Journal of Experimental Botany
doi:10.1093/jxb/erx055

FLOWERING NEWSLETTER REVIEW

Floral transitions in wheat and barley: interactions between photoperiod, abiotic stresses, and nutrient status

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Received 30 August 2016; Editorial decision 1 February 2017; Accepted 1 February 2017

Editor: Lars Hennig, Swedish University of Agricultural Sciences

Abstract

The timing of plant reproduction has a large impact on yield in crop plants. Reproductive development in temperate cereals comprises two major developmental transitions. During spikelet initiation, the identity of the shoot meristem switches from the vegetative to the reproductive stage and spikelet primordia are formed on the apex. Subsequently, floral morphogenesis is initiated, a process strongly affected by environmental variation. Recent studies in cereal grasses have suggested that this later phase of inflorescence development controls floret survival and abortion, and is therefore crucial for yield. Here, we provide a synthesis of the early morphological and the more recent genetic studies on shoot development in wheat and barley. The review explores how photoperiod, abiotic stress, and nutrient signalling interact with shoot development, and pinpoints genetic factors that mediate development in response to these environmental cues. We anticipate that research in these areas will be important in understanding adaptation of cereal grasses to changing climate conditions.

Key words: Abiotic stress, barley, floral transition, floret development, nutrient, photoperiod, wheat.

Floral transitions in wheat and barley

The timing of reproductive development has a major effect on yield in cereal crops such as wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.). As seeds are of high agronomic importance, a better understanding of the developmental processes that determine potential seed number could enhance the efficiency of breeding programmes aimed at improving grain yield.

Here, we review the phenology and genetics of pre-anthesis development of barley and wheat. We argue that the plasticity of spike development is controlled by interactions between photoperiod, abiotic stresses, and nutrient availability which function as potent signals to modify development in wheat and barley. Developmental decisions in turn affect source–sink relationships and eventually spike architecture and yield.

The phenology of reproductive development in response to environmental cues

Most of our knowledge on the genetic control of reproductive development stems from the model dicot plant *Arabidopsis thaliana*. These studies have focused on the genetic control of

the vegetative to reproductive phase transition (Andrés and Coupland, 2012). In contrast to Arabidopsis, where floral transition and flowering take place within a short period of time, in cereal crops such as wheat and barley, several weeks may pass between the initiation of the first spikelet primordia and flowering. The shoot apex of barley and wheat develops inside the leaf sheath and can therefore only be assessed upon microscopic dissection of the plant. During the last stage of pre-anthesis development, the spike is pushed out of the flag leaf sheath, a stage referred to as 'heading'. Within a few days after heading, anthesis or flowering (pollination) take place. The flowers of cereals develop on a specialized short branch called a spikelet which carries one (barley) or more (wheat) florets and form on opposite sides of the central rachis. Consequently, wheat and barley form branchless spike-shaped inflorescences in which spikelets represent the fundamental building blocks, comprising one or more florets.

The shoot apex is already formed in the embryo, and changes in form and complexity during development, as at first, leaves and, later, flowers are formed (Kirby and Appleyard, 1987). Pre-anthesis development can be classified into three major phases based on morphological changes of the shoot apical meristem: the vegetative phase, the early reproductive phase, and the late reproductive phase (Slafer and Rawson, 1994; González *et al.*, 2002). A quantitative scale for barley development based on the morphogenesis of the shoot apex and the carpel of the most advance flower per spike is provided by Waddington *et al.* (1983).

During the vegetative phase, the apex is conical in shape and initiates leaves. As development proceeds, the apex becomes more cylindrical in shape, indicating that the initiation of spikelet primordia has begun. Spikelet primordia become visible at the double ridge stage. The lower ridge represent a leaf primordium, the further development of which is largely suppressed. The upper ridge eventually differentiates into a spikelet. In wheat, the final number of spikelets is determined by the formation of a terminal spikelet when the last initiated primordia, instead of becoming spikelet primordia, develop into floret primordia. In contrast, the barley inflorescence is indeterminate and spikelet primordia initiation continues until shortly after initiation of the pistil primordia (Waddington *et al.*, 1983). Reproductive development is commonly subdivided into the early reproductive phase during which spikelet primordia are initiated and a late reproductive phase during which stem internodes elongate and the floret primordia develop into flowers. The duration of the vegetative and early reproductive phases determines the number of spikelet primordia initiated on the shoot apex, while the late reproductive phase determines how many spikelet primordia develop fertile florets (Alqudah and Schnurbusch, 2014; Digel *et al.*, 2015). The late reproductive phase during stem elongation shows the strongest plasticity in response to internal and external factors and therefore has a large impact on the number of grains, the most important component of cereal yield (Miralles *et al.*, 2000; González *et al.*, 2003; Slafer, 2003; Reynolds *et al.*, 2009; Sreenivasulu and Schnurbusch, 2012).

Barley and wheat are facultative long-day plants and characterized by two major growth types: winter and spring. Winter growth types are defined as genotypes which show accelerated flowering after vernalization, a prolonged exposure to cold temperature. In contrast, spring barley does not respond to vernalization and flowers in the absence of vernalization. However, there exists a continuous gradation regarding spring and winter growth habits that range from typical spring to extreme winter (vernalization requirement) (Enomoto, 1929; Saisho *et al.*, 2011). Wild barley (*H. vulgare* ssp. *spontaneum*) and wild wheat (*T. monoccocum*), the progenitors of cultivated barley and wheat, have a winter growth habit, indicating that the winter growth habit is ancestral in these cereals (Campoli and von Korff, 2014). In addition to variation in vernalization response, wheat and barley vary in their photoperiod response, the acceleration of flowering in response to long days of >12 h of light per day.

Different phenological phases of pre-anthesis development vary in their sensitivity to vernalization and photoperiod depending on the growth type (Fig. 1). In winter barley, vernalization affects flowering time, predominantly by reducing the duration of the vegetative phase (Griffiths *et al.*, 1985;

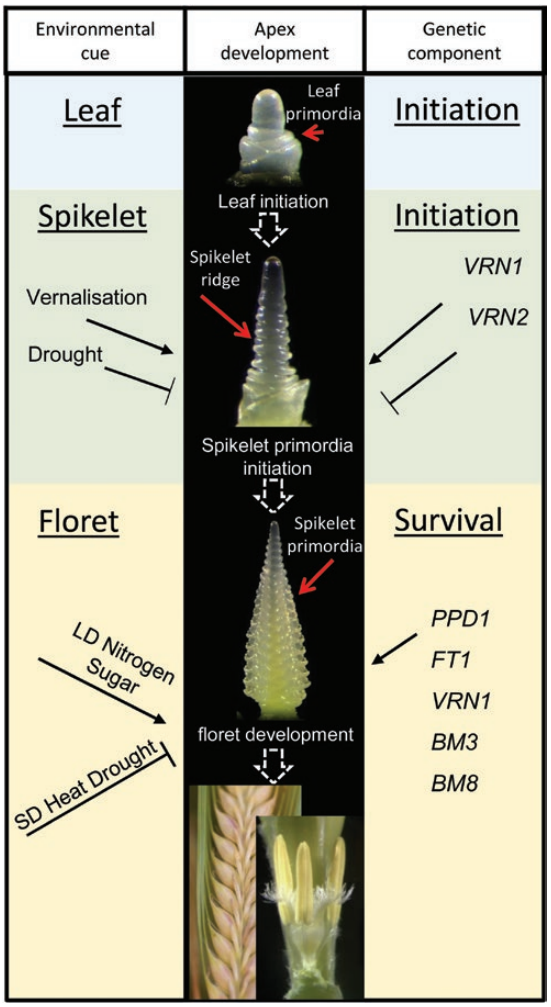

Fig. 1. Schematic representation of the development of the shoot apical meristem in response to different environmental cues in barley. The effects of environmental factors on spikelet primordia initiation and floret survival are given on the left-hand side. The effects of major genetic components on the timing of spikelet initiation and on floret survival are indicated on the right-hand side of the diagram.

Roberts *et al.*, 1988; González *et al.*, 2002), but strong effects of vernalization on inflorescence development were also reported (González *et al.*, 2002). The effect of photoperiod on pre-anthesis development depends on the growth habit, the vernalization treatment, and the intrinsic photoperiod response of the genotype. In the absence of vernalization, photoperiod has no effect on the duration of the vegetative phase, but accelerates the subsequent reproductive phases in winter barley. In spring barley and vernalized winter barley, long days shorten the vegetative phase, but mainly accelerate the late reproductive phase of stem elongation (Roberts *et al.*, 1988; Miralles and Richards, 2000; Digel *et al.*, 2015, 2016). In addition, shifting barley plants at defined developmental stages from long to short days suggested that the beginning of stem elongation and floral development only occurs under long days. Under short days, barley plants initiated floret primordia, while stem elongation and spike development were strongly impaired and the shoot apical meristem was aborted at the early stem elongation phase. In addition, Batch and Morgan (1974) showed that a transfer of barley plants from long to short days at a late developmental stage induced male sterility and floral abortion. Consequently, in conditions where floral induction is marginal, such as short photoperiods, the apex might initiate spikelet primordia, but floral development may not continue. Floral development in wheat and barley thus resembles a two-phase system, with the initiation of spikelet primordia on the apex, which is then followed by floral morphogenesis only if external and internal conditions are favourable (Aspinall, 1966).

These earlier physiological studies of shoot apex development in wheat and barley have often neglected genetic differences in photoperiod and vernalization response between genotypes, also because information on causative genes and gene variants was not available. However, in recent years, flowering time genes and functional variants have been identified in wheat and barley. This knowledge should now be used to dissect how individual genes interact with environmental cues to control different pre-anthesis phases.

Genetic control of developmental transitions in wheat and barley

The major flowering time regulators in wheat and barley are part of a complex network that interacts with environmental cues to control distinct developmental phases. For a comprehensive overview on flowering time genes and pathways in barley and wheat, please refer to Campoli and von Korff (2014).

The effect of major flowering time regulators on individual phases of spike development is depicted in Fig. 1. Vegetative to reproductive phase transition in wheat and barley is controlled by VERNALIZATION1 (VRN1) and VERNALIZATION2 (VRN2) (Yan *et al.*, 2003, 2004; von Zitzewitz *et al.*, 2005). VRN1 (APETALA1/FRUITFUL-like) is a MADS-box transcription factor which controls the vernalization requirement in winter wheat and barley in interaction with VRN2 (Yan *et al.*, 2003, 2004). The *VRN2* locus encodes duplicated ZCCT (zinc finger and CCT domain) proteins and is a strong inhibitor of flowering under long-day conditions before winter (Yan *et al.*, 2004). Up-regulation of *VRN2* is controlled by *HvCO1* and *HvCO2*, the barley homologues of the Arabidopsis photoperiod response gene *CONSTANS* under long days in barley (Mulki and von Korff, 2016). During vernalization, *VRN1* is up-regulated and represses *VRN2* expression in the leaf (Sasani *et al.*, 2009). In spring barley and wheat, insertions and deletions in the first intron of *VRN1* cause an up-regulation of the gene independently of vernalization (Fu *et al.*, 2005; von Zitzewitz *et al.*, 2005; Cockram *et al.*, 2007; Szucs *et al.*, 2007). In addition, spring wheat and barley genotypes lack a functional copy of VRN2 due to loss-of-function mutations in the *VRN2* coding sequence or due to naturally occurring deletions of the entire *VRN2* locus (Yan *et al.*, 2004; Dubcovsky *et al.*, 2005). High *VRN2* and low *VRN1* expression levels correlate with a delay in spikelet initiation (Pearce *et al.*, 2013). However, *VRN1* is probably also involved in inflorescence development as its expression in the shoot apical meristem is strongly correlated with the expression of floral homeotic genes (Digel *et al.*, 2015). A key regulator of inflorescence development under long days is encoded by the *PHOTOPERIOD1* gene (*Ppd-H1*, *Ppd-A1*, *Ppd-B1*, *Ppd-D1*; Turner *et al.*, 2005; Beales *et al.*, 2007; Wilhelm *et al.*, 2009; Díaz *et al.*, 2012). *PPD1* encodes a PSEUDO-RESPONSE-REGULATOR (PRR) protein, which is homologous to the Arabidopsis *PRR3*/*PRR7* of the circadian clock, and characterized by a pseudoreceiver and a CCT (CONSTANS, CONSTANS-like, and TOC1) domain. The ancestral, dominant form of *PPD1* confers an acceleration of flowering under increasing day length. Barley and wheat carry different natural polymorphisms which modify the response to long days. In barley, a recessive mutation in the CCT domain of *ppd-H1* has been selected in spring cultivars grown in northern agricultural areas. This variant leads to a minor delay in the vegetative to reproductive phase transition, but a strong delay of the late reproductive development in spring barley (Alqudah *et al.*, 2014; Digel *et al.*, 2015). In addition, the mutated variant increases the number of spikelet primordia on the shoot apex and the number of seeds per spike under favourable conditions (Digel *et al.*, 2015). Similarly to barley, loss-of-function deletions in the wheat *ppd1* homeologous series delay flowering time under long days (Shaw *et al.*, 2013). In addition, in wheat, insertions and deletions in the promoters of *Ppd-A1a* and *Ppd-D1a* cause their constitutive up-regulation and early flowering under long and short days (Beales *et al.*, 2007; Wilhelm *et al.*, 2009; Nishida *et al.*, 2013). A latitudinal cline in the distribution of the functional variation at *PPD1* in barley and wheat indicates that this gene has a strong adaptive effect on yield (Worland *et al.*, 1998; Cockram *et al.*, 2007). The expression of *PPD1* is repressed in the night by *EARLY FLOWERING 3* (*ELF3*) and *LUX ARRHYTHMO* (*LUX*), and mutations in both genes lead to a constitutive up-regulation of *PPD1* and photoperiod-independent early flowering in wheat and barley (Faure *et al.*, 2012; Mizuno *et al.*, 2012; Zakhrabekova *et al.*, 2012; Campoli *et al.*, 2013; Alvarez *et al.*, 2016). In Arabidopsis, *ELF3* and *LUX* form, together with *EARLY FLOWERING 4* (*ELF4*), 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 ~24 h and controls plants' adaptation to daily and seasonal changes in the environment (Müller *et al.*, 2014; Johansson and Staiger, 2015). In addition, the expression of *PPD1* is induced and dependent on *PHYTOCHROME C* (*PHYC*). Tetraploid wheat plants homozygous for loss-of-function mutations in all *PHYC* copies flowered significantly later under long days, while a hypermorphic *phyC* allele in barley induced *PPD1* expression and caused early flowering under long and short days (Chen *et al.*, 2014; Pankin *et al.*, 2014). Consequently, PPD1 mediates the light input into the flowering time pathway as controlled by components of the circadian clock and PHYC.

Under long days, PPD1 induces the expression of *VRN3*, a homologue of Arabidopsis *FLOWERING LOCUS T* (*FT*) and rice *Hd3a* (Turner *et al.*, 2005; Campoli *et al.*, 2012*a*, *b*). FT and Hd3a proteins translocate from the leaves through the phloem to the shoot apical meristem, where these proteins induce the switch from vegetative to reproductive growth (Corbesier *et al.*, 2007; Tamaki *et al.*, 2007). Expression of *HvFT1* in the leaf correlates with an up-regulation of *Vrn-H1* and the related MADS-box transcription factors *BM3* and *BM8* in the shoot apical meristem (Digel *et al.*, 2015). Barley carries five different *FT*-like genes: *FT1* (*VRN3*), *FT2*, *FT3*, *FT4*, and *FT5* (Faure *et al.*, 2007). Similar to Arabidopsis, *FT*-like genes in cereals have been described as central regulators of the transition from vegetative to reproductive growth (Kojima *et al.*, 2002; Li and Dubcovsky, 2008). However, a recent study in barley demonstrated that natural variation at *Ppd-H1* and associated variation in the expression of *HvFT1* had a major effect on inflorescence development and floret fertility, but did not strongly affect the timing of vegetative to reproductive phase transition (Digel *et al.*, 2015). This finding is consistent with previously reported effects of *Ppd-D1* on increasing floret fertility in wheat (Worland *et al.*, 1998). Two recent studies have shown that the application of gibberellin under short days accelerated the spikelet initiation in wheat and barley, but both species failed to produce seeds under short days, suggesting that in addition to gibberellin, a signal that is generated only under long days is necessary for floret fertility in these temperate crops (Pearce *et al.*, 2013; Boden *et al.*, 2014).

In summary, different pre-anthesis phases of development are controlled by different genes and environmental signals. Vernalization and the vernalization genes *VRN1* and *VRN2* are dominant over the photoperiod response pathway and control vegetative to reproductive phase transition, but are also involved in the early and late reproductive development. Floral cues such as photoperiod and the photoperiod response regulators PPD1, FT, and the downstream component VRN1 are associated with inflorescence development, survival, and abortion of floret primordia. The genetic control of photoperiod and vernalization response is known, but how these genetic pathways interact with other environmental factors such as abiotic stresses is a topic of current and future interest. In the following, we discuss the possible interactions between photoperiod response, abiotic stress, and nutrient availability and signalling, and their effects on wheat and barley development.

Reproductive development under abiotic stresses

Phenology of reproductive development under abiotic stresses

The genetic control of photoperiod and vernalization response is well characterized in wheat and barley. However, abiotic stresses, which are predicted to increase in frequency, duration, and severity due to climate change, also have a huge impact on cereal reproductive development (Saini and Westgate, 1999; Barnabás *et al.*, 2008; Dai, 2012; Stocker *et al.*, 2013). In particular, post-transition reproductive development, which is critical for determining the number of fertile florets and grain number, is very susceptible to drought and heat (Saini and Westgate, 1999; Campoli and von Korff, 2014; Slafer *et al.*, 2014). Understanding the physiology and genetic control of drought and heat tolerance in cereal crops has received much attention over the last years (Saini and Westgate, 1999; Baum *et al.*, 2007; Barnabás *et al.*, 2008; von Korff *et al.*, 2008; Guo *et al.*, 2009; Farooq *et al.*, 2012; Bita and Gerats, 2013; Rollins *et al.*, 2013). However, these studies on abiotic stress tolerance have often neglected the interactions of stress responses with plant phenology. Increasing evidence suggests that stress responses depend on the developmental stage of the plant. On the other hand, reproductive development itself is regulated by abiotic stresses (Conti *et al.*, 2014; Riboni *et al.*, 2014; Kazan and Lyons, 2016). Consequently, abiotic stresses need to be viewed as developmental signals rather than only as damaging to plant structures. Understanding the molecular basis for stress-induced changes in reproductive development will play a crucial part to ensure future yield stability of temperate cereals. In the following, we provide an overview of the physiological effects of drought and heat on barley and wheat development and the scarce knowledge on the genetic integration of heat and drought signals into the developmental pathways in temperate cereals.

The developing reproductive structures of temperate cereals are protected by the enveloping leaf sheath and are therefore usually less exposed to direct consequences of drought and heat stresses, such as a reduction in relative water content, compared with vegetative tissues (Saini and Westgate, 1999). The effects of abiotic stresses on reproductive development are, therefore, largely dependent on the stress resistance mechanisms of the vegetative plant organs and signals originating there.

The physiological effects of abiotic stresses on cereal development vary between different studies as a consequence of the timing and severity of the stress (e.g. Nicholls and May, 1963; Husain and Aspinall, 1970). Drought and heat stress reduce the grain number per spike by modulating the duration

of pre-anthesis development and by disturbing several sensitive events around anthesis that include male and female meiosis and fertilization (Zavadskaja and Skazkin, 1960; Bingham, 1966; Saini and Westgate, 1999; Barnabás *et al.*, 2008; Ji *et al.*, 2010; Bita and Gerats, 2013; Stratonovitch and Semenov, 2015). While most studies have evaluated the effects of drought and high temperatures on flowering and grain filling, we will focus our review on the effects of these two stresses on pre-anthesis development (Fig. 1).

Phenology of reproductive development under drought

Early flowering and seed set allow crops to escape terminal drought in many Mediterranean environments. Mediterranean barley and wheat varieties and their wild progenitors are consequently primarily winter types with rapid flowering in response to an increase in photoperiod (Campoli and von Korff, 2014; Drosse *et al.*, 2014; Al-Ajlouni *et al.*, 2016). However, in environments where drought does not limit the duration of the growing season, but affects plants in early growth phases, a delay of development coupled with drought avoidance/enhanced water use efficiency is favourable over a drought escape strategy (Schmalenbach *et al.*, 2014; Kooyers, 2015). This correlates well with the selection of late flowering wheat and barley varieties for cultivation in northern latitudes where terminal droughts are less likely to occur (Worland *et al.*, 1998; Turner *et al.*, 2005; Jones *et al.*, 2008).

Drought itself may alter the timing of reproductive development. Many plant species are induced to flower following drought stress, which results in a drought escape response (Riboni *et al.*, 2013; Kazan and Lyons, 2016). However, studies on the microscopic development of wheat and barley have most commonly reported a delay of reproductive development under drought. Nicholls and May (1963) found that drought delayed inflorescence development and reduced the rate of spikelet primordia induction compared with control conditions. Similarly, Husain and Aspinall (1970) reported that drought at early developmental stages delayed reproductive development and suppressed the response of the apical meristem to an increase in the photoperiod. The authors suggested that the rapid inhibition of primordium formation on the apex during a period of water deficit resulted from changes in leaf metabolism rather than from a fall in the water potential of the apical tissues. Similar to drought, osmotic stress rapidly and completely inhibited both apical elongation and the formation of new primordia, while the development of lateral primordia on the apex, although slowed by water stress, was not completely inhibited (Singh *et al.*, 1973). A recent study showed that the effects of drought on flowering time are genotype dependent (Al-Ajlouni *et al.*, 2016). A panel of 11 genotypes which differed in their allelic status at the major flowering time genes *Ppd-H1* and *Vrn-H1* were subjected to drought at the seedling or stem elongation phase or kept under control conditions, and flowering time and yield parameters were scored. The barley genotypes with a winter *vrn-H1* or a mutated *ppd-H1* allele displayed a strong delay in flowering when drought was applied at the seedling stage. In contrast, barley cultivars with a spring *Vrn-H1* and a dominant *Ppd-H1* allele did not show an altered development when stress was applied at the seedling stage or their development was accelerated when stress was applied at the stem elongation phase. Drought stress thus probably interacts with major flowering time genes such as *PPD1* and *VRN1*, and possibly other external cues such as temperature and photoperiod to adjust seasonal flowering behaviour in cereals. In the model species Arabidopsis, it was found that the circadian clock and photoperiod pathways probably interact with drought response to control developmental plasticity. In Arabidopsis, drought escape only occurs under inductive long days. It is controlled by the circadian clock gene *GIGANTEA* (*GI*), the photoperiod response gene *CONSTANS* (*CO*), the floral integrator genes *FT* and *TWIN SISTER OF FT* (*TSF*), and the drought-related phytohormone abscisic acid (ABA; Riboni *et al.*, 2013, 2016). ABA probably controls drought escape via the potentiation of florigen-like genes in a photoperiodic manner. *aba1* mutants are impaired in ABA biosynthesis and display reduced accumulations of FT and TSF transcripts, especially under drought conditions (Riboni *et al.*, 2013). Similarly, in the short-day crop rice, the photoperiod response factors *EARLY HEADING DATE 1* (*Ehd1*), *Hd3a*, and *RICE FLOWERING LOCUS T 1* (*RFT1*) integrate drought response signals to co-ordinate reproductive development (Galbiati *et al.*, 2016; Zhang *et al.*, 2016). The result is a delay in flowering also under inductive short days.

In addition to photoperiod pathway components, drought response in Arabidopsis is also controlled by an miRNA 169 (miR169) and its target, a *NUCLEAR FACTOR-YA* (*NF-YA*) subunit (Xu *et al.*, 2014). NF-Ys are heterotrimeric transcription factors that bind to the highly abundant CCAAT motif in eukaryotic promotors. In plants, each subunit is encoded by multiple genes, many of which have previously been shown to regulate diverse processes such as embryo development, stress responses, and flowering time (Petroni *et al.*, 2012). *NF-YA* mRNA cleavage results in reduced expression of the vernalization gene and floral repressor *FLOWERING LOCUS C* (*FLC*), and accelerates flowering in Arabidopsis (Xu *et al.*, 2014). Stress responsiveness of miR169 and its targets is conserved between mono- and dicotyledonous plant species and has recently been demonstrated in barley (Zhao *et al.*, 2009; Zhang *et al.*, 2011; Xu *et al.*, 2014; Ferdous *et al.*, 2017). Furthermore, NF-Y subunits in Einkorn wheat (*T. monococcum*) interact with several known flowering regulators including the floral inducers PPD1 and the repressor VRN2 through their CCT domains (Li *et al.*, 2011). Whether the miR169–*NF-Y* regulon for stress-regulated flowering is conserved in the temperate cereals needs to be verified.

In barley and wheat, information on the genetic control of development in response to drought is scarce. However, the photoperiod response gene *PPD1* is induced by osmotic stress, and was associated with an induction of stress response genes (Habte *et al.*, 2014). In Arabidopsis, the *PPD1* homologues *PRR* genes have already been associated with abiotic stress tolerance (Nakamichi *et al.*, 2016). As in Arabidopsis, the promoter of *Ppd-H1* of barley contains a number of ABA-responsive elements (ABREs) (Habte *et al.*, 2014), suggesting that *PPD1* integrates stress and photoperiod signals.

The integration of drought and photoperiod signals might present an adaptive advantage for temperate cereals because it enables the perception of drought as a seasonal signal to adapt development to terminal summer droughts. Compared with variation in photoperiod, which does not change over the years, the integration of stress signals into the flowering pathways enables the fine-tuning of flowering time to fluctuations in water availability.

Future studies need to identify genetic factors controlling developmental plasticity in response to drought and characterize the interactions between drought and other environmental cues in barley and wheat.

Phenology of reproductive development under different ambient temperatures

For evaluation of the effects of temperature on development, it is important to distinguish between cold, ambient temperature, and heat. The control of reproductive development in response to cold temperature termed vernalization is reviewed in detail in Dennis *et al.* (2009) and Greenup *et al.* (2009) and is not a topic of the current review. Ambient temperature thresholds have been well defined for wheat (reviewed in Porter and Gawith, 1999) and depend on the specific plant organ, developmental phase, and genotype. At temperatures >37 °C, growth is arrested, and temperatures of >40–45 °C are lethal in wheat. However, optimal temperatures range between 17 °C and 23 °C, and temperatures beyond this range may already elicit stress responses. Here, we want to review the effects of ambient temperatures including temperatures of >23 °C on barley and wheat reproductive development. In wheat, an increase in temperature from 10 °C to 19 °C accelerated reproductive development, while temperature regimes >19 °C delayed terminal spikelet initiation and reduced the number of spikelet primordia in wheat (Slafer and Rawson, 1994). Temperatures below and above the optimal growth temperatures therefore delay growth and reproductive development. In addition, detailed physiological studies have demonstrated that the effects of ambient temperature on development are strongly dependent on the photoperiod. Hemming *et al.* (2012) reported that an increase of temperature from 15 °C to 25 °C accelerated development under long days and delayed early development under short days in a winter barley cultivar. Rawson and Richards (1993) have tested the effects of different photoperiods and ambient temperatures (33.3/20 °C and 20/12 °C, day/night) on development in wheat isolines differing at *Ppd-H1*, *VRN1*, *VRN2*, *VRN3*, and *VRN4*. Under short days of 9 h light, an increase in temperature delayed the appearance of double ridges, but accelerated the later development up to ear emergence. In contrast, under long photoperiods of 13 h, high temperatures shortened the time to double ridges and slowed down the production of spikelet primordia. Similarly, a high ambient temperature of 30 °C delayed the spikelet inititation in barley, and the effect was dependent on the photoperiod and light intensity (Aspinall, 1969). These studies in wheat and barley indicated that the effects of ambient temperature changes depend on the temperature range, the genotype, and the photoperiod.

Also in Arabidopsis, the temperature and photoperiod pathways interact to control reproductive development. High temperature accelerated flowering and overcame the delay in flowering commonly observed under short photoperiods by up-regulating the floral integrator gene *FT* (Halliday *et al.*, 2003; Balasubramanian *et al.*, 2006). In addition, recent studies have identified *ELF3* as an essential component of the ambient temperature response (Thines and Harmon, 2010). Elevated temperatures during dark inhibit the EC by an unknown mechanism (Thines *et al.*, 2014; Mizuno *et al.*, 2014*a*, *b*; Box *et al.*, 2015; Raschke *et al.*, 2015), leading to increased expression of *PHYTOCHROME-INTERACTING FACTOR 4* (*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). A recent study has shown that activation of FT and early flowering under high temperatures in short days depends on the co-ordinate functions of CONSTANS, PIF4/5, and the high temperature-dependent deactivation of the floral repressor SHORT VEGETATIVE PHASE (SVP) in the meristem (Fernández *et al.*, 2016). In addition, temperature-dependent splicing of *FLOWERING LOCUS M* (*FLM*; *MAF1*) results in two major splice forms, that either facilitate or inhibit *SVP* dependent repression of *FT* (Balasubramanian *et al.*, 2006; Posé *et al.*, 2013; Sureshkumar *et al.*, 2016). Consequently, transcription factors from the photoperiod and thermosensory flowering pathways converge on the transcriptional regulation of the floral integrator *FT* to control reproductive development under high temperatures.

In temperate cereals, the molecular basis of developmental plasticity in response to ambient temperature has long remained elusive. Hemming *et al.* (2012) found no clear candidates for the genetic control of inflorescence development under high ambient temperatures. We have shown recently that in barley high ambient temperatures of 28 °C compared with 20 °C accelerated or delayed reproductive development depending on the photoperiod response gene *Ppd-H1* and its upstream night-time repressor *HvELF3* (Ejaz and von Korff, 2017). Spring barley genotypes with the mutated *ppd-H1* allele showed a delay in flowering and reduced the numbers of florets and seeds per spike under high vs. control temperatures. In contrast, introgression lines with the wild-type *Ppd-H1* or a mutant *Hvelf3* allele showed accelerated floral development and maintained the seed number under high ambient temperatures. In contrast to Arabidopsis, high ambient temperature repressed the expression of *HvFT1* independently of the genotype. The regulation of *BARLEY MADS-box* genes *Vrn-H1*, *HvBM3*, and *HvBM8* under high ambient temperature was genotype dependent and correlated with the *Ppd-H1*- and *HvELF3*-dependent effect of high temperature on flowering. In addition, structural variation in the first intron of *Vrn-H1* controlled reproductive development under high ambient temperatures. The full-length winter allele was strongly down-regulated, and spikelet initiation did not occur under high ambient temperatures of 28 °C in a spring genotype with an introgression of a

winter *vrn-H1* allele. Consequently, the expression regulation of the BM genes controlled ambient temperature response in barley. Similarly, a recent study has revealed that natural variation in the first intron of the *MADS-box* gene *FLM* and consequent expression variation was responsible for differential temperature response in Arabidopsis (Lutz *et al.*, 2015). Structural variation in related MADS-box transcription factors may play a role in temperature adaptation across different species. In Arabidopsis, substantial variation in the thermosensitive response is mediated by natural variation at the vernalization gene *FLC* that functions as a potent suppressor of thermal induction (Balasubramanian *et al.*, 2006). The barley homologue *HvOS2* is up-regulated under high ambient temperature in a *Vrn-H1*-dependent manner and may also be involved in floral repression under high ambient temperatures (Greenup *et al.*, 2010; Hemming *et al.*, 2012; Ejaz and von Korff, 2017).

In conclusion, the timing of reproductive development is strongly affected by drought and heat stresses. So far, only few studies have explored the genetic control of pre-anthesis development in response to heat and drought. These suggested that developmental plasticity in response to drought and heat is mediated by the photoperiod response and vernalization pathways. The modification of these pathways by abiotic stresses might be a strategy to adapt seasonal development to short-term fluctuations in water availability and ambient temperatures.

Importance of nutrient signalling in the context of development

Sucrose and nitrogen availability are crucial throughout the whole plant life cycle. Different plant organs and developmental phases have different nutrient sources and requirements. The initial seedling growth is supported by stored nutrients in the endosperm. As the seedling develops, mature leaves are the source of sucrose from photosynthesis. Sucrose from the leaf is initially used for newly developing leaves and, upon the transition to reproductive growth, translocated to developing shoot apical meristems through the phloem. In addition, there is a strong remobilization of nutrients, particularly nitrogen, from the senescing leaves to the developing shoot apical meristem. The assimilation, translocation, partitioning, and storage of nutrients in the plant are commonly referred to as source–sink interactions; they can be enhanced by increasing either the source, sink, or the translocation capacity, and therefore their manipulation is determinant for high crop productivity (Yu *et al.*, 2015). Efficient nutrient allocation and appropriate source–sink interactions are critical throughout the whole of reproductive development. Increasing evidence demonstrates that photoperiod and abiotic stresses affect reproductive development by impacting on the source–sink relationships and on nutrient availability to developing reproductive structures. Here, we explore the scarce knowledge on the interactions between photoperiod, stress, and nutrient availability, and their effects on pre-anthesis development.

Nutrient availability influences floret survival

Crop plants initiate a large number of primordia, probably because the metabolic cost required to initiate floret primordia is low compared with that required to maintain floret growth to the stage of a fertile floret. However, only a certain proportion of those primordia develop into fertile florets. Floret survival is thus far more relevant than floret initiation in the determination of the final number of fertile florets, and the reason why some spikelets die and others become fertile is still under debate in the literature. A higher number of fertile florets per spike has been associated with an increased duration of the late reproductive phase in wheat and barley, possibly because extending this phase reduces the competition between spike and stem for limited assimilates, thereby increasing the number of fertile florets (Miralles *et al.*, 2000; González *et al.*, 2003; Isidro *et al.*, 2011; Guo and Schnurbusch, 2015; Guo *et al.*, 2015, 2016). The number of fertile florets is also regulated by autophagy, a self-degradative process by which cell organelles are eliminated (Glick *et al.*, 2010). For example, floret autophagy in wheat was shown to be triggered by sugar starvation generated by development, as accelerated plant development leads to increased carbohydrate consumption (Ghiglione *et al.*, 2008). Accordingly, culturing detached wheat spikes in sucrose solution increased the grain number per spike (Waters *et al.*, 1984). It was also shown that nitrogen fertilization controls floret fertility. In durum wheat (*Triticum durum*), floret initiation was not affected by different nitrogen fertilization regimes, but higher nitrogen fertilization accelerated the rate of floret development and improved the survival rate of florets (Ferrante *et al.*, 2010).

Interestingly, it was shown that increasing the light duration or intensity improved nutrient availability to the developing spike, possibly because of higher photosynthetic rates and carbon acquisition (González *et al.*, 2005). However, a recent study in barley suggested that photoperiod or genetic variation in photoperiod sensitivity may also affect the transport of nutrients to the spike. This came from the observation that many transcripts associated with the transport of sugars, amino acids, metal ions, and phosphate were up-regulated in the leaf at early reproductive stages in fast developing, photoperiod-responsive barley genotypes with high floret fertility (Digel *et al.*, 2015). Higher fertility was associated with the induction of *HvFT1* in the leaf and *HvFT2* in the meristem, and shown to be dependent on long-day photoperiods and allelic variation at *Ppd-H1*. The identified nutrient transporters were co-regulated with *HvFT1* expression in the leaf, suggesting that developmental signals affect source–sink relationships that lead to higher floret fertility (Digel *et al.*, 2015). One of those genes is involved in iron uptake from the soil to the roots, and interestingly its orthologue in Arabidopsis is *YELLOW STRIPE LIKE 3*, which has also been associated with flower fertility because mutants are impaired in the ability to remobilize iron from senescing leaves to the developing flowers (Waters *et al.*, 2006).

In addition, abiotic stresses impact on the nutrient balance in plants. First, drought may result in stomatal closure and, therefore, a reduction in photosynthesis and carbon

acquisition. Secondly, soil water deficits generally lead to an accumulation of carbon in the leaves for osmotic adjustment and to an increased transport of carbons to the roots (Hummel *et al.*, 2010). Abiotic stresses may thus reduce the transport of nutrients to the developing spike. In maize, sensitivity of female organs to drought stress has been attributed to problems with carbohydrate transport and metabolism. When comparing well-watered with drought-treated plants, carbohydrate transport to ovaries decreased in drought conditions and expression of carbohydrate (e.g. starch and sucrose) metabolism genes was altered (Mäkelä *et al.*, 2005; Kakumanu *et al.*, 2012). In wheat, anther development shows a high susceptibility to drought, and male gametophyte sterility is induced even under moderate water stress conditions (Saini, 1997; Saini and Westgate, 1999). The disruption of pollen development under drought correlated with changes in sugar metabolism within the anthers (Dorion *et al.*, 1996; Koonjul *et al.*, 2005). Genetic variability for drought tolerance of anther development was correlated with a potential to maintain carbohydrate allocation and sink strength in the reproductive organs in wheat (Ji *et al.*, 2010). Consequently, photoperiod and abiotic stresses control spike development by modifying nutrient availability in developing flower organs.

Increasing evidence suggests that nutrient availability is important not only to sustain development and growth but also in triggering developmental decisions. Sugars and nitrogen function both as metabolic sources and as signalling molecules (Sheen *et al.*, 1999; Smeekens and Hellmann, 2014), as exemplified by the dual role of hexose kinases as sugar sensors as well as part of developmental pathways to control gene expression (Granot *et al.*, 2013). In Arabidopsis, mutations in genes of key enzymes in sugar and starch metabolism such as *HEXOKINASE1* (*HXK1*) and *PHOSPHOGLUCOMUTASE1* (*PGM1*) affect various aspects of development, including flowering (Paul *et al.*, 2008).

In addition, TREHALOSE-6-PHOSPHATE (T6P) functions as a signalling molecule that relays information about carbohydrate availability to other signalling pathways, and the disruption of T6P metabolism causes a wide range of developmental phenotypes (van Dijken *et al.*, 2004; Lunn *et al.*, 2006; Ponnu *et al.*, 2011). A reduction of *TREHALOSE-6-PHOSPHATE SYNTHASE* (*TPS1*) expression levels caused a down-regulation of *FT* in the leaf and extremely late flowering in Arabidopsis (Wahl *et al.*, 2013). A recent study has shown that the overexpression of the rice *TPP1*, an enzyme responsible for the dephosphorylation of T6P to trehalose, in developing maize ears resulted in an increased yield stability, translated in increased kernel number and weight. The transgenic plants had low T6P and high sucrose levels when compared with the wild-type plants, suggesting an improved sink function of these tissues that translated into higher yield (Nuccio *et al.*, 2015; Smeekens, 2015). In cereals, T6P was also shown to accumulate during grain filling, probably related to increased sucrose supply (Martínez-Barajas *et al.*, 2011).

Furthermore, nitrogen levels modify flowering time in plants, with nitrogen limitation often inducing early flowering (Bernier *et al.*, 1993; Loeppky and Coulman, 2001; Castro Marín *et al.*, 2011; Liu *et al.*, 2013). Accordingly, high-nitrate conditions repress positive regulators of flowering such as *FT* and *APETALA1* (*AP1*), and the *GID1B* gibberellic acid (GA) receptor and induce negative regulators of GA signalling in Arabidopsis (Richter *et al.*, 2010; Kant *et al.*, 2011). These results are consistent with nitrate availability controlling members of the photoperiod pathway and the GA pathway at different levels (GA biosynthesis, perception, and signalling) to determine the timing of vegetative to reproductive phase change.

In summary, sugars and other nutrients are essential as sources of energy but also as signalling molecules and metabolic sensors of the plant energy status. Photoperiod, ambient temperature, and drought alter nutrient availability and distribution in the plant and may thus impact on spike development. In addition, nutrients trigger developmental decisions by controlling the expression of flowering time regulators. The involvement of flowering time genes in the remobilization and transport of nutrients and assimilates from source to sink organs as well as the control of flowering time genes by plant primary metabolism is not yet well explored in cereals and is an exciting avenue for future research.

Conclusion and future perspectives

Earlier physiological studies have dissected the effects of environmental cues on different phases of spike development in barley and wheat. These have found that spikelet initiation and floral morphogenesis are at least partly under different environmental and genetic control. Future studies need to elucidate further the genetic and molecular control of pre-anthesis in response to environmental cues that change source–sink relationships. Recent advances in the establishment of genomic and genetic resources (International Barley Genome Sequencing Consortium, 2012) and high-throughput metabolomic, proteomic, and transcriptome platforms now provide the basis to unravel the genetic, molecular, and metabolic regulation of spike development in barley and wheat. This information needs to be coupled with detailed physiological studies to better understand the genetic control of nutrient transport in the context of reproductive development in barley and wheat.

Acknowledgements

This work was supported by the Max Planck Society and by DFG grants SPP1530 ('Flowering time control: from natural variation to crop improvement'), the Excellence Cluster EXC10208, and the GRK2064 (Water use efficiency and drought stress responses: From Arabidopsis to Barley).

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