd-H1*-dependent variation in flowering time under different ambient temperatures correlated with relative expression levels of the barley *MADS*-box genes *VERNALIZATION1 (HvVRN1), HvBM3*, and *HvBM8* in the leaf (Ejaz and von Korff, 2017). The authors show that *Ppd-H1* interacts with regulatory variation at *HvVRN1*. *Ppd-H1* only accelerated floral development in the background of a spring *HvVRN1* allele with a deletion in the regulatory intron. The full-length winter *Hvvrn1* allele was strongly down regulated and flowering was delayed by high temperatures irrespective of *Ppd-H1* (Ejaz and von Korff, 2017). These authors establish that the photoperiodic and vernalization pathways interact to control flowering time and floret fertility in response to ambient temperature in barley.

1.6 Conclusions

Overall, these studies demonstrate that temperate cereals wheat and barley integrate high ambient temperature signals on the primary developmental cues of photoperiod and vernalization during the late reproductive phase of stem elongation. Moreover, high temperatures are not stress temperatures, act as environmental signals of reproductive development under inductive photoperiods, and reinforce their effect on genotypes of winter growth habits prior and after the cold periods in grasses. These findings imply that warm temperature episodes during winter and early spring would lead to the active growth (Kiss et al., 2017). These morpho-physiological responses of high ambient temperature affect floral fertility and yield by altering the duration of anther development. Moreover, high-temperature changes hormone homeostasis, sugar-starch metabolism and lipid composition in grasses. This chapter also highlights that more research is needed to investigate the underlying molecular control of these adaptive responses to high temperature in temperate cereals.

1.7 References

Abiko M, Akibayashi K, Sakata T, Kimura M, Kihara M, Itoh K, Asamizu E, Sato S, Takahashi H, Higashitani A (2005) High-temperature induction of male sterility during barley (Hordeum vulgare L.) anther development is mediated by transcriptional inhibition. Sex Plant Reprod **18**: 91–100

Abou-Elwafa SF, Amein KA (2016) Genetic Diversity and Potential High Temperature Tolerance in Barley (*Hordeum vulgare*). World J Agric Res **4**: 1–8

Alqudah AM, Schnurbusch T (2014) Awn primordium to tipping is the most decisive developmental phase for spikelet survival in barley. Funct Plant Biol **41**: 424

Andersson MX, Hamberg M, Kourtchenko O, Brunnström Å, McPhail KL, Gerwick WH, Göbel C, Feussner I, Ellerström M (2006) Oxylipin Profiling of the Hypersensitive Response in *Arabidopsis thaliana*. J Biol Chem **281**: 31528–31537

Angus JF, Mackenzie DH, Morton R, Schafer CA (1981) Phasic development in field crops II. Thermal and photoperiodic responses of spring wheat. F Crop Res **4**: 269–283

Appendino ML, Slafer GA (2003) Earliness per se and its dependence upon temperature in diploid wheat lines differing in the major gene Eps-A^m 1 alleles. J Agric Sci **141**: 149–154

Aspinall D (1969) The effects of day length and light intensity on the growth of barley. Interactions between the effects of temperature, photoperiod, and the spectral composition of the light source. Aust J biol Sci **22**: 53–67

Ausín I, Alonso-Blanco C, Martínez-Zapater J-M (2005) Environmental regulation of flowering. Int J Dev Biol **49**: 689–705

Ayala-Ochoa A, Vargas-Suárez M, Loza-Tavera H, León P, Jiménez-García LF, Sánchez-de-Jiménez E (2004) In maize, two distinct ribulose 1,5-bisphosphate carboxylase/ oxygenase activase transcripts have different day/night patterns of expression. Biochimie **86**: 439–449

Balasubramanian S, Sureshkumar S, Lempe J, Weigel D (2006) Potent induction of Arabidopsis thaliana flowering by elevated growth temperature. PLoS Genet **2**: e106

Barton MK (2010) Twenty years on: The inner workings of the shoot apical meristem, a developmental dynamo. Dev Biol **341**: 95–113

Becker A, Theißen G (2003) The major clades of MADS-box genes and their role in the development and evolution of flowering plants. Mol Phylogenet Evol **29**: 464–489

Benlloch R, Berbel A, Serrano-Mislata A, Madue F (2007) Floral Initiation and Inflorescence Architecture: A Comparative View. Ann Bot. doi: 10.1093/aob/mcm146

Bennett MD, Smith JB, Kemble R (1972) The effect of temperature on meiosis and pollen development in wheat and rye. Can J Genet Cytol **14**: 615–624

Bernier G, Kinet J-M, Sachs RM (1981) The physiology of flowering. CRC Press Boca Raton

Berry J, Bjorkman O (1980) Photosynthetic response and adaptation to temperature in

higher plants. Annu Rev Plant Physiol **31**: 491–543

Bhusal N, Kumar AS, Sharma P, Sareen S, Zhang A (2017) Mapping QTLs for grain yield components in wheat under heat stress. doi: 10.1371/journal.pone.0189594

Bilger W, Schreiber U, Lange OL (1987) Chlorophyll fluorescence as an indicator of heat induced limitation of photosynthesis in Arbutus unedo L. Plant Response to Stress. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 391–399

Blázquez MA, Ahn JH, Weigel D (2003) A thermosensory pathway controlling flowering time in Arabidopsis thaliana. Nat Genet **33**: 168–171

Blázquez MA, Green R, Nilsson O, Sussman MR, Weigel D (1998) Gibberellins Promote Flowering of Arabidopsis by Activating the LEAFY Promoter. Plant Cell **10**: 791–800

Boden SA, Kavanová M, Finnegan EJ, Wigge PA (2013) Thermal stress effects on grain yield in Brachypodium distachyon occur via H2A. Z-nucleosomes. Genome Biol **14**: R65

Boikoglou E, Ma Z, von Korff M, Davis AM, Nagy F, Davis SJ (2011) Environmental Memory from a Circadian Oscillator: The Arabidopsis thaliana Clock Differentially integrates Perception of Photic vs. Thermal Entrainment. Genetics **189**: 655–664

Bonnett OT (1935) The Development Of The Barley Spike. J. Agric. Res. **51**:451-457

Börner A, Buck-Sorlin GH, Hayes PM, Malyshev S, Korzun V (2002) Molecular mapping of major genes and quantitative trait loci determining flowering time in response to photoperiod in barley. Plant Breed **121**: 129–132

Borràs-Gelonch G, Denti M, B Thomas WT, Romagosa I (2012) Genetic control of pre-heading phases in the Steptoe × Morex barley population under different conditions of photoperiod and temperature. Euphytica **183**: 303–321

von Bothmer R. (1995) An ecogeographical study of the genus Hordeum. International Board for Plant Genetic Resources

von Bothmer R, Komatsuda T (2011) Barley Origin and Related Species. Barley Prod Improv Uses. doi: 10.1002/9780470958636.ch2

Box MSS, Huang BEE, Domijan M, Jaeger KEE, Khattak AKK, Yoo SJJ, Sedivy ELL, Jones DMM, Hearn TJJ, Webb AARAR, et al (2014) ELF3 Controls Thermoresponsive Growth in Arabidopsis. Curr Biol **25**: 1–6

Braun HJ, Atlin G, and Payne T (2010) Multi-location testing as a tool to identify plant response to global climate change. Climate change and crop production.

Briggs DE (1978) Barley. doi: DOl: 10.1007/978-94-009-5715-2

Brooking IR, Kirby EJM (1981) Interrelationships between stem and ear development in winter wheat: the effects of a Norin 10 dwarfing gene, Gai/Rht2. J Agric Sci **97**: 373-381

Bullrich L, Appendino M, Tranquilli G, Lewis S, Dubcovsky J (2002) Mapping of a thermo-sensitive earliness per se gene on Triticum monococcum chromosome $1A^m$. Theor Appl Genet **105**: 585–593

Campoli C, Drosse B, Searle I, Coupland G, Von Korff M (2012) Functional

characterisation of HvCO1, the barley (Hordeum vulgare) flowering time ortholog of CONSTANS. Plant J **69**: 868–880

Campoli C, Pankin A, Drosse B, Casao CM, Davis SJ, Von Korff M (2013) 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 Phytol **199**: 1045–1059

Capovilla G, Schmid M, Posé D (2014) Control of flowering by ambient temperature. J Exp Bot **66**: 59–69.

Casal JJ (2012) Shade Avoidance. The Arab Book.**e0157** doi: 10.1199/tab.0157

Cattivelli L, Ceccarelli S, Romagosa I, Stanca M (2011) Abiotic Stresses in Barley: Problems and Solutions. Barley Prod Improv Uses. Blackwell Publishing Ltd.pp 282–306. ISBN: 978-0-813-80123-0

Chen A, Li C, Hu W, Lau MY, Lin H, Rockwell NC, Martin SS, Jernstedt JA, Lagarias JC, Dubcovsky J (2014) PHYTOCHROME C plays a major role in the acceleration of wheat flowering under long-day photoperiod. Proc Natl Acad Sci **111**: 10037–10044

Chen J, Burke JJ, Xin Z, Xu C, Velten J (2006) Characterization of the Arabidopsis thermosensitive mutant atts02 reveals an important role for galactolipids in thermotolerance. Plant, Cell Environ **29**: 1437–1448

Choi K, Kim J, Hwang H-J, Kim S, Park C, Kim SY, Leea and I (2011) The FRIGIDA Complex Activates Transcription of FLC, a Strong Flowering Repressor in Arabidopsis, by Recruiting Chromatin Modification Factors. Plant Cell **11**: 445–458

Collins NC, Tuberosa R (2008) Editor's Choice Series on the Next Generation of Biotech Crops Quantitative Trait Loci and Crop Performance under Abiotic Stress: Where Do We Stand? Plant Physiol **147**: 469–486

Corbesier L, Vincent C, Jang S, Fornara F, Fan Q, Searle I, Giakountis A, Farrona S, Gissot L, Turnbull C (2007) FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. Science (80-) **316**: 1030–1033

Crawford AJ, McLachlan DH, Hetherington AM, Franklin and KA (2012) High temperature exposure increases plant cooling capacity. Curr Biol **22**: R396–R397

Das S, Krishnan P, Nayak M, Ramakrishnan B (2014) High temperature stress effects on pollens of rice (Oryza sativa L.) genotypes. Environ Exp Bot **101**: 36–46

Digel B, Pankin A, von Korff M (2015) Global Transcriptome Profiling of Developing Leaf and Shoot Apices Reveals Distinct Genetic and Environmental Control of Floral Transition and Inflorescence Development in Barley. Plant Cell **27**: 2318–2334

Digel B, Tavakol E, Verderio G, Tondelli A, Xu X, Cattivelli L, Rossini L, von Korff M (2016) Photoperiod-H1 (Ppd-H1) controls leaf size in barley. Plant Physiol DOI:10.1104/pp.16.00977

Dodd AN, Salathia N, Hall A, Kévei E, Tóth R, Nagy F, Hibberd JM, Millar AJ, Webb A a R (2005) Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. Science **309**: 630–633

Dolferus R, Ji X, Richards RA (2011) Abiotic stress and control of grain number in cereals. Plant Sci **181**: 331–341

Dubcovsky J, Chen C, Yan L (2005) Molecular characterization of the allelic variation at the VRN-H2 vernalization locus in barley. Mol Breed **15**: 395–407

E. S. Coen EMM (1991) The War of whorls: genetic interactions controlling flower development. Nature **353**: 31-37

Ejaz M, von Korff M (2017) The Genetic Control of Reproductive Development under High Ambient Temperature. Plant Physiol **173**: 294–306

Ellis RH, Roberts EH, Summerfield RJ, Cooper JP (1988) Environmental control of flowering in barley (Hordeum vulgare L.). II. Rate of developement as a function of temperature and photoperiod and its modification by low-temperature vernalization. Ann Bot **62**: 145–158

Ellis RH, Summerfield RJ, Roberts EH, Cooper JP (1989) Environmental Control of Flowering in Barley (Hordeum vulgare). III. Analysis of Potential Vernalization Responses, and Methods of Screening Germplasm for Sensitivity to Photoperiod and Temperature. Ann Bot **63**: 687–704

Emebiri LC (2013) QTL dissection of the loss of green colour during post-anthesis grain maturation in two-rowed barley. Theor Appl Genet **126**: 1873–1884

Evans LT (1960) The influence of environmental conditions on inflorescence development in some long-day grasses. New Phytol. **59**, 163-174

Faris DG, Guitard AA (1969) Yield and primary culm yield components of two spring barley cultivars as influenced by temperature, daylength and growth stage. Can J Plant Sci **49**: 701–713

Farré EM, Harmer SL, Harmon FG, Yanovsky MJ, Kay SA (2005) Overlapping and Distinct Roles of PRR7 and PRR9 in the Arabidopsis Circadian Clock. Curr Biol **15**: 47–54

Fischer RA (1985) Number of kernels in wheat crops and the influence of solar radiation and temperature. J agric Sci, Camb **105**: 447–461

Fitter AH, Fitter RSR (2002) Rapid changes in flowering time in British plants. Science (80-) **296**: 1689–1691

Forster BP, Franckowiak JD, Lundqvist U, Lyon J, Pitkethly I, Thomas WTB (2007) The Barley Phytomer. Ann Bot **100**: 725–723

Franckowiak JD, Lundqvist U (2012) Descriptions of Barley Genetic Stocks for 2012. Barley Genet Newsl **42**: 36–173

Gasser CS, Broadhvest J, Hauser BA (1998) Genetic analysis of ovule development. Annu Rev Plant Physiol Plant Mol Biol **49**: 1–24

Gol L, Tomé F, Von Korff M (2017) Floral transitions in wheat and barley: interactions between photoperiod, abiotic stresses, and nutrient status. J Exp Bot **68**: 1399–1410

González FG, Slafer GA, Miralles DJ (2002) Vernalization and photoperiod responses in wheat pre-flowering reproductive phases. F Crop Res **74**: 183–195

González FG, Slafer GA, Miralles DJ (2003) Grain and floret number in response to photoperiod during stem elongation in fully and slightly vernalized wheats. F Crop Res **81**: 17–27

Gous PW, Meder R, Fox GP (2015) Near infrared spectral assessment of stay- green barley genotypes under heat stress. J Near Infrared Spectrosc. doi: 10.1255/jnirs.1163

Gregory FG, Purvis ON (1948) Reversal of vernalization by high temperature. Nature **161**: 859-860

Gregory ONP and FG (1945) Devernalisation by high temperature. Nature 155(3926):113-114

Grundy J, Stoker C, Carré IA (2015) Circadian regulation of abiotic stress tolerance in plants. Front Plant Sci **6**: 648 doi.org/10.3389/fpls.2015.00648

Guo Y, Gan S-S (2012) Convergence and divergence in gene expression profiles induced by leaf senescence and 27 senescence-promoting hormonal, pathological and environmental stress treatments. Plant Cell Environ **35**: 644–655

Gürel F, Öztürk ZN, Uçarlı C, Rosellini D (2016) Barley Genes as Tools to Confer Abiotic Stress Tolerance in Crops. Front Plant Sci **7**: 1137

Habte E, Müller LM, Shtaya M, Davis SJ, Von Korff M (2014) Osmotic stress at the barley root affects expression of circadian clock genes in the shoot. Plant, Cell Environ **37**: 1321–1337

Hartmann U, Höhmann S, Nettesheim K, Wisman E, Saedler H, Huijser P (2000) Molecular cloning of SVP: a negative regulator of the floral transition in Arabidopsis. Plant J **21**: 351–60

Hayes, P.M., A. Castro, L. Marquez-Cedillo, A. Corey, C. Henson, B.L. Jones, J. Kling, D. Mather, I. Matus, C. Rossi and KS (2003) Diversity in barley : (Hordeum vulgare), In Bothmer van R., et al. (eds) Diversity in Barley. elsevier Amsterdam, pp 201- 226.

Hemming M, Trevaskis B (2011) Make hay when the sun shines: The role of MADS-box genes in temperature-dependant seasonal flowering responses. plant Sci **180**: 447–453

Hemming MN, Fieg S, Peacock WJ, Dennis ES, Trevaskis B (2009) Regions associated with repression of the barley (Hordeum vulgare) VERNALIZATION1 gene are not required for cold induction. Mol Genet Genomics **282**: 107–117

Hemming MN, Peacock WJ, Dennis ES, Trevaskis B (2008) Low-temperature and daylength cues are integrated to regulate FLOWERING LOCUS T in barley. Plant Physiol **147**: 355–366

Hemming MN, Walford S a., Fieg S, Dennis ES, Trevaskis B (2012) Identification of High-Temperature-Responsive Genes in Cereals. Plant Physiol **158**: 1439–1450

Herrero E, Davis SJ (2012) Time for a nuclear meeting: Protein trafficking and chromatin dynamics intersect in the plant circadian system. Mol Plant **5**: 554–565

Ishibashi T, Dryhurst D, Rose KL, Shabanowitz J, Hunt DF, Ausió J (2009) Acetylation of Vertebrate H2A.Z and Its Effect on the Structure of the Nucleosome. Biochemistry **48**: 5007–5017

Jagadish SVK, Craufurd PQ, Wheeler TR (2007) High temperature stress and spikelet fertility in rice (Oryza sativa L.). J Exp Bot **58**: 1627–1635

Jain M, Prasad PVV, Boote KJ, Hartwell Jr AL, Chourey PS (2007) Effects of season-long high temperature growth conditions on sugar-to-starch metabolism in developing microspores of grain sorghum (Sorghum bicolor L. Moench). Planta **227**: 67–79

Jiao Y, Meyerowitz EM (2010) Cell-type specific analysis of translating RNAs in developing flowers reveals new levels of control. Mol. Syst. Biol. 6: doi:10.1038/msb.2010.76

Jordan DR, Hunt CH, Cruickshank AW, Borrell AK, Henzell RG (2012) The Relationship Between the Stay-Green Trait and Grain Yield in Elite Sorghum Hybrids Grown in a Range of Environments. Crop Sci **52**: 1153

Kamioka M, Takao S, Suzuki T, Taki K, Higashiyama T, Kinoshita T, Nakamichi N (2016) Direct Repression of Evening Genes by CIRCADIAN CLOCK-ASSOCIATED1 in the Arabidopsis Circadian Clock. Plant Cell **28**: 696–711

Karsai I, Igartua E, Casas a. M, Kiss T, Soós V, Balla K, Bedő Z, Veisz O (2013) Developmental patterns of a large set of barley (Hordeum vulgare) cultivars in response to ambient temperature. Ann Appl Biol **162**: 309–323

Kellogg EA (2007) Floral displays: genetic control of grass inflorescences. Curr Opin Plant Biol **10**: 26–31

Kemp DR, Culvenor RA (1994) Improving the grazing and drought tolerance of temperate perennial grasses Improving the grazing and drought tolerance of temperate perennial grasses. New Zeal J Agric Res **37**: 365–378

Kim JY, Mahé A, Guy S, Brangeon J, Roche O, Chourey PS, Prioul JL (2000) Characterization of two members of the maize gene family, Incw3 and Incw4, encoding cell-wall invertases. Gene **245**: 89–102

Kim K, Portis AR (2005) Temperature dependence of photosynthesis in Arabidopsis plants with modifications in Rubisco activase and membrane fluidity. Plant Cell Physiol **46**: 522–530

Kim W-Y, Fujiwara S, Suh S-S, Kim J, Kim Y, Han L, David K, Putterill J, Nam HG, Somers DE (2007) ZEITLUPE is a circadian photoreceptor stabilized by GIGANTEA in blue light. Nature **449**: 356–360

Kiss T, Dixon LE, Soltész A, Bányai J, Mayer M, Balla K, Allard V, Galiba G, Slafer GA, Griffiths S, et al (2017) Effects of ambient temperature in association with photoperiod on phenology and on the expressions of major plant developmental genes in wheat (*Triticum aestivum* L.). Plant Cell Environ **40**: 1629–1642

Kobza J, Edwards GE (1987) Influences of leaf temperature on photosynthetic carbon metabolism in wheat. Plant Physiol **83**: 69–74

Koini M a., Alvey L, Allen T, Tilley C a., Harberd NP, Whitelam GC, Franklin K a. (2009) High Temperature-Mediated Adaptations in Plant Architecture Require the bHLH Transcription Factor PIF4. Curr Biol **19**: 408–413

Koiwa H, Barb AW, Xiong L, Li F, McCully MG, Lee B -h., Sokolchik I, Zhu J, Gong Z,

Reddy M, et al (2002) C-terminal domain phosphatase-like family members (AtCPLs) differentially regulate Arabidopsis thaliana abiotic stress signaling, growth, and development. Proc Natl Acad Sci **99**: 10893–10898

Koiwa H, Hausmann S, Bang WY, Ueda A, Kondo N, Hiraguri A, Fukuhara T, Bahk JD, Yun D-J, Bressan RA, et al (2004) Arabidopsis C-terminal domain phosphatase-like 1 and 2 are essential Ser-5-specific C-terminal domain phosphatases. Proc Natl Acad Sci **101**: 14539–14544

Kresowich S, Barbazuk B, Bedell JA, Al. E (2005) Towards sequencing the Sorghum genome. A US National Science Foundation-spon- sored workshop report. Plant Physiol **134**: 912–918

Kumar A, Li C, Portis Jr AR (2009) Arabidopsis thaliana expressing a thermostable chimeric Rubisco activase exhibits enhanced growth and higher rates of photosynthesis at moderately high temperatures. Photosynth Res **100**: 143–153

Kumar SV, Lucyshyn D, Jaeger KE, Alós E, Alvey E, Harberd NP, Wigge PA (2012) Transcription factor PIF4 controls the thermosensory activation of flowering. Nature **484**: 242–245

Kumar SV, Wigge P a. (2010) H2A.Z-Containing Nucleosomes Mediate the Thermosensory Response in Arabidopsis. Cell **140**: 136–147

Kurek I, Chang TK, Bertain SM, Madrigal A, Liu L, Lassner MW, Zhu G (2007) Enhanced Thermostability of *Arabidopsis* Rubisco Activase Improves Photosynthesis and Growth Rates under Moderate Heat Stress. Plant Cell **19**: 3230–3241

Large EC (1954) Growth stages in cereals illustration of the Feekes scale. Plant Pathol **3**: 128–129

Larkindale J, Huang B (2004) Changes of lipid composition and saturation level in leaves and roots for heat-stressed and heat-acclimated creeping bentgrass (Agrostis stolonifera). Environ Exp Bot **51**: 57–67

Law RD, Crafts-Brandner SJ (2001) High Temperature Stress Increases the Expression of Wheat Leaf Ribulose-1,5-Bisphosphate Carboxylase/oxygenase Activase Protein. Arch Biochem Biophys **386**: 261–267

Lee H-J, Jung J-H, Llorca LC, Kim S-G, Lee S, Baldwin IT, Park C-M (2014) FCA mediates thermal adaptation of stem growth by attenuating auxin action in Arabidopsis. Nat Commun. doi: 10.1038/ncomms6473

Lee JH, Ryu H-S, Chung KS, Posé D, Kim S, Schmid M, Ahn JH (2013) Regulation of temperature-responsive flowering by MADS-box transcription factor repressors. Science (80-) **342**: 628–632

Lee JH, Yoo SJ, Park SH, Hwang I, Lee JS, Ahn JH (2007) Role of SVP in the control of flowering time by ambient temperature in Arabidopsis. Genes Dev **21**: 397–402

Legnaioli T, Cuevas J, Mas P (2009) TOC1 functions as a molecular switch connecting the circadian clock with plant responses to drought. EMBO J **28**: 3745–3757

Lewis S, Faricelli ME, Appendino ML, Valárik M, Dubcovsky J (2008) The chromosome region including the earliness per se locus Eps-Am1 affects the duration of early

developmental phases and spikelet number in diploid wheat. J Exp Bot **59**: 3595–3607

Li D, Liu C, Shen L, Wu Y, Chen H, Robertson M, Helliwell CA, Ito T, Meyerowitz E, Yu H (2008) A repressor complex governs the integration of flowering signals in Arabidopsis. Dev Cell **15**: 110–120

Lobell DB, Gourdji SM (2012) The influence of climate change on global crop productivity. Plant Physiol **160**: 1686–1697

Lu SX, Webb CJ, Knowles SM, Kim SHJ, Wang Z, Tobin EM (2012) CCA1 and ELF3 Interact in the control of hypocotyl length and flowering time in Arabidopsis. Plant Physiol **158**: 1079–1088

McClung CR, Davis SJ (2010) Ambient thermometers in plants: From physiological outputs towards mechanisms of thermal sensing. Curr Biol **20**: R1086–R1092

Michaels SD, Amasino RM (2000) Memories of winter: vernalization and the competence to flower. Plant Cell Environ **23**: 1145–1153

Millar CB, Xu F, Zhang K, Grunstein M (2006) Acetylation of H2AZ Lys 14 is associated with genome-wide gene activity in yeast. Genes Dev **20**: 711–22

Miralles DJ, Katz SD, Colloca A, Slafer GA (1998) Floret development in near isogenic wheat lines differing in plant height. F Crop Res **59**: 21–30

Miralles DJ, Richards RA (2000) Responses of Leaf and Tiller Emergence and Primordium Initiation in Wheat and Barley to Interchanged Photoperiod. Ann Bot **85**: 655–663

Molisch H (1928) Der Lebensdauer der Pflanzen. EH Fulling, transl, 1938 65:

del Moral LG, Miralles DJ, and Slafer G (2002) Barley Science: Recent Advances from Molecular Biology to Agronomy of Yield ... - Gustavo A Slafer, Jose Luis Molina-Cano, Roxana Savin, Jose Luis Araus, Ignacio Romagosa - Google Books. Food Product Press, New York

Murai K, Miyamae M, Kato H, Takumi S, Ogihara Y (2003) WAP1, a wheat APETALA1 homolog, plays a central role in the phase transition from vegetative to reproductive growth. Plant Cell Physiol **44**: 1255–1265

Nakamichi N, Kiba T, Kamioka M, Suzuki T, Yamashino T, Higashiyama T, Sakakibara H, Mizuno T, Kay SA (2012) Transcriptional repressor PRR5 directly regulates clock-output pathways. doi: 10.1073/pnas.1205156109

Narayanan S, Tamura PJ, Roth MR, Prasad P V, Welti R (2016) Wheat leaf lipids during heat stress: I. High day and night temperatures result in major lipid alterations. Plant Cell Environ **39**: 787–803

Nishida H, Ishihara D, Ishii M, Kaneko T, Kawahigashi H, Akashi Y, Saisho D, Tanaka K, Handa H, Takeda K (2013) Phytochrome C is a key factor controlling long-day flowering in barley. Plant Physiol **163**: 804–814

Nusinow DA, Helfer A, Hamilton EE, King JJ, Imaizumi T, Schultz TF, Farré EM, Kay SA (2011) The ELF4-ELF3-LUX complex links the circadian clock to diurnal control of hypocotyl growth. Nature **475**: 398–402

Oliver SN, Finnegan EJ, Dennis ES, Peacock WJ, Trevaskis B (2009) Vernalization-induced flowering in cereals is associated with changes in histone methylation at the VERNALIZATION1 gene. Proc Natl Acad Sci **106**: 8386–8391

Oshino T, Abiko M, Saito R, Ichiishi E, Endo M, Kawagishi-Kobayashi M, Higashitani A (2007) Premature progression of anther early developmental programs accompanied by comprehensive alterations in transcription during high-temperature injury in barley plants. Mol Genet Genomics **278**: 31–42

Oshino T, Miura S, Kikuchi S, Hamada K, Yano K, Watanabe M, Higashitani A (2011) Auxin depletion in barley plants under high-temperature conditions represses DNA proliferation in organelles and nuclei via transcriptional alterations. Plant Cell Environ **34**: 284–290

Ougham H, Armstead I, Howarth C, Galyuon I, Donnison I, Thomas H (2007) The Genetic Control of Senescence Revealed by Mapping Quantitative Trait Loci. Annu. Plant Rev. John Wiley & Sons, Ltd, Chichester, UK, pp 171–201

Pankin A, Campoli C, Dong X, Kilian B, Sharma R, Himmelbach A, Saini R, Davis SJ, Stein N, Schneeberger K (2014) 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. **198**: 383-396.

Pestsova E, Röder M (2002) Microsatellite analysis of wheat chromosome 2D allows the reconstruction of chromosomal inheritance in pedigrees of breeding programmes. Theor Appl Genet **106**: 84–91

Pokhilko A, Fernández AP, Edwards KD, Southern MM, Halliday KJ, Millar AJ (2012) The clock gene circuit in Arabidopsis includes a repressilator with additional feedback loops. Mol Syst Biol **8**: 574

Porter JR, Gawith M (1999) Temperatures and the growth and development of wheat: a review. Eur J Agron **10**: 23–36

Posé D, Verhage L, Ott F, Yant L, Mathieu J, Angenent GC, Immink RGH, Schmid M (2013) Temperature-dependent regulation of flowering by antagonistic FLM variants. Nature **503**: 414–7

Purvis ON, Gregory FG (1952) Studies in Vernalisation XII. The Reversibility by High Temperature of the Vernalised Condition in Petkus Winter Rye. Ann Bot **16**: 1–21

Quinn, P. J, Williams WP (1985) Environmentally-induced changes in chloroplast membranes and their effects on photosynthetic function. In Photosynthetic mechanisms and the environment., Editors: J Barber, NR Baker. Publisher: Elsevier, Amsterdam pp 1-47

Radin JW, Lu Z, Percy RG, Zeiger E (1994) Genetic variability for stomatal conductance in Pima cotton and its relation to improvements of heat adaptation. Proc Natl Acad Sci U S A **91**: 7217–7221

Rang ZW, Jagadish SVK, Zhou QM, Craufurd PQ, Heuer S (2011) Effect of high temperature and water stress on pollen germination and spikelet fertility in rice. Environ Exp Bot **70**: 58–65

Ratcliffe OJ, Kumimoto RW, Wong BJ, Riechmann JL (2003) Analysis of the

Arabidopsis MADS AFFECTING FLOWERING Gene Family: MAF2 Prevents Vernalization by Short Periods of Cold. Plant Cell **15**: 1159–1169

Rawson H (1971) An upper limit for spikelet number per ear in wheat as controlled by photoperiod. Aust J Agric Res **22**: 537-546

Rawson HM (1970) Spikelet number, its control and relation to yield per ear in wheat. Aust J biol Sci **23**: 1–15

Rawson HM, Richards RA (1993) Effects of high temperature and photoperiod on floral development in wheat isolines differing in vernalisation and photoperiod genes. F Crop Res **32**: 181–192

Rawson HM, Zajac M (1993) Effects of higher temperatures, photoperiod and seed vernalisation on development in two spring wheats. Funct Plant Biol **20**: 211–222

Reeves PH, Coupland G (2000) Response of plant development to environment: control of flowering by daylength and temperature. Curr Opin Plant Biol **3**: 37–42

Reynolds M, Foulkes J, Furbank R, Griffiths S, King J, Murchie E, Parry M, Slafer G (2012) Achieving yield gains in wheat. Plant, Cell Environ **35**: 1799–1823

Reynolds M, Foulkes MJ, Slafer GA, Berry P, Parry MAJ, Snape JW, Angus WJ (2009) Raising yield potential in wheat. J Exp Bot **60**: 1899–1918

Reynolds M, Tuberosa R, Jan Leach by, Greenland A (2008) Translational research impacting on crop productivity in drought-prone environments This review comes from a themed issue on Plant Biotechnology Edited. Curr Opin Plant Biol **11**: 171–179

Riechmann JL, Heard J, Martin G, Reuber L, Jiang C, Keddie J, Adam L, Pineda O, Ratcliffe OJ, Samaha RR, et al (2000) Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. Science **290**: 2105–10

Robertson DS (1985) A possible technique for isolating genic DNA for quantitative traits in plants. J Theor Biol **117**: 1–10

Roberts EH, Summerfield RJ, Cooper JP, Ellis RH (1988) 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. Ann Bot **62**: 127–144

Rollins J a., Habte E, Templer SE, Colby T, Schmidt J, Von Korff M (2013) Leaf proteome alterations in the context of physiological and morphological responses to drought and heat stress in barley (Hordeum vulgare L.). J Exp Bot **64**: 3201–3212

Ronald JA and, Davis JS (2017) Making the Clock Tick: The Transcriptional Landscape of the Plant Circadian Clock. Fac 1000 Res 6: 951

Ruwali K, Upadhyaya Y, & Matai B (1988) Adaptation of rainfed wheats under diverse ecological conditions. Proc. Int. Congr. Plant Physiol. New Delhi, India, pp 863–869

S. Tzay-Fa (2000) Effect of day and night temperature variation and of high temperature on devernalization in radish. http://www.actahort.org/books/514/514_17.htm

Saidi Y, Finka A, Goloubinoff P (2011) Heat perception and signalling in plants: a tortuous

path to thermotolerance. New Phytol **190**: 556–565

Saini H, Sedgley M, Aspinall D (1983) Effect of Heat Stress During Floral Development on Pollen Tube Growth and Ovary Anatomy in Wheat (*Triticum aestivum* L.). Aust J Plant Physiol **10**: 137´-144

Saini H, Sedgley M, Aspinall D (1984) Development Anatomy in Wheat of Male Sterility Induced by Heat Stress, Water Deficit or Abscisic Acid. Aust J Plant Physiol **11**: 243-253

Saini HS, Aspinall D (1982) Abnormal sporogenesis in wheat (Triticum aestivum L.) induced by short periods of high temperature. Ann Bot **49**: 835–846

Sakata T, Oda S, Tsunaga Y, Shomura H, Kawagishi-Kobayashi M, Aya K, Saeki K, Endo T, Nagano K, Kojima M (2014) Reduction of gibberellin by low temperature disrupts pollen development in rice. Plant Physiol **164**: 2011–2019

Sakata T, Oshino T, Miura S, Tomabechi M, Tsunaga Y, Higashitani N, Miyazawa Y, Takahashi H, Watanabe M, Higashitani A (2010) Auxins reverse plant male sterility caused by high temperatures. Proc Natl Acad Sci **107**: 8569–8574

Sakata T, Takahashi H, Nishiyama I, Higashitani A (2000) Effects of high temperature on the development of pollen mother cells and microspores in barley Hordeum vulgare L. J Plant Res **113**: 395–402

Sakuma S, Salomon B, Komatsuda T (2011) The Domestication Syndrome Genes Responsible for the Major Changes in Plant Form in the Triticeae Crops. **52**: 738–749

Salvucci ME, Crafts-Brandner SJ, Salvucci ME (2004) Relationship between the heat tolerance of photosynthesis and the thermal stability of rubisco activase in plants from contrasting thermal environments. Plant Physiol **134**: 1460–70

Salvucci ME, Loo FJ van de, Stecher D (2003) Two isoforms of Rubisco activase in cotton, the products of separate genes not alternative splicing. Planta **216**: 736–744

Salvucci ME, Portis AR, Ogren WL (1985) A soluble chloroplast protein catalyzes ribulosebisphosphate carboxylase/oxygenase activation in vivo. Photosynth Res **7**: 193–201

Sanchez A, Shin J, Davis SJ (2011) Abiotic stress and the plant circadian clock. Plant Signal Behav **6**: 223–231

Satake T, Yoshida S (1978) High Temperature-Induced Sterility in Indica Rices at Flowering. Crop Sci **47**: 6–17

Scortecci KC, Michaels SD, Amasino RM (2001) Identification of a MADS-box gene, FLOWERING LOCUS M, that represses flowering. Plant J **26**: 229–236

Seemann JR, Berry JA, Downton WJ (1984) Photosynthetic response and adaptation to high temperature in desert plants : a comparison of gas exchange and fluorescence methods for studies of thermal tolerance. Plant Physiol **75**: 364–8

Shanmugavadivel P, Sv AM, Prakash C, Mk R, Tiwari R, Mohapatra T, Singh NK (2017) High Resolution Mapping of QTLs for Heat Tolerance in Rice Using a 5K SNP Array. Rice. doi: 10.1186/s12284-017-0167-0

Sharkey TD, Badger MR, von Caemmerer S, Andrews TJ (2001) Increased heat

sensitivity of photosynthesis in tobacco plants with reduced Rubisco activase. Photosynth Res **67**: 147–156

Sharman BC (1947) The Biology and Developmental Morphology of the Shoot Apex in the GRAMINEAE. New Phytol **46**: 20–34

Shaw LM, Turner AS, Laurie DA (2012) The impact of photoperiod insensitive Ppd-1a mutations on the photoperiod pathway across the three genomes of hexaploid wheat (Triticum aestivum). Plant J **71**: 71–84

Shen Q, Fu L, Dai F, Jiang L, Zhang G, Wu D (2016) Multi-omics analysis reveals molecular mechanisms of shoot adaption to salt stress in Tibetan wild barley. BMC Genomics **17**: 889 DOI 10.1186/s12864-016-3242-9

Shirdelmoghanloo H, Taylor JD, Lohraseb I, Rabie H, Brien C, Timmins A, Martin P, Mather DE, Emebiri L, Collins NC (2016) A QTL on the short arm of wheat (Triticum aestivum L.) chromosome 3B affects the stability of grain weight in plants exposed to a brief heat shock early in grain filling. DOI 10.1186/s12870-016-0784-6

Shitsukawa N, Ikari C, Shimada S, Kitagawa S, Sakamoto K, Saito H, Ryuto H, Fukunishi N, Abe T, Takumi S (2007) The einkorn wheat (Triticum monococcum) mutant, maintained vegetative phase, is caused by a deletion in the VRN1 gene. Genes Genet Syst **82**: 167–170

Simpson GG, Gendall AR, Dean C (1999) when to switch to flowering. Annu Rev Cell Dev Biol **99**: 519–50

Slafer GA (2003) Genetic basis of yield as viewed from a crop physiologist's perspective. Ann Appl Biol **142**: 117–128

Slafer GA, Rawson HM (1996) Responses to photoperiod change with phenophase and temperature during wheat development. F Crop Res **46**: 1–13

Slafer GA, Savin R (1991) Developmental Base Temperature in Different Phenological Phases of Wheat (Triticum aestivum). J Exp Bot **42**: 1077–1082

Slafer G, Rawson H (1994) Sensitivity of Wheat Phasic Development to Major Environmental Factors: a Re-Examination of Some Assumptions Made by Physiologists and Modellers. Aust J Plant Physiol **21**: 393

Sreenivasulu N, Schnurbusch T (2012) A genetic playground for enhancing grain number in cereals. Trends Plant Sci **17**: 91–101

Suárez-López P, Wheatley K, Robson F, Onouchi H, Valverde F, Coupland G (2001) CONSTANS mediates between the circadian clock and the control of flowering in Arabidopsis. Nature **410**: 1116–1120

Tanaka W, Pautler M, Jackson D, Hirano H-Y (2013) Grass Meristems II: Inflorescence Architecture, Flower Development and Meristem Fate. Plant Cell Physiol **54**: 313–324

Theißen G, Melzer R, Rümpler F (2016) MADS-domain transcription factors and the floral quartet model of flower development: linking plant development and evolution. Development **143**: 3259–3271

Theißen G, Saedler H (2001) Plant biology: Floral quartets. Nature **409**: 469–471

Thines B, Harmon FG (2010) Ambient temperature response establishes ELF3 as a required component of the core Arabidopsis circadian clock. Proc Natl Acad Sci **107**: 3257–3262

Thomas H, Ougham H (2014) The stay-green trait. J Exp Bot **65**: 3889–3900

Tingle JN, Faris DG, Ormrod DP (1970) Effects of temperature, light, and variety in controlled environments on floret number and fertility in barley. Crop Sci **10**: 26–28

To K-Y, Suen D-F, Chen S-CG (1999) Molecular characterization of ribulose-1,5-bisphosphate carboxylase/oxygenase activase in rice leaves. Planta **209**: 66–76

Todd GW (1982) Photosynthesis and Respiration of Vegetative and Reproductive Parts of Wheat and Barley Plants in Response to Increasing Temperature. Proc Oklahoma **62**: 57–62

Tranquilli G, Dubcovsky J (2000) Epistatic interaction between vernalization genes Vrn-Am1 and Vrn-Am2 in diploid wheat. J Hered **91**: 304–306

Trevaskis B, Tadege M, Hemming MN, Peacock WJ, Dennis ES, Sheldon C (2007) Short vegetative phase-like MADS-box genes inhibit floral meristem identity in barley. Plant Physiol **143**: 225–235

Turck F, Fornara F, Coupland G (2008) Regulation and identity of florigen: FLOWERING LOCUS T moves center stage. Annu Rev Plant Biol **59**: 573–594

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

Valdés-Mora F, Song JZ, Statham AL, Strbenac D, Robinson MD, Nair SS, Patterson KI, Tremethick DJ, Stirzaker C, Clark SJ (2012) Acetylation of H2A.Z is a key epigenetic modification associated with gene deregulation and epigenetic remodeling in cancer. Genome Res **22**: 307–21

Vargas M, van Eeuwijk FA, Crossa J, Ribaut J-M (2006) Mapping QTLs and QTL × environment interaction for CIMMYT maize drought stress program using factorial regression and partial least squares methods. Theor Appl Genet **112**: 1009–1023

Verma V, Foulkes MJ, Worland AJ, Sylvester-Bradley R, Caligari PDS, Snape JW (2004) Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. Euphytica **135**: 255–263

Vijayalakshmi K, Fritz A, Paulsen, GM, Bai, G., Pandravada, S., & Gill B (2010) Modeling and mapping QTL for senescence-related traits in winter wheat under high temperature. Mol Breed **26**: 163–175

Visioni A, Tondelli A, Francia E, Pswarayi A, Malosetti M, Russell J, Thomas W, Waugh R, Pecchioni N, Romagosa I, et al (2013) Genome-wide association mapping of frost tolerance in barley (Hordeum vulgare L.). BMC Genomics **14**: 424

Waddington SR, Cartwright PM, Wall PC (1983) A quantitative scale of spike initial and pistil development in barley and wheat. Ann Bot **51**: 119–130

Walster HL (1920) Formative Effect of High and Low Temperatures upon Growth of Barley: A Chemical. Source Bot Gaz **69**: 97–126

Wardlaw I, Sofield I, Cartwright P (1980) Factors Limiting the Rate of Dry Matter

Accumulation in the Grain of Wheat Grown at High Temperature. Aust J Plant Physiol **7**: 387-400

Weberling F (1992) Morphology of Flowers and Inflorescences. CUP Archive

Weis E (1981) The temperature-sensitivity of dark-inactivation and light-activation of the ribulose-1,5-bisphosphate car□ylase in spinach chloroplasts. FEBS Lett **129**: 197–200

Werneke JM, Chatfield JM, Ogren WL (1989) Alternative mRNA splicing generates the two ribulosebisphosphate carboxylase/oxygenase activase polypeptides in spinach and Arabidopsis. Plant Cell **1**: 815–825

Wigge PA (2013) Ambient temperature signalling in plants. Curr Opin Plant Biol **16**: 661–666

Yan L, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, SanMiguel P, Bennetzen JL, Echenique V, Dubcovsky J (2004) The wheat VRN2 gene is a flowering repressor down-regulated by vernalization. Science **303**: 1640–1644

Zadoks JC, CHANG TT, KONZAK CF (1974) A decimal code for the growth stages of cereals. Weed Res **14**: 415–421

Zagotta MT, Hicks KA, Jacobs CI, Young JC, Hangarter RP, Meeks-Wagner DR (1996) The Arabidopsis ELF3 gene regulates vegetative photomorphogenesis and the photoperiodic induction of flowering. Plant J **10**: 691–702

Zakhrabekova S, Gough SP, Braumann I, Müller AH, Lundqvist J, Ahmann K, Dockter C, Matyszczak I, Kurowska M, Druka A (2012) Induced mutations in circadian clock regulator Mat-a facilitated short-season adaptation and range extension in cultivated barley. Proc Natl Acad Sci **109**: 4326–4331

Zhang B, Wu S, Zhang Y 'e, Xu T, Guo F, Tang H, Li X, Wang P, Qian W, Xue Y, et al (2016) A High Temperature-Dependent Mitochondrial Lipase EXTRA GLUME1 Promotes Floral Phenotypic Robustness against Temperature Fluctuation in Rice (Oryza sativa L.). PLoS Genet. doi: 10.1371/journal.pgen.1006152

Zhang N, Kallis RP, Ewy RG, and Archie R. Portis J (2002) Light limitation of photosynthesis and activation of ribulose bisphosphate carboxylase in wheat seedlings. Proc Natl Acad Sci **78**: 2985–2989

Zheng B, Chenu K, Chapman SC (2016) Velocity of temperature and flowering time in wheat–assisting breeders to keep pace with climate change. Glob Chang Biol **22**: 921–933

Zheng G, Tian B, Zhang F, Tao F, Li W (2011) Plant adaptation to frequent alterations between high and low temperatures: remodelling of membrane lipids and maintenance of unsaturation levels. Plant Cell Environ **34**: 1431–1442

Zohary D, Hopf M (1993) Domestication of plants in the Old World. The origin and spread of cultivated plants in West Asia, Europe and the Nile Valley. Oxford University Press

V. Chapter 2-Original Research: The Genetic Control of Reproductive Development under High Ambient Temperature

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Authors:

Mahwish Ejaz and Maria von Korff*

* Address for correspondence to korff@mpipz.mpg.de.

Affiliations:

Max Planck Institute for Plant Breeding Research, D-50829 Cologne, Germany (M.E., M.v.K.);
Institute of Plant Genetics, Heinrich-Heine-University, 40225 Düsseldorf, Germany (M.E., M.v.K.);
Cluster of Excellence on Plant Sciences "From Complex Traits towards Synthetic Modules," 40225 Düsseldorf, Germany (M.v.K.)

Contributions:

M.E. and M.v.K. conceived and designed the experiments;
M.E. carried out all the experiments and analyzed the data;
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The Genetic Control of Reproductive Development under High Ambient Temperature[1][OPEN]

Mahwish Ejaz and Maria von Korff*

Max Planck Institute for Plant Breeding Research, D-50829 Cologne, Germany (M.E., M.v.K.); Institute of Plant Genetics, Heinrich-Heine-University, 40225 Düsseldorf, Germany (M.E., M.v.K.); and Cluster of Excellence on Plant Sciences "From Complex Traits towards Synthetic Modules," 40225 Düsseldorf, Germany (M.v.K.)

ORCID IDs: 0000-0002-3857-5665 (M.E.); 0000-0002-6816-586X (M.v.K.).

Abstract

Ambient temperature has a large impact on reproductive development and grain yield in temperate cereals. However, little is known about the genetic control of development under different ambient temperatures. Here, we demonstrate that in barley (*Hordeum vulgare*), high ambient temperatures accelerate or delay reproductive development depending on the photoperiod response gene *PHOTOPERIOD1* (*Ppd-H1*) and its upstream regulator *EARLY FLOWERING3* (*HvELF3*). A natural mutation in *Ppd-H1* prevalent in spring barley delayed floral development and reduced the number of florets and seeds per spike, while the wild-type *Ppd-H1* or a mutant *Hvelf3* allele accelerated floral development and maintained the seed number under high ambient temperatures. High ambient temperature delayed the expression phase and reduced the amplitude of clock genes and repressed the floral integrator gene *FLOWERING LOCUS T1* independently of the genotype. *Ppd-H1*-dependent variation in flowering time under different ambient temperatures correlated with relative expression levels of the *BARLEY MADS*-box genes *VERNALIZATION1* (*HvVRN1*), *HvBM3*, and *HvBM8* in the leaf. Finally, we show that *Ppd-H1* interacts with regulatory variation at *HvVRN1*. *Ppd-H1* only accelerated floral development in the background of a spring *HvVRN1* allele with a deletion in the regulatory intron. The full-length winter *Hvvrn1* allele was strongly down-regulated, and flowering was delayed by high temperatures irrespective of *Ppd-H1*. Our findings demonstrate that the photoperiodic and vernalization pathways interact to control flowering time and floret fertility in response to ambient temperature in barley.

Introduction

Climate models predict that an increase in global average temperature will have large impacts on crop yield (Lobell et al., 2011). High temperatures are particularly critical during plant reproductive development and affect flowering time, flower fertility, and seed set. To sustain high crop yields under changing climatic conditions, it is important to understand the genetic basis of plant development in response to ambient temperature.

Temperature-dependent flowering is regulated by the vernalization and ambient temperature pathways. Whereas vernalization requires long periods of cold during the winter, the ambient temperature pathway modulates flowering in response to short-term temperature changes (Wigge, 2013). Research in the model plant Arabidopsis (*Arabidopsis thaliana*), a facultative long-day (LD) plant, has demonstrated that the temperature and photoperiod pathways interact to control reproductive development. For example, high temperature accelerates flowering and overcomes the delay in flowering commonly observed under short photoperiods in Arabidopsis (Balasubramanian et al., 2006). Early flowering in response to high temperature was correlated with an increase in the expression of the floral integrator gene *FLOWERING LOCUS T* (*FT*) independently of day length (Halliday et al., 2003; Balasubramanian et al., 2006). The FT protein acts as a long-distance signal (florigen) that conveys the information to induce flowering from leaves to the shoot meristem (Corbesier et al., 2007; Jaeger and Wigge, 2007; Kobayashi and Weigel, 2007; Mathieu et al., 2007; Tamaki et al., 2007). In addition, recent studies have identified *EARLY FLOWERING3* (*ELF3*), a repressor of light signals to the circadian clock as an essential component of ambient temperature response (Thines and Harmon, 2010). *ELF3* forms together with *ELF4* and *LUX ARRHYTHMO* (*LUX*), the so-called "evening complex" (EC) that functions as a night-time repressor of gene expression in the circadian clock of Arabidopsis (Nusinow et al., 2011; Herrero et al., 2012). The circadian clock is an autonomous oscillator that produces endogenous biological rhythms with a period of about 24 h and consists of at least three interlocking feedback loops. The core loops comprise (1) the inhibition of EC

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* Address for correspondence to korff@mpipz.mpg.de.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Maria von Korff (korff@mpipz.mpg.de).

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genes by CIRCADIAN CLOCK ASSOCIATED1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) late at night, (2) the inhibition of *PSEUDO RESPONSE REGULATOR* (*PRR*) genes by the EC early at night, and (3) the inhibition of *LHY/CCA1* by TIMING OF CAB EXPRESSION1/PRR1 in the morning (Pokhilko et al., 2012).

Several independent studies have recently found that elevated temperatures, specifically during dark periods, inhibit the activity of the EC by an unknown mechanism (Box et al., 2015; Mizuno et al., 2014; Thines et al., 2014; Raschke et al., 2015), leading to increased expression of *PHYTOCHROME-INTERACTING FACTOR4* (*PIF4*; Koini et al., 2009). PIF4 binding to the promoter of *FT* and consequent transcriptional activation of *FT* is promoted by an improved chromatin accessibility through temperature-dependent histone modifications at the *FT* promoter (Kumar and Wigge, 2010; Kumar et al., 2012). However, high temperature also accelerated flowering in *pif4* mutants under long photoperiods, suggesting that a *PIF4*-independent thermoresponsive flowering pathway acts through components of the photoperiod pathway (Koini et al., 2009; Press et al., 2016).

In addition, the MADS-box genes *SHORT VEGETATIVE PHASE*-like (*SVP*), *FLOWERING LOCUS C* (*FLC*), and *FLOWERING LOCUS M* (*FLM*; *MAF1*) play a role in the thermosensory regulation of flowering in Arabidopsis (Balasubramanian et al., 2006; Lee et al., 2007; Gu et al., 2013). Loss of function of either *SVP* or *FLM* results in partial temperature-insensitive early flowering (Balasubramanian et al., 2006; Lee et al., 2007, 2013; Posé et al., 2013). Moreover, *FLM* is subject to temperature-dependent alternative splicing (Balasubramanian et al., 2006; Sureshkumar et al., 2016) resulting in two major splice forms, that either facilitate or inhibit *SVP* dependent repression of *FT*, and the floral homeotic genes *SUPPRESSOR OF OVEREXPRESSION1* and *SEPALLATA* (Posé et al., 2013). Interestingly, a structural polymorphism in the first intron of *FLM* affects its expression, splicing, and also regulates flowering predominantly at lower ambient temperatures (Lutz et al., 2015). Such structural polymorphisms within the first intron are typical within the family of MADS-box transcription factor genes and play an important role for expression variation and possibly adaptation to different environments across different species (Hong et al., 2003; Distelfeld et al., 2009; Schauer et al., 2009; Yoo et al., 2011).

While flowering-time control in response to temperature is well described in Arabidopsis, little is known about the genetic determinants of ambient temperature response in cereal grasses (Bullrich et al., 2002; Appendino and Slafer, 2003; Lewis et al., 2008; Hemming et al., 2012). In barley (*Hordeum vulgare*), a complex interplay between day length and temperature in the regulation of flowering has been reported. Under LD conditions, barley plants accelerated reproductive development at 25°C compared with 15°C, whereas the opposite was the case under short days (SDs) (Hemming et al., 2012). In contrast to Arabidopsis, the transcript level of the barley homolog of *FT* was not influenced by temperature, and no clear candidate genes for the integration of thermal signals into the flowering-time pathways have been identified so far (Hemming et al., 2012). Barley is a facultative LD plant and is characterized by winter and spring growth habits as determined by natural variation at the two vernalization genes, *VERNALIZATION1* (*HvVRN1*; *HvBM5a*) and *HvVRN2* (Yan et al., 2003, 2004; Trevaskis et al., 2006). Winter types accelerate flowering after a prolonged period of cold (vernalization), whereas spring barley does not respond to vernalization. The MADS-box gene *HvVRN1* is characterized by a series of different deletions and insertions in the first regulatory intron, which has been linked to differences in vernalization response and flowering behavior (Hemming et al., 2009). Photoperiod response, rapid flowering under LDs, is determined by natural variation at the *PHOTOPERIOD-H1* (*Ppd-H1*) gene, which is homologous to the *PRR* genes of the circadian clock in Arabidopsis (Turner et al., 2005). The wild-type allele is prevalent in winter barley, while a natural mutation in the conserved CCT domain of *Ppd-H1* causes a delay in flowering under LDs and is predominant in spring barley from cultivation areas with long growing seasons (Turner et al., 2005; von Korff et al., 2006, 2010; Wang et al., 2010). *Ppd-H1* induces flowering under LDs by up-regulating *HvFT1*, the barley homolog of FT in Arabidopsis (Turner et al., 2005; Campoli et al., 2012b). *Ppd-H1* is repressed during the night by *HvELF3*, *HvLUX1*, and *PHYTOCHROME C*, and mutations in these genes result in a day-neutral up-regulation of *HvFT1* and early flowering (Faure et al., 2012; Zakhrabekova et al., 2012; Campoli et al., 2013; Pankin et al., 2014). Consequently, the major vernalization and photoperiod response genes are known in barley, whether these also play a role for thermoresponsive flowering is not known.

In Arabidopsis, commonly used macroscopic indicators of reproductive phase change or floral transition are time to bolting or rosette leaf number under the first open floral bud (Pouteau and Albertini, 2009). Under optimal conditions, floral transition, bolting, and flowering are well correlated in Arabidopsis. In barley, most stages of reproductive development, including flowering, occur within the leaf sheath and can therefore only be scored upon dissection of the shoot. Waddington et al. (1983) developed a quantitative scale for barley and wheat development based on the morphogenesis of the shoot apex and carpels. This scale is based on the progression of the most advanced floret primordium and carpel of the inflorescence. The enlargement of the apical dome at Waddington stage (W) 1.0 represents an apex that is transitioning to a reproductive state and indicates the end of the vegetative phase. The emergence of the first floret primordia on the shoot apex at the double ridge stage (W2.0) specifies a reproductive shoot apical meristem (SAM). At the stamen primordium stage (W3.5), the first floral organ primordia differentiate, and stem elongation initiates. In barley, the induction of floret primordia on the

inflorescence continues until the awn primordium stage (W5.0). Anthesis and pollination of the most advanced floret occurs at the last stage of the Waddington scale (W10.0). This last step can be scored macroscopically because it is marked by the emergence of awn tips from the top of the leaf sheath (heading). Most commonly, flowering is scored as heading in barley. However, the different phases of shoot apex development differ in their sensitivity to environmental cues and are controlled by different genetic factors, so that floral transition and flowering may not be correlated and separated in time by many weeks (Digel et al., 2015). Variation in the timing of different developmental phases in turn affects the number of floret primordia, fertile flowers, and seeds per spike (Digel et al., 2015). To better understand the effects of temperature on development, it is therefore important to investigate the effects of environmental and genetic variation on individual phases of shoot apex development.

The objective of present study was to elucidate the genetic control of reproductive development under high ambient temperature in barley. We show that high ambient temperature delays the phase and reduces the amplitude of clock gene expression. Further, we demonstrate that under high ambient temperature, flowering time and seed number are controlled by interactions between *Ppd-H1* and *HvVRN1* and correlate with expression levels of the *BARLEY MADS*-box genes *HvBM3* and *HvBM8* in the leaf. These findings provide new insights into the genetic and molecular control of flowering time and inflorescence development under high ambient temperature in barley.

RESULTS

High Ambient Temperature Delays Reproductive Development and Reduces Seed Set in Spring Barley

To examine the effect of high ambient temperature on flowering in barley, we scored the spring barley genotypes Bowman and Scarlett for days to flowering under control (20°C/16°C) and high temperatures (28°C/24°C) in LDs. These genotypes carry a mutated *ppd-H1* allele, a functional *HvELF3* allele, and a spring *HvVRN1* allele and therefore do not respond to vernalization and are late flowering under LDs. Flowering was significantly delayed in both Bowman and Scarlett under high as compared to control temperatures (Fig. 1A). In addition, high temperature reduced floret and seed number per spike in both genotypes (Fig. 1, B and C). The total number of florets and seeds per spike were reduced in Bowman by 19% and 34% and in Scarlett by 30% and 74%, respectively, at high compared to control temperatures (Fig. 1, B and C). Under short-day condition (8 h light/16 h dark), Bowman and Scarlett plants never flowered neither under control nor under high ambient temperature conditions (data not shown).

The effect of increased temperature on floral development was evaluated by monitoring the progression

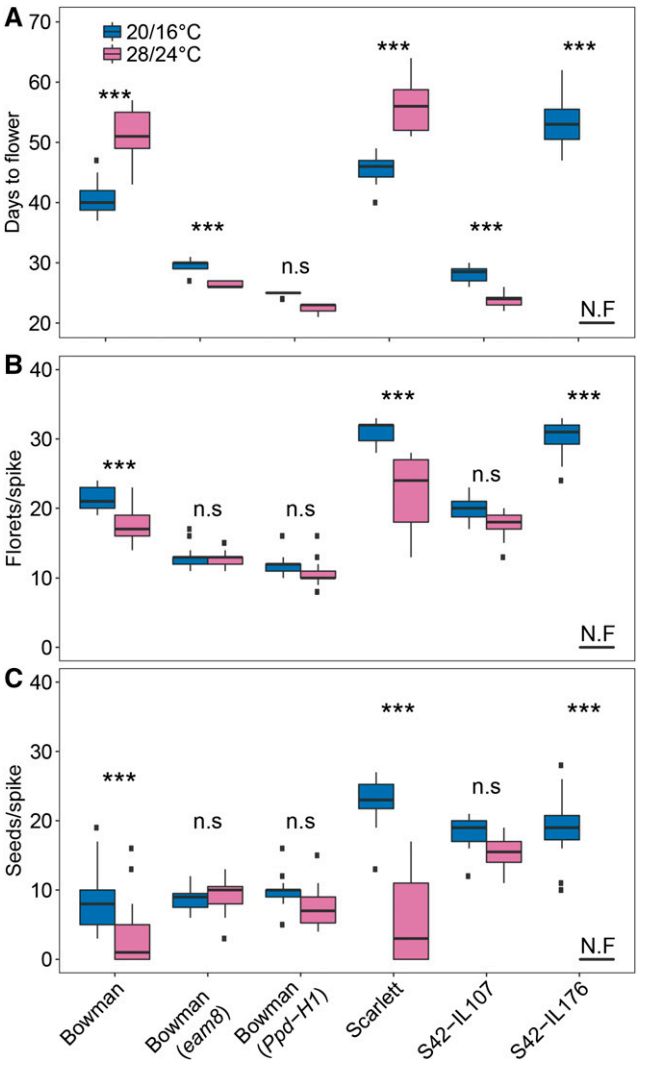

Figure 1. High ambient temperature affects flowering time, floret, and seed number per main spike in barley. Days to flower (A), the number of florets (B), and the number of seeds per main spike (C) under control (blue; 20°C/16°C, day/night) and high ambient temperatures (pink; 28°C/24°C, day/night) in the spring barley varieties Bowman and Scarlett and the derived ILs Bowman(*eam8*) (*Hvelf3*), Bowman(*Ppd-H1*), S42-IL107 (*Ppd-H1*), and S42-IL176 (*Hvvrn1*). Flowering time, floret, and seed number were recorded for 20 plants per genotype and treatment under LDs (16 h light/8 h night). N.F indicates nonflowering plants. Statistical differences were calculated by a two-factorial ANOVA and a post-hoc Tukey's multiple comparison test: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; n.s = nonsignificant.

of the main shoot apex (MSA) in Bowman and Scarlett plants grown at 20°C/16°C and 28°C/24°C according to the Waddington scale (Waddington et al., 1983). Microscopic dissection of the MSA revealed that high temperature did not have a strong effect on floral transition (W2.0), but greatly delayed the late reproductive phase of inflorescence development (after W3.5) both in Bowman and Scarlett (Fig. 2, A and C). In summary, high ambient temperature primarily delayed inflorescence development and reduced the number of

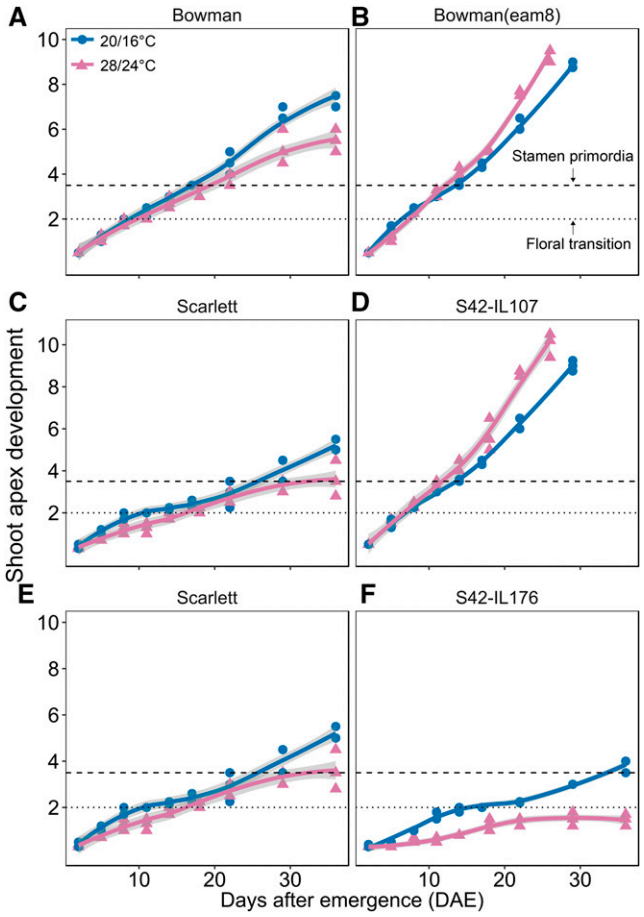

Figure 2. High ambient temperature affects MSA development in barley. Microscopic development of the MSA was scored under control (blue; 20°C/16°C, day/night) and high ambient (pink; 28°C/24°C, day/night) temperatures every 3 d according to the Waddington scale (Waddington et al., 1983). MSA development was delayed under high compared to control temperature in Bowman (A) and Scarlett (C and E), accelerated in Bowman(*eam8*) (B) and S42-IL107 (D), and further delayed floral transition in S42-IL176 (F). Three or four plants per genotype were dissected at each time point in each treatment under LDs (16 h light/8 h night). Statistical differences ($P < 0.05$) were calculated using a polynomial regression model at 95% confidence interval (Loess smooth line).

seeds per spike in the spring barley genotypes Bowman and Scarlett.

High Ambient Temperature Accelerates Flowering Time in Genotypes with a Nonfunctional *Hvelf3* Allele and a Dominant *Ppd-H1* Allele

In Arabidopsis, the circadian clock and photoperiod pathways modulate ambient temperature responses to regulate flowering. Therefore, we further characterized reproductive development in introgression lines (ILs) with a nonfunctional *Hvelf3* or dominant *Ppd-H1* alleles under control and high ambient temperatures. *HvELF3* is a component of the EC in Arabidopsis and represses *Ppd-H1* expression in the night in barley (Faure et al., 2012). Therefore, the barley *Hvelf3* mutant genotype is characterized by high expression of *Ppd-H1* during the night (Faure et al., 2012). The IL Bowman(*eam8*) carrying a nonfunctional *Hvelf3* allele in the background of Bowman and the ILs S42-IL107 and Bowman(*Ppd-H1*) with the wild-type *Ppd-H1* gene in the background of Scarlett and Bowman were analyzed along with the parental genotypes for flowering time, floret fertility, and seed set. In addition, the microscopic development of the MSA was evaluated in Scarlett, Bowman, S42-IL107, and Bowman(*eam8*) under control and high ambient temperatures.

Microscopic dissection of the MSA revealed that in contrast to Bowman with a delayed development under high temperatures, Bowman(*eam8*) showed an accelerated MSA development at 28°C/24°C compared to 20°C/16°C (Fig. 2B). As a result, Bowman(*eam8*) plants flowered on average 5 d earlier at high compared to control temperatures (Fig. 1A).

Since *HvELF3* might control flowering time through its downstream target *Ppd-H1*, we evaluated if variation at *Ppd-H1* mediated the flowering response under high ambient temperature. In contrast to the parental lines, S42-IL107 and Bowman(*Ppd-H1*) plants flowered on average 7 and 2 d earlier under high ambient compared to control temperatures (Fig. 2D). The dissection of the MSA in Scarlett and S42-IL107 revealed that high ambient temperature accelerated in particular the phase of stem elongation and inflorescence development (Fig. 2D). In addition, the ANOVA for floret and seed number revealed a significant interaction between *Ppd-H1* and *HvELF3* with temperature (Supplemental Table S1). High ambient temperatures caused a larger reduction in floret and seed number in Bowman and Scarlett than in the ILs Bowman(*eam8*), S42-IL107, and Bowman(*Ppd-H1*) (Fig. 1, B and C; Supplemental Table S1).

Taken together, high ambient temperature affected inflorescence development and flowering time in a *HvELF3*- and *Ppd-H1*-dependent manner. Quantitative variation in the reduction of seed number under high ambient temperatures was dependent on *HvELF3* and *Ppd-H1*.

Variation at *HvVRN1* Affects Reproductive Development under High Ambient Temperature

Natural variation in the length of the first regulatory intron of *HvVRN1* has a strong effect on vernalization response in barley. Therefore, we examined whether this variation also affected the response to ambient temperature variation in barley. For this purpose, we compared the development of Scarlett with that of S42-IL176. Scarlett carries a spring *HvVRN1* allele with a deletion in the first regulatory intron, whereas S42-IL176 carries an introgression of the full-length winter *Hvvrn1* allele. Although high ambient temperature delayed reproductive development in both genotypes,

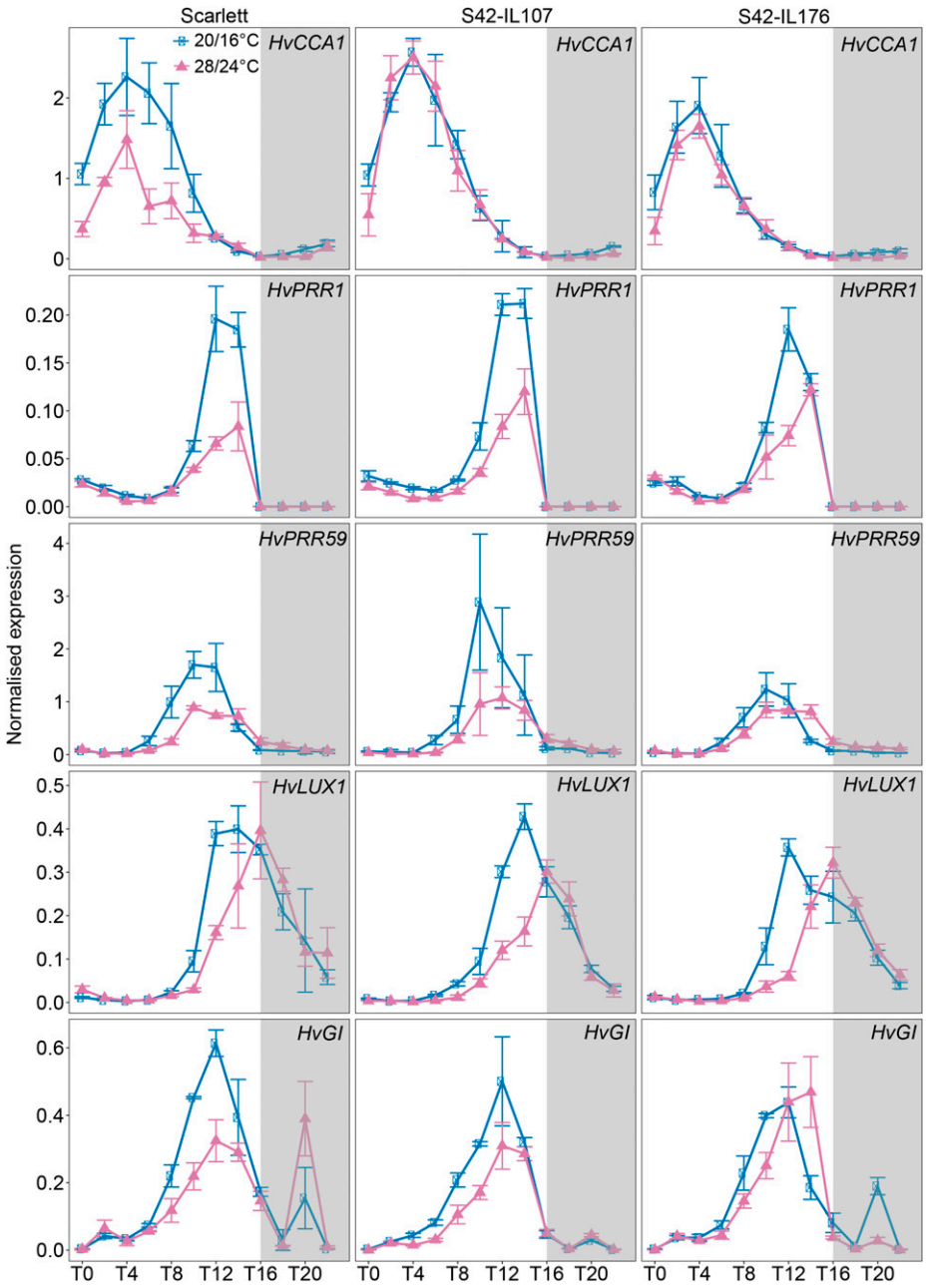

Figure 3. Diurnal expression patterns of circadian clock genes in Scarlett, S42-IL107, and S42-IL176 under control and high ambient temperatures. Diurnal expression of circadian clock genes was assayed every 2 h for 24 h under control (blue; 20°C/16°C, day/night) and high ambient (pink; 28°C/24°C, day/night) temperatures under LDs (16 h light/8 h night). Gray boxes indicate nights. Error bars indicate ± SD of three biological replicates.

the effect was more pronounced in S42-IL176, which did not undergo floral transition and did not flower until 160 d after emergence (DAE) when the experiment was stopped (Fig. 2F, 1A). Consequently, the full-length intron of *Hvvrn1* was correlated with a strong delay in floral transition under high ambient temperatures. In order to assess if variation at *HvVRN1* was also associated with inflorescence development in response to ambient temperature, we shifted Scarlett and S42-IL176 plants from 20°C/16°C to 28°C/24°C only after floral transition (W2.0). Under these conditions, the IL with the winter *Hvvrn1* allele also showed a strong delay in inflorescence development under high ambient temperatures compared to control conditions (Supplemental Fig. S1B). Flowering was delayed by about 2 weeks under 28°C/24°C compared to 20°C/16°C. However, S42-IL176 plants were able to produce flowers and seeds, when the temperature treatment was started after floral transition (Supplemental Fig. S1C).

High Ambient Temperature Affects the Expression of Clock Genes

To further characterize the *Ppd-H1*-, *HvELF3*-, and *HvVRN1*-dependent effects of high temperature on barley development, we analyzed the expression of

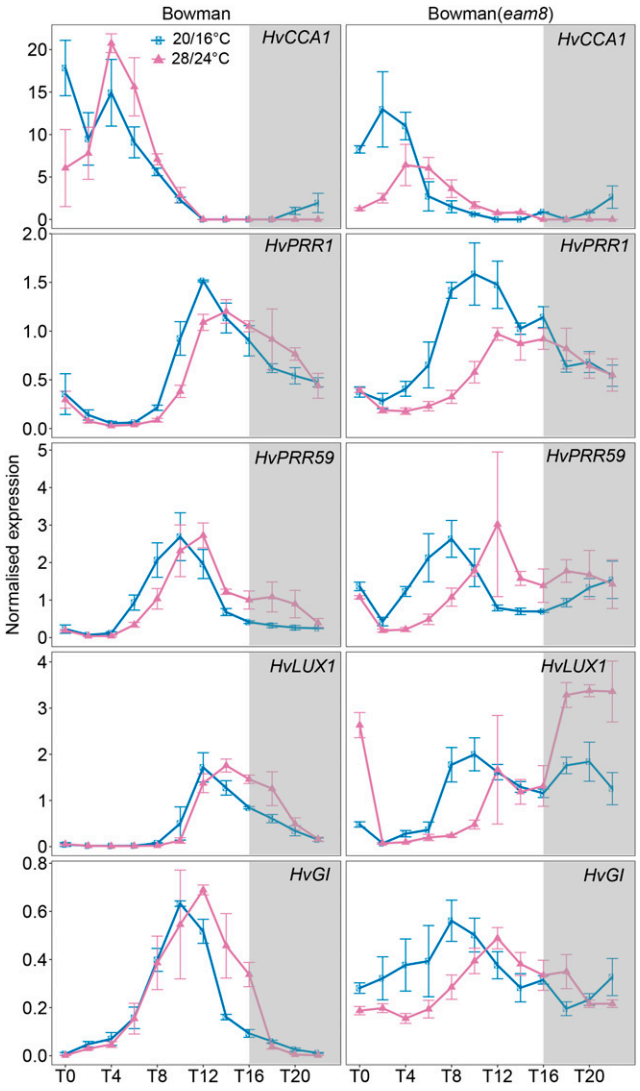

Figure 4. Diurnal expression of circadian clock genes in Bowman and Bowman(*eam8*) under control and high ambient temperatures. Diurnal expression of circadian clock genes was assayed every 2 h for 24 h under control (blue; 20°C/16°C, day/night) and high ambient (pink; 28°C/24°C, day/night) temperatures under LDs (16 h light/8 h night) are shown. Gray boxes indicate nights. Error bars indicate ± SD of three biological replicates.

barley genes from the circadian clock, photoperiod, and vernalization response pathways in the parental and ILs. Because the barley clock is plastic under abiotic stresses, we first tested the effects of high ambient temperature on variation in the diurnal pattern of clock gene expression. Under control conditions, the circadian clock genes showed a diurnal pattern of expression with clock genes peaking at different times of the day, corroborating previous results (Campoli et al., 2012b; Habte et al., 2014). The expression phase of clock genes did not differ between the parental lines Scarlett, and the ILs S42-IL107 and S42-IL176, suggesting that *Ppd-H1* and *HvVRN1* did not affect diurnal clock oscillations. By contrast, the expression phase and shape of clock genes were significantly different between Bowman and Bowman(*eam8*). The expression phase of the clock genes in Bowman(*eam8*) was advanced by 2 h. The expression peaks were less defined and broader in Bowman(*eam8*) than in Bowman. Moreover, Bowman(*eam8*) exhibited higher levels of *Ppd-H1* expression at most time points during the day compared to Bowman. Consequently, the loss-of-function mutation in *HvELF3* affected the diurnal pattern of clock gene expression and caused a strong increase in *Ppd-H1* expression independent of the ambient temperature.

High ambient temperatures caused a decrease in the expression of clock genes, as seen for most clock genes in Scarlett and for *HvCCA1* and *HvPRR1* in Bowman (Figs. 3 and 4). Furthermore, the expression phase of clock genes was delayed by 4 h under high ambient temperature compared to control conditions in Scarlett and Bowman. This reduction in expression amplitude and the shift in the expression phase were also observed in all ILs, suggesting that temperature affected the phase of clock gene expression independently of the genotype.

High Ambient Temperature Reduces Expression of Flowering Time Genes

As the clock genes are putative upstream regulators of flowering-time genes, we investigated whether the temperature-dependent changes in clock gene expression correlated with changes in the expression of flowering-time genes. As observed for the clock genes, most flowering-time regulators showed a significantly lower expression under high ambient temperature. *Ppd-H1* exhibited a reduction in expression in Scarlett, Scarlett-derived ILs, and Bowman(*eam8*), but not in Bowman under high ambient temperature. The expression levels of *HvCO1*, the barley homolog of the major Arabidopsis photoperiod response gene CONSTANS, were reduced, and the peak expression was delayed by approximately 4 h under high ambient temperature in Scarlett and Scarlett-derived ILs (Supplemental Fig. S4). While in Bowman *HvCO1* expression peaked at dusk (T16) under control temperature, it showed an expression peak in the night at T20 under high ambient temperature (Supplemental Fig. S5). This suggested that *HvCO1* expression was controlled by the clock and a temperature-dependent phase shift of clock genes. However, no consistent changes in the level and peak time of *HvCO1* expression were observed in Bowman and Bowman(*eam8*) (Supplemental Fig. S5).

The expression levels of *HvFT1*, a putative target of *Ppd-H1*, were significantly down-regulated under high temperature in all genotypes. In addition, *HvFT1* expression levels were overall significantly different between genotypes with higher transcript abundance in

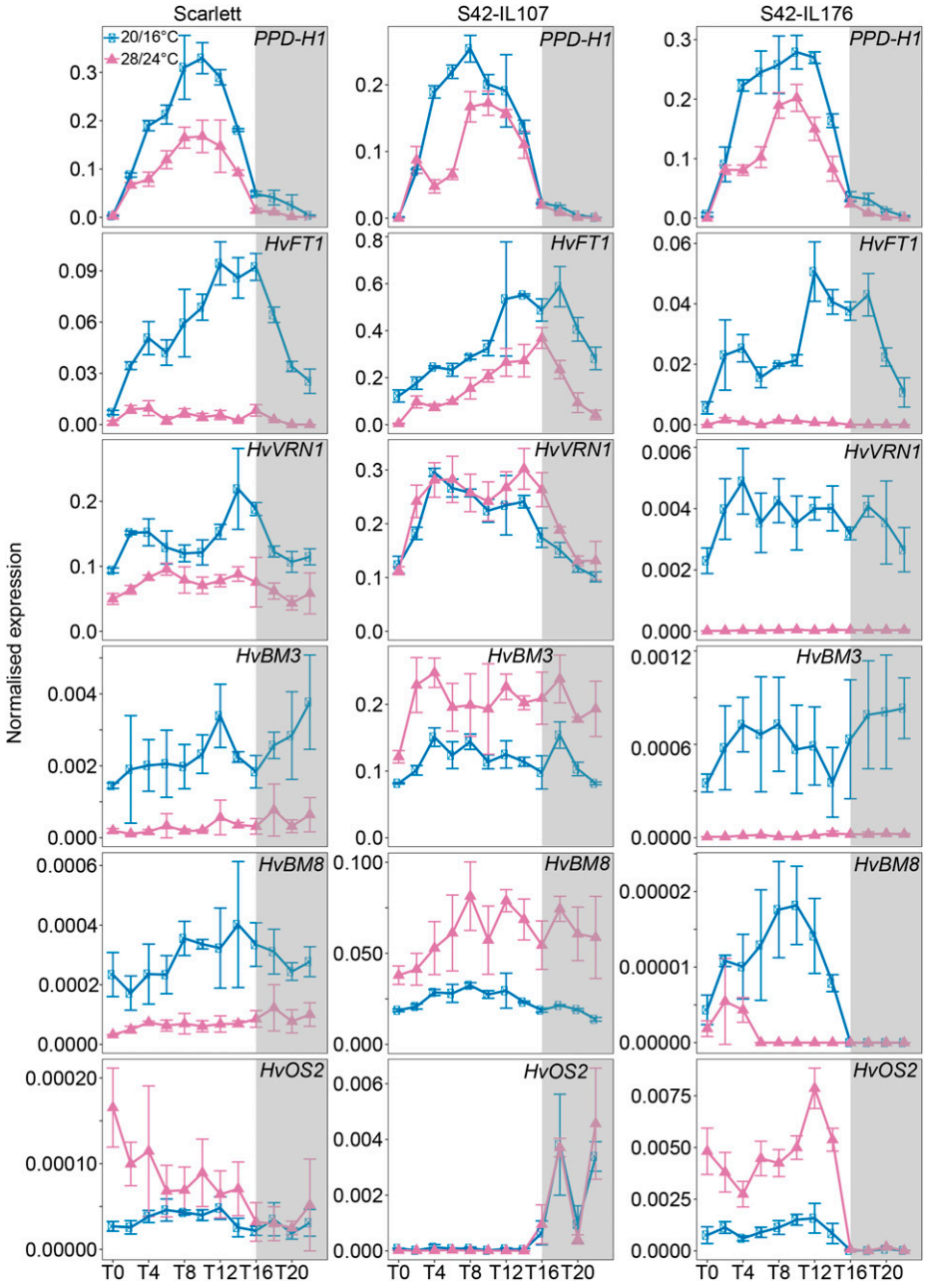

Figure 5. High ambient temperature affects the expression of flowering-time genes in Scarlett, S42-IL107, and S42-IL176 under control and high ambient temperatures. Diurnal expression of flowering-time genes was assayed every 2 h for 24 h under control (blue; 20°C/16°C, day/night), and high ambient (pink; 28°C/24°C, day/night) temperatures under LDs (16 h light/8 h night) are shown. Gray boxes indicate nights. Error bars indicate ± SD of three biological replicates.

S42-IL107 and Bowman(*eam8*) and lower transcript levels in S42-IL176 compared to the parental lines (Figs. 5 and 6).

The MADS-box genes *HvVRN1*, *HvBM3*, and *HvBM8* were also strongly down-regulated under high versus control temperatures. In S42-IL176, the expression levels of the winter *Hvvrn1* allele were 90-fold lower, while the expression levels of the spring *HvVRN1* allele in Scarlett were only 2-fold lower under high ambient compared to control temperature (Fig. 5). This suggested that the winter allele of *HvVRN1* was repressed by high ambient temperatures (Fig. 5). The expression patterns of *HvBM3* and *HvBM8* were comparable to those of *HvVRN1* with a stronger temperature-dependent down-regulation in S42-IL176 compared to Scarlett. In contrast, S42-IL107 with a dominant *Ppd-H1* allele exhibited an up-regulation of *HvVRN1*, *HvBM3*, and *HvBM8* under high compared to control temperatures (Fig. 5). In Bowman(*eam8*), expression levels of the *HvBM* genes were approximately 10-fold higher compared to Bowman under control and high ambient temperature conditions. In addition, *HvVRN1* and *HvBM3* were only slightly down-regulated under high versus control temperatures, while expression of *HvBM8* was not significantly different between control and high-temperature conditions (Fig. 6). *HvOS2*, a

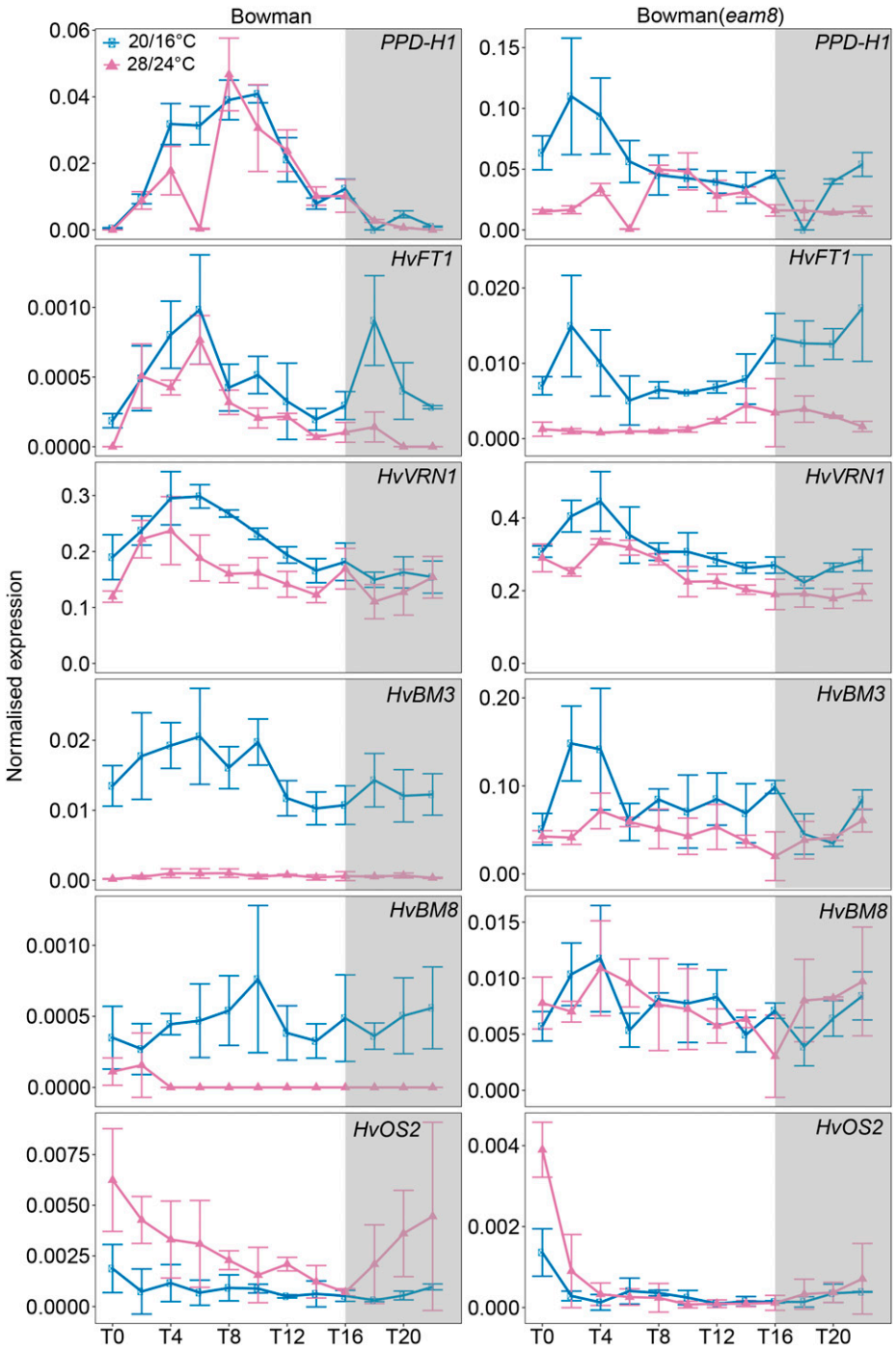

Figure 6. Diurnal expression of flowering-time genes in Bowman and Bowman(*eam8*) under control and high ambient temperatures. Diurnal expression of flowering-time genes sampled every 2 h for 24 h under control (blue; 20°C/16°C, day/night) and high ambient (pink; 28°C/24°C, day/night) temperatures under LDs (16 h light/8 h night) are shown. Gray boxes indicate nights. Error bars indicate ± SD of three biological replicates.

repressor of flowering and homolog of the major Arabidopsis vernalization gene *FLC* (Greenup et al., 2010, Ruelens et al., 2013), was up-regulated under high versus control temperatures and was controlled by *Ppd-H1*, *HvELF3*, and *HvVRN1*. Expression levels of *HvOS2* were up-regulated under high ambient temperature in Scarlett and Bowman, but very low during the day in S42-IL107 and Bowman(*eam8*) under both temperatures. *HvOS2* expression levels were further increased under high temperatures in S42-IL176 with the winter *Hvvrn1* allele and no detectable expression of *Hvvrn1*. *HvOS2* expression levels were consequently negatively correlated with *HvVRN1* expression and controlled by ambient temperature.

Variation at *Ppd-H1* and *HvELF3*, therefore, correlated with the temperature-dependent regulation of the MADS-box transcription factor genes. It is interesting to note, that in S42-IL107, the expression patterns of *HvFT1* and the *HvBM* genes were not correlated under the different temperature regimes, as the *HvBM* genes

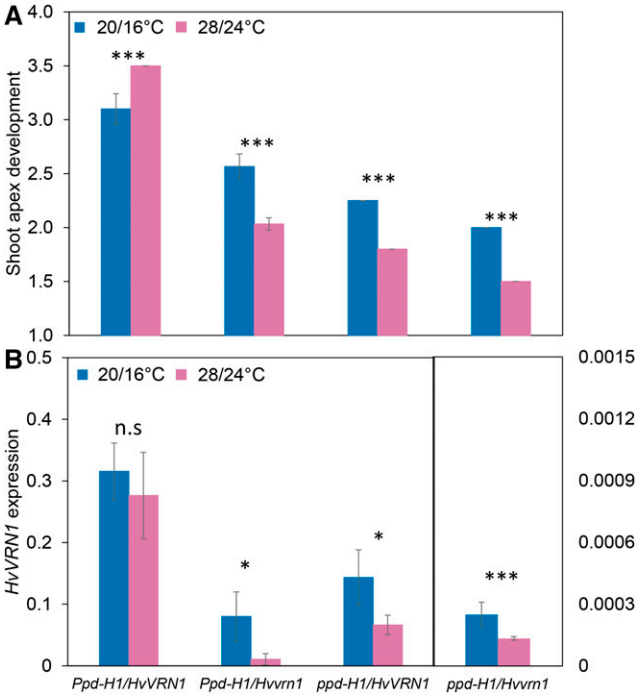

Figure 7. *Ppd-H1* and *HvVRN1* interact to control the development of the MSA under different ambient temperatures. Microscopic changes in MSA development were scored under control (blue; 20°C/16°C, day/night) and high ambient (pink; 28°C/24°C, day/night) temperatures under LDs (16 h light/8 h night) in F3 families derived from a cross between the winter barley Igri and the spring barley Golden Promise. Selected F3 families segregated for *Ppd-H1* and *HvVRN1* and were fixed for the spring alleles at *HvVRN2* (deleted) and *HvFT1*. Early MSA development was accelerated under high temperature in *Ppd-H1/HvVRN1* and delayed in *Ppd-H1/Hvvrn1*, *ppd-H1/HvVRN1*, and *ppd-H1/Hvvrn1*. Significant differences were determined by a two-way ANOVA and a Tukey HSD pairwise comparison test: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$., n.s = nonsignificant.

were up-regulated, but *HvFT*1 was down-regulated under high compared to control temperatures. The expression patterns of the *HvBM* genes, but not of *HvFT1*, correlated with the differential flowering time in response to high ambient temperatures. Low expression of the *HvBM* genes under high temperatures in Scarlett and Bowman coincided with a delay in reproductive development, while accelerated inflorescence development in S42-IL107 correlated with an up-regulation of the *HvBM* genes under high ambient versus control temperatures. In Bowman(*eam8*) with accelerated development under high temperatures, the expression of *HvBM3* and *HvBM8* was strongly increased compared to Bowman and not very different between temperature regimes. In S42-IL176 with a winter *Hvvrn1* allele, a complete down-regulation of *HvFT1* and *HvBM* genes correlated with a strong delay in reproductive development, as this genotype did not undergo floral transition under high temperatures.

Taken together, the wild-type *Ppd-H1* and a loss-of-function *Hvelf3* allele correlated with an accelerated development under high compared to control temperatures and a higher expression of *HvBM* genes under high compared to control temperatures. In addition, variation in the regulatory region of the first intron in *HvVRN1* controlled the expression of *HvVRN1* itself, of the related *HvBM* genes and reproductive development under high ambient temperatures.

Ppd-H1 and *HvVRN1* Interact to Control Inflorescence Development under High Ambient Temperatures

Our results showed that variation at *Ppd-H1* was correlated with the expression of *HvBM* genes including *HvVRN1* under high compared to control temperatures. Therefore, we examined if *Ppd-H1* and *HvVRN1* interacted to control reproductive development under different ambient temperatures. For this purpose, we analyzed MSA development and gene expression of *HvVRN1* in F_3 families selected from a cross between the winter barley variety Igri and the spring barley variety Golden Promise. The F3 families were segregating for variation at *Ppd-H1* and *HvVRN1* but were fixed for the spring alleles at the other major flowering loci *HvVRN2* and *HvFT1*. Reproductive development was delayed under high ambient temperatures in F_3 plants with a spring *ppd-H1* allele irrespective of the *HvVRN1* allele as seen for Scarlett and S42-IL176. In addition, under high ambient temperature, the dominant *Ppd-H1* allele accelerated development in the background of a spring *HvVRN1* allele, as observed for S42-IL107. F_3 plants carrying a winter *Hvvrn1* allele and a wild-type *Ppd-H1* allele exhibited a delay in MSA development under high ambient temperature compared to control conditions (Fig. 7A). Consequently, *Ppd-H1* interacted with *HvVRN1* to control the development under high temperatures, where only plants with a dominant *Ppd-H1* and a spring *HvVRN1* allele showed an accelerated development under high versus control temperatures. The expression of the spring *HvVRN1* allele was not affected in the presence of a dominant *Ppd-H1* allele under high ambient versus control temperatures. However, the winter *Hvvrn1* allele was down-regulated in the *Ppd-H1* and *ppd-H1* backgrounds under high compared to control temperature (Fig. 7B). The winter *Hvvrn1* allele was stronger down-regulated than the spring *HvVRN1* allele as shown for Scarlett and S42-IL176. These results indicated that *Ppd-H1* interacts with *HvVRN1*, where a dominant *Ppd-H1* allele only accelerated floral development under high ambient temperature in the background of a spring *HvVRN1* allele.

DISCUSSION

Understanding how ambient temperature controls plant development and eventually grain yield in crop plants is gaining importance in the light of a predicted increase in average global temperatures. The circadian clock influences plant adaptation to different abiotic

stresses and controls many different output traits, including plant development. Furthermore, the circadian clock itself is altered in response to changing environmental conditions. For example, osmotic stress increased the amplitude and advanced the expression phase of clock genes in barley, and high salinity resulted in a lengthening of the circadian period in wheat (Erdei et al., 1998; Habte et al., 2014). We found that an increase in ambient temperature from 20°C/16°C to 28°C/24°C decreased expression levels and delayed the phase of clock gene expression. Although the clock is temperature-compensated and maintains an approximately 24 h period over a range of ambient temperatures (Pittendrigh, 1954; Gould et al., 2006; Salomé et al., 2010), previous studies have reported changes in the expression phase and amplitude of oscillator components under different temperatures. For example, in Arabidopsis peak expression levels of *CCA1* and *LHY* RNA rhythms increased in amplitude as temperatures decreased from 17°C to 12°C (Gould et al., 2006; Mizuno et al., 2014). Temperatures of above 30°C are considered as heat stress for temperate cereals (Barnabás et al., 2008). However, an induction of a stress response when increasing the temperature from 20°C to 28°C cannot be excluded. Therefore, the observed alterations in clock oscillations in this work may be related to changes in the level of stress-response hormones. In Arabidopsis, application of the stress hormone abscisic acid lengthened the period of the Arabidopsis clock (Hanano et al., 2006), probably through evolutionary conserved ABA-responsive elements (ABREs) ABREs present in the promoters of *TIMING OF CAB EXPRESSION1*, LHY, and CCA1 (Bieniawska et al., 2008; Spensley et al., 2009; Picot et al., 2010; Habte et al., 2014). In addition, the heat shock transcription factor *HsfB2b* repressed transcription of *PRR7* at high temperatures and in response to drought (Kolmos et al., 2014). Salomé et al. (2010) and Kolmos et al. (2014) found that the *PRR* genes are important for the temperature compensation of the clock in Arabidopsis, as high temperature led to overcompensation and lengthening of the period in a *HsfB2b* overexpression line or double *prr7/9* mutant. In our study, the changes in clock gene expression under high ambient temperature were also observed in S42-IL107 and Bowman(*eam8*), suggesting that these temperature-mediated changes of the clock were not controlled by the *PRR* homolog *Ppd-H1* or its upstream regulator *HvELF3*. In addition, the down-regulation of all *PRR* genes under high ambient temperature suggested that the repressive EC consisting of *HvELF3*, *HvELF4*, and *HvLUX1* was not reduced in its activity under high temperature in barley as demonstrated for Arabidopsis (Mizuno et al., 2014).

Although the function of clock plasticity under different environmental conditions is not well understood, it may affect the expression of different clock output genes and related traits. We observed that the altered clock expression patterns correlated with changes in the diurnal expression patterns of flowering-time genes. Similar to the reduction in the expression amplitudes of clock genes, the expression levels of the majority of flowering-time genes including *Ppd-H1* and its downstream target *HvFT1* were strongly reduced under high ambient temperatures. However, in contrast to the clock genes, temperature-dependent changes in the expression of flowering-time genes were controlled by *Ppd-H1*, *HvELF3*, and *HvVRN1*. *Ppd-H1* and *HvFT1* transcripts were reduced under high compared to the control temperatures in all genotypes. In contrast, relative expression patterns of *BARLEY MADS-box* (*BM*) genes *HvBM3*, *HvVRN1* (*HvBM5a*), and *HvBM8* were genotype and condition specific. While in Scarlett *HvVRN1*, *HvBM3*, and *HvBM8* were down-regulated, they were not down-regulated or even up-regulated under high versus control temperature in S42-IL107 and Bowman(*eam8*). This indicated that *Ppd-H1* and *HvELF3* controlled the relative expression levels of *BM* genes under different ambient temperature conditions. *HvBM3* and *HvBM8* are known targets of *Ppd-H1* under LD conditions and their expression patterns correlate with the development of the inflorescence (Digel et al., 2015, 2016). In this study, we show that the effect of *Ppd-H1* on *HvBM3* and *HvBM8* expression and flowering time was temperature dependent. Scarlett and Bowman with a mutated *ppd-H1* allele showed a relatively lower expression of *HvBM3*, *HvVRN1*, and *HvBM8* and a delay in floral development under high versus control temperatures. S42-IL107, with a wild type *Ppd-H1* allele, exhibited a relatively higher expression of *HvBM3*, *HvVRN1*, and *HvBM8* and was characterized by a faster inflorescence development under high versus control temperatures. Interestingly, functional variation at *Ppd-H1* also had a strong effect on the number of florets and seeds per main spike under high ambient temperatures. While in Scarlett and Bowman the mutated *ppd-H1* allele was correlated with a strong reduction of the number of seeds per main spike, S42-IL107 did not show a significant reduction in seed number under high temperatures. This suggested that *Ppd-H1* affected floret fertility and seed set under high ambient temperatures, possibly by controlling the rate of development of the inflorescence.

A previous study found that high ambient temperatures accelerated flowering time under LDs, but delayed development under SDs in a winter barley with a wild-type *Ppd-H1* allele (Hemming et al., 2012). The authors suggested an interaction between the photoperiod and thermosensitive pathway. Our study demonstrates that this interaction is mediated by *Ppd-H1*, which is functional under LDs, but not under SDs (Digel et al., 2015). Furthermore, we show that the effect of *Ppd-H1* on early reproductive development under high temperatures is dependent on *HvVRN1*. Only in the background of a spring *HvVRN1* allele or after up-regulation of *Hvvrn1* by vernalization, the wild-type *Ppd-H1* allele is capable of accelerating early reproductive development under high ambient temperatures.

Among the *BM* genes, *HvVRN1* has been extensively characterized for its role in vernalization response. The

winter *HvVRN1* allele is up-regulated by a prolonged exposure to cold to allow flowering after winter. Our results suggest that *HvVRN1* expression is negatively regulated by high ambient temperature, and this down-regulation of the winter *Hvvrn1* allele correlated with a strong delay in reproductive development. The full-length winter *Hvvrn1* allele in S42-IL176 was more strongly down-regulated by high ambient temperature compared to the spring *HvVRN1* allele with a deletion in the first intron. Interestingly, a recent study has revealed that natural variation in the first intron of the *MADS-box* gene *FLM* was responsible for differential temperature response in Arabidopsis (Lutz et al., 2015). Consequently, structural variation in related *MADS-box* transcription factors may play a role in temperature adaptation across different species. In Arabidopsis, high ambient temperature accelerates plant development and growth. However, different Arabidopsis ecotypes show substantial variation in the thermosensitive response mediated by natural variation at the vernalization gene and floral repressor *FLC*. High expression levels of *FLC* in autonomous pathway mutants functioned as a potent suppressor of thermal induction (Balasubramanian et al., 2006). *HvOS2*, the putative barley homolog of *FLC*, was up-regulated under high ambient temperature in a *HvVRN1*-dependent manner. The barley vernalization gene and floral inducer *HvVRN1*, in turn, was down-regulated by high temperature, and this correlated with a down-regulation of *HvBM3* and *HvBM8* and a delay in floral development. Different vernalization genes might, therefore, mediate thermosensitive flowering across different species.

CONCLUSION

Our study demonstrates that an interaction of *Ppd-H1* and *HvVRN1* controls reproductive development and the number of seeds per spike under high ambient temperatures. These genetic interactions between *Ppd-H1* and *HvVRN1* are important to consider for breeding barley better adapted to climate change.

MATERIALS AND METHODS

Plant Material, Growth Conditions, and Phenotyping

Flowering time, development of the SAM, flower fertility, and seed set were scored in the spring cultivars Bowman and Scarlett and four derived ILs. Bowman and Scarlett are characterized by a mutation in the CCT domain of *Ppd-H1* and the spring allele (*HvVRN1-1*; Hemming et al., 2009) at the vernalization response gene *HvVRN1*. The IL Bowman(*eam8*.w) carries a base pair mutation leading to a premature stop codon in *HvELF3*, orthologous to *ELF3* in Arabidopsis (*Arabidopsis thaliana*; Faure et al., 2012). Bowman(*Ppd-H1*) carries an introgression of the dominant *Ppd-H1* allele from wild barley (*Hordeum vulgare*; Druka et al., 2011). The Scarlett derived ILs S42-IL107 and S42-IL176 carry a dominant allele of *Ppd-H1* and a recessive winter *Hvvrn1* allele, respectively, both derived from wild barley (von Korff et al., 2006; Schmalenbach et al., 2008; Wang et al., 2010). In addition, development of the MSA and expression of *HvVRN1* were analyzed in selected F_3 families derived from a cross between the winter barley Igri and the spring barley Golden Promise. These F_3 families segregated for natural variation at *Ppd-H1* and *HvVRN1* and were fixed for the spring alleles at *HvFT1* and *VRN-H2* (locus deleted, Mulki et al., 2016).

For scoring of shoot apex development, flowering time, floret number, and seed number per spike seeds were stratified at 4°C for 5 d for even germination followed by a transfer to controlled growth chambers with day/night temperatures of 20°C/16°C or 24°C/28°C, a light intensity of ~300 μM and long photoperiods (LD; 16 h light/8 h dark). Light and temperature were monitored throughout the experiments using WatchDog series 1000 light sensors. Plants were fertilized once per week, and trays were shuffled twice a week to normalize for position effects. Plants were either shifted to high ambient temperatures after stratification or after floral transition as determined by the formation of a double ridge SAM (Waddington et al., 1983). Experiments were replicated two or three times using different randomizations to minimize the environmental effects.

The MSA of three to four representative plants per genotype (Bowman, Bowman(*eam8*), Scarlett, S42-IL107, and S42-IL176) and treatment were dissected every 3 to 7 d starting from the third DAE until 36 DAE. At each time point, the developmental stage of the MSA was determined according to the quantitative scale of Waddington et al. (1983), which rates the development of the most advanced floret primordium. Images of apices were obtained using the Nikon imaging software and a stereo microscope (Nikon SMZ18) equipped with a digital camera (Nikon digital sight DS-U3). The apex was dissected with a microsurgical stab knife (5 mm blade at 15° [SSC#72-1551], Sharepoint, Surgical Specialties) under the stereo microscope to confirm the developmental stage of each harvested MSA. In addition, morphological phenotypes of the main shoot, i.e. heading date (at Z49; Zadoks et al., 1974), the number of florets per spike, and the number of grains per spike, were recorded during development and at plant maturity for 20 plants per genotype.

Leaf Sampling, RNA Extraction, and Gene Expression Analysis

For the analysis of diurnal expression variation in clock and flowering-time genes in Scarlett, S42-IL107, S42-IL176, Bowman, and Bowman(*eam8*.w), plants were grown in 96-well trays (Einheitserde) under day/night temperatures of 20°C/16°C or 24°C/28°C, a light intensity of ~300 μM and long photoperiods (LD; 16 h light/8 h dark). Leaf samples were harvested 21 DAE at 2 h intervals starting from the onset of light (ZT0) to the end of the night (T22). For all genotypes and treatment conditions, three biological replicates of two pooled plants were sampled per time point. Total RNA extraction, cDNA synthesis, and quantitative real-time-PCRs using gene-specific primers as detailed in Supplemental Table S2 were performed as explained in Campoli et al. (2012a, 2012b). The expression of target genes was normalized against the geometric mean of the three internal controls *HvACTIN*, *HvGAPDH*, and *HvβTUBULIN* (Supplemental Table S1). Two technical replicates were used for each sample, and each data point was quantified based on the titration curve for each target gene and normalized against the geometric mean of the three housekeeping genes using the LightCycler 480 Software (Roche; version 1.5).

Statistical Analysis

Significant differences in flowering time, floret, and seed number were calculated with a two-factorial ANOVA with the factors genotype and temperature treatment. In addition, least square means for each gene by temperature combination were calculated followed by a Tukey's multiple comparison test. Significant differences in *HvVRN1* expression were calculated with an ANOVA including temperature treatment, *HvVRN1*, and *Ppd-H1* genotype and all possible interaction effects. Statistical differences in the MSA development between temperature regimes were calculated using a polynomial regression model at 95% confidence interval (Loess smooth line).

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. *HvVRN1* affects reproductive development in response to ambient temperature after floral transition.

Supplemental Figure S2. Diurnal expression of circadian clock genes *HvPRR73* and *HvPRR95* in Scarlett, S42-IL107, and S42-IL176 under control and high ambient temperatures.

Supplemental Figure S3. Diurnal expression of circadian clock genes *HvPRR73* and *HvPRR95* in Bowman and Bowman(*eam8*) under control and high ambient temperatures.

Supplemental Figure S4. High ambient temperature down-regulates the expression of the flowering-time gene *HvCO1* in Scarlett, S42-IL107, and S42-IL176.

Supplemental Figure S5. Effect of high ambient temperature on diurnal expression of the flowering-time gene *HvCO1* in Bowman and Bowman(*eam8*).

Supplemental Table S1. Two-factorial ANOVA and least square means for heading date, floret, and seed number.

Supplemental Table S2. List of primers used in this study.

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LITERATURE CITED

Appendino M, Slafer GA (2003) Earliness per se and its dependence upon temperature in diploid wheat lines differing in the allelic constitution of a major gene Eps-A^{m}1 alleles. J Agric Sci **141:** 149–154

Balasubramanian S, Sureshkumar S, Lempe J, Weigel D (2006) Potent induction of Arabidopsis thaliana flowering by elevated growth temperature. PLoS Genet **2:** e106

Barnabás B, Jäger K, Fehér A (2008) The effect of drought and heat stress on reproductive processes in cereals. Plant Cell Environ **31:** 11–38

Bieniawska Z, Espinoza C, Schlereth A, Sulpice R, Hincha DK, Hannah MA (2008) Disruption of the Arabidopsis circadian clock is responsible for extensive variation in the cold-responsive transcriptome. Plant Physiol **147:** 263–279

Box MS, Huang BE, Domijan M, Jaeger KE, Khattak AK, Yoo SJ, Sedivy EL, Jones DM, Hearn TJ, Webb AA, et al (2015) ELF3 controls thermoresponsive growth in Arabidopsis. Curr Biol **25:** 194–199

Bullrich L, Appendino L, Tranquilli G, Lewis S, Dubcovsky J (2002) Mapping of a thermo-sensitive earliness per se gene on Triticum monococcum chromosome 1A(m). Theor Appl Genet **105:** 585–593

Campoli C, Drosse B, Searle I, Coupland G, von Korff M (2012a) Functional characterisation of HvCO1, the barley (Hordeum vulgare) flowering time ortholog of CONSTANS. Plant J **69:** 868–880

Campoli C, Pankin A, Drosse B, Casao CM, Davis SJ, von Korff M (2013) 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 Phytol **199:** 1045–1059

Campoli C, Shtaya M, Davis SJ, von Korff M (2012b) Expression conservation within the circadian clock of a monocot: natural variation at barley Ppd-H1 affects circadian expression of flowering time genes, but not clock orthologs. BMC Plant Biol **12:** 97

Corbesier L, Vincent C, Jang S, Fornara F, Fan Q, Searle I, Giakountis A, Farrona S, Gissot L, Turnbull C, et al (2007) FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. Science **316:** 1030–1033

Digel B, Pankin A, von Korff M (2015) Global transcriptome profiling of developing leaf and shoot apices reveals distinct genetic and environmental control of floral transition and inflorescence development in barley. Plant Cell **27:** 2318–2334

Digel B, Tavakol E, Verderio G, Tondelli A, Xu X, Cattivelli L, Rossini L, von Korff M (2016) Photoperiod-H1 (Ppd-H1) controls leaf size. Plant Physiol **172:** 405–415

Distelfeld A, Li C, Dubcovsky J (2009) Regulation of flowering in temperate cereals. Curr Opin Plant Biol **12:** 178–184

Druka A, Sato K, Muehlbauer GJ (2011) Genome Analysis: The state of knowledge of barley genes. *In* SE Ulrich, ed, Barley: Production, Improvement, and Uses, John Wiley and Sons, New York, pp. 85–111

Erdei L, Szegletes Z, Barabás KN, Pestenácz A, Fülöp K, Kalmár L, Kovács A, Tóth B, Dér A (1998) Environmental stress and the biological clock in plants: changes of rhythmic behavior of carbohydrates, antioxidant enzymes and stomatal resistance by salinity. J Plant Physiol **152:** 265–271

Faure S, Turner AS, Gruszka D, Christodoulou V, Davis SJ, von Korff M, Laurie DA (2012) Mutation at the circadian clock gene EARLY MATURITY 8 adapts domesticated barley (Hordeum vulgare) to short growing seasons. Proc Natl Acad Sci USA **109:** 8328–8333

Gould PD, Locke JC, Larue C, Southern MM, Davis SJ, Hanano S, Moyle R, Milich R, Putterill J, Millar AJ, et al (2006) The molecular basis of temperature compensation in the Arabidopsis circadian clock. Plant Cell **18:** 1177–1187

Greenup AG, Sasani S, Oliver SN, Talbot MJ, Dennis ES, Hemming MN, Trevaskis B (2010) ODDSOC2 is a MADS box floral repressor that is down-regulated by vernalization in temperate cereals. Plant Physiol, **153:** 1062–1073

Gu X, Le C, Wang Y, Li Z, Jiang D, Wang Y, He Y (2013) Arabidopsis FLC clade members form flowering-repressor complexes coordinating responses to endogenous and environmental cues. Nat Commun **4:** 1947

Habte E, Müller LM, Shtaya M, Davis SJ, von Korff M (2014) Osmotic stress at the barley root affects expression of circadian clock genes in the shoot. Plant Cell Environ **37:** 1321–1327

Halliday KJ, Salter MG, Thingnaes E, Whitelam GC (2003) Phytochrome control of flowering is temperature sensitive and correlates with expression of the floral integrator FT. Plant J **33:** 875–885

Hanano S, Domagalska MA, Nagy F, Davis SJ (2006) Multiple phytohormones influence distinct parameters of the plant circadian clock. Genes Cells **11:** 1381–1392

Hemming MN, Fieg S, Peacock WJ, Dennis ES, Trevaskis B (2009) Regions associated with repression of the barley (Hordeum vulgare) VERNALIZATION1 gene are not required for cold induction. Mol Genet Genomics **282:** 107–117

Hemming MN, Walford SA, Fieg S, Dennis ES, Trevaskis B (2012) Identification of high-temperature-responsive genes in cereals. Plant Physiol **158:** 1439–1450

Herrero E, Kolmos E, Bujdoso N, Yuan Y, Wang M, Berns MC, Uhlworm H, Coupland G, Saini R, Jaskolski M, et al (2012) EARLY FLOWERING4 recruitment of EARLY FLOWERING3 in the nucleus sustains the Arabidopsis circadian clock. Plant Cell **24:** 428–443

Hong RL, Hamaguchi L, Busch MA, Weigel D (2003) Regulatory elements of the floral homeotic gene AGAMOUS identified by phylogenetic footprinting and shadowing. Plant Cell **15:** 1296–1309

Jaeger KE, Wigge PA (2007) FT protein acts as a long-range signal in Arabidopsis. Curr Biol **17:** 1050–1054

Karsai I, Igartua E, Casas A, Kiss T, Soós V, Balla K, Bedő Z, Veisz O (2013) Developmental patterns of a large set of barley (Hordeum vulgare) cultivars in response to ambient temperature. Ann Appl Biol **162:** 309–323

Kobayashi Y, Weigel D (2007) Move on up, it's time for change—mobile signals controlling photoperiod-dependent flowering. Genes Dev **21:** 2371–2384

Koini MA, Alvey L, Allen T, Tilley CA, Harberd NP, Whitelam GC, Franklin KA (2009) High temperature-mediated adaptations in plant architecture require the bHLH transcription factor PIF4. Curr Biol **19:** 408–413

Kolmos E, Chow BY, Pruneda-Paz JL, Kay SA (2014) HsfB2b-mediated repression of PRR7 directs abiotic stress responses of the circadian clock. Proc Natl Acad Sci USA **111:** 16172–16177

Kumar SV, Lucyshyn D, Jaeger KE, Alós E, Alvey E, Harberd NP, Wigge PA (2012) Transcription factor PIF4 controls the thermosensory activation of flowering. Nature **484:** 242–245

Kumar SV, Wigge PA (2010) H2A.Z-containing nucleosomes mediate the thermosensory response in Arabidopsis. Cell **140:** 136–147

Lee JH, Ryu H-S, Chung KS, Posé D, Kim S, Schmid M, Ahn JH (2013) Regulation of temperature-responsive flowering by MADS-box transcription factor repressors. Science **342:** 628–632

Lee JH, Yoo SJ, Park SH, Hwang I, Lee JS, Ahn JH (2007) Role of SVP in the control of flowering time by ambient temperature in Arabidopsis. Genes Dev **21:** 397–402

Lewis S, Faricelli ME, Appendino ML, Valárik M, Dubcovsky J (2008) The chromosome region including the earliness per se locus Eps-Am1 affects the duration of early developmental phases and spikelet number in diploid wheat. J Exp Bot **59:** 3595–3607

Lobell DB, Schlenker W, Costa-Roberts J (2011) Climate trends and global crop production since 1980. Science **333:** 616–620

Lutz U, Posé D, Pfeifer M, Gundlach H, Hagmann J, Wang C, Weigel D, Mayer KF, Schmid M, Schwechheimer C (2015) Modulation of ambient temperature-dependent flowering in Arabidopsis thaliana by natural variation of FLOWERING LOCUS M. PLoS Genet **11:** e1005588

Mathieu J, Warthmann N, Küttner F, Schmid M (2007) Export of FT protein from phloem companion cells is sufficient for floral induction in Arabidopsis. Curr Biol **17:** 1055–1060

Mizuno T, Nomoto Y, Oka H, Kitayama M, Takeuchi A, Tsubouchi M, Yamashino T (2014) Ambient temperature signal feeds into the circadian clock transcriptional circuitry through the EC night-time repressor in Arabidopsis thaliana. Plant Cell Physiol **55:** 958–976

Mulki MA, von Korff M (2016) CONSTANS controls floral repression by up-regulating VERNALIZATION2 (VRN-H2) in barley. Plant Physiol **170:** 325–337

Nusinow DA, Helfer A, Hamilton EE, King JJ, Imaizumi T, Schultz TF, Farré EM, Kay SA (2011) The ELF4-ELF3-LUX complex links the circadian clock to diurnal control of hypocotyl growth. Nature **475:** 398–402

Pankin A, Campoli C, Dong X, Kilian B, Sharma R, Himmelbach A, Saini R, Davis SJ, Stein N, Schneeberger K, et al (2014) 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 **198:** 383–396

Picot E, Krusche P, Tiskin A, Carré I, Ott S (2010) Evolutionary analysis of regulatory sequences (EARS) in plants. Plant J **64:** 165–176

Pittendrigh CS (1954) On temperature independence in the clock system controlling emergence time in Drosophila. Proc Natl Acad Sci USA **40:** 1018–1029

Pokhilko A, Fernández AP, Edwards KD, Southern MM, Halliday KJ, Millar AJ (2012) The clock gene circuit in Arabidopsis includes a repressilator with additional feedback loops. Mol Syst Biol **8:** 574

Posé D, Verhage L, Ott F, Yant L, Mathieu J, Angenent GC, Immink RG, Schmid M (2013) Temperature-dependent regulation of flowering by antagonistic FLM variants. Nature **503:** 414–417

Pouteau S, Albertini C (2009) The significance of bolting and floral transitions as indicators of reproductive phase change in Arabidopsis. J Exp Bot **60:** 3367–3377

Press MO, Lanctot A, Queitsch C (2016) PIF4 and ELF3 act independently in Arabidopsis thaliana thermoresponsive flowering. PLoS ONE **11:** e0161791

Raschke A, Ibañez C, Ullrich KK, Anwer MU, Becker S, Glöckner A, Trenner J, Denk K, Saal B, Sun X, et al (2015) Natural variants of ELF3 affect thermomorphogenesis by transcriptionally modulating PIF4-dependent auxin response genes. BMC Plant Biol **15:** 197

Ruelens P, de Maagd RA, Proost S, Theißen G, Geuten K, Kaufmann K (2013) FLOWERING LOCUS C in monocots and the tandem origin of angiosperm-specific MADS-box genes. Nat Commun **4:** doi/10.1038/ncomms3280

Salomé PA, Weigel D, McClung CR (2010) The role of the Arabidopsis morning loop components CCA1, LHY, PRR7, and PRR9 in temperature compensation. Plant Cell **22:** 3650–3661

Schauer SE, Schlüter PM, Baskar R, Gheyselinck J, Bolaños A, Curtis MD, Grossniklaus U (2009) Intronic regulatory elements determine the divergent expression patterns of AGAMOUS-LIKE6 subfamily members in Arabidopsis. Plant J **59:** 987–1000

Schmalenbach I, Körber N, Pillen K (2008) Selecting a set of wild barley introgression lines and verification of QTL effects for resistance to powdery mildew and leaf rust. Theor Appl Genet **117:** 1093–1106

Spensley M, Kim J-Y, Picot E, Reid J, Ott S, Helliwell C, Carré IA (2009) Evolutionarily conserved regulatory motifs in the promoter of the Arabidopsis clock gene LATE ELONGATED HYPOCOTYL. Plant Cell **21:** 2606–2623

Sureshkumar S, Dent C, Seleznev A, Tasset C, Balasubramanian S (2016) Nonsense-mediated mRNA decay modulates FLM-dependent thermosensory flowering response in Arabidopsis. Nat Plants **2:** 16055

Tamaki S, Matsuo S, Wong HL, Yokoi S, Shimamoto K (2007) Hd3a protein is a mobile flowering signal in rice. Science **316:** 1033–1036

Thines B, Harmon FG (2010) Ambient temperature response establishes ELF3 as a required component of the core Arabidopsis circadian clock. Proc Natl Acad Sci USA **107:** 3257–3262

Thines BC, Youn Y, Duarte MI, Harmon FG (2014) The time of day effects of warm temperature on flowering time involve PIF4 and PIF5. J Exp Bot **65:** 1141–1151

Trevaskis B, Hemming MN, Peacock WJ, Dennis ES (2006) HvVRN2 responds to daylength, whereas HvVRN1 is regulated by vernalization and developmental status. Plant Physiol **140:** 1397–1405

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

von Korff M, Léon J, Pillen K (2010) Detection of epistatic interactions between exotic alleles introgressed from wild barley (H. vulgare ssp. spontaneum). Theor Appl Genet **121:** 1455–1464

von Korff M, Wang H, Léon J, Pillen K (2006) AB-QTL analysis in spring barley: II. Detection of favourable exotic alleles for agronomic traits introgressed from wild barley (H. vulgare ssp. spontaneum). Theor Appl Genet **112:** 1221–1231

Waddington S, Cartwright P, Wall P (1983) A quantitative scale of spike initial and pistil development in barley and wheat. Ann Bot **51:** 119–130

Wang G, Schmalenbach I, von Korff M, Léon J, Kilian B, Rode J, Pillen K (2010) Association of barley photoperiod and vernalization genes with QTLs for flowering time and agronomic traits in a BC2DH population and a set of wild barley introgression lines. Theor Appl Genet **120:** 1559–1574

Wigge PA (2013) Ambient temperature signalling in plants. Curr Opin Plant Biol **16:** 661–666

Yan L, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, SanMiguel P, Bennetzen JL, Echenique V, Dubcovsky J (2004) The wheat VRN2 gene is a flowering repressor down-regulated by vernalization. Science **303:** 1640–1644

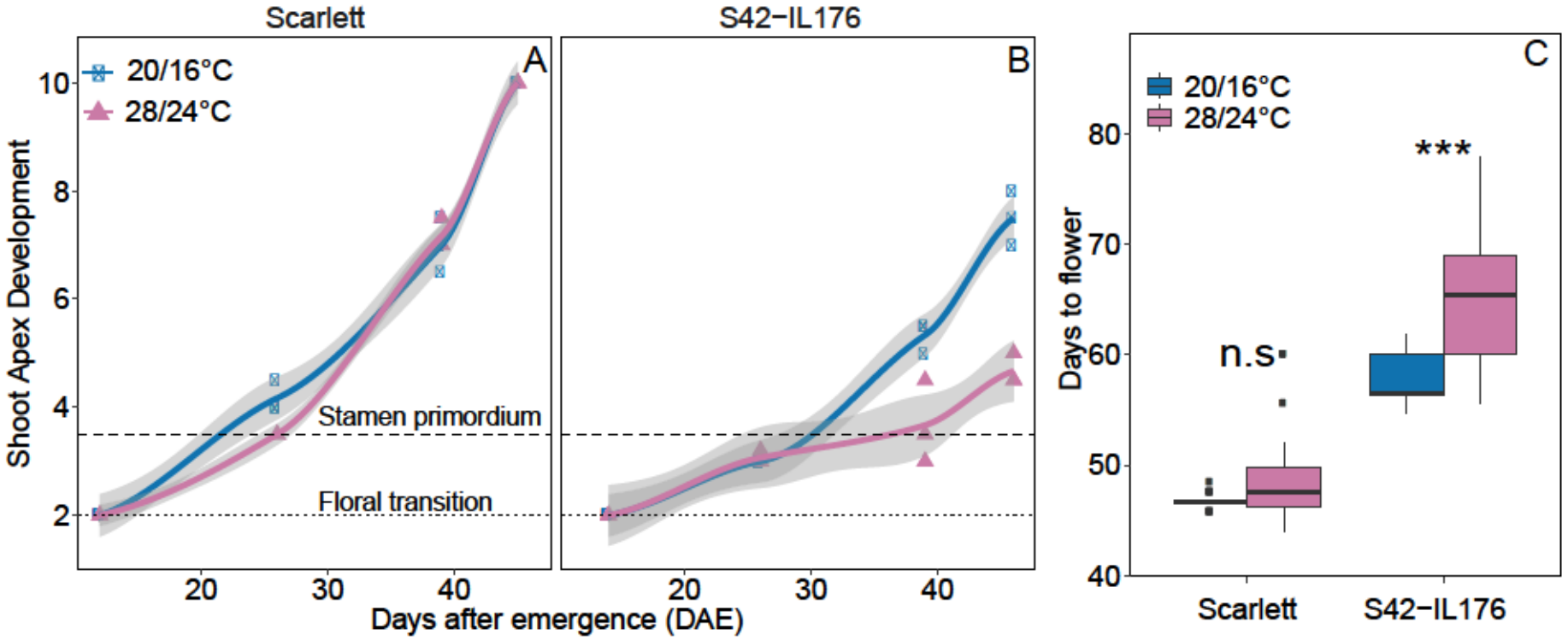
Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of the wheat vernalization gene VRN1. Proc Natl Acad Sci USA **100:** 6263–6268

Yoo SK, Wu X, Lee JS, Ahn JH (2011) AGAMOUS-LIKE 6 is a floral promoter that negatively regulates the FLC/MAF clade genes and positively regulates FT in Arabidopsis. Plant J **65:** 62–76

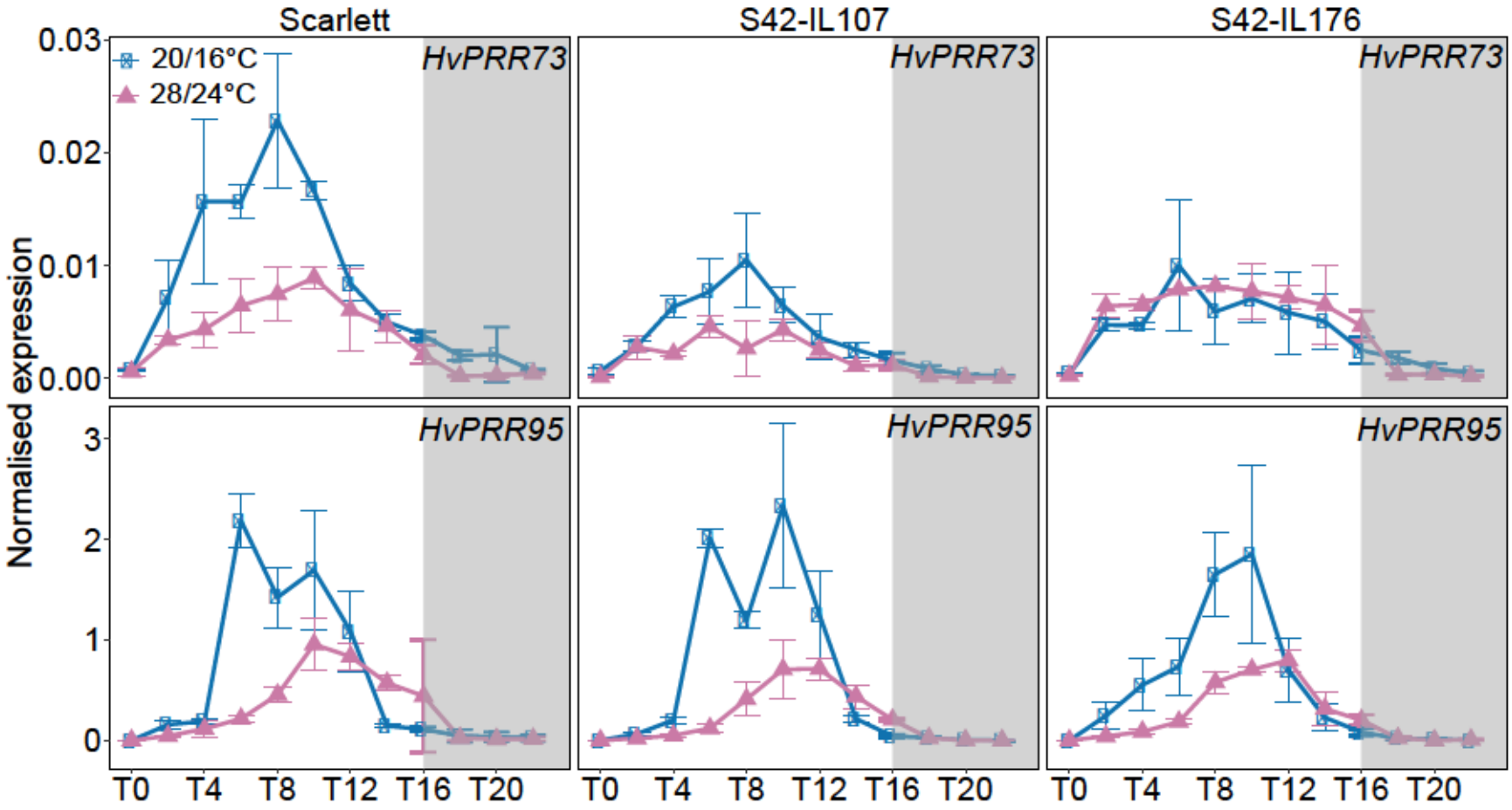
Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res **14:** 415–421

Zakhrabekova S, Gough SP, Braumann I, Müller AH, Lundqvist J, Ahmann K, Dockter C, Matyszczak I, Kurowska M, Druka A, et al (2012) Induced mutations in circadian clock regulator Mat-a facilitated short-season adaptation and range extension in cultivated barley. Proc Natl Acad Sci USA **109:** 4326–4331

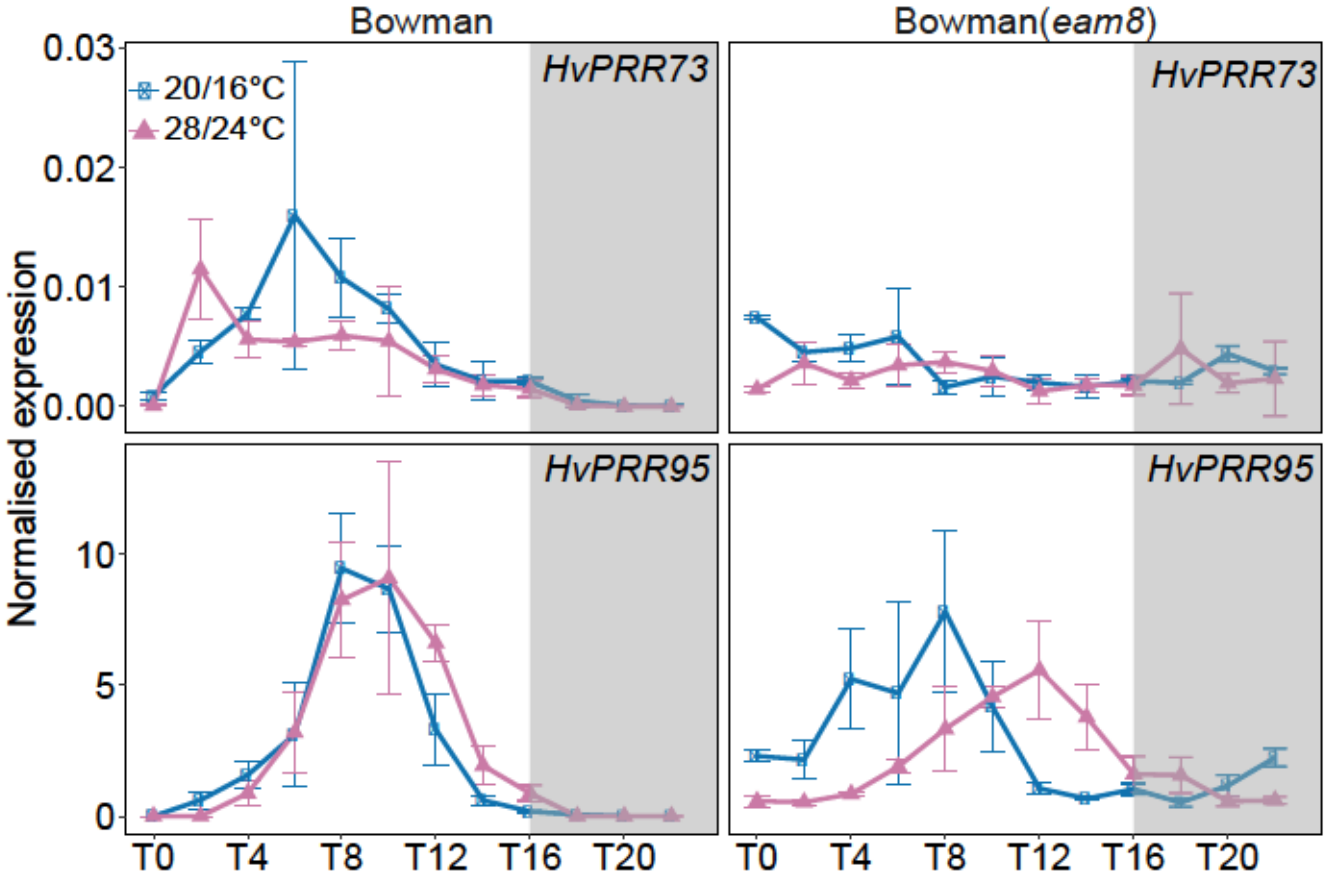
Supplemental Data



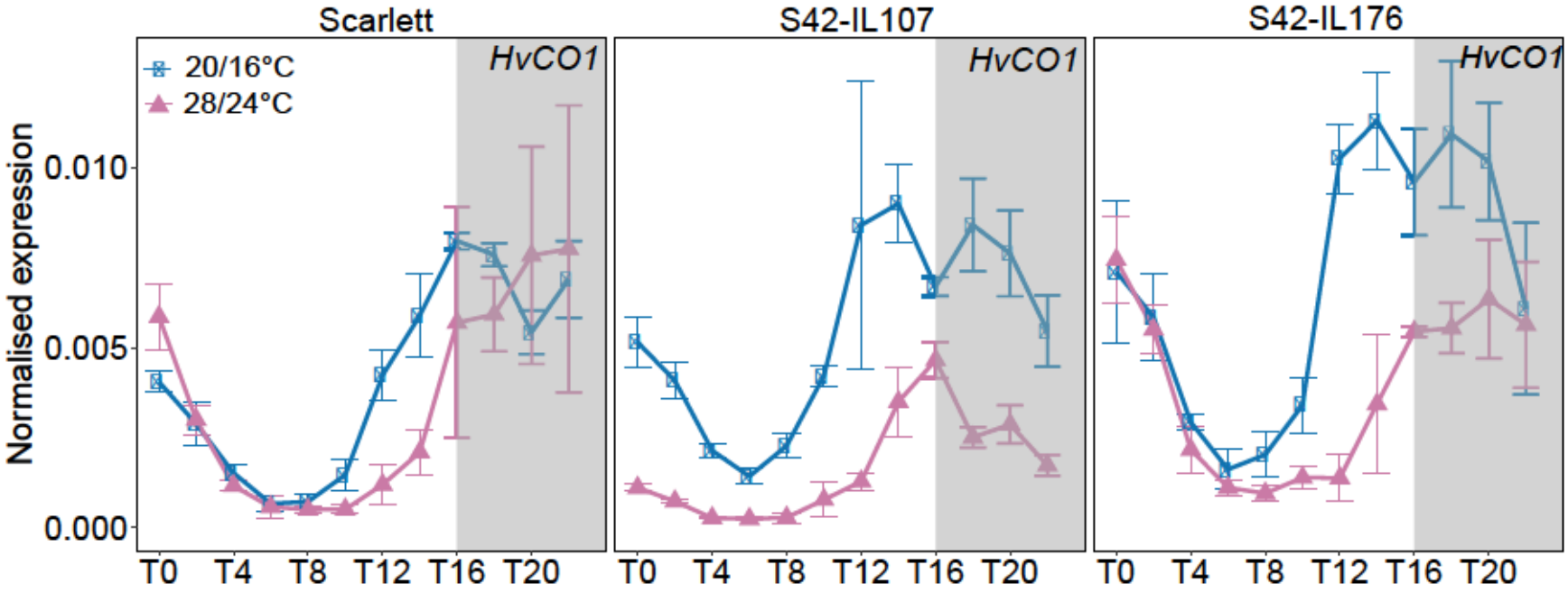
Supplementary Figure 1: *HvVRN1* affects reproductive development in response to ambient temperature after floral transition. Development of the main shoot apex (MSA) was scored under control (blue) and high ambient (pink) temperatures every ten days according to the Waddington scale (Waddington et al., 1983). MSA development was not affected under high compared to control temperatures in Scarlett (A) and delayed inflorescence development in the derived introgression line S42-IL176 (*Hvvrn1*) (B). Plants were grown at control temperature (blue, 20/16°C, day/night) and transferred to high temperature (pink, 28/24°C, day/night) at floral transition (W2.0). 3-4 plants per genotype were dissected at each time point in each treatment under long days (16h light/8h night). Statistical differences were calculated using a polynomial regression model at a 95% confidence interval (Loess smooth line). (C) Days to flowering of the MSA under control (blue, 20/16°C, day/night) and high ambient temperatures (pink, 28/24°C, day/night) in the spring barley variety Scarlett and the derived introgression line S42-IL176 (*Hvvrn1*). Flowering time was recorded for 6-8 plants per genotype and treatment. Statistical differences were calculated by an ANOVA and a posthoc Tukeys HSD pairwise comparison test: *P <0.05, **P <0.01, ***P <0.001, n.s=non-significant.



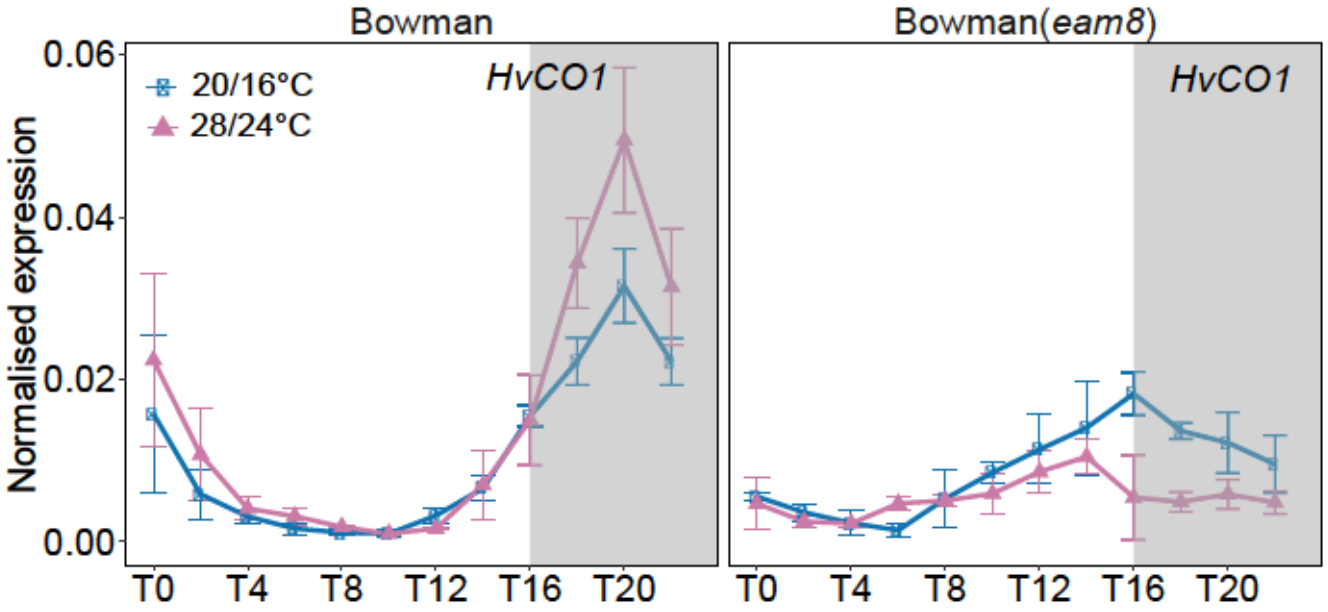
Supplementary Figure 2: Diurnal expression of circadian clock genes *HvPRR73* and *HvPRR95* in Scarlett, S42-IL107, and S42-IL176 under control and high ambient temperatures. Gene expression was assayed every two hours for 24 hours under control (blue, 20/16°C, day/night) and high ambient (pink, 28/24°C, day/night) temperatures under long days (16h light/8h night). Grey boxes indicate nights. Error bars indicate ±SD of three biological replicates.



Supplementary Figure 3: Diurnal expression of circadian clock genes *HvPRR73* and *HvPRR95* in Bowman and Bowman(*eam8*) under control and high ambient temperatures Gene expression was assayed every two hours for 24 hours under control (blue, 20/16°C, day/night) and high ambient (pink, 28/24°C, day/night) temperatures under long days (16h light/8h night). Grey boxes indicate nights. Error bars indicate ±SD of three biological replicates.



Supplementary Figure 4: High ambient temperature downregulates the expression of flowering time gene *HvCO1* in Scarlett, S42-IL107, and S42-IL176. Diurnal expression of *HvCO1* was assayed every two hours for 24 hours under control (blue, 20/16°C, day/night) and high ambient (pink, 28/24°C, day/night) temperatures under long days (16h light/8h night). Grey boxes indicate nights. Error bars indicate ±SD of three biological replicates.



Supplementary Figure 5: Effect of high ambient temperature on diurnal expression of flowering time gene *HvCO1* in Bowman and Bowman(*eam8*). Diurnal expression of *HvCO1* was assayed every two hours for 24 hours under control (blue, 20/16°C, day/night) and high ambient (pink, 28/24°C, day/night) temperatures under long days (16h light/8h night). Grey boxes indicate nights. Error bars indicate ±SD of three biological replicates.

Supplementary Table 1: A) Two-factorial ANOVA, F values and significances (**p<0.01, *** p<0.001, ns = non-significant) and B) Least square means for heading date, floret and seed number for each genotype (P = Parental genotype, Scarlett or Bowman, V = Introgression line for *HvELF3*, *PPD-H1* or *HvVRN1*) by environment combination (C = Control, H= High ambient temperatures). Small letters indicate significant differences (p<0.05).

A

Factor	Heading	Floret number	Seed number
	F Value	F Value	F Value
HvELF3			
Temperature	44***	27***	10**
HvELF3	1102***	310***	15***
*HvELF3**Temp	178***	22***	15***
Ppd-H1			
Temperature	50***	35***	53***
PPD-H1	2098***	117***	12***
*PPD-H1**Temperature	189***	10***	19***
HvVRN1			
Temperature	6995***	732***	363***
HvVRN1	6131***	236***	23***
*HvVRN1**Temp	4617***	235***	1 ns

B

Factor	P/C	P/H	V/C	V/H
HvELF3				
Heading	40^{a}	51^{c}	29.8^{b}	26^{d}
Floret number	21^{a}	17c	13^{b}	13^{b}
Seed number	9^{a}	3^{b}	9^{a}	9^{a}
PPD-H1				
Heading	42^{a}	52^{b}	26^{c}	23^{d}
Floret number	26^{a}	19^{c}	16^{b}	14^{b}
Seed number	15^{a}	4^{c}	14^{ab}	11^{b}
HvVRN1				
Heading	46^{a}	56^{c}	52^{b}	$>106^{d}$
Floret number	30^{a}	22^{b}	30^{a}	0^{c}
Seed number	23^{a}	5^{c}	19^{b}	0^{d}

Supplementary Table 2: List of q-PCR primers used in this study.

Gene ID	Gene name	Forward primer sequence	Reverse primer sequence	Source
AY145451	*HvACTIN*	CGT GTT GGA TTC TGG TGA TG	AGC CAC ATA TGC GAG CTT CT	Campoli et al.2012a
AJ249143	*HvBM3*	GCC GTC ACC AGC ACA AGC AA	CCC CAT TCA CCC TGT AGC AAA GA	Digel et al. 2015
AJ249146	*HvBM8*	CCA CAG CAG CCG ACA CCT A	TGC CTT TGG GGG AGA AGA CG	Digel et al. 2015
JN603242	*HvCCA1*	CCT GGA ATT GGA GAT GGA GA	TGA GCA TGG CTT CTG ATT TG	Campoli et al.2012b
AF490468	*HvCO1*	CTG CTG GGG CTA GTG CTT AC	CCT TGT TGC ATA ACG TGT GG	Campoli et al.2012a
DQ100327	*HvFT1*	GGT AGA CCC AGA TGC TCC AA	TCG TAG CAC ATC ACC TCC TG	Campoli et al.2012a
AK362208	*HvGAPDH*	GTG AGG CTG GTG CTG ATT ACG	AGT GGT GCA GCT AGC ATT TGA GAC	unpublished
AY740524	*HvGI*	TCA GTT AGA GCT CCT GGA AGT	GGT AGT TTG GGC TTT GGA TG	Campoli et al.2012b
Hv.20312	*HvLUX1*	AAT TCA GTC CAC GGA TGC TC	CTT CAC TTC AGC TCC CCT TG	Campoli et al.2012
HM130525	*HvOS2*	CAA TGC TGA TGA CTC AGA TGC T	CGCTATTTCGTTGCGCCAAT	Green up et al. 2010
JN603243	*HvPRR1*	GAG CAT AGC ATG GCA CTT CA	TGT CTT TCC TCG GAA ATT GG	Campoli et al.2012b
AK361360	*HvPRR59*	GAA ATT CCG CAT GAA AAG GA	TTC CGC ATC TTC TGT TGT TG	Campoli et al.2012b
AK376549	*HvPRR73*	GCG CCG TAG AGA ATC AGA AC	CAT GTC GGG TAC AGT CAT CG	Campoli et al.2012b
AK252005	*HvPRR95*	CAG AAC TCC AGT GTC GCA AA	TGC TGT TGC CAG AGT TGT TC	Campoli et al.2012b
Y09741	*HvßTUBLIN*	GTG CAT GGT TCT TGA CAA CG	GCA TGT GAC TCC ACT CAT GG	unpublished
AY750995	*HvVRN1*	CTG AAG GCG AAG GTT GAG AC	TTC TCC TCC TGC AGT GAC CT	Campoli et al.2012a
AY970701	*PPD-H1*	GAT GGA TTC AAA GGC AAG GA	GAA CAA TTG GCT CCT CCA AA	Campoli et al.2012a

VI. Abbreviation

Abbreviation	Explanation
%	percent
≤	less and equal
≥	greater and equal
°C	degree Celsius
2D	two dimentional
ABREs	ABA-responsive elements
AM	auxiliary meristems
ANOVA	analysis of variance
AP1	APETALA1
bHLB	basic helix-loop-helix
Bt2	Brittle 2
C^{14}	isotope of carbon
C^3	C_3 carbon fixation
C^4	C_4 carbon fixation
CCA1	CIRCADIAN CLOCK ASSOCIATED1
CCT	CONSTANS, CO-like, and TOC1
CDK	cyclin-dependent kinase
CTD	C-terminal domain
CWI	CELL WALL INVERTASE
DAE	days after emergence
eam8	early maturity 8
EC	evening complex
EG1	EXTRA GLUME1
ELF3	EARLY FLOWERING3
ELF4	EARLY FLOWERING4
F3	third filial generation
FLC	FLOWERING LOCUS C
FLD	FLOWERING LOCUS D
FLM	FLOWERING LOCUS M
FM	floral meristems
FRI	FRIGIDA
FT	FLOWERING LOCUS T
GA	gibberellins
GAox3	GA oxidase 3
GBSS1	Granule bound starch synthase-1
GI	GIGANTEA
h	hour
H3	Histone 3
H4	Histone 4
HsfB2b	heat shock factors B2b
Hv	Hordeum vulgare

Abbreviation	Explanation
HvBM3	Barley MADS-box 3
HvBM5	Barley MADS-box 5
HvBM8	Barley MADS-box 8
HvPpd1	Dominant photoperiod 1 allele in barley
Hvppd1	Recessive mutated photoperiod 1 allele in barley
HvVRN1	Vernalization 1 gene/also referred to the spring allele
Hvvrn1	Winter allele of Vernalization 1
HvVRN2	Vernalization 2 gene in barley
IL	Introgression lines
IM	inflorescence meristem
LD	long day
LHY	LATE ELONGATED HYPOCOTYL
LRP	late reproductive phase
LUX	LUX ARRHYTHMO
MADS-box	MCM1, AGAMOUS, DEFICIENS, SRF
MAF	MADS AFFECTING FLOWERING
Mha1	Plasma membrane H+ATPase gene
mRNA	messenger RNA
MSA	main shoot apex
MST1	Monosaccharide transporter gene
MYB	myeloblastosis
NIR	near infrared
no.	number
Ox-lipids	oxidized acyl chains
PGM	pollen grain mitosis
PIF4	PHYTOCHROME INTERACTING FACTOR 4
PMC	Pollen mother cells
Pn	net photosynthesis
PPD1	Photoperiod 1 referred to wheat
PPD-D1	Photoperiod 1 allele in wheat
Ppd-H1	Barley Photoperiod 1
PRR	PSEUDO-RESPONSE REGULATOR
PSII	photosystem II
QTL	quantitative trait loci
RCA	Rubisco activase
RILs	recombinant Inbred Lines
RNA	ribonucleic acid
ROS	reactive oxygen species
RPB1	RNA polymerase II; the largest subunit
SAM	shoot apical meristem
SbIncw1	Sorghum cell wall invertase gene 123
SbIvr2	Sorghum vacuolar invertase gene
SD	short day
Sh1	Shrunken 1

Abbreviation	Explanation
SM	spikelet meristems
SNP	single nucleotide polymorphism
SOC1	SUPPRESSOR OF CONSTANS 1
SU1	Sugary 1
Sus1	Sucrose synthase 1
SVP	SHORT VEGETATIVE PHASE
Ta	Triticum aestivum
TFs	transcription factors
TOC1	TIMING OF CAB EXPRESSION1
TSM	triple spikelet meristem
UGPase	UDP-pyrophosphorylase gene
uM	micro moles
VRN1	vernalization 1
VRN2	vernalization 2
W	Waddington
ZCCT	zinc finger and CCT domain
ZT	zeitgeber
α	alpha
β	beta
δ	delta
ϕ	phi

VII. Eidesstaatliche Erklärung

Ich versichere, dass ich die von mir vorgelegte Dissertaion selbständig verfasst und keine anderen als die angegebenen Hilfsmittel genutzt habe – einschließlich Tabellen, Karten und Abbildungen. Alle wörtlich oder inhaltlich übernommenen Stellen habe ich als solche gekennzeichnet.

Ich versichere außerdem, dass ich die vorgelegte Dissertation nur in diesem und keinem anderen Promotionsverfahren eingereicht habe, sowie, dass ich eine Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde. Die Bestimmungen des Promotionsverfahrens sind mir bekannt.

Die von mir vorgelegte Dissertation ist von Prof. Dr. Maria von Korff Schmisung und Prof. Dr. Peter Westhoff betreut worden.

Ich versichere, dass ich alle Angaben wahrheitsgemäß nach bestem Wissen und Gewissen gemacht habe.

Köln, _____ _____

Mahwish Ejaz

VIII. Acknowledgements

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