Influence of drought on the reproductive development of barley (*Hordeum vulgare* L.)

Inaugural-Dissertation

zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultät
der Heinrich-Heine-Universität Düsseldorf

vorgelegt von

Leonard Gol
aus Leverkusen

Düsseldorf, Juli 2020

aus dem Institut für Pflanzengenetik
der Heinrich-Heine-Universität Düsseldorf
und assoziiert mit dem
Department für Entwicklungsbiologie der Pflanzen
des Max-Planck-Instituts für Pflanzenzüchtungsforschung in Köln

Gedruckt mit der Genehmigung der
Mathematisch-Naturwissenschaftlichen Fakultät der
Heinrich-Heine-Universität Düsseldorf

Berichterstatter:

1. Professor Dr. Maria von Korff Schmising

2. Professor Dr. Rüdiger Simon

Tag der mündlichen Prüfung: 30.09.2020

I Eidesstattliche Erklärung

Eidesstattliche Erklärung zur Dissertation mit dem Titel:
„Influence of drought on the reproductive development of barley (*Hordeum vulgare* L.)"

Ich versichere an Eides statt, dass die Dissertation von mir selbständig und ohne unzulässige fremde Hilfe unter Beachtung der „Grundsätze zur Sicherung guter wissenschaftlicher Praxis an der Heinrich-Heine-Universität Düsseldorf" erstellt worden ist.

Außerdem versichere ich, dass ich diese Dissertation nur in diesem und keinem anderen Promotionsverfahren eingereicht habe und dass diesem Promotionsverfahren kein gescheitertes
Promotionsverfahren vorausgegangen ist.

Köln, 12.10.2020
Ort, Datum

Unterschrift

This work is funded by the DFG Research Training Group GRK2064 (Water use efficiency and drought stress responses: From Arabidopsis to Barley).

II Contents

III Summary

Variation in inflorescence development and flowering time has a major impact on reproductive success and yield in cereal crops, including barley *(Hordeum v. vulgare* L.). The genetic control of flowering time in response to photoperiod and vernalization is well described. In contrast, effects of drought on developmental timing and inflorescence development are poorly characterized and knowledge on the responsible genetic factors is limited. The aim of this work is to understand how reproductive development of barley is controlled by drought stress and if the central photoperiod response gene *PHOTOPERIOD-H1 (Ppd-H1)* modulates reproductive development in response to drought.

In the first chapter of my thesis, I reviewed the available literature on the effect of photoperiod and abiotic stresses on reproductive development in cereals. Here, I describe that effects of abiotic stress factors on plant development have received less attention than stress resistance mechanisms, e.g. evaporation resistance, and are therefore less well understood. Studies in the model species Arabidopsis (*Arabidopsis thaliana* L.) and rice (*Oryza sativa* L.) showed that often the same signaling pathways that control flowering in response to photoperiod and vernalization also fine-tune reproductive development in response to abiotic stress. Specifically, photoperiod response genes mediate development in response to drought. The same genetic factors may therefore integrate different seasonal cues to fine-tune development and adapt reproductive timing to seasonal and short-term environmental variation.

For the second chapter of my thesis, I therefore investigated the effect of drought on spike development in barley genotypes carrying natural variants at the major photoperiod response gene *Ppd-H1*.

For this purpose, I established and applied reproducible drought stress assays in soil. This facilitated a detailed analysis of the effect of drought on the shoot architecture and inflorescence development. To investigate the effect of *Ppd-H1,* three different spring barley cultivars which carry a mutated variant of *ppd-H1* with reduced function and derived introgression lines carrying the wild-type *Ppd-H1* allele were used. I first applied drought starting from early vegetative growth and lasting until maturity to study effects of drought on the transition from vegetative to reproductive development as well as floral progression.

Second, I subjected the plants to a severe transient drought treatment followed by rewatering during floral development.

I examined the effects of drought on the microscopic development of the shoot apex and numerous other developmental phenotypes including tiller and spike number, plant height, vegetative and reproductive biomass and leaf size. The study revealed that a continuous mild drought stress delayed floral development in genotypes with the mutated *ppd-H1* allele, whereas reproductive development was not altered by drought in the introgression lines. Furthermore, drought stress reduced the number of spikelets per spike and severely lowered the number of spikes per plant specifically in the spring barley genotypes compared to the introgression lines. The transient and severe stress slowed down development in all genotypes. However, after rewatering plants carrying the wild-type *Ppd-H1* allele accelerated development and flowered simultaneously with the control plants. In contrast, spring barley genotypes did not accelerate development and flowered significantly later.

I performed gene expression analyses on a diurnal and developmental time scale to identify genes downstream of *Ppd-H1* that correlate with the drought and *Ppd-H1*-dependent differences in development. Drought had only small effects on the diurnal expression levels of circadian clock genes and these were independent of variation at *Ppd-H1*. However, drought strongly reduced the expression of the flowering promotors *FLOWERING LOCUS T1* and the BARLEY MADS-box genes *BM3* and *BM8.* The downregulation of *BM3* and *BM8* correlated with the drought-induced differences in development between the parental spring barley genotypes and introgression lines.

In conclusion, with my work I could demonstrate that drought modulates barley development and thereby shoot and spike architectures. The photoperiod response gene *Ppd-H1* integrates drought and photoperiod cues to adjust the expression of *FT1*, *BM3* and *BM8* and thereby strongly influences developmental timing and other agronomically relevant characteristics, including the number of spikes per plant. My work provides new insights into the phenology and genetic control of reproductive development under drought in barley.

IV Zusammenfassung

Unterschiede in der Infloreszenzentwicklung und im Blühzeitpunkt haben einen entscheidenden Einfluss auf den reproduktiven Erfolg und den Ertrag von Getreide, inklusive Gerste (*Hordeum v. vulgare* L.). Die genetische Kontrolle des Blühzeitpunkts in Antwort auf die Photoperiode und Vernalisation sind umfassend charakterisiert. Im Gegensatz dazu ist der Einfluss von Trockenstress auf die Entwicklungsgeschwindigkeit und Infloreszenzentwicklung nur unvollständig beschrieben und wenig ist über die verantwortlichen genetischen Faktoren bekannt. Das Ziel dieser Arbeit ist es, zu verstehen, wie die reproduktive Entwicklung von Gerste von Trockenstress beeinflusst wird und ob das Gen *PHOTOPERIOD-H1 (Ppd-H1)*, welches eine zentrale Rolle für die Antwort auf die Photoperiode spielt, die reproduktive Entwicklung auch in Antwort auf Trockenstress moduliert.

Im ersten Kapitel meiner Dissertation habe ich die Literatur, die sich mit dem Einfluss der Photoperiode und abiotischer Stressfaktoren auf die reproduktive Entwicklung von Getreide beschäftigt, zusammengefasst und analysiert. Hierbei zeige ich auf, dass die Einflüsse abiotischer Stressfaktoren auf die pflanzliche Entwicklung weniger Aufmerksamkeit erfahren haben als andere Mechanismen der Stressresistenz, wie zum Beispiel Verdunstungsresistenz, und daher bisher weniger gut verstanden sind. Studien mit den Modellpflanzen Arabidopsis (*Arabidopsis thaliana* L.) und Reis (*Oryza sativa* L.) zeigten jedoch, dass oft die gleichen Signalkaskaden, welche die reproduktive Entwicklung in Antwort auf die Photoperiode und Vernalisation kontrollieren, auch für die präzise Anpassung der reproduktiven Entwicklung in Antwort auf Trockenstress verantwortlich sind. Insbesondere beeinflussen Gene der Antwort auf die Photoperiode auch die Entwicklung unter Trockenstress. Die gleichen genetischen Faktoren integrieren daher möglicherweise verschiedene saisonale Umwelteinflüsse, um die Sprossentwicklung sowohl an saisonale als auch an kurzfristige Umweltveränderungen anzupassen.

Für das zweite Kapitel meiner Dissertation habe ich daher den Einfluss von Trockenstress auf die Ährenentwicklung in Gerstelinien untersucht, die sich am Photoperiodegen *Ppd-H1* unterscheiden.

Zu diesem Zweck habe ich eine Methode entwickelt, mit der ich viele Pflanzen gleichzeitig einem gleichmäßigen Trockenstress unterziehen konnte. Dies ermöglichte mir

eine detaillierte Analyse des Einflusses von Trockenstress auf die Sprossarchitektur und die Entwicklung der Infloreszenz. Um den Einfluss von *Ppd-H1* zu untersuchen, nutzte ich drei verschiedene Sommergerstensorten, welche eine mutierte Variante von *ppd-H1* mit verminderter Funktion tragen, und abgeleitete Introgressionslinien mit dem Wildtyp Allel *Ppd-H1*. Die drei Sommergerstenlinien und abgeleiteten Introgressionslinien wurden unter zwei verschiedenen Trockenstressbedingungen getestet. Zuerst wurden die Pflanzen einem milden Trockenstress von der frühen vegetativen Entwicklung bis zur Erntereife ausgesetzt, um die Effekte von Trockenstress sowohl auf den Übergang von vegetativer zu reproduktiver Entwicklung als auch auf die weitere Infloreszenzentwicklung zu untersuchen. Außerdem behandelte ich dieselben Genotypen Pflanzen mit einem vorrübergehenden, schwerwiegenden Trockenstress gefolgt von der Wiederherstellung normaler Bewässerung während der Infloreszenzentwicklung.

Ich untersuchte den Einfluss von Trockenstress auf die mikroskopische Entwicklung der Sprossspitze und zahlreiche andere Merkmale der Sprossentwicklung, wie der Anzahl der Seitentriebe und Ähren, der Wuchshöhe, der vegetativen und reproduktiven Biomasse sowie der Blattgröße. Meine Untersuchung zeigte auf, dass ein milder, kontinuierlicher Trockenstress die Blütenentwicklung in Genotypen mit einem mutierten *ppd-H1* Allel verlangsamte, während die reproduktive Entwicklung in den Introgressionslinien nicht beeinflusst wurde. Des Weiteren reduzierte Trockenstress die Anzahl der Ährchen pro Ähre und sorgte für eine starke Verringerung der Anzahl von Ähren pro Pflanze speziell in den untersuchten Sommergersten im Vergleich zu den Introgressionslinien. Der vorrübergehende, schwerwiegende Trockenstress verlangsamte die Entwicklung aller Genotypen. Allerdings beschleunigten Pflanzen, welche das Wildtyp Allel von *Ppd-H1* tragen, ihre Entwicklung, nachdem die normale Bewässerung wieder aufgenommen wurde und blühten zeitgleich mit durchgehend gewässerten Kontrollpflanzen. Im Gegensatz dazu beschleunigten die Sommergersten ihre Entwicklung nicht und blühten zu einem signifikant späteren Zeitpunkt.

Ich führte Genexpressionsanalysen über diurnale und die Entwicklung umfassende Zeitskalen durch, um *Ppd-H1* nachgeschaltete Gene zu identifizieren, welche mit den Trockenstress- und *Ppd-H1*-abhängigen Unterschieden der Entwicklung korrelieren. Trockenstress zeigte nur einen geringen Einfluss auf die diurnalen Expressionslevel von Genen der zirkadianen Uhr und diese waren zudem unabhängig von der Variation an *Ppd-H1*. Allerdings reduzierte Trockenstress stark die Expression der blühinduzierenden

Gene *FLOWERING LOCUS T1* und den BARLEY MADS-box Genen *BM3* und *BM8.* Die Repression von *BM3* und *BM8* korrelierte dabei mit den Trockenstress-induzierten Unterschieden in der Entwicklung zwischen den elterlichen Sommergersten und den Introgressionslinien.

Zusammengefasst konnte ich mit meiner Arbeit zeigen, dass Trockenstress die Entwicklung von Gerste und dadurch auch die Spross- und Ährenarchitektur moduliert. *Ppd-H1,* als zentrales Gen der Antwort auf die Photoperiode, integriert Signale von Trockenstress und Photoperiode, um die Expression von *FT1, BM3* und *BM8* anzupassen und übt dadurch einen starken Einfluss auf die Entwicklungsgeschwindigkeit und andere agronomisch bedeutsame Charakteristika wie die Anzahl von Ähren pro Pflanze aus. Meine Arbeit liefert neue Erkenntnisse über die Phänologie und die genetische Kontrolle der reproduktiven Entwicklung unter Trockenstress in Gerste.

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

The following manuscript was published in the Journal of Experimental Botany in April, 2017.

Authors:

Leonard Gol[1], Filipa Tomé[1,2,3] and Maria von Korff[1,2,3,]

Affiliations:

[1] Max Planck Institute for Plant Breeding Research, D-50829, Cologne, Germany

[2] Institute of Plant Genetics, Heinrich-Heine-University, D-40225 Düsseldorf, Germany

[3] Cluster of Excellence on Plant Sciences 'From Complex Traits towards Synthetic Modules', D-40225 Düsseldorf, Germany

Contributions:

The manuscript was written by Leonard Gol and Filipa Tomé with help from Maria von Korff. Leonard Gol wrote the section on development under different abiotic stresses. Filipa Tomé wrote the section on nutrient signaling in the context of development.

Journal of Experimental Botany, Vol. 68, No. 7 pp. 1399–1410, 2017
doi:10.1093/jxb/erx055

FLOWERING NEWSLETTER REVIEW

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

Leonard Gol[1,*], Filipa Tomé[1,2,3*] and Maria von Korff[1,2,3,†]

[1] Max Planck Institute for Plant Breeding Research, D-50829, Cologne, Germany
[2] Institute of Plant Genetics, Heinrich-Heine-University, D-40225 Düsseldorf, Germany
[3] Cluster of Excellence on Plant Sciences 'From Complex Traits towards Synthetic Modules', D-40225 Düsseldorf, Germany

* These authors contributed equally to this work.
† Correspondence: korff@mpipz.mpg.de

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;

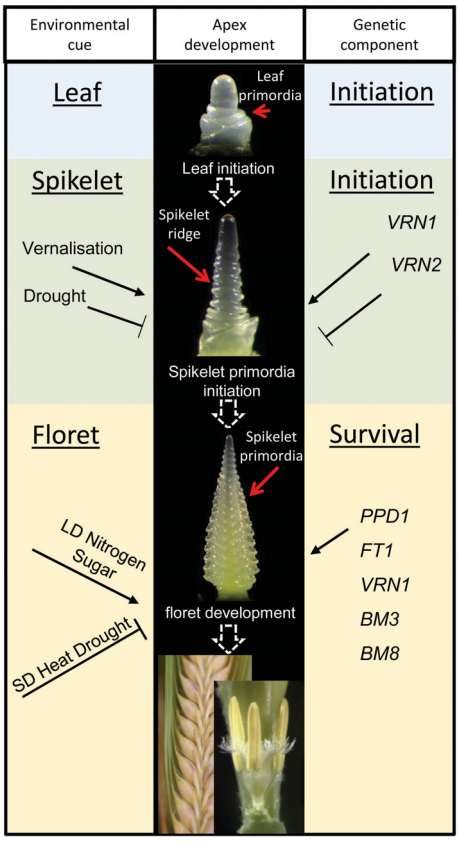


Fig. 1. Schematic representation of the development of the shoot apical meristem in response to different environmental cues in barley. The effects of environmental factors on spikelet primordia initiation and floret survival are given on the left-hand side. The effects of major genetic components on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

References

Al-Ajlouni Z, Al-Abdallat A, Al-Ghzawi A, Ayad J, Abu Elenein J, Al-Quraan N, Baenziger P. 2016. Impact of pre-anthesis water deficit on yield and yield components in barley (*Hordeum vulgare* L.) plants grown under controlled conditions. Agronomy **6,** 33.

Alqudah AM, Schnurbusch T. 2014. Awn primordium to tipping is the most decisive developmental phase for spikelet survival in barley. Functional Plant Biology **41,** 424–436.

Alqudah AM, Sharma R, Pasam RK, Graner A, Kilian B, Schnurbusch T. 2014. Genetic dissection of photoperiod response based on GWAS of pre-anthesis phase duration in spring barley. PLoS One **9,** e113120.

Alvarez MA, Tranquilli G, Lewis S, Kippes N, Dubcovsky J. 2016. Genetic and physical mapping of the earliness per se locus Eps-A (m) 1 in Triticum monococcum identifies *EARLY FLOWERING 3* (*ELF3*) as a candidate gene. Functional and Integrative Genomics **16,** 365–382.

Andrés F, Coupland G. 2012. The genetic basis of flowering responses to seasonal cues. Nature Reviews. Genetics **13,** 627–639.

Aspinall D. 1966. Effects of day length and light intensity on growth of barley IV. Genetically controlled variation in response to photoperiod. Australian Journal of Biological Sciences **19,** 517.

Aspinall D. 1969. The effects of day length and light intensity on the growth of barley. 6. Interactions between the effects of temperature, photoperiod, and the spectral composition of the light source. Australian Journal of Bological Sciences **22,** 153–6724.

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

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

Batch JJ, Morgan DG. 1974. Male sterility induced in barley by photoperiod. Nature **250,** 165–167.

Baum M, Von Korff M, Guo P, *et al*. 2007. Molecular approaches and breeding strategies for drought tolerance in barley. In: Varshney R, Tuberosa R, eds. Genomic assisted crop improvement: Vol. 2: genomics applications in crops. Dordrecht, The Netherlands: Springer, 51–79.

Beales J, Turner A, Griffiths S, Snape JW, Laurie DA. 2007. A pseudo-response regulator is misexpressed in the photoperiod insensitive *Ppd-D1a* mutant of wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics **115,** 721–733.

Bernier G, Havelange A, Houssa C, Petitjean A, Lejeune P. 1993. Physiological signals that induce flowering. The Plant Cell **5,** 1147–1155.

Bingham J. 1966. Varietal response in wheat to water supply in the field, and male sterility caused by a period of drought in a glasshouse experiment. Annals of Applied Biology **57,** 365–377.

Bita CE, Gerats T. 2013. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Frontiers in Plant Science **4,** 273.

Boden SA, Weiss D, Ross JJ, Davies NW, Trevaskis B, Chandler PM, Swain SM. 2014. *EARLY FLOWERING3* regulates flowering in spring barley by mediating gibberellin production and *FLOWERING LOCUS T* expression. The Plant Cell **26,** 1557–1569.

Box MS, Huang BE, Domijan M, *et al*. 2015. *ELF3* controls thermoresponsive growth in *Arabidopsis*. Current Biology **25,** 194–199.

Campoli C, Drosse B, Searle I, Coupland G, von Korff M. 2012*a*. Functional characterisation of *HvCO1*, the barley (*Hordeum vulgare*) flowering time ortholog of *CONSTANS*. The Plant Journal **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 Phytologist **199,** 1045–1059.

Campoli C, Shtaya M, Davis SJ, von Korff M. 2012*b*. 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 Biology **12,** 97.

Campoli C, von Korff M. 2014. Genetic control of reproductive development in temperate cereals. In: Fornara F, ed. Advances in botanical research: Vol. 72. The molecular genetics of floral transition and flower development. New York: Academic Press, 131–158.

Castro Marín I, Loef I, Bartetzko L, Searle I, Coupland G, Stitt M, Osuna D. 2011. Nitrate regulates floral induction in Arabidopsis, acting independently of light, gibberellin and autonomous pathways. Planta **233,** 539–552.

Chen A, Li C, Hu W, *et al*. 2014. Phytochrome C plays a major role in the acceleration of wheat flowering under long-day photoperiod. Proceedings of the National Academy of Sciences, USA **111,** 10037–10044.

Cockram J, Jones H, Leigh FJ, O'Sullivan D, Powell W, Laurie DA, Greenland AJ. 2007. Control of flowering time in temperate cereals: genes, domestication, and sustainable productivity. Journal of Experimental Botany **58,** 1231–1244.

Conti L, Galbiati M, Tonelli C. 2014. ABA and the floral transition. In: Zhang D-P, ed. Abscisic acid: metabolism, transport and signaling. Dordrecht, The Netherlands: Springer, 137–175.

Corbesier L, Vincent C, Jang S, *et al*. 2007. FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. Science **316,** 1030–1033.

Dai A. 2012. Increasing drought under global warming in observations and models. Nature Climate Change **3,** 52–58.

Dennis ES, Peacock WJ, Michaels S, *et al*. 2009. Vernalization in cereals. Journal of Biology **8,** 57.

Díaz A, Zikhali M, Turner AS, *et al*. 2012. Copy number variation affecting the *Photoperiod-B1* and *Vernalization-A1* genes is associated with altered flowering time in wheat (*Triticum aestivum*). PLoS One **7,** e33234.

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. The 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 Physiology **172,** 405–415.

Dorion S, Lalonde S, Saini HS. 1996. Induction of male sterility in wheat by meiotic-stage water deficit is preceded by a decline in invertase activity and changes in carbohydrate metabolism in anthers. Plant Physiology **111,** 137–145.

Drosse B, Campoli C, Mulki A, Von Korff M. 2014. Genetic control of reproductive development. In: Kumlehn J, Stein N. eds. Biotechnological approaches to barley improvement, biotechnology in agriculture and forestry. Berlin, Heidelberg: Springer, 81–99.

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

Ejaz M, von Korff M. 2017. The genetic control of reproductive development under high ambient temperature. Plant Physiology **173,** 294–306.

Enomoto N. 1929. On the physiological difference between the spring and winter types in wheat and barley. Journal of the Imperial Agricultural Experimental Station **1,** 107–38 (in Japanese with English summary).

Farooq M, Hussain M, Wahid A, Siddique KHM. 2012. Drought stress in plants: an overview. In: Aroca R, ed. Plant responses to drought stress. Berlin: Springer, 1–33.

Faure S, Higgins J, Turner A, Laurie DA. 2007. The FLOWERING LOCUS T-like gene family in barley (Hordeum vulgare). Genetics **176,** 599–609.

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. Proceedings of the National Academy of Sciences, USA **109,** 8328–8333.

Ferdous J, Sanchez-Ferrero JC, Langridge P, Milne L, Chowdhury J, Brien C, Tricker PJ. 2017. Differential expression of microRNAs and potential targets under drought stress in barley. Plant, Cell and Environment **40,** 11–24.

Fernández V, Takahashi Y, Le Gourrierec J, Coupland G. 2016. Photoperiodic and thermosensory pathways interact through *CONSTANS* to promote flowering at high temperature under short days. The Plant Journal **86,** 426–440.

Ferrante A, Savin R, Slafer GA. 2010. Floret development of durum wheat in response to nitrogen availability. Journal of Experimental Botany **61,** 4351–4359.

Fu D, Szucs P, Yan L, Helguera M, Skinner JS, von Zitzewitz J, Hayes PM, Dubcovsky J. 2005. Large deletions within the first intron in VRN-1 are associated with spring growth habit in barley and wheat. Molecular Genetics and Genomics **273,** 54–65.

Galbiati F, Chiozzotto R, Locatelli F, Spada A, Genga A, Fornara F. 2016. Hd3a, RFT1 and Ehd1 integrate photoperiodic and drought stress

signals to delay the floral transition in rice. Plant, Cell and Environment **39,** 1982–1993.

Ghiglione HO, Gonzalez FG, Serrago R, Maldonado SB, Chilcott C, Curá JA, Miralles DJ, Zhu T, Casal JJ. 2008. Autophagy regulated by day length determines the number of fertile florets in wheat. The Plant Journal **55,** 1010–1024.

Glick D, Barth S, Macleod KF. 2010. Autophagy: cellular and molecular mechanisms. Journal of Pathology **221,** 3–12.

González FG, Slafer GA, Miralles DJ. 2002. Vernalization and photoperiod responses in wheat pre-flowering reproductive phases. Field Crops Research **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. Field Crops Research **81,** 17–27.

González FG, Slafer GA, Miralles DJ. 2005. Photoperiod during stem elongation in wheat: is its impact on fertile floret and grain number determination similar to that of radiation? Functional Plant Biology **32,** 181–188.

Granot D, David-Schwartz R, Kelly G. 2013. Hexose kinases and their role in sugar-sensing and plant development. Frontiers in Plant Science **4,** 44.

Greenup A, Peacock WJ, Dennis ES, Trevaskis B. 2009. The molecular biology of seasonal flowering-responses in Arabidopsis and the cereals. Annals of Botany **103,** 1165–1172.

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 Physiology **153,** 1062–1073.

Griffiths FEW, Lyndon RF, Bennett MD. 1985. The effects of vernalization on the growth of the wheat shoot apex. Annals of Botany **56,** 501–511.

Guo P, Baum M, Grando S, *et al*. 2009. Differentially expressed genes between drought-tolerant and drought-sensitive barley genotypes in response to drought stress during the reproductive stage. Journal of Experimental Botany **60,** 3531–3544.

Guo Z, Chen D, Alqudah AM, Röder MS, Ganal MW, Schnurbusch T. 2016. Genome-wide association analyses of 54 traits identified multiple loci for the determination of floret fertility in wheat. New Phytologist (in press).

Guo Z, Chen D, Schnurbusch T. 2015. Variance components, heritability and correlation analysis of anther and ovary size during the floral development of bread wheat. Journal of Experimental Botany **66,** 3099–3111.

Guo Z, Schnurbusch T. 2015. Variation of floret fertility in hexaploid wheat revealed by tiller removal. Journal of Experimental Botany **66,** 5945–5958.

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 and Environment **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. The Plant Journal **33,** 875–885.

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

Herrero E, Kolmos E, Bujdoso N, *et al*. 2012. EARLY FLOWERING4 recruitment of EARLY FLOWERING3 in the nucleus sustains the Arabidopsis circadian clock. The Plant Cell **24,** 428–443.

Hummel I, Pantin F, Sulpice R, *et al*. 2010. Arabidopsis plants acclimate to water deficit at low cost through changes of carbon usage: an integrated perspective using growth, metabolite, enzyme, and gene expression analysis. Plant Physiology **154,** 357–372.

Husain I, Aspinall AD. 1970. Water stress and apical morphogenesis in barley. Annals of Botany **34,** 393–407.

International Barley Genome Sequencing Consortium. 2012. A physical, genetic and functional sequence assembly of the barley genome. Nature **491,** 711–6.

Isidro J, Alvaro F, Royo C, Villegas D, Miralles DJ, García del Moral LF. 2011. Changes in duration of developmental phases of durum wheat caused by breeding in Spain and Italy during the 20th century and its impact on yield. Annals of Botany **107,** 1355–1366.

Ji X, Shiran B, Wan J, Lewis DC, Jenkins CL, Condon AG, Richards RA, Dolferus R. 2010. Importance of pre-anthesis anther sink strength for maintenance of grain number during reproductive stage water stress in wheat. Plant, Cell and Environment **33,** 926–942.

Johansson M, Staiger D. 2015. Time to flower: interplay between photoperiod and the circadian clock. Journal of Experimental Botany **66,** 719–730.

Jones H, Leigh FJ, Mackay I, *et al*. 2008. Population-based resequencing reveals that the flowering time adaptation of cultivated barley originated east of the Fertile Crescent. Molecular Biology and Evolution **25,** 2211–2219.

Kakumanu A, Ambavaram MM, Klumas C, Krishnan A, Batlang U, Myers E, Grene R, Pereira A. 2012. Effects of drought on gene expression in maize reproductive and leaf meristem tissue revealed by RNA-Seq. Plant Physiology **160,** 846–867.

Kant S, Peng M, Rothstein SJ. 2011. Genetic regulation by NLA and microRNA827 for maintaining nitrate-dependent phosphate homeostasis in arabidopsis. PLoS Genetics **7,** e1002021.

Kazan K, Lyons R. 2016. The link between flowering time and stress tolerance. Journal of Experimental Botany **67,** 47–60.

Kirby E, Appleyard M. 1987. Cereal development guide. Kenilworth: Arable Unit, National Agricultural Centre.

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. Current Biology **19,** 408–413.

Kojima S, Takahashi Y, Kobayashi Y, Monna L, Sasaki T, Araki T, Yano M. 2002. Hd3a, a rice ortholog of the Arabidopsis FT gene, promotes transition to flowering downstream of Hd1 under short-day conditions. Plant and Cell Physiology **43,** 1096–1105.

Koonjul PK, Minhas JS, Nunes C, Sheoran IS, Saini HS. 2005. Selective transcriptional down-regulation of anther invertases precedes the failure of pollen development in water-stressed wheat. Journal of Experimental Botany **56,** 179–190.

Kooyers NJ. 2015. The evolution of drought escape and avoidance in natural herbaceous populations. Plant Science **234,** 155–162.

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.

Li C, Distelfeld A, Comis A, Dubcovsky J. 2011. Wheat flowering repressor VRN2 and promoter CO2 compete for interactions with NUCLEAR FACTOR-Y complexes. The Plant Journal **67,** 763–773.

Li C, Dubcovsky J. 2008. Wheat FT protein regulates VRN1 transcription through interactions with FDL2. The Plant Journal **55,** 543–554.

Liu T, Li Y, Ren J, Qian Y, Yang X, Duan W, Hou X. 2013. Nitrate or NaCl regulates floral induction in *Arabidopsis thaliana*. Biologia **68,** 215–222.

Loeppky HA, Coulman BE. 2001. Residue removal and nitrogen fertilization affects tiller development and flowering in meadow bromegrass. Agronomy Journal **93,** 891.

Lunn JE, Feil R, Hendriks JH, *et al*. 2006. Sugar-induced increases in trehalose 6-phosphate are correlated with redox activation of ADPglucose pyrophosphorylase and higher rates of starch synthesis in *Arabidopsis thaliana*. Biochemical Journal **397,** 139–148.

Lutz U, Posé D, Pfeifer M, *et al*. 2015. Modulation of ambient temperature-dependent flowering in *Arabidopsis thaliana* by natural variation of *FLOWERING LOCUS M*. PLoS Genetics **11,** e1005588.

Mäkelä P, McLaughlin JE, Boyer JS. 2005. Imaging and quantifying carbohydrate transport to the developing ovaries of maize. Annals of Botany **96,** 939–949.

Martínez-Barajas E, Delatte T, Schluepmann H, *et al*. 2011. Wheat grain development is characterized by remarkable trehalose 6-phosphate accumulation pregrain filling: tissue distribution and relationship to SNF1-related protein kinase1 activity. Plant Physiology **156,** 373–381.

Miralles D, Richards RA. 2000. Responses of leaf and tiller emergence and primordium initiation in wheat and barley to interchanged photoperiod. Annals of Botany **85,** 655–663.

Miralles DJ, Richards RA, Slafer GA. 2000. Duration of the stem elongation period influences the number of fertile florets in wheat and barley. Functional Plant Biology **27,** 931–940.

Mizuno T, Kitayama M, Oka H, Tsubouchi M, Takayama C, Nomoto Y, Yamashino T. 2014*a*. The EC night-time repressor plays a crucial role in modulating circadian clock transcriptional circuitry by conservatively double-checking both warm-night and night-time-light signals in a synergistic manner in *Arabidopsis thaliana*. Plant and Cell Physiology **55,** 2139–2151.

Mizuno N, Nitta M, Sato K, Nasuda S. 2012. A wheat homologue of *PHYTOCLOCK 1* is a candidate gene conferring the early heading phenotype to einkorn wheat. Genes and Genetic Systems **87,** 357–367.

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

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

Müller LM, von Korff M, Davis SJ. 2014. Connections between circadian clocks and carbon metabolism reveal species-specific effects on growth control. Journal of Experimental Botany **65,** 2915–2923.

Nakamichi N, Takao S, Kudo T, Kiba T, Wang Y, Kinoshita T, Sakakibara H. 2016. Improvement of Arabidopsis biomass and cold, drought and salinity stress tolerance by modified circadian clock-associated PSEUDO-RESPONSE REGULATORs. Plant and Cell Physiology **57,** 1085–1097.

Nicholls P, May L. 1963. Studies on the growth of the barley apex I. Interrelationships between primordium formation, apex length, and spikelet development. Australian Journal of Biological Sciences **16,** 561.

Nishida H, Yoshida T, Kawakami K, Fujita M, Long B, Akashi Y, Laurie DA, Kato K. 2013. Structural variation in the 5′ upstream region of photoperiod-insensitive alleles *Ppd-A1a* and *Ppd-B1a* identified in hexaploid wheat (*Triticum aestivum* L.), and their effect on heading time. Molecular Breeding **31,** 27–37.

Nuccio ML, Wu J, Mowers R, *et al*. 2015. Expression of trehalose-6-phosphate phosphatase in maize ears improves yield in well-watered and drought conditions. Nature Biotechnology **33,** 862–869.

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, *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.

Paul MJ, Primavesi LF, Jhurreea D, Zhang Y. 2008. Trehalose metabolism and signaling. Annual Review of Plant Biology **59,** 417–441.

Pearce S, Vanzetti LS, Dubcovsky J. 2013. Exogenous gibberellins induce wheat spike development under short days only in the presence of *VERNALIZATION1*. Plant Physiology **163,** 1433–1445.

Petroni K, Kumimoto RW, Gnesutta N, Calvenzani V, Fornari M, Tonelli C, Holt BF 3rd, Mantovani R. 2012. The promiscuous life of plant NUCLEAR FACTOR Y transcription factors. The Plant Cell **24,** 4777–4792.

Ponnu J, Wahl V, Schmid M. 2011. Trehalose-6-phosphate: connecting plant metabolism and development. Frontiers in Plant Science **2,** 70.

Porter JR, Gawith M. 1999. Temperatures and the growth and development of wheat: a review. European Journal of Agronomy **10,** 23–36.

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.

Raschke A, Ibañez C, Ullrich KK, *et al*. 2015. Natural variants of *ELF3* affect thermomorphogenesis by transcriptionally modulating *PIF4*-dependent auxin response genes. BMC Plant Biology **15,** 197.

Rawson HM, Richards RA. 1993. Effects of high temperature and photoperiod on floral development in wheat isolines differing in vernalisation and photoperiod genes. Field Crops Research **32,** 181–192.

Reynolds M, Foulkes MJ, Slafer GA, Berry P, Parry MA, Snape JW, Angus WJ. 2009. Raising yield potential in wheat. Journal of Experimental Botany **60,** 1899–1918.

Riboni M, Galbiati M, Tonelli C, Conti L. 2013. *GIGANTEA* enables drought escape response via abscisic acid-dependent activation of the florigens and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS*. Plant Physiology **162,** 1706–1719.

Riboni M, Robustelli Test A, Galbiati M, Tonelli C, Conti L. 2014. Environmental stress and flowering time: the photoperiodic connection. Plant Signaling and Behavior **9,** e29036.

Riboni M, Robustelli Test A, Galbiati M, Tonelli C, Conti L. 2016. ABA-dependent control of *GIGANTEA* signalling enables drought escape via up-regulation of *FLOWERING LOCUS T* in *Arabidopsis thaliana*. Journal of Experimental Botany **67,** 6309–6322.

Richter R, Behringer C, Müller IK, Schwechheimer C. 2010. The GATA-type transcription factors GNC and GNL/CGA1 repress gibberellin signaling downstream from DELLA proteins and PHYTOCHROME-INTERACTING FACTORS. Genes and Development **24,** 2093–2104.

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. Annals of Botany **62,** 127–144.

Rollins JA, 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.). Journal of Experimental Botany **64,** 3201–3212.

Saini HS. 1997. Effects of water stress on male gametophyte development in plants. Sexual Plant Reproduction **10,** 67–73.

Saini HS, Westgate ME. 1999. Reproductive development in grain crops during drought. Advances in Agronomy **68,** 59–96.

Saisho D, Ishii M, Hori K, Sato K. 2011. Natural variation of barley vernalization requirements: implication of quantitative variation of winter growth habit as an adaptive trait in East Asia. Plant and Cell Physiology **52,** 775–784.

Sasani S, Hemming MN, Oliver SN, *et al*. 2009. The influence of vernalization and daylength on expression of flowering-time genes in the shoot apex and leaves of barley (*Hordeum vulgare*). Journal of Experimental Botany **60,** 2169–2176.

Schmalenbach I, Zhang L, Reymond M, Jiménez-Gómez JM. 2014. The relationship between flowering time and growth responses to drought in the Arabidopsis Landsberg *erecta* × Antwerp-1 population. Frontiers in Plant Science **5,** 609.

Shaw LM, Turner AS, Herry L, *et al*. 2013. Mutant alleles of *Photoperiod-1* in wheat (*Triticum aestivum* L.) that confer a late flowering phenotype in long days PLoS One **8,** e79459.

Sheen J, Zhou L, Jang JC. 1999. Sugars as signaling molecules. Current Opinion in Plant Biology **2,** 410–418.

Singh TN, Paleg IG, Aspinall D. 1973. Stress metabolism I. Nitrogen metabolism and growth in the barley plant during water stress. Australian Journal of Biological Sciences **26,** 45–56.

Slafer GA. 2003. Genetic basis of yield as viewed from a crop physiologist's perspective. Annals of Applied Biology **142,** 117–128.

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. Australian Journal of Plant Physiology **21,** 393.

Slafer GA, Savin R, Sadras VO. 2014. Coarse and fine regulation of wheat yield components in response to genotype and environment. Field Crops Research **157,** 71–83.

Smeekens S. 2015. From leaf to kernel: trehalose-6-phosphate signaling moves carbon in the field. Plant Physiology **169,** 912–913.

Smeekens S, Hellmann HA. 2014. Sugar sensing and signaling in plants. Frontiers in Plant Science **5,** 113.

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

Stocker T, Qin D, Plattner G, Tignor M, Allen S. 2013. Climate change 2013: the physical science basis. Cambridge: Cambridge University Press.

Stratonovitch P, Semenov MA. 2015. Heat tolerance around flowering in wheat identified as a key trait for increased yield potential in Europe under climate change. Journal of Experimental Botany **66,** 3599–3609.

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

Szucs P, Skinner JS, Karsai I, Cuesta-Marcos A, Haggard KG, Corey AE, Chen TH, Hayes PM. 2007. Validation of the *VRN-H2/VRN-H1* epistatic model in barley reveals that intron length variation in *VRN-H1* may account for a continuum of vernalization sensitivity. Molecular Genetics and Genomics **277,** 249–261.

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. Proceedings of the National Academy of Sciences, 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. Journal of Experimental Botany **65,** 1141–1151.

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.

van Dijken AJ, Schluepmann H, Smeekens SC. 2004. Arabidopsis trehalose-6-phosphate synthase 1 is essential for normal vegetative growth and transition to flowering. Plant Physiology **135,** 969–977.

von Korff M, Grando S, Del Greco A, This D, Baum M, Ceccarelli S. 2008. Quantitative trait loci associated with adaptation to Mediterranean dryland conditions in barley. Theoretical and Applied Genetics **117,** 653–669.

von Zitzewitz J, Szucs P, Dubcovsky J, *et al*. 2005. Molecular and structural characterization of barley vernalization genes. Plant Molecular Biology **59,** 449–467.

Waddington SR, Cartwright PM, Wall PC. 1983. A quantitative scale of spike initial and pistil development in barley and wheat. Annals of Botany **51,** 119–130.

Wahl V, Ponnu J, Schlereth A, *et al*. 2013. Regulation of flowering by trehalose-6-phosphate signaling in *Arabidopsis thaliana*. Science **339,** 704–707.

Waters BM, Chu HH, Didonato RJ, Roberts LA, Eisley RB, Lahner B, Salt DE, Walker EL. 2006. Mutations in Arabidopsis *yellow stripe-like1* and *yellow stripe-like3* reveal their roles in metal ion homeostasis and loading of metal ions in seeds. Plant Physiology **141,** 1446–1458.

Waters SP, Martin P, Lee BT. 1984. The influence of sucrose and abscisic acid on the determination of grain number in wheat. Journal of Experimental Botany **35,** 829–840.

Wilhelm EP, Turner AS, Laurie DA. 2009. Photoperiod insensitive *Ppd-A1a* mutations in tetraploid wheat (*Triticum durum* Desf.). Theoretical and Applied Genetics **118,** 285–294.

Worland AJ, Borner A, Korzun V, Li WM, Petrovic S, Sayers EJ. 1998. The influence of photoperiod genes on the adaptability of European winter wheats. Euphytica **100,** 385–394.

Xu MY, Zhang L, Li WW, Hu XL, Wang MB, Fan YL, Zhang CY, Wang L. 2014. Stress-induced early flowering is mediated by miR169 in Arabidopsis thaliana. Journal of Experimental Botany **65,** 89–101.

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*. Proceedings of the National Academy of Sciences, USA **100,** 6263–6268.

Yu SM, Lo SF, Ho TH. 2015. Source–sink communication: regulated by hormone, nutrient, and stress cross-signaling. Trends in Plant Science **20,** 844–857.

Zakhrabekova S, Gough SP, Braumann I, *et al*. 2012. Induced mutations in circadian clock regulator *Mat-a* facilitated short-season adaptation and range extension in cultivated barley. Proceedings of the National Academy of Sciences, USA **109,** 4326–4331.

Zavadskaja I, Skazkin F. 1960. Microsporogenesis in barley with deficient soil moisture and application of nitrogen at different developmental periods. Proceedings of the USSR Academy of Sciences **131,** 692–694.

Zhang C, Liu J, Zhao T, *et al*. 2016. A drought-inducible transcription factor delays reproductive timing in rice. Plant Physiology **171,** 334–343.

Zhang X, Zou Z, Gong P, Zhang J, Ziaf K, Li H, Xiao F, Ye Z. 2011. Over-expression of microRNA169 confers enhanced drought tolerance to tomato. Biotechnology Letters **33,** 403–409.

Zhao B, Ge L, Liang R, Li W, Ruan K, Lin H, Jin Y. 2009. Members of miR-169 family are induced by high salinity and transiently inhibit the NF-YA transcription factor. BMC Molecular Biology **10,** 29.

VI *Ppd-H1* integrates drought stress signals to control spike development and flowering time in barley

The following manuscript was published in the Journal of Experimental Botany in May, 2020.

Authors:

Leonard Gol[1,2], Einar B. Haraldsson[1], Maria von Korff[1,2,3,*]

Affiliations:

[1] Institute for Plant Genetics, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany

[2] Max-Planck-Institute for Plant Breeding Research, Cologne, Germany

[3] Cluster of Excellence on Plant Sciences, "SMART Plants for Tomorrows Needs", Heinrich-Heine University Düsseldorf, Düsseldorf, Germany

Contributions:

Leonard Gol and Maria von Korff conceived the project and planned the experiments. Leonard Gol performed all experiments, except experiments with Golden Promise which were performed by Leonard Gol and Einar Baldvin Haraldsson. Leonard Gol wrote the manuscript with help from Maria von Korff.

Supplementary data:

Supplementary Data S1.:" Genotyping of introgression lines used in this study." is available online at the Journal of Experimental Botany: https://academic.oup.com/jxb/advance-article/doi/10.1093/jxb/eraa261/5847724

Journal of Experimental Botany
doi:10.1093/jxb/eraa261 Advance Access Publication 27 May 2020
This paper is available online free of all access charges (see https://academic.oup.com/jxb/pages/openaccess for further details)

RESEARCH PAPER

Ppd-H1 integrates drought stress signals to control spike development and flowering time in barley

Leonard Gol[1,2], Einar B. Haraldsson[1] and Maria von Korff[1,2,3,*]

[1] Institute for Plant Genetics, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany
[2] Max-Planck-Institute for Plant Breeding Research, Cologne, Germany
[3] Cluster of Excellence on Plant Sciences, 'SMART Plants for Tomorrows Needs', Heinrich-Heine University Düsseldorf, Düsseldorf, Germany

* Correspondence: maria.korff.schmising@hhu.de

Received 26 March 2020; Editorial decision 19 May 2020; Accepted 21 May 2020

Editor: Matt Jones, University of Glasgow, UK

Abstract

Drought impairs growth and spike development, and is therefore a major cause of yield losses in the temperate cereals barley and wheat. Here, we show that the photoperiod response gene *PHOTOPERIOD-H1* (*Ppd-H1*) interacts with drought stress signals to modulate spike development. We tested the effects of a continuous mild and a transient severe drought stress on developmental timing and spike development in spring barley cultivars with a natural mutation in *ppd-H1* and derived introgression lines carrying the wild-type *Ppd-H1* allele from wild barley. Mild drought reduced the spikelet number and delayed floral development in spring cultivars but not in the introgression lines with a wild-type *Ppd-H1* allele. Similarly, drought-triggered reductions in plant height, and tiller and spike number were more pronounced in the parental lines compared with the introgression lines. Transient severe stress halted growth and floral development; upon rewatering, introgression lines, but not the spring cultivars, accelerated development so that control and stressed plants flowered almost simultaneously. These genetic differences in development were correlated with a differential down-regulation of the flowering promotors *FLOWERING LOCUS T1* and the BARLEY MADS-box genes *BM3* and *BM8.* Our findings therefore demonstrate that *Ppd-H1* affects developmental plasticity in response to drought in barley.

Keywords: Barley, development, drought, flowering, *FLOWERING LOCUS T*, MADS-box genes, photoperiod, stress.

Introduction

Global warming increases the frequency and intensity of severe water scarcity events, which negatively affect the yield of rain-fed crops such as barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) (Xie *et al.*, 2018; Kahiluoto *et al.*, 2019). Drought during reproductive development impairs spike development and floret fertility, and is therefore a major cause of yield losses in these temperate cereals (Gol *et al.*, 2017). At present, strategies to breed cereal varieties with improved yield under drought are limited due to a lack of knowledge of the genetic factors that control inflorescence and flower development under drought conditions. Understanding the plasticity and genetic control of stress-induced changes in reproductive development will be crucial to ensure future yield stability of temperate cereals.

Abbreviations: ABA, abscisic acid; FC, field capacity; LD, long day; MSA, main shoot apex; RWC, relative water content; SAM, shoot apical meristem; SD, short day; SWC, soil water content.

The model plant *Arabidopsis thaliana* accelerates reproductive development under drought, a response that has been termed drought escape. In Arabidopsis, drought escape is triggered under inductive long-day (LD) conditions and is controlled by components of the circadian clock and the photoperiod response pathway (Riboni *et al.*, 2013, 2016). Under drought conditions, the phytohormone abscisic acid (ABA) modulates the activity and signalling of the clock gene *GIGANTEA* (*GI*) and consequently its ability to activate *FLOWERING LOCUS T* (*FT*) under long photoperiods (Riboni *et al.*, 2013, 2016). The FT protein acts as a florigenic signal, moving long distances from the leaf to the shoot apical meristem (SAM) to induce the floral transition (Abe *et al.*, 2005; Wigge *et al.*, 2005; Corbesier *et al.*, 2007; Jaeger and Wigge, 2007; Mathieu *et al.*, 2007; Tamaki *et al.*, 2007; Jaeger *et al.*, 2013). Under non-inductive short days (SDs), ABA delays flowering by repressing the flowering-promoting MADS-box gene *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*), encoding a transcription factor integrating floral cues in the shoot meristem (Riboni *et al.*, 2016). In addition, it was shown that ABA-responsive element (ABRE)-binding factors (ABFs) interact with NUCLEAR FACTOR Y subunit C (NF-YC) 3/4/9 to promote flowering by inducing *SOC1* transcription under drought conditions (Hwang *et al.*, 2019). On the other hand, ABSCISIC ACID-INSENSITIVE 3/4/5 bZIP transcription factors involved in ABA signalling repress flowering by up-regulating the floral repressor and vernalization gene *FLOWERING LOCUS C* (*FLC*) (Wang *et al.*, 2013; Shu *et al.*, 2016). Consequently, drought cues depend on the photoperiod and interact with photoperiod response and vernalization genes to modulate flowering time in Arabidopsis. In contrast to Arabidopsis, rice (*Oryza sativa* L.) shows a delay in flowering in response to drought under inductive photoperiods, and this delay is accompanied by a down-regulation of the florigenic signals *HEADING DATE 3a* (*Hd3a*) and *RICE FLOWERING LOCUS T 1* (*RFT1*) (Galbiati *et al.*, 2016; Zhang *et al.*, 2016). Consequently, the developmental response to drought varies within and between species, and is linked to the differential regulation of *FT*-like genes (Kazan and Lyons, 2016). However, the effects of drought on reproductive development and genetic components that modulate this response are not known in most crop species including the important temperate crop barley.

Barley germplasm is characterized by high genetic diversity and variation in response to abiotic stresses. While elite cultivars tend to be more stress susceptible, wild and landrace barley genotypes are well adapted to drought-prone environments and therefore represent a valuable resource for improving stress tolerance in elite barley (Baum *et al.*, 2007; von Korff *et al.*, 2008; Rollins *et al.*, 2013*b*; Templer *et al.*, 2017). It was demonstrated that yield stability in the field was associated with the major photoperiod response gene *PHOTOPERIOD H1* (*Ppd-H1*) and the vernalization gene *VERNALIZATION 1* (*VRN1*) (von Korff *et al.*, 2008; Rollins *et al.*, 2013*a*; Al-Ajlouni *et al.*, 2016; Wiegmann *et al.*, 2019). These findings suggested that the timing of reproductive development is crucial to maximize yield formation under harsh environmental conditions. However, it is not known if and how these floral regulators interact with stress cues to modulate development. *Ppd-H1*, a barley homologue of the PSEUDO RESPONSE REGULATOR (PRR) genes from the Arabidopsis circadian clock, induces the expression of *FLOWERING LOCUS T1* (*FT1*), a homologue of Arabidopsis *FT* and rice *Hd3a* under LDs (Turner *et al.*, 2005; Corbesier *et al.*, 2007; Tamaki *et al.*, 2007; Campoli *et al.*, 2012*a*, *b*). In barley, the up-regulation of *FT1* in the leaf is correlated with induction of the MADS-box genes *VRN1* (*BM5a*), *BARLEY MADS-box 3* (*BM3*) and *BM8*, barley homologues of Arabidopsis *APETALA1/FRUITFUL* (*AP1/FUL*), and the acceleration of inflorescence development (Schmitz *et al.*, 2000; Trevaskis *et al.*, 2007; Digel *et al.*, 2015). Homologues of *Ppd-H1/PRR37* function in the circadian clock in Arabidopsis and rice (Makino *et al.*, 2001; Murakami *et al.*, 2003; Turner *et al.*, 2005). The circadian clock is an internal timekeeper that allows plants to anticipate predictable changes in the environment and controls a number of output traits including development and stress responses (Sanchez *et al.*, 2011; Müller *et al.*, 2014; Johansson and Staiger, 2015). In Arabidopsis, the central oscillator is composed of negative transcriptional feedback loops: the rise of *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*) and *LATE ELONGATED HYPOCOTYL* (*LHY*) late at night inhibits the evening complex genes *EARLY FLOWERING 3* (*ELF3*), *EARLY FLOWERING 4* (*ELF4*), and *LUX*, which in turn repress the *PRR* genes at night. Barley homologues of these clock genes have been identified and their interactions are largely conserved in barley (Campoli *et al.*, 2012*b*; Müller *et al.*, 2020). Accordingly, elements of the evening complex genes repress *Ppd-H1* at night and thereby control the photoperiod-dependent up-regulation of *FT1* (Faure *et al.*, 2012; Mizuno *et al.*, 2012; Zakhrabekova *et al.*, 2012; Campoli *et al.*, 2013; Alvarez *et al.*, 2016). In spring barley grown in northern latitudes, a recessive mutation in the CONSTANS, CONSTANS-like, and TOC1 (CCT) domain of *ppd-H1* has been selected (Jones *et al.*, 2008). This *ppd-H1* allele delays flowering under LDs and thereby improves yield in temperate environments with long growing seasons (Cockram *et al.*, 2007; Alqudah *et al.*, 2014; Digel *et al.*, 2015). In contrast, early flowering in response to LDs promoted by the wild-type *Ppd-H1* allele was associated with improved yield under Mediterranean environments with terminal stress (Wiegmann *et al.*, 2019). However, it is not known if the two *Ppd-H1* variants also interact with stress cues to modulate reproductive development.

Here, we provide a detailed analysis of barley development under drought. We show that variation at *Ppd-H1* interacts with drought to control flowering time, grain yield, as well as the expression of *FT1* and the downstream MADS-box genes *BM3* and *BM8*.

Materials and methods

Plant materials, growth conditions, and phenotyping

Drought responses were scored in the spring barley (*H. vulgare* L.) cultivars Scarlett, Golden Promise, and Bowman, and their derived introgression lines S42-IL107 (Scarlett), GP-fast (Golden Promise), and BW281 (Bowman). Scarlett, Golden Promise, and Bowman carry a natural mutation in the CCT domain of *Ppd-H1*, that causes a delay in flowering

under LD conditions (Turner *et al.*, 2005). The derived introgression lines S42-IL107 and BW281 carry a dominant *Ppd-H1* allele introgressed from wild and winter barley, respectively (Druka *et al.*, 2011; Schmalenbach *et al.*, 2011). GP-fast was created via crossing of Golden Promise to the winter barley cultivar Igri, followed by two rounds of backcrossing to Golden Promise to reduce the size of the introgression.

The three spring barley cultivars and derived introgression lines were genotyped with the Barley 50k iSelect SNP Array at TraitGenetics GmbH (Gatersleben, Germany) (Bayer *et al.*, 2017). Chromosomal positions for each marker were obtained from the POPSEQ_2017 genetic map (Cantalapiedra *et al.*, 2015; Mascher *et al.*, 2017). Sizes of the introgressions were calculated based on half the distance between the markers flanking donor introgressions and the first polymorphic markers within the introgressions (Supplementary Fig. S1; Supplementary Data S1 at *JXB* online).

We conducted two different drought experiments. First, a continuous drought treatment was applied by a controlled dry down of the soil to a soil water content (SWC) of 15% of field capacity (FC), and this FC was maintained until plant maturity. In a second experiment, a transient drought treatment was applied by withholding water for eight consecutive days during floral development followed by rewatering to control levels. Both experiments were performed in a controlled-environment chamber under 60% relative humidity. Individual grains were sown in 7 cm×7 cm×8 cm black plastic pots; 40 pots (5×8 rows) per tray. Genotypes were distributed randomly on each tray and rearranged after each sampling to maintain the initial planting density. Additionally, trays were rotated and shuffled at least twice per week. Each pot was filled with exactly 150 g of soil mixture. A mixture of 93% (v/v) Einheitserde ED73 (Einheitserde Werkverband e.V., Sinntal_Altengronau, Germany), 6.6% (v/v) sand, and 0.4% (v/v) Osmocote exact standard 3–4M (Scotts Company LLC), was freshly prepared before sowing. This porous soil mixture with high organic matter content was selected to further aid the even distribution of moisture in the soil. Grains were stratified in well-watered soil at 4 °C in the dark for at least 4 d. Plants were then germinated under SD conditions (8 h, 22 °C day; 16 h, 18 °C night; photosynthetically active radiation ~250 μM m^{-2} s^{-1}). For the continuous drought treatment, water was withheld after germination until the SWC reached 15% FC, while the control plants were watered to maintain 70% FC. The desired SWC of 15% FC was reached after 10 d when all plants were transferred from SDs to LDs and kept under LDs for the rest of the experiment (16 h, 22 °C day; 8 h, 18 °C night; photosynthetically active radiation ~250 μM m^{-2} s^{-1}). For the application of severe transient drought, plants of Scarlett and S42-IL107 were germinated under SD conditions and shifted to LDs after 10 d. All plants were kept at 70% FC until they had reached the awn primordium stage [Waddington stage 3 (W3)]. Then watering was stopped for eight consecutive days. SWC in the pots reached a relative water content (RWC) of 8% FC on the eighth day. Control plants were kept at 70% FC during this time. Subsequently, all drought-treated pots were rewatered to control levels of 70% FC. FC was calculated from the difference in weight of fully hydrated and oven-dried soil. SWC was measured gravimetrically (Coleman, 1947). Pots were soaked with water and subsequently left to drain by gravity until their weight remained stable; this was set as 100% FC. Dry weight was measured after pots were dried in a drying cabinet at ~60°C until their weight remained stable. Measurements of FC were corrected for the biomass accumulation of growing plants as the experiments progressed by subtracting the weight of harvested plants from the measured soil weight. The weight of pots was checked daily and all plants were watered daily to maintain the same SWC throughout development. At least three replicate plants of all six genotypes were sown and germinated for each sampling time point.

The development of the main shoot apex (MSA) was scored in accordance with the stages described by Waddington *et al.* (1983) that is based on the progression of inflorescence initiation and then the most advanced floret primordium and pistil of the inflorescence. At W2 the first spikelets initiate and the MSA transitions to a reproductive inflorescence. The first floral organ primordia differentiate and stem elongation initiates at the stamen primordium stage (W3.5). New spikelet primordia are continuously initiated until about W5, which then mature into florets until anthesis and pollination at W10. MSA dissection was performed with microsurgical stab knives (SSC#72-1551; Sharpoint, Surgical Specialties Corporation). Images of developing apices were obtained using a Nikon stereo microscope (Nikon SMZ18), Nikon DS-U3 controller unit, and a Nikon DS-Fi2 digital camera. Nikon NIS-Elements software was used for image acquisition. Heading date was scored at Zadoks stage Z49 when first awns became visible, otherwise also referred to as tipping (Zadoks *et al.*, 1974; Alqudah and Schnurbusch, 2017). Spike number, the number of grains per spike, the number of grains per plant, and thousand kernel weight (TKW) were scored at harvest.

Leaf RWC was determined from measurements of fresh, turgid, and dry weight of leaf sections from the middle part of the youngest fully expanded leaf. Turgid weight was measured after soaking the leaf sections in deionized water at 4 °C overnight in the dark. Dry weight of leaf sections was measured after drying at 70°C. The RWC was then calculated as (Smart and Bingham, 1974).

RNA extraction and gene expression analysis

Sections from the middle of the youngest fully emerged leaf were sampled for the developmental time courses at Zeitgeber time 8 (ZT8). Sampling was started on the first day after transfer to LDs in the continuous drought treatment and as soon as water was withheld in the severe drought experiment. Sampling was continued until flowering for both treatments. Samples for the diurnal expression analyses were harvested every 4 h starting at ZT0, with one additional sampling at ZT22. RNA extraction, reverse transcription, and quantitative real-time PCR (qRT-PCR) were performed as previously described (Campoli *et al.*, 2012*a*, *b*; Digel *et al.*, 2015). Several combinations of reference genes were tested for each experiment, and the genes with the most stable expression were chosen for normalization. The geometric mean of *Actin* and *ADP-ribosylation factor 1-like protein* (*ADP*) absolute expression was used for the calculation of relative gene expression levels for the developmental time courses. The geometric mean of *ADP* and *Glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) absolute expression was used for the calculation of relative gene expression levels for the diurnal time course. Normalization was performed by dividing target gene expression values by the obtained mean of the reference genes.

Statistical analysis

All statistical analyses were performed with R (R Core Team, 2020). Polynomial regressions (Loess smooth line) were calculated using second-degree polynomials and an alpha of 0.75, with a 95% confidence interval. Student's *t*-test assuming two-tailed distribution and equal variance was used to compare group means for control and drought treatments at each time point of the time course analyses with a significance cut-off of $P<0.05$. Significant differences in trait expression between treatments and genotypes were compared by Kruskal–Wallis ANOVA followed by Conover–Iman test for multiple comparisons and Bonferroni correction with a significance cut-off of $P<0.05$.

Results

Drought interacts with Ppd-H1 *to modulate flowering time*

We aimed to characterize the effects of drought on the timing of reproductive development and on shoot and spike morphology. In addition, we tested if the major photoperiod response gene *Ppd-H1* controlled reproductive development in response to drought. We quantified the effects of drought on developmental timing, growth, and inflorescence morphology in the spring barley genotypes Scarlett, Golden Promise, and Bowman with a natural mutation in the CCT domain of *Ppd-H1* and in the derived introgression lines S42-IL107 (Scarlett), GP-fast

(Golden Promise), and BW281 (Bowman) that carry wild-type *Ppd-H1* alleles introgressed from wild barley (*H. vulgare* ssp. *spontaneum*) or winter barley (Supplementary Fig. S1) (Druka *et al.*, 2011; Schmalenbach *et al.*, 2011).

We developed an assay to apply drought starting from early vegetative growth and lasting until maturity. With this assay, drought effects on the transition of vegetative to reproductive development and on floral progression were examined. Heading date, scored as a proxy for flowering time, was significantly delayed in all parental spring barley genotypes (Supplementary Fig. S2). Heading date was delayed by 11 d in Scarlett, by 13 d in Golden Promise, and by 3 d in Bowman under drought compared with control conditions (Fig. 1A). In contrast, heading date was not significantly different under drought compared with control conditions in S42-IL107 and GP-fast, and was significantly accelerated in BW281. In the parental genotypes, the number of spikes per plant was strongly reduced under drought; all plants produced only a maximum of three spikes under drought compared with >10 spikes under control conditions (Fig. 1B). The introgression lines produced on average 5–6 spikes per plant under drought compared with twice as many under control conditions, and thus significantly more under drought compared with the parental genotypes. Drought also reduced the number of grains per spike in all genotypes (Fig. 1C). However, there were no consistent differences in the reduction of grain number between *Ppd-H1* variants. The reductions in the number of spikes per plant and grains per spike resulted in a severely reduced number of grains per plant under drought (Fig. 1D). Total grain numbers under drought were significantly higher in the introgression lines S42-IL107 and BW281 than in the parental lines and not significantly different between Golden Promise and GP-fast. Drought did not strongly influence the TKW. Total yield per genotype was therefore primarily determined by the grain number (Fig. 1E).

We further investigated at which stage drought reduced final grain number and evaluated the effects of drought on spikelet versus grain number. Drought reduced the number of initiated spikelets in Scarlett, S42-IL107, Golden Promise, and Bowman by between 9% in Bowman to 18% in S42-IL107, while spikelet numbers were not significantly different between control and drought in BW281 and GP-fast (Fig. 1F). Furthermore, not all spikelets on the main spike developed grains. Under control conditions, the number of grains compared with initiated spikelets was reduced by 34–37% in the introgression lines and by 37% in Bowman, 50% in Scarlett, and 62% in Golden Promise. Consequently, in S42-IL107, GP-fast, and BW281, a higher percentage of spikelets developed grains compared with Scarlett, Golden Promise, and Bowman, respectively. Under drought conditions, the number of grains per spikelet was even more strongly reduced in all genotypes compared with control conditions, except for Golden Promise and S42-IL107. Under drought, relative grain numbers compared with spikelet numbers were reduced by 88% in Scarlett, by 64% in GP-fast, and by 56% and 57% in Bowman and BW281, respectively. Consequently, the reduction in grain number per spike under drought was primarily caused by an abortion of florets or floret sterility rather than a decrease in spikelet numbers.

Development of the MSA was scored after microdissection according to the scale established by Waddington *et al.* (1983) (Supplementary Fig. S3). The timing of spikelet initiation was not significantly altered by drought in any of the genotypes (Fig. 2A). However, drought delayed floral progression in the parental genotypes, but not in the introgression lines. Similarly, stem elongation, measured as plant height, was strongly reduced under drought in the three parental genotypes, but was less affected in the introgression lines (Fig. 2B). Variation at *Ppd-H1* and drought also had strong effects on the progression of tiller development (Fig. 2C). The introgression lines developed significantly fewer tillers than the parental lines under control and drought conditions. Drought delayed the development of tillers in Scarlett, Bowman, and BW281, but tiller development was not significantly different in S42-IL107, Golden Promise, and GP-fast. Consequently, drought had a much stronger effect on spike number than tiller number, demonstrating that the plants produced tillers during drought that did not develop a spike (Fig. 1B). The faster reproductive development in the introgression lines correlated with a reduced biomass accumulation compared with the parental lines under control and drought conditions. Drought reduced fresh weight biomass in all lines, and the relative reductions were similar between the parental genotypes and their respective introgression line. For example, 34 d after emergence, an ~70% reduction in biomass was observed in both Scarlett and S42-IL107 (Fig. 2D). We did not observe any effect of drought on the phyllochron and the number of leaves on the main culm, but leaf size was strongly reduced under drought (Supplementary Fig. S4). Leaf RWC was not altered under drought in any of the tested lines, indicating that all plants responded to the reduced water availability through a growth reduction and thus avoided tissue dehydration (Fig. 2E).

The induction of spikelets on the MSA terminated earlier in the introgression lines which therefore formed fewer spikelets compared with their respective parents. The introgression lines initiated spikelets until W4–5 while the parental lines formed new spikelets until W5–6 (Fig. 3). Under drought, the initiation of spikelets was slowed down in the parental lines, so that fewer spikelets were initiated under drought than under control conditions. However, in the introgression lines, there was no significant difference in the initiation of spikelet primordia between control and drought conditions. While the parental lines initiated more spikelets than the introgression lines, a higher proportion of spikelets did not develop florets in the parental genotypes, compared with the introgression lines. The introgression lines initiated fewer spikelets under control conditions, but drought did not reduce spikelet number further in these lines. The differences between spikelet number and grain number observed in the introgression lines (Fig. 1F) were therefore due to low floret fertility and not a failure in developing florets.

Taken together, *Ppd-H1* controlled the drought-induced changes in reproductive development, shoot and spike morphology, and plant height. Elite spring barley with a mutation in *ppd-H1* displayed a strong delay in floral development and reductions in plant height and the number of spikelets initiated on the main inflorescence under drought, whereas these traits

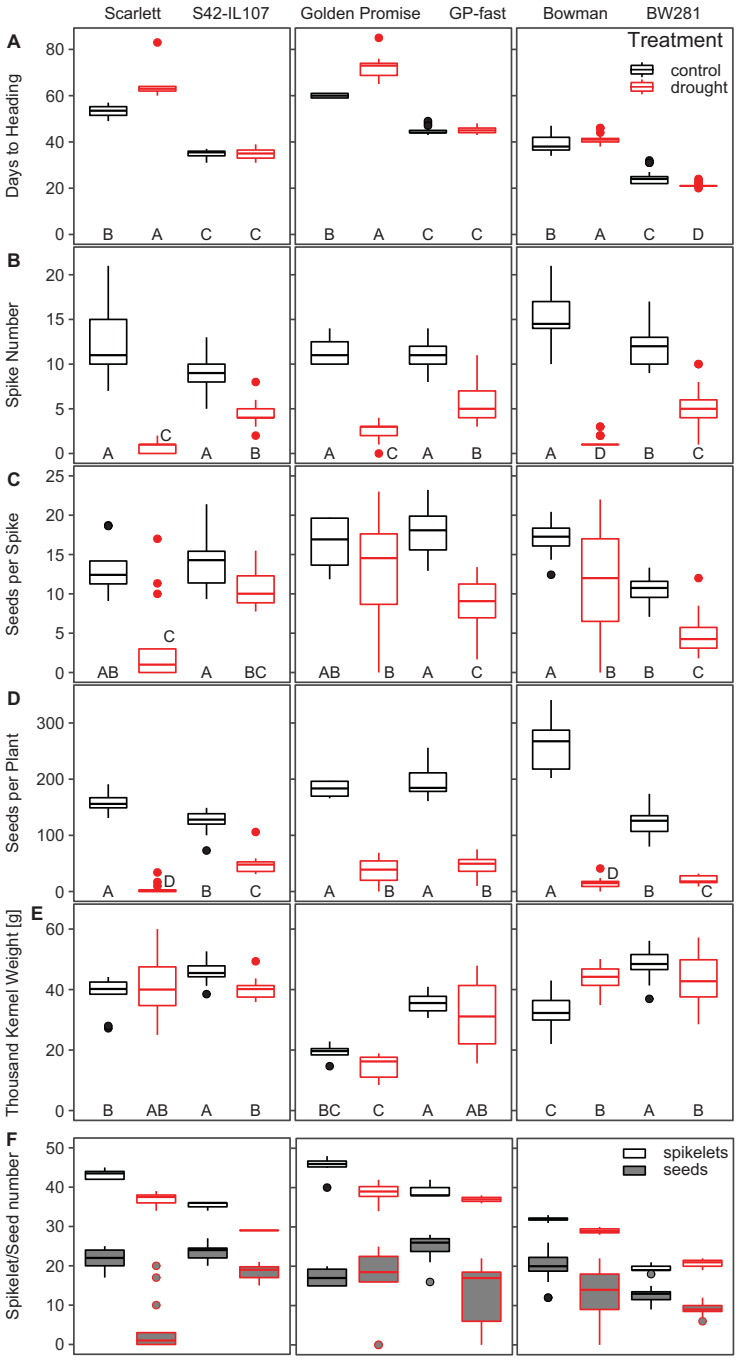


Fig. 1. Continuous drought affects heading date, and shoot and spike morphology in barley. Days to heading (A), spike number per plant (B), grain number per spike (C), the number of grains per plant (D), thousand kernel weight (TKW) (E), and the maximum number of developed spikelets (unfilled boxes) and the number of grains (grey box) (F) were scored under control (black) and drought (red) conditions under LDs (16 h light/8 h night) in the spring barley cultivars Scarlett, Golden Promise, and Bowman, and the derived introgression lines S42-IL107, GP-fast, and BW281. Statistical groups were assigned using Kruskal–Wallis ANOVA and post-hoc Conover–Iman test and Bonferroni correction. Different letters indicate that groups differ ($P<0.05$).

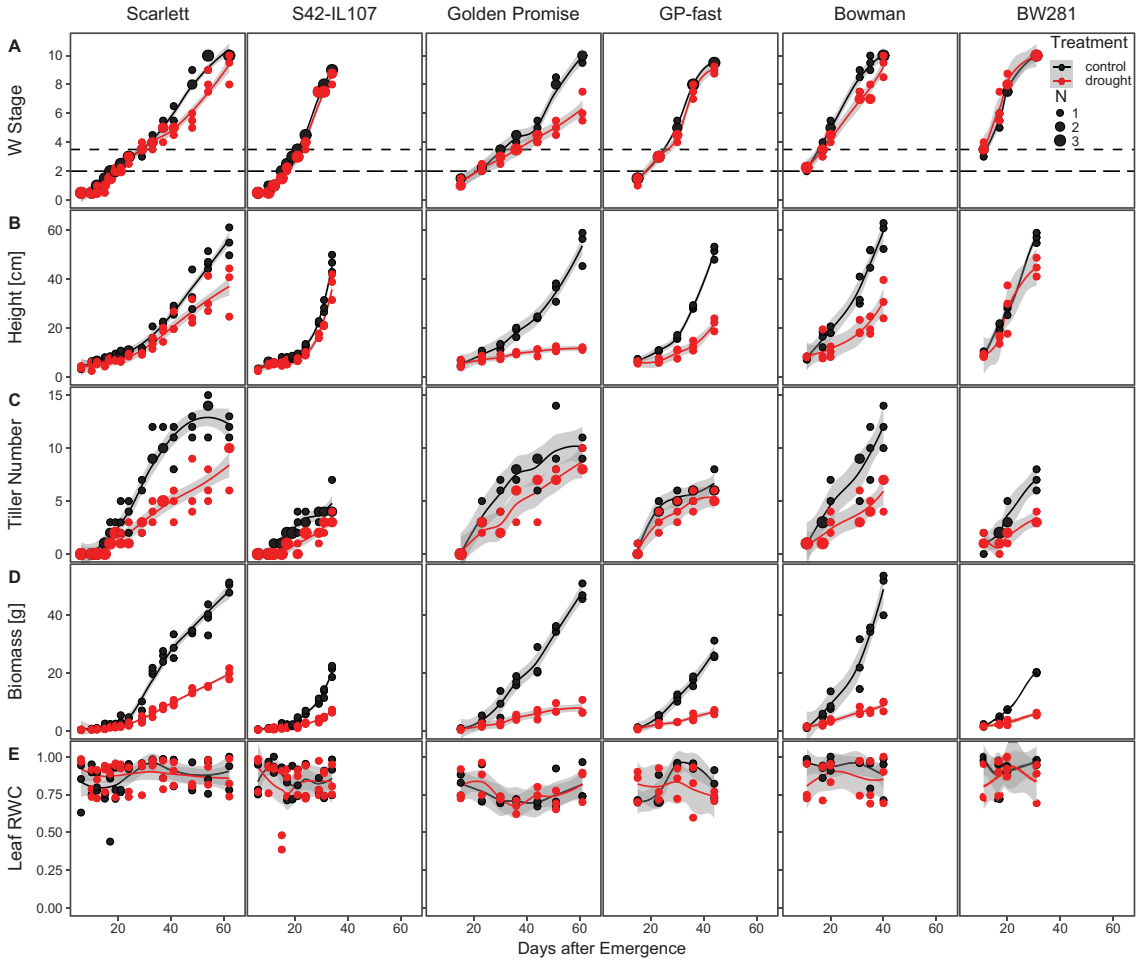


Fig. 2. Continuous drought delays floral development in spring barley. Development of the main shoot apex (MSA) (A), plant height (B), the number of tillers (C), fresh weight biomass (D), and leaf relative water content (RWC) (E) were scored during development under control (black) and drought (red) conditions under LDs (16 h light/8 h night) in the spring barley cultivars Scarlett, Golden Promise, and Bowman, and their derived introgression lines S42-IL107, GP-fast, and BW281 according to the scale by Waddington *et al.* (1983). Dot sizes indicate the number of overlapping samples. Trend lines were calculated using a polynomial regression (Loess smooth line); grey areas show the 95% confidence interval.

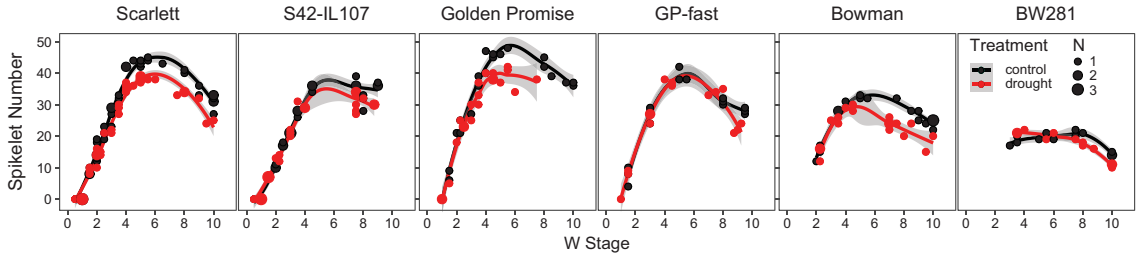


Fig. 3. Continuous drought affects tillering and spikelet number in barley. The number of spikelets on the main shoot apex (MSA) (A) were scored during development under control (black) and drought (red) conditions under LDs (16 h light/8 h night) in the spring barley cultivars Scarlett, Golden Promise, and Bowman, and the derived introgression lines S42-IL107, GP-fast, and BW281. Dot sizes indicate the number of overlapping samples. Trend lines were calculated using a polynomial regression (Loess smooth line); grey areas show the 95% confidence interval.

were scarcely affected under drought in the introgression lines with a wild-type *Ppd-H1* allele. Finally, drought had a strong detrimental effect on floret fertility which resulted in a reduction of grains independent of the *Ppd-H1* genotype.

Ppd-H1 *affects the plasticity of reproductive development in response to a transient drought stress*

The severity, duration, and timing of drought events are highly variable in nature. We therefore tested if the observed effects of drought on reproductive development are dependent on the timing and severity of the stress. In addition, we investigated if *Ppd-H1* also affected the plasticity of development in response to a transient drought stress followed by a recovery phase. Under severe drought, reproductive development stopped completely in Scarlett for the duration of the stress treatment and resumed after rewatering (Fig. 4A). However, the delay in development was maintained after the stress treatment, and stressed plants flowered significantly later than control plants. In S42-IL107, reproductive development only slowed down after the onset of drought stress and did not stop completely. After rewatering, reproductive development even accelerated so that control and stressed plants flowered almost at the same time (Fig. 4A; Supplementary Fig. S5). Tiller development was also halted in both genotypes upon the onset of stress, but both genotypes resumed tiller development after rewatering, and tiller numbers were not significantly different between control and stress conditions at flowering. Spikelet numbers were not strongly altered during development because at the onset of drought (W3) the majority of spikelets had already initiated. Drought, however, still caused a small reduction in spikelet initiation in both genotypes. The treatment completely stopped biomass accumulation in both genotypes already after 2 d of withholding water. On the eighth day, when the drought level was most severe, control plants of both Scarlett and S42-IL107 had accumulated almost nine times as much biomass compared with drought-stressed samples. The reductions in fresh biomass were also caused by a strong decline in the leaf RWC upon application of the severe drought stress (Fig. 4D, E). However, after rewatering, RWC levels rapidly increased again and were similar to RWC levels in control plants 6 d after rewatering in both genotypes. While RWC levels fully recovered after rewatering and stressed plants resumed growth, fresh weight biomass was significantly lower in stressed compared with control plants at flowering.

Taken together, transient severe stress applied during stem elongation also delayed floral development as observed under mild stress. Interestingly, the introgression line but not the parental line accelerated reproductive development after rewatering. Stressed and control S42-IL107 plants flowered nearly simultaneously, suggesting that *Ppd-H1* affects the developmental plasticity in response to drought.

Drought alters the expression of clock and floral regulator genes in barley

Components of the circadian clock play important roles in the control of flowering time regulators in barley. Additionally,

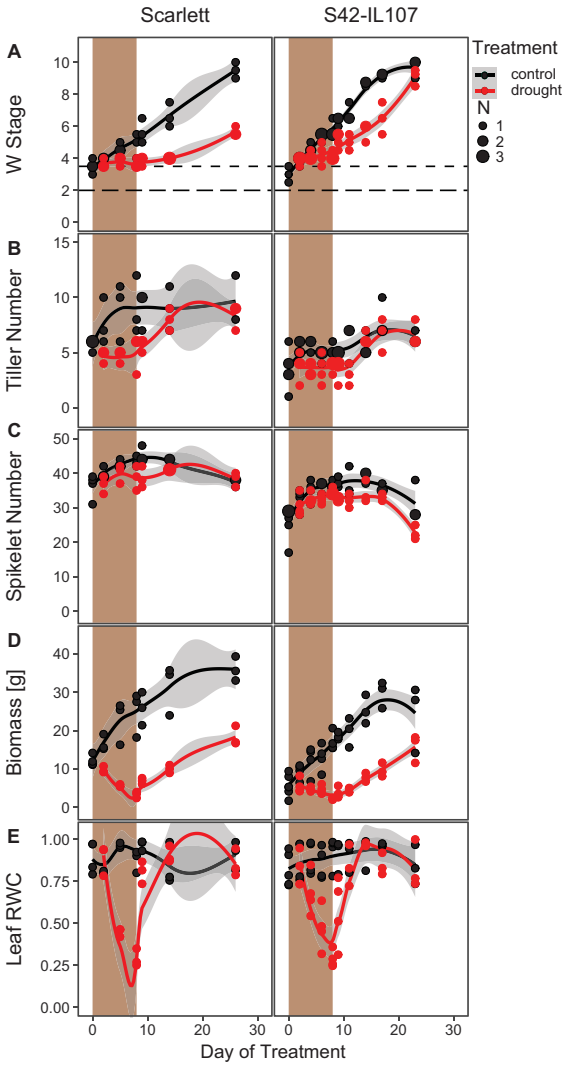


Fig. 4. Severe drought delays MSA development in barley. MSA development (A), the number of tillers (B), the number of spikelets (C), fresh weight biomass (D), and leaf RWC (E) were scored under control (black) and severe drought (red) conditions and during recovery. Shaded areas indicate the period during which plants were not watered. Dot sizes indicate the number of overlapping samples. Trend lines were calculated using a polynomial regression (Loess smooth line), grey areas show the 95% confidence interval.

previous studies have found that abiotic stresses alter the diurnal gene expression of core clock genes and clock-regulated genes in barley (Habte *et al.*, 2014; Ford *et al.*, 2016; Ejaz and von Korff, 2017). We therefore examined whether reduced SWC affected reproductive development through alterations in the diurnal expression patterns of clock and flowering time genes. For this purpose, leaf samples of Scarlett and S42-IL107 plants grown under control and continuous mild drought conditions were harvested every 4 h over 24 h at the stamen primordium stage (≥W3.5).

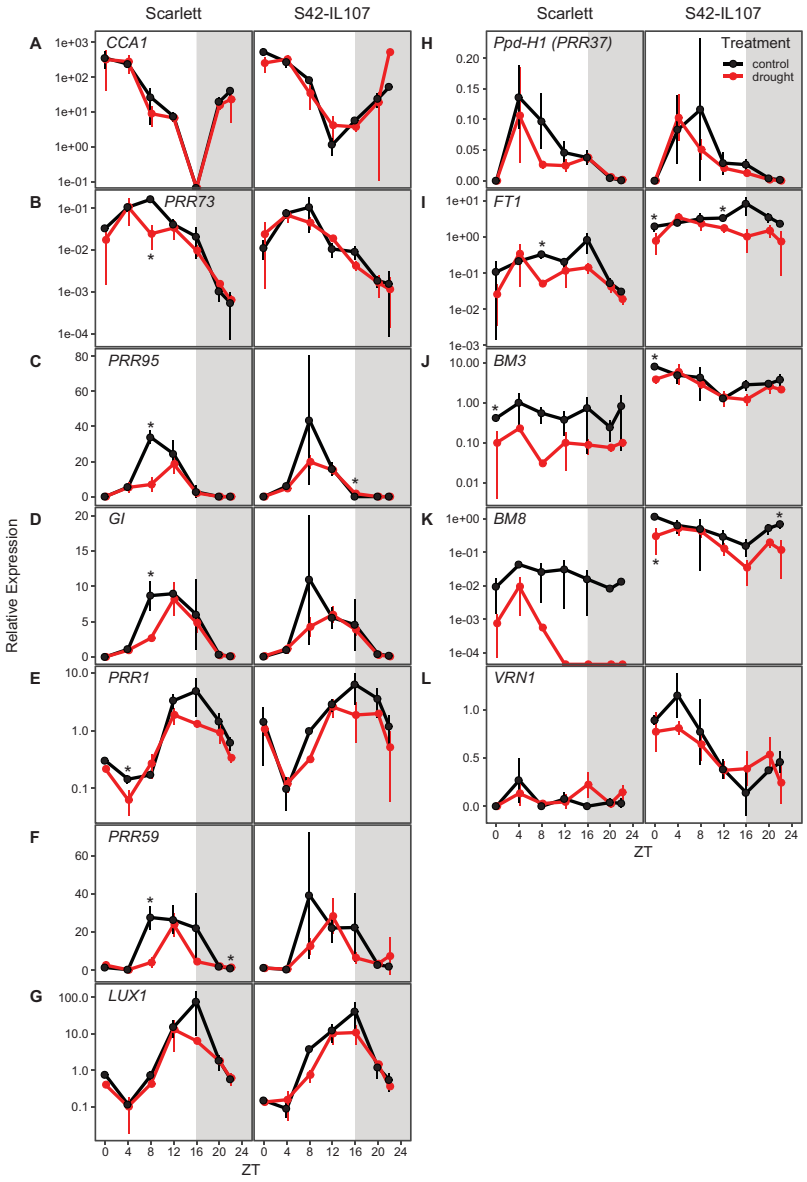


Fig. 5. Continuous drought reduces the transcript levels of circadian clock and flowering time genes in barley. Diurnal gene expression of circadian clock genes was measured every 4 h for 24 h with an additional sampling at ZT22 under control (black) and drought (red) conditions under LDs (16 h light/8 h night) in the spring barley cultivars Scarlett and the derived introgression line S42-IL107. Grey areas indicate darkness. Error bars indicate ±SD of three biological replicates; an asterisk indicates a significant difference between control and drought at the respective time point (*t*-test, $P<0.05$).

We investigated the expression of known barley core clock genes (Campoli *et al.*, 2012*b*; Müller *et al.*, 2020), with expression peaks at different times of the day (Fig. 5). The expression levels of the morning-expressed *CCA1* and the evening-expressed *LUX1* were not consistently altered between drought and control conditions (Fig. 5A, G). Expression levels of *PRR59*, *PRR73*, *PRR95*, and *GIGANTEA* (*GI*) were down-regulated at ZT8 under drought compared with control conditions in Scarlett (Fig. 5B–D, F). Drought also affected the peak time of expression of some clock transcripts. The expression peaks of *PRR95* and *GI* were delayed by 4 h, while expression peaks of *PRR1* and *LUX1* were advanced by 4 h in both genotypes. There were no consistent differences in the expression levels and patterns of clock genes between Scarlett and S42-IL107 under both conditions. Similar to the clock genes, the floral regulator genes and putative downstream targets of

Ppd-H1 were down-regulated under drought. Expression of *Ppd-H1* itself was not strongly affected under drought in either genotype (Fig. 5H). However, the expression levels of floral regulator genes differed between the genotypes under control and drought conditions. Expression levels of *FT1*, and the barley MADS-box genes *VRN1*, *BM3*, and *BM8*, were overall higher in S42-IL107 than in Scarlett under both conditions (Fig. 5I–L). Drought reduced *FT1* transcript levels in both genotypes, in particular at the evening peak time of expression. However, expression of *FT1* under drought was at all time points higher in S42-IL107 than in Scarlett. *BM3* and *BM8* were down-regulated under drought specifically in Scarlett at the majority of time points (Fig. 5K). In S42-IL107, transcript levels of *BM3* and *BM8* were not strongly altered between control and drought conditions.

In summary, drought decreased the expression levels of clock genes and floral regulator genes, and affected the peak time of expression of evening-expressed clock genes. Expression patterns of clock genes were similar between Scarlett and S42-IL107 under control and drought conditions; genetic variation at *Ppd-H1* (*PRR37*) therefore did not affect the diel expression patterns of clock genes. However, expression of floral regulator genes was significantly different between Scarlett and S42-IL107 under control and drought conditions. In addition, expression levels of floral regulator genes were more strongly altered under drought in Scarlett than in S42-IL107, demonstrating that *Ppd-H1* interacted with drought to control reproductive development and expression levels of major flowering time genes in barley.

Ppd-H1 *alters the effect of drought on flowering time gene expression during development*

We further investigated how *Ppd-H1* and drought affected expression of floral regulator genes during development in all six genotypes. Transcript levels of floral regulator genes were investigated in leaf samples from plants analysed for developmental traits as shown in Fig. 2. The youngest fully developed leaf was harvested at ZT8 in all genotypes starting from the first day after transfer to LDs until flowering. At transfer to LDs, all genotypes had formed a reproductive inflorescence at the double ridge stage (W2), with the exception of BW281, which was already at the awn primordium stage (W3).

The expression levels of *Ppd-H1* were not strongly altered by the treatment or *Ppd-H1* variant, with the exception of Golden Promise where *Ppd-H1* transcript levels were significantly higher under control than drought conditions (W3.5–W5.5) (Fig. 6A). In contrast, *FT1* expression levels were down-regulated under drought in all genotypes (Fig. 6B). *FT1* transcript levels increased during development and this increase was slowed down under drought, in particular in the parental line Scarlett. In Golden Promise, no *FT1* transcript was detected under drought at any time point. In the introgression lines, *FT1* expression levels were only significantly different between conditions at single time points in S42-IL107 and GP-fast, and were not changed in BW281. These differences in *FT1* transcript levels under drought correlated with the observed delay in floral progression in the parental genotypes as compared with the introgression lines under drought versus control conditions (Fig. 6A). Transcript levels of the vernalization gene *VRN1* were higher in the introgression than parental lines, but not significantly different between control and drought conditions (Fig. 6C). Transcript levels of *BM3* and *BM8* increased during development in all genotypes, and this increase was delayed and reduced under drought in Scarlett, Golden Promise, and Bowman, but not significantly different in S42-IL107 and GP-fast under drought versus control treatments. In BW281, *BM3* expression levels increased faster and to higher levels under drought compared with control conditions which correlated with the acceleration in floral development under drought in this line (Fig. 6D, E).

We also tested the effects of the transient severe drought stress on the expression of floral regulator genes in Scarlett and S42-IL107 (Fig. 7). During the transient drought treatment, transcript levels of *Ppd-H1*, *FT1*, *BM3*, and *BM8* were strongly down-regulated compared with control conditions in both genotypes (Fig. 7A, B, D, E). In Scarlett, the down-regulation of these flowering inducers extended long into the recovery phase, even after leaf RWC had returned to control levels. In S42-IL107, transcript levels of floral inducers recovered rapidly after rewatering and eventually reached the same levels as observed under control conditions. Transcript levels of *VRN1* were down-regulated after the transient drought stress in both genotypes, but matched *VRN1* expression levels in control plants at flowering (Fig. 7C).

In summary, both mild continuous and severe transient drought reduced the transcript levels of flowering inducers. However, reductions in transcript levels were stronger in the parental than in the introgression lines with a wild-type *Ppd-H1* allele. *Ppd-H1* therefore modulated expression of floral inducers in response to drought in barley. In addition, transcript levels rapidly recovered after a transient drought stress to control levels in the introgression line but not the parental line, suggesting that *Ppd-H1* affected transcriptional homeostasis in response to drought.

Discussion

Ppd-H1 was identified as a photoperiod response gene that controls adaptation to different environments by modulating flowering time in response to LDs (Turner *et al.*, 2005; Cockram *et al.*, 2007; Jones *et al.*, 2008; Wiegmann *et al.*, 2019). Here, we demonstrate that *Ppd-H1* also integrates drought stress signals to modulate floral development in barley. Drought delayed floral development in the parental genotypes with a mutated *ppd-H1* allele, while reproductive development was not affected by drought in genotypes with a wild-type *Ppd-H1* allele (Figs 1, 2). This variation in developmental timing in response to drought was linked to variation in the number of initiated spikelet primordia on the main shoot. Spikelet initiation was reduced in the parental lines, but not in the introgression lines under drought (Fig. 3). Similarly, drought-triggered reductions in plant height, and tiller and spike number were more pronounced in the parental lines compared with the introgression lines. Under the severe transient stress, reproductive

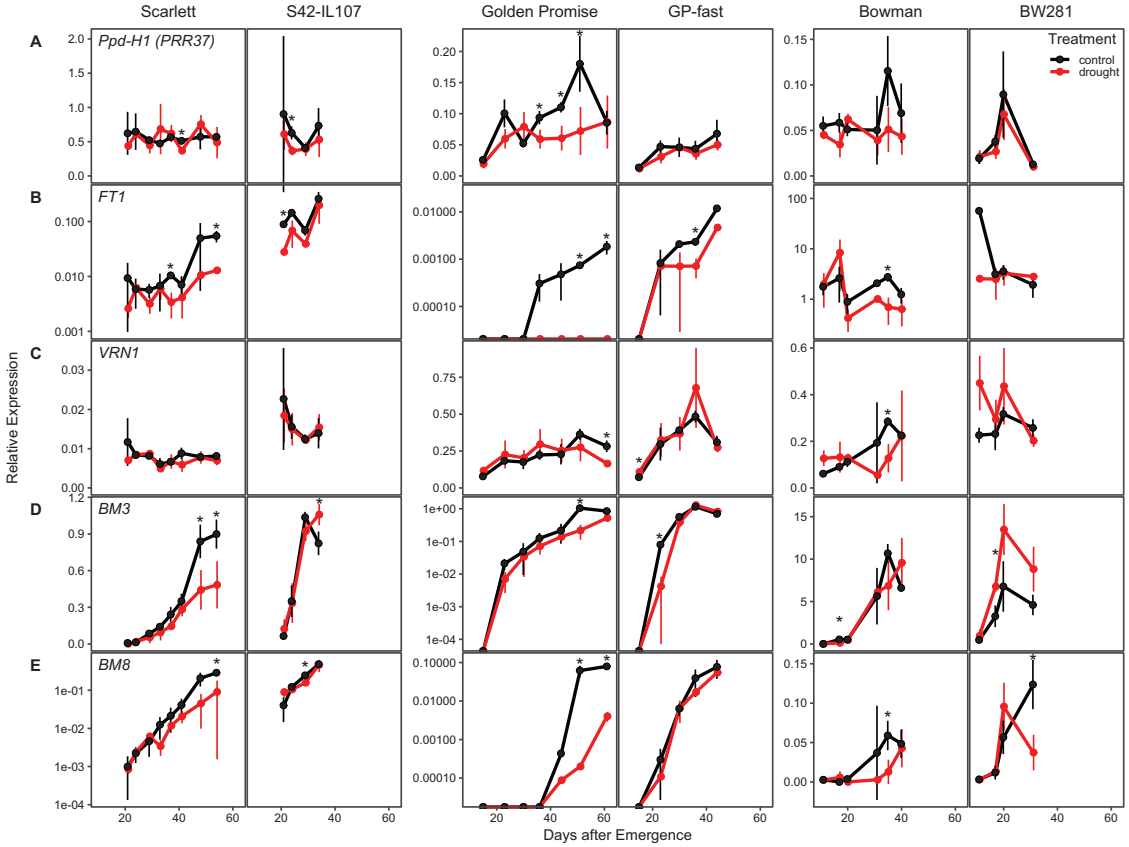


Fig. 6. Continuous drought affects the expression of flowering time genes during development in barley. Transcript levels of flowering time genes was measured during development under control (black) and drought (red) conditions under LDs (16 h light/8 h night) in the spring barley cultivars Scarlett, Golden Promise, and Bowman, and the derived introgression lines S42-IL107, GP-fast, and BW281. Error bars indicate ±SD of three biological replicates; an asterisk indicates a significant difference between control and drought at the respective time point (*t*-test, $P<0.05$).

development slowed down in all genotypes; however, upon rewatering, the introgression line with a wild-type *Ppd-H1* allele accelerated development so that control and stressed plants flowered simultaneously. In contrast, parental lines flowered significantly later after a transient stress than plants under control conditions (Fig. 4). Taken together, the results demonstrated that *Ppd-H1* interacts with drought to control the development and morphology of the shoot and spike. *Ppd-H1* has already been associated with a number of shoot- and spike-related traits in barley and acts as a key gene to coordinate the development of different plant organs with reproductive timing (Digel *et al.*, 2015, 2016; Alqudah *et al.*, 2016, 2018; Ejaz and von Korff, 2017; Pham *et al.*, 2019; Shaaf *et al.*, 2019). Furthermore, our results suggested that *Ppd-H1* controlled the plasticity of reproductive development in response to drought. The parental lines with a mutation in *ppd-H1* displayed a high trait variance between treatments and thus developmental plasticity. In contrast, the introgression lines exhibited a higher trait stability under drought, in particular for developmental timing and spikelet initiation, while biomass reductions under drought were comparable between genotypes. The identification of genes/alleles maintaining trait stability in response to environmental perturbations is interesting for breeding genotypes with high yield stability under global climatic changes and higher frequencies of extreme weather events.

The most plastic trait under drought in all genotypes was grain number. Drought caused a minor reduction in the number of spikelet primordia and in the number of spikelets/florets, but a major reduction in the final grain number (Fig. 1). This suggested that drought reduced grain number primarily by affecting floret fertility and tiller number. It has already been described that water deficit impairs pollen development. Altered tapetal degeneration and associated changes in nutrient provision and signalling have been identified as the primary causes for cellular defects in pollen maturation under drought stress (Saini *et al.*, 1984; Lalonde *et al.*, 1997; Saini, 1997; Saini and Westgate, 1999; Aloni *et al.*, 2001; Pressman *et al.*, 2002; Barnabás *et al.*, 2008; De Storme and Geelen, 2014). Moreover, drought interferes with ovary survival or early grain development, potentially by restricting expansive growth, and thereby reduces the number of grains per spike (Guo *et al.*, 2016; Oury *et al.*, 2016*a*, *b*; Turc and Tardieu,

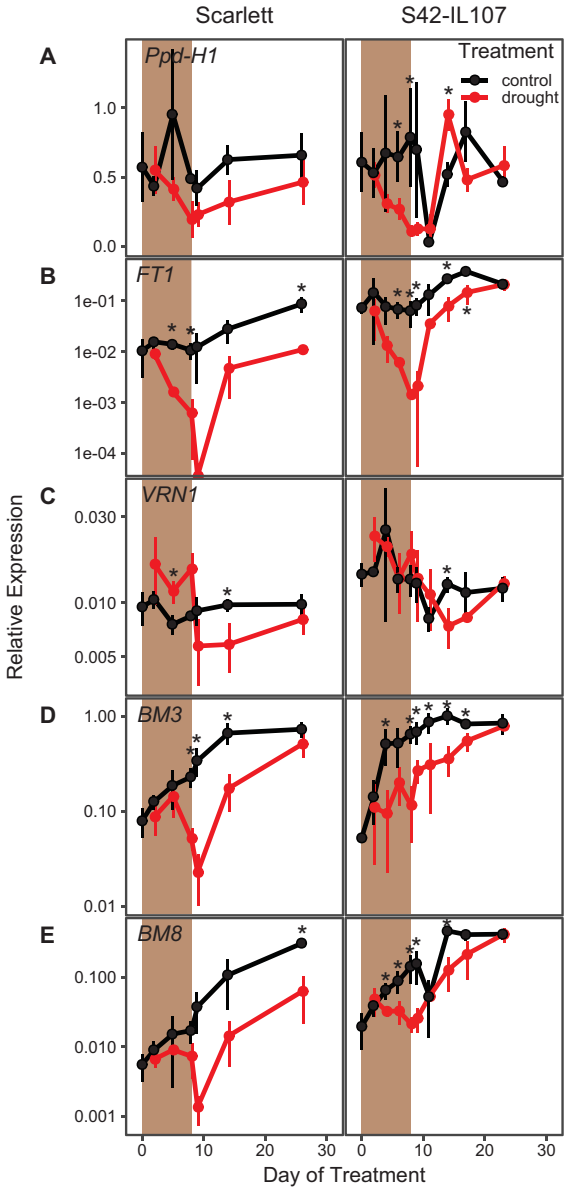


Fig. 7. Severe drought interacts with *Ppd-H1* to control transcript levels of flowering time genes during stress and recovery. Transcript levels of flowering time genes were measured during development under control (black) and drought (red) conditions under LDs (16 h light/8 h night) in the spring barley cultivar Scarlett and the derived introgression line S42-IL107. Shaded areas indicate the period during which plants were not watered; error bars indicate ±SD of three biological replicates; an asterisk indicates a significant difference between control and drought at the respective time point (*t*-test, $P<0.05$).

2018). In contrast to grain number, TKW was not very variable between drought and control conditions. We concluded that floral development is most susceptible to drought, while spikelet initiation as well as grain filling were less affected. These effects in controlled-environment chambers correspond to observations in field-grown wheat, where yield differences between environments were primarily controlled by variation in grain number while TKW was relatively stable across environments (Slafer *et al.*, 2014, 2015). Our results therefore underline the importance of floral development and fertility for yield under drought, which supports recent studies that challenge the central importance of 'terminal drought' as the main cause for losses in cereal yield in drought-prone Mediterranean regions (Savin *et al.*, 2015).

The circadian clock controls genes of the photoperiod response pathway, and *Ppd-H1* itself is a barley homologue of an Arabidopsis clock gene (Faure *et al.*, 2012; Campoli *et al.*, 2013). Furthermore, the circadian clock controls stress adaptation and is itself regulated by stress cues (Liu *et al.*, 2013; Tamaru *et al.*, 2013; Habte *et al.*, 2014; Grundy *et al.*, 2015; Lee *et al.*, 2016; Ejaz and von Korff, 2017; Guadagno *et al.*, 2018). We consequently tested if drought interacted with variation at *Ppd-H1* to affect the expression of barley clock genes. Indeed, drought marginally affected the amplitude and phase of clock gene expression. Clock gene transcripts were down-regulated under drought; however, variation at *Ppd-H1* had no consistent effects on clock gene expression, under either control or drought conditions (Fig. 5). This supports earlier studies which demonstrated that the natural mutation in *ppd-H1* did not affect the expression of other barley clock homologues either under control conditions or under osmotic and/or high temperature stress (Campoli *et al.*, 2012*b*; Habte *et al.*, 2014; Ejaz and von Korff, 2017). However, we cannot exclude that drought might have interacted with *Ppd-H1* to affect clock proteins post-transcriptionally (Más *et al.*, 2003; Kiba *et al.*, 2007). Like the clock genes, the transcripts of the flowering time genes *FT1*, *BM3*, and *BM8* were reduced under drought during floral development (Figs 5, 6). Similarly, in rice, the *FT* homologues *Hd3a* and *RFT1* were down-regulated under drought stress and this correlated with a delay in floral transition under inductive SDs (Galbiati *et al.*, 2016). In contrast, in Arabidopsis, drought induces early flowering through the ABA-dependent stimulation of GI or of ABFs that trigger *SOC1* and *FT* transcriptional activation (Riboni *et al.*, 2016; Hwang *et al.*, 2019). On the other hand, it was also shown that ABSCISIC ACID-INSENSITIVE 4 (ABI4), a key component in the ABA signalling pathway, negatively regulated floral transition by directly promoting expression of the floral repressor *FLC*. Interestingly, the barley vernalization gene *VRN1* was not consistently altered in expression under drought, suggesting that the vernalization response pathway is not involved in transmitting drought signals in barley. However, all genotypes carry spring alleles at *VRN1*; future research therefore needs to test the response of the winter *vrn1* allele to drought and its effects on flowering.

Because negative and positive effects of drought and ABA on flowering time were observed, it was suggested that different levels of stress may elicit different developmental responses. A moderate level of drought and ABA levels may delay floral transition, allowing for flowering to occur after the stress, while a severe drought stress and high ABA levels promote flowering and drought escape to maximize reproductive success (Shu

et al., 2018). However, we found that both mild and severe stress resulted in a delay in flowering time. Differential responses to drought were rather genetically controlled where *Ppd-H1* controlled the drought-dependent down-regulation of *FT1*, *BM3*, and *BM8*, and correlated differences in reproductive development. Furthermore, after a transient drought stress, *FT1*, *BM3*, and *BM8* transcript levels recovered fast after rewatering and eventually matched those under control conditions in the introgression but not in the parental line (Fig. 7). Consequently, *Ppd-H1* also affected transcript homeostasis after a severe transient perturbation by stress. In contrast to reports from rice and Arabidopsis, drought did not strongly impact the timing of spikelet initiation but slowed down and impaired floral development and fertility. *FT1*, *BM3*, and *BM8* have already been linked to inflorescence and floral development in barley, wheat, and rice (Digel *et al.*, 2015; Wu *et al.*, 2017; Callens *et al.*, 2018; Shaw *et al.*, 2019). In rice, simultaneous knockdown of *OsMADS14* (*VRN1*, *FUL1*), *OsMADS15* (*BM3*, *FUL2*), and *OsMAD18* (*BM8*, *FUL3*) resulted in floral reversion and the formation of lateral vegetative tillers (Kobayashi *et al.*, 2012). Similarly, triple wheat *vrn1ful2ful3* mutants formed vegetative tillers instead of spikelets on lateral meristems and displayed a reduced stem elongation (Li *et al.*, 2019). Reduced transcript levels of *FT1*, *BM3*, and *BM8* might therefore have contributed to an impaired floral development and decreased stem elongation in the drought-stressed plants in our study. It has been shown in barley and rice, that *FT* homologues have positive effects on gibberellin (GA) biosynthesis or stem responsiveness to GA and thus stem elongation (Pearce *et al.*, 2013; Gómez-Ariza *et al.*, 2019). Reduced *FT1* transcript levels might therefore have contributed to a reduction in stem elongation under drought; Golden Promise with the strongest *FT1* down-regulation under drought was also characterized by the strongest reduction in plant height.

In summary, our results demonstrate that *Ppd-H1* integrates photoperiod and drought stress signals to control reproductive timing and the plasticity of shoot and spike morphology in response to drought in barley. These differential responses to drought are linked to a differential down-regulation of *FT1*, *BM3*, and *BM8* transcripts in the leaf. Future studies need to elucidate linked transcriptional changes in the inflorescences and further dissect the effects of drought on floral organ development. Furthermore, results obtained in this study under controlled conditions need to be verified under field conditions.

Supplementary data

Supplementary data are available at *JXB* online.

Table S1. Oligonucleotides used in this study.

Data S1. Genotyping of introgression lines used in this study.

Fig. S1. Genetic map of *Ppd-H1* introgression in spring barley backgrounds, introgression size in centiMorgans (cM), and the polymorphic markers flanking the insertions.

Fig. S2. Flowering morphology in Golden Promise and Bowman background under continuous drought.

Fig. S3. MSA and pistil morphology in Golden Promise background.

Fig. S4. Continuous drought affects leaf size but not the phyllochron in barley.

Fig. S5. Severe drought delays flowering in barley.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

LG and MvK conceived the project and planned the experiments. LG performed all experiments, except experiments with Golden Promise which were performed by LG and EBH. LG and MvK wrote the manuscript.

Acknowledgements

We warmly thank Kerstin Luxa, Caren Dawidson, Andrea Lossow, and Thea Rütjes for excellent technical assistance. This work was funded by the Max Planck Society and the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany´s Excellence Strategy—EXC-2048/1—Project ID: 390686111 and the GRK 2064: Water use efficiency and drought stress responses: From Arabidopsis to Barley. Deutsche Forschungsgemeinschaft (DFG) - Projektnummer 252965955.

References

Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K, Araki T. 2005. FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. Science **309**, 1052–1056.

Al-Ajlouni Z, Al-Abdallat A, Al-Ghzawi A, Ayad J, Abu Elenein J, Al-Quraan N, Baenziger P. 2016. Impact of pre-anthesis water deficit on yield and yield components in barley (*Hordeum vulgare* L.) plants grown under controlled conditions. Agronomy **6**, 33.

Aloni B, Peet M, Pharr M, Karni L. 2001. The effect of high temperature and high atmospheric CO_2 on carbohydrate changes in bell pepper (*Capsicum annuum*) pollen in relation to its germination. Physiologia Plantarum **112**, 505–512.

Alqudah AM, Koppolu R, Wolde GM, Graner A, Schnurbusch T. 2016. The genetic architecture of barley plant stature. Frontiers in Genetics **7**, 117.

Alqudah AM, Schnurbusch T. 2017. Heading date is not flowering time in spring barley. Frontiers in Plant Science **8**, 896.

Alqudah AM, Sharma R, Pasam RK, Graner A, Kilian B, Schnurbusch T. 2014. Genetic dissection of photoperiod response based on GWAS of pre-anthesis phase duration in spring barley. PLoS One **9**, e113120.

Alqudah AM, Youssef HM, Graner A, Schnurbusch T. 2018. Natural variation and genetic make-up of leaf blade area in spring barley. Theoretical and Applied Genetics **131**, 873–886.

Alvarez MA, Tranquilli G, Lewis S, Kippes N, Dubcovsky J. 2016. Genetic and physical mapping of the earliness per se locus *Eps-A (m) 1* in *Triticum monococcum* identifies *EARLY FLOWERING 3* (*ELF3*) as a candidate gene. Functional & Integrative Genomics **16**, 365–382.

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

Baum M, Von Korff M, Guo P, *et al*. 2007. Molecular approaches and breeding strategies for drought tolerance in barley. In: Varshney R, Tuberosa R, eds. Genomic assisted crop improvement: Vol. 2: Genomics applications in crops. Dordrecht: Springer, 51–79.

Bayer MM, Rapazote-Flores P, Ganal M, *et al*. 2017. Development and evaluation of a barley 50k iSelect SNP array. Frontiers in Plant Science **8**, 1792.

Callens C, Tucker MR, Zhang D, Wilson ZA. 2018. Dissecting the role of MADS-box genes in monocot floral development and diversity. Journal of Experimental Botany **69**, 2435–2459.

Campoli C, Drosse B, Searle I, Coupland G, von Korff M. 2012*a*. Functional characterisation of *HvCO1*, the barley (*Hordeum vulgare*) flowering time ortholog of CONSTANS. The Plant Journal **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 Phytologist **199**, 1045–1059.

Campoli C, Shtaya M, Davis SJ, von Korff M. 2012*b*. 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 Biology **12**, 97.

Cantalapiedra CP, Boudiar R, Casas AM, Igartua E, Contreras-Moreira B. 2015. BARLEYMAP: physical and genetic mapping of nucleotide sequences and annotation of surrounding loci in barley. Molecular Breeding **35**, 13.

Cockram J, Jones H, Leigh FJ, O'Sullivan D, Powell W, Laurie DA, Greenland AJ. 2007. Control of flowering time in temperate cereals: genes, domestication, and sustainable productivity. Journal of Experimental Botany **58**, 1231–1244.

Coleman E. 1947. A laboratory procedure for determining the field capacity of soils. Soil Science **63**, 277–284.

Corbesier L, Vincent C, Jang S, *et al*. 2007. FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis*. Science **316**, 1030–1033.

De Storme N, Geelen D. 2014. The impact of environmental stress on male reproductive development in plants: biological processes and molecular mechanisms. Plant, Cell & Environment **37**, 1–18.

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. The 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 Physiology **172**, 405–415.

Druka A, Franckowiak J, Lundqvist U, *et al*. 2011. Genetic dissection of barley morphology and development. Plant Physiology **155**, 617–627.

Ejaz M, von Korff M. 2017. The genetic control of reproductive development under high ambient temperature. Plant Physiology **173**, 294–306.

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. Proceedings of the National Academy of Sciences, USA **109**, 8328–8333.

Ford B, Deng W, Clausen J, Oliver S, Boden S, Hemming M, Trevaskis B. 2016. Barley (*Hordeum vulgare*) circadian clock genes can respond rapidly to temperature in an *EARLY FLOWERING 3*-dependent manner. Journal of Experimental Botany **67**, 5517–5528.

Galbiati F, Chiozzotto R, Locatelli F, Spada A, Genga A, Fornara F. 2016. *Hd3a, RFT1* and *Ehd1* integrate photoperiodic and drought stress signals to delay the floral transition in rice. Plant, Cell & Environment **39**, 1982–1993.

Gol L, Tomé F, von Korff M. 2017. Floral transitions in wheat and barley: interactions between photoperiod, abiotic stresses, and nutrient status. Journal of Experimental Botany **68**, 1399–1410.

Gómez-Ariza J, Brambilla V, Vicentini G, *et al*. 2019. A transcription factor coordinating internode elongation and photoperiodic signals in rice. Nature Plants **5**, 358–362.

Grundy J, Stoker C, Carré IA. 2015. Circadian regulation of abiotic stress tolerance in plants. Frontiers in Plant Science **6**, 648.

Guadagno CR, Ewers BE, Weinig C. 2018. Circadian rhythms and redox state in plants: till stress do us part. Frontiers in Plant Science **9**, 247.

Guo Z, Slafer GA, Schnurbusch T. 2016. Genotypic variation in spike fertility traits and ovary size as determinants of floret and grain survival rate in wheat. Journal of Experimental Botany **67**, 4221–4230.

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 & Environment **37**, 1321–1327.

Hwang K, Susila H, Nasim Z, Jung JY, Ahn JH. 2019. *Arabidopsis* ABF3 and ABF4 transcription factors act with the NF-YC complex to regulate *SOC1* expression and mediate drought-accelerated flowering. Molecular Plant **12**, 489–505.

Jaeger KE, Pullen N, Lamzin S, Morris RJ, Wigge PA. 2013. Interlocking feedback loops govern the dynamic behavior of the floral transition in *Arabidopsis*. The Plant Cell **25**, 820–833.

Jaeger KE, Wigge PA. 2007. FT protein acts as a long-range signal in *Arabidopsis*. Current Biology **17**, 1050–1054.

Johansson M, Staiger D. 2015. Time to flower: interplay between photoperiod and the circadian clock. Journal of Experimental Botany **66**, 719–730.

Jones H, Leigh FJ, Mackay I, Bower MA, Smith LM, Charles MP, Jones G, Jones MK, Brown TA, Powell W. 2008. Population-based resequencing reveals that the flowering time adaptation of cultivated barley originated east of the Fertile Crescent. Molecular Biology and Evolution **25**, 2211–2219.

Kahiluoto H, Kaseva J, Balek J, *et al*. 2019. Decline in climate resilience of European wheat. Proceedings of the National Academy of Sciences, USA **116**, 123–128.

Kazan K, Lyons R. 2016. The link between flowering time and stress tolerance. Journal of Experimental Botany **67**, 47–60.

Kiba T, Henriques R, Sakakibara H, Chua NH. 2007. Targeted degradation of PSEUDO-RESPONSE REGULATOR5 by an SCFZTL complex regulates clock function and photomorphogenesis in *Arabidopsis thaliana*. The Plant Cell **19**, 2516–2530.

Kobayashi K, Yasuno N, Sato Y, Yoda M, Yamazaki R, Kimizu M, Yoshida H, Nagamura Y, Kyozuka J. 2012. Inflorescence meristem identity in rice is specified by overlapping functions of three *AP1/FUL*-like MADS box genes and *PAP2*, a *SEPALLATA* MADS box gene. The Plant Cell **24**, 1848–1859.

Lalonde S, Beebe DU, Saini HS. 1997. Early signs of disruption of wheat anther development associated with the induction of male sterility by meiotic-stage water deficit. Sexual Plant Reproduction **10**, 40–48.

Lee HG, Mas P, Seo PJ. 2016. MYB96 shapes the circadian gating of ABA signaling in *Arabidopsis*. Scientific Reports **6**, 17754.

Li C, Lin H, Chen A, Lau M, Jernstedt J, Dubcovsky J. 2019. Wheat VRN1, FUL2 and FUL3 play critical and redundant roles in spikelet development and spike determinacy. Development **146**, dev175398.

Liu T, Carlsson J, Takeuchi T, Newton L, Farré EM. 2013. Direct regulation of abiotic responses by the Arabidopsis circadian clock component PRR7. The Plant Journal **76**, 101–114.

Makino S, Matsushika A, Kojima M, Oda Y, Mizuno T. 2001. Light response of the circadian waves of the APRR1/TOC1 quintet: when does the quintet start singing rhythmically in Arabidopsis? Plant & Cell Physiology **42**, 334–339.

Más P, Kim WY, Somers DE, Kay SA. 2003. Targeted degradation of TOC1 by ZTL modulates circadian function in *Arabidopsis thaliana*. Nature **426**, 567–570.

Mascher M, Gundlach H, Himmelbach A, *et al*. 2017. A chromosome conformation capture ordered sequence of the barley genome. Nature **544**, 427–433.

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

Mizuno N, Nitta M, Sato K, Nasuda S. 2012. A wheat homologue of *PHYTOCLOCK 1* is a candidate gene conferring the early heading phenotype to einkorn wheat. Genes & Genetic Systems **87**, 357–367.

Müller L, Mombaerts L, Pankin A, Davis SJ, Webb AA, Goncalves J, von Korff M. 2020. Differential effects of day–night cues and the circadian clock on the barley transcriptome. Plant Physiology (in press).

Müller LM, von Korff M, Davis SJ. 2014. Connections between circadian clocks and carbon metabolism reveal species-specific effects on growth control. Journal of Experimental Botany **65**, 2915–2923.

Murakami M, Ashikari M, Miura K, Yamashino T, Mizuno T. 2003. The evolutionarily conserved *Os*PRR quintet: rice pseudo-response regulators implicated in circadian rhythm. Plant & Cell Physiology **44**, 1229–1236.

Oury V, Caldeira CF, Prodhomme D, Pichon JP, Gibon Y, Tardieu F, Turc O. 2016. Is change in ovary carbon status a cause or a consequence of maize ovary abortion in water deficit during flowering? Plant Physiology **171**, 997–1008.

Oury V, Tardieu F, Turc O. 2016. Ovary apical abortion under water deficit is caused by changes in sequential development of ovaries and in silk growth rate in maize. Plant Physiology **171**, 986–996.

Pearce S, Vanzetti LS, Dubcovsky J. 2013. Exogenous gibberellins induce wheat spike development under short days only in the presence of *VERNALIZATION1*. Plant Physiology **163**, 1433–1445.

Pham AT, Maurer A, Pillen K, Brien C, Dowling K, Berger B, Eglinton JK, March TJ. 2019. Genome-wide association of barley plant growth under drought stress using a nested association mapping population. BMC Plant Biology **19**, 134.

Pressman E, Peet MM, Pharr DM. 2002. The effect of heat stress on tomato pollen characteristics is associated with changes in carbohydrate concentration in the developing anthers. Annals of Botany **90**, 631–636.

R Core Team. 2020. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Riboni M, Galbiati M, Tonelli C, Conti L. 2013. *GIGANTEA* enables drought escape response via abscisic acid-dependent activation of the florigens and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS*. Plant Physiology **162**, 1706–1719.

Riboni M, Robustelli Test A, Galbiati M, Tonelli C, Conti L. 2016. ABA-dependent control of GIGANTEA signalling enables drought escape via up-regulation of *FLOWERING LOCUS T* in *Arabidopsis thaliana*. Journal of Experimental Botany **67**, 6309–6322.

Rollins JA, Drosse B, Mulki MA, Grando S, Baum M, Singh M, Ceccarelli S, von Korff M. 2013*a*. Variation at the vernalisation genes *Vrn-H1* and *Vrn-H2* determines growth and yield stability in barley (*Hordeum vulgare*) grown under dryland conditions in Syria. Theoretical and Applied Genetics **126**, 2803–2824.

Rollins JA, Habte E, Templer SE, Colby T, Schmidt J, von Korff M. 2013*b*. Leaf proteome alterations in the context of physiological and morphological responses to drought and heat stress in barley (*Hordeum vulgare* L.). Journal of Experimental Botany **64**, 3201–3212.

Saini HS. 1997. Effects of water stress on male gametophyte development in plants. Sexual Plant Reproduction **10**, 67–73.

Saini H, Sedgley M, Aspinall D. 1984. Development anatomy in wheat of male sterility induced by heat stress, water deficit or abscisic acid. Functional Plant Biology **11**, 243.

Saini HS, Westgate ME. 1999. Reproductive development in grain crops during drought. Advances in Agronomy **68**, 59–96.

Sanchez A, Shin J, Davis SJ. 2011. Abiotic stress and the plant circadian clock. Plant Signaling & Behavior **6**, 223–231.

Savin R, Slafer GA, Cossani CM, Abeledo LG. 2015. Cereal yield in Mediterranean-type environments: challenging the paradigms on terminal drought, the adaptability of barley vs wheat and the role of nitrogen fertilization. In: Sadras VO, Calderini DF, eds. Crop physiology. Applications for genetic improvement and agronomy, 2nd edn. Academic Press, 141–158.

Schmalenbach I, March TJ, Bringezu T, Waugh R, Pillen K. 2011. High-resolution genotyping of wild barley introgression lines and fine-mapping of the threshability locus *thresh-1* using the illumina goldengate assay. G3 (Bethesda, Md.) **1**, 187–196.

Schmitz J, Franzen R, Ngyuen TH, Garcia-Maroto F, Pozzi C, Salamini F, Rohde W. 2000. Cloning, mapping and expression analysis of barley MADS-box genes. Plant Molecular Biology **42**, 899–913.

Shaaf S, Bretani G, Biswas A, Fontana IM, Rossini L. 2019. Genetics of barley tiller and leaf development. Journal of Integrative Plant Biology **61**, 226–256.

Shaw LM, Lyu B, Turner R, Li C, Chen F, Han X, Fu D, Dubcovsky J. 2019. *FLOWERING LOCUS T2* regulates spike development and fertility in temperate cereals. Journal of Experimental Botany **70**, 193–204.

Shu K, Chen Q, Wu Y, Liu R, Zhang H, Wang S, Tang S, Yang W, Xie Q. 2016. ABSCISIC ACID-INSENSITIVE 4 negatively regulates flowering through directly promoting Arabidopsis *FLOWERING LOCUS C* transcription. Journal of Experimental Botany **67**, 195–205.

Shu K, Luo X, Meng Y, Yang W. 2018. Toward a molecular understanding of abscisic acid actions in floral transition. Plant & Cell Physiology **59**, 215–221.

Slafer GA, Kantolic AG, Appendino ML, Tranquilli G, Miralles DJ, Savin R. 2015. Genetic and environmental effects on crop development determining adaptation and yield. In: Sadras VO, Calderini DF, eds. Crop physiology. Applications for genetic improvement and agronomy, 2nd edn. Academic Press, 285–319.

Slafer GA, Savin R, Sadras VO. 2014. Coarse and fine regulation of wheat yield components in response to genotype and environment. Field Crops Research **157**, 71–83.

Smart RE, Bingham GE. 1974. Rapid estimates of relative water content. Plant Physiology **53**, 258–260.

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

Tamaru T, Hattori M, Ninomiya Y, *et al*. 2013. ROS stress resets circadian clocks to coordinate pro-survival signals. PLoS One **8**, e82006.

Templer SE, Ammon A, Pscheidt D, *et al*. 2017. Metabolite profiling of barley flag leaves under drought and combined heat and drought stress reveals metabolic QTLs for metabolites associated with antioxidant defense. Journal of Experimental Botany **68**, 1697–1713.

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 Physiology **143**, 225–235.

Turc O, Tardieu F. 2018. Drought affects abortion of reproductive organs by exacerbating developmentally driven processes via expansive growth and hydraulics. Journal of Experimental Botany **69**, 3245–3254.

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, Grando S, Del Greco A, This D, Baum M, Ceccarelli S. 2008. Quantitative trait loci associated with adaptation to Mediterranean dryland conditions in barley. Theoretical and Applied Genetics **117**, 653–669.

Waddington SR, Cartwright PM, Wall PC. 1983. A quantitative scale of spike initial and pistil development in barley and wheat. Annals of Botany **51**, 119–130.

Wang Y, Li L, Ye T, Lu Y, Chen X, Wu Y. 2013. The inhibitory effect of ABA on floral transition is mediated by ABI5 in *Arabidopsis*. Journal of Experimental Botany **64**, 675–684.

Wiegmann M, Maurer A, Pham A, *et al*. 2019. Barley yield formation under abiotic stress depends on the interplay between flowering time genes and environmental cues. Scientific Reports **9**, 6397.

Wigge PA, Kim MC, Jaeger KE, Busch W, Schmid M, Lohmann JU, Weigel D. 2005. Integration of spatial and temporal information during floral induction in *Arabidopsis*. Science **309**, 1056–1059.

Wu F, Shi X, Lin X, Liu Y, Chong K, Theißen G, Meng Z. 2017. The ABCs of flower development: mutational analysis of *AP1/FUL*-like genes in rice provides evidence for a homeotic (A)-function in grasses. The Plant Journal **89**, 310–324.

Xie W, Xiong W, Pan J, Ali T, Cui Q, Guan D, Meng J, Mueller ND, Lin E, Davis SJ. 2018. Decreases in global beer supply due to extreme drought and heat. Nature Plants **4**, 964–973.

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

Zakhrabekova S, Gough SP, Braumann I, *et al*. 2012. Induced mutations in circadian clock regulator *Mat-a* facilitated short-season adaptation and range extension in cultivated barley. Proceedings of the National Academy of Sciences, USA **109**, 4326–4331.

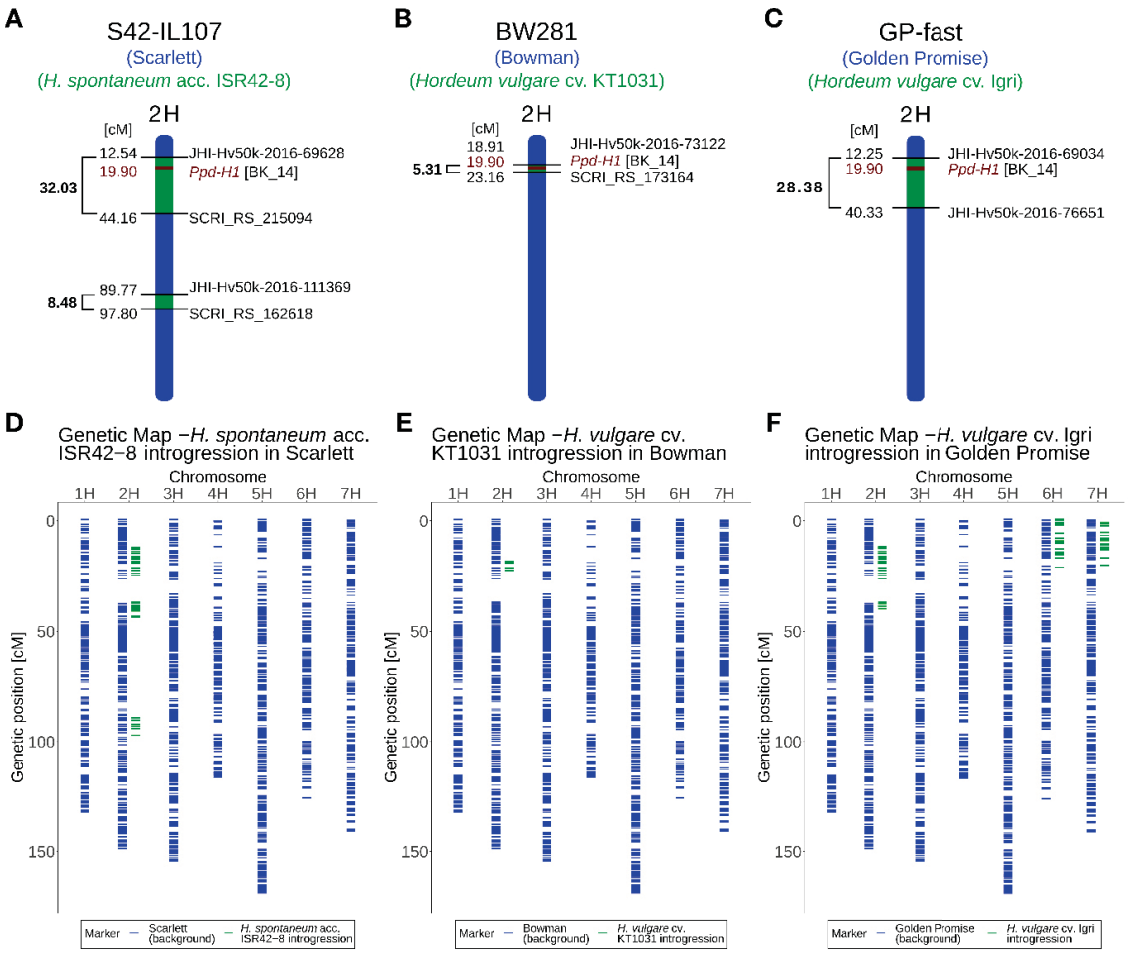
Zhang C, Liu J, Zhao T, *et al*. 2016. A drought-inducible transcription factor delays reproductive timing in rice. Plant Physiology **171**, 334–343.

6 Supplementary data

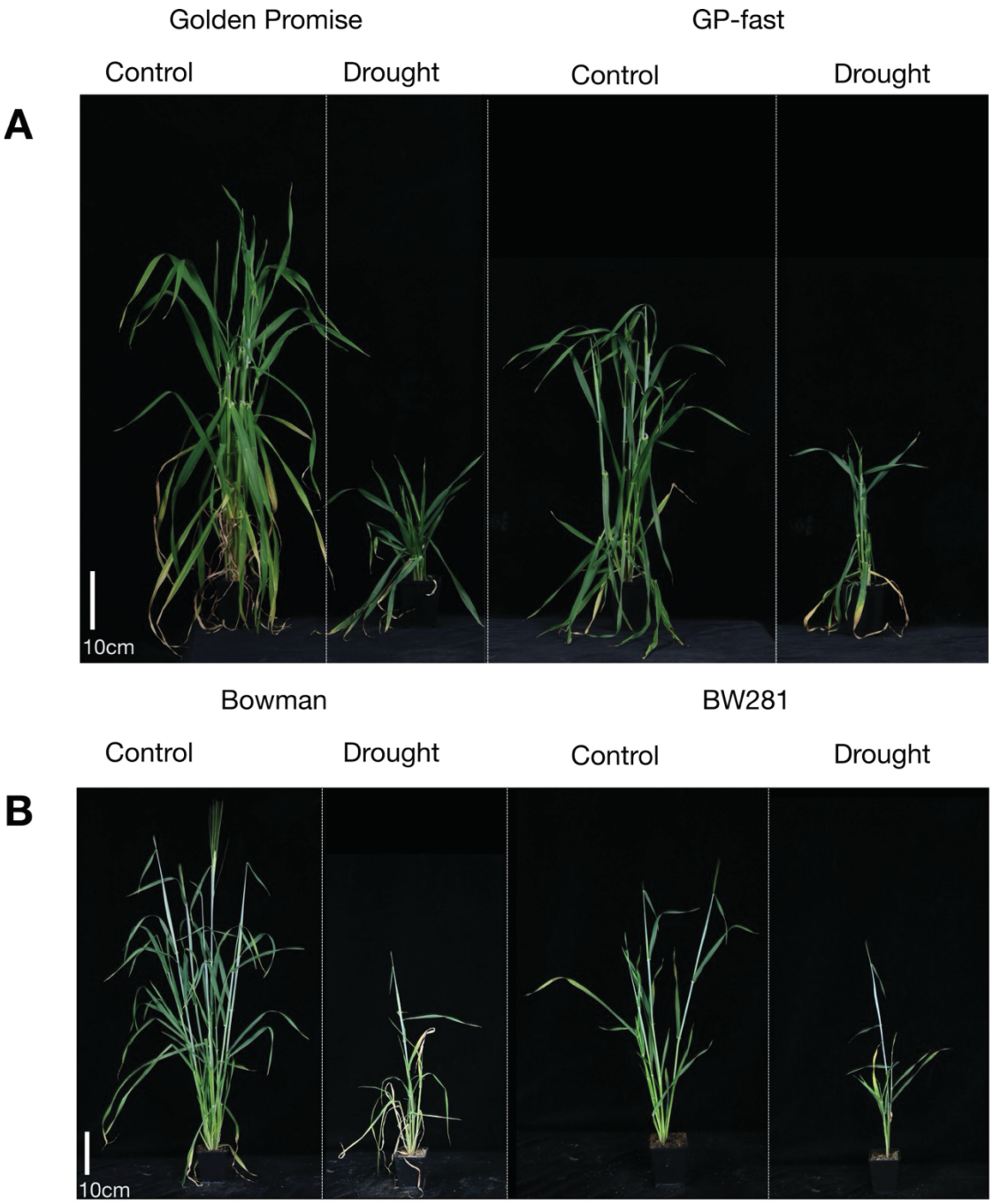
Supplementary Data S1.:" Genotyping of introgression lines used in this study." is available online at the Journal of Experimental Botany: https://academic.oup.com/jxb/advance-article/doi/10.1093/jxb/eraa261/5847724

Supplemental Table 1: Oligonucleotides used in this study

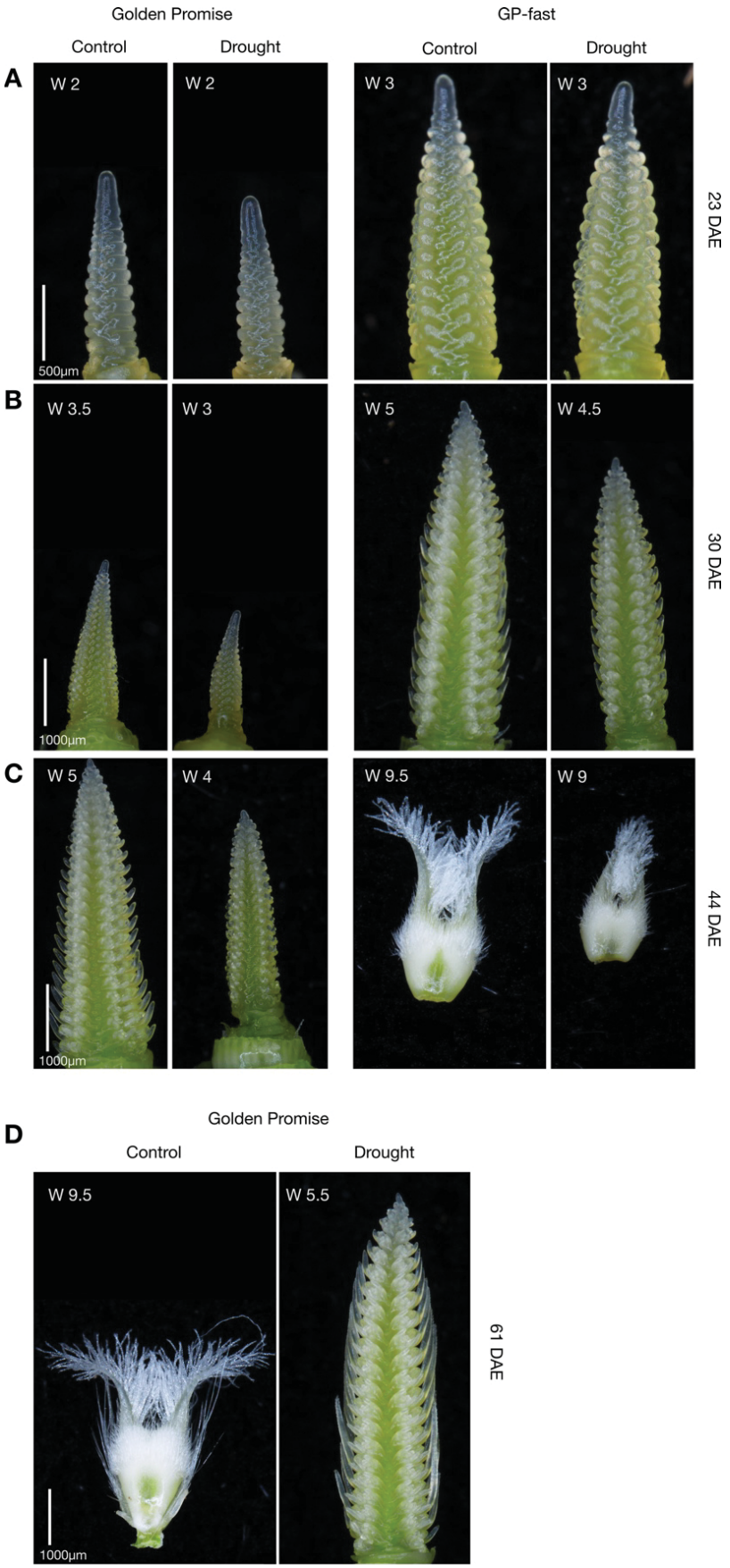
Gene ID	Gene name	Forward primer sequence	Reverse primer sequence	Source
AJ249143	*HvBM3*	GCC GTC ACC AGC ACA AGC AA	CCC CAT TCA CCC TGT AGC AAA GA	Digel *et al.,* 2015
AJ249146	*HvBM8*	CCA CAG CAG CCG ACA CCT A	TGC CTT TGG GGG AGA AGA CG	Digel *et al*., 2015
AK362208	*HvGAPDH*	GTG AGG CTG GTG CTG ATT ACG	AGT GGT GCA GCT AGC ATT TGA GAC	Ejaz and von Korff, 2016
AK376549	*HvPRR73*	GCG CCG TAG AGA ATC AGA AC	CAT GTC GGG TAC AGT CAT CG	Campoli *et al*., 2012b
AY145451	*HvACTIN*	CGT GTT GGA TTC TGG TGA TG	AGC CAC ATA TGC GAG CTT CT	Campoli *et al*., 2012a
AY740524	*HvGI*	TCA GTT AGA GCT CCT GGA AGT	GGT AGT TTG GGC TTT GGA TG	Campoli *et al*., 2012b
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
DQ100327	*HvFT1*	GGT AGA CCC AGA TGC TCC AA	TCG TAG CAC ATC ACC TCC TG	Campoli *et al*., 2012a
AY150676	*HvABI5*	CGC GCT GAA GTA TTG AAA CA	CAC CAG AAC GTT GCA GCT TA	Kobayashi *et al*., 2008
AJ508228.2	*HvADP*	GCT CTC CAA CAA CAT TGC CAA C	GAG ACA TCC AGC ATC ATT CAT TCC	Ferdous *et al*., 2015
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
JN603242	*HvCCA1*	CCT GGA ATT GGA GAT GGA GA	TGA GCA TGG CTT CTG ATT TG	Campoli *et al*., 2012b
AK252005	*HvPRR95*	CAG AAC TCC AGT GTC GCA AA	TGC TGT TGC CAG AGT TGT TC	Campoli *et al*., 2012b
Hv.20312	*HvLUX1*	AAT TCA GTC CAC GGA TGC TC	CTT CAC TTC AGC TCC CCT TG	Campoli *et al*., 2012b



Supplemental Figure 1 Genetic map of *Ppd-H1* introgression in spring barley backgrounds, introgression size in centimorgan (cM) and the polymorphic markers flanking the insertions. Donor parents of wild-type *Ppd-H1* allele (green) introgressed into their respective background cultivars (blue). Introgression lines were genotyped with the Barley 50k iSelect SNP Array (Bayer *et al.*, 2017). Location of introgressed *Ppd-H1* allele and flanking regions on chromosome 2H (A-C), and overview of all chromosomes of the three introgression lines (D-F). Mapping positions of markers were obtained from the POPSEQ_2017 map (Cantalapiedra *et al.*, 2015; Mascher *et al.*, 2017), and the sizes of introgressed regions were calculated from the midway point between flanking SNPs. There are additional donor introgressions in GP-fast on chromosome 6H and 7H, 22.34 cM and 19.90 cM, respectively.

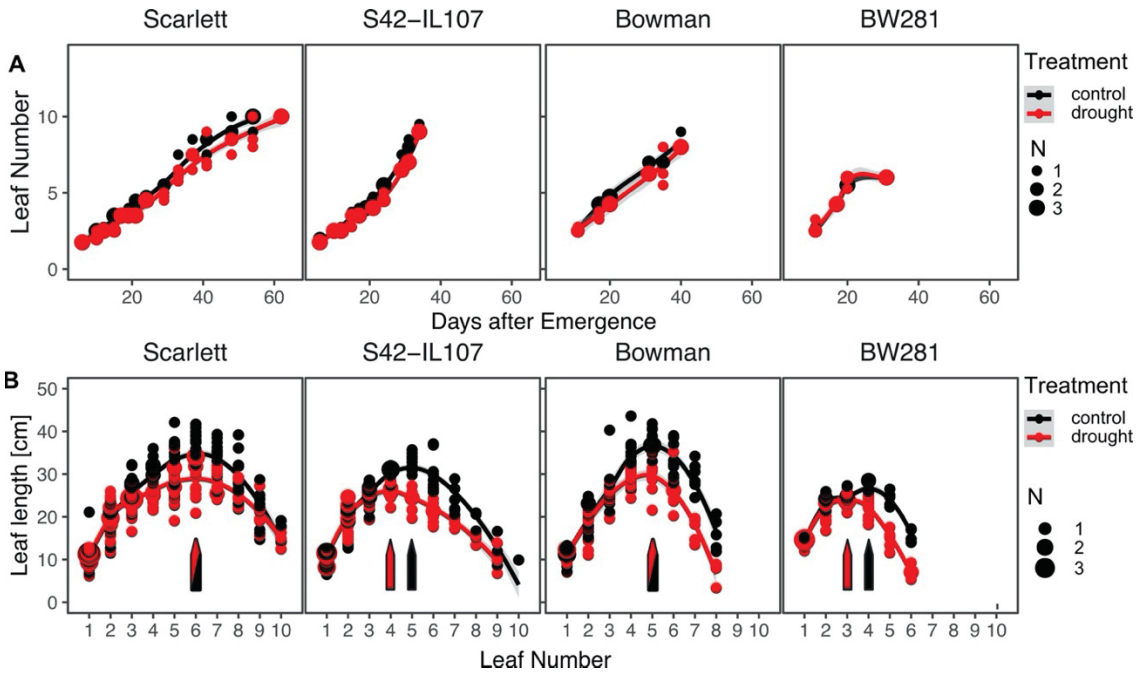


Supplemental Figure 2 Flowering morphology in Golden Promise and Bowman background under continuous drought. Representative plants of Golden Promise and GP-fast (A) plants at heading under control conditions after 63 and 43 DAE respectively. Representative plants of Bowman and BW281 (B) at heading under control conditions after 43 and 35 DAE respectively. Plants were grown under LDs (16 h light/8 h night) under either drought or control conditions.

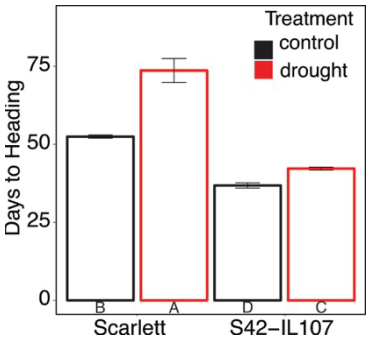


Supplemental Figure 3 MSA and pistil morphology in Golden Promise background.

Representative images of MSAs or pistils of Golden Promise and GP-fast 23 DAE (A), 30 DAE (B), 44 DAE (C) and 62 DAE (D). No images of GP-fast are presented for 62 DAE because plants had already flowered. Plants were grown under LDs (16 h light/8 h night) under either drought or control conditions.



Supplemental Figure 4 Continuous drought affects leaf size but not the phyllochron in barley. Leaf emergence (A) and length of fully emerged leaves (B) were scored over development under control (black) and drought (red) conditions in LDs (16 h light/8 h night) in the spring barley cultivars Scarlett and Bowman and their derived introgression lines S42-IL107 and BW281. Dot sizes indicate the number of overlapping samples. Trend lines were calculated using a polynomial regression (Loess smooth line), grey areas show 95% confidence interval.



Supplemental Figure 5 Severe drought delays flowering in barley. Days to heading was scored under control (black) and drought (red) conditions in LDs (16 h light/8 h night) in the spring barley cultivar Scarlett and the derived introgression line S42-IL107. Statistical groups were assigned using Kruskal-Wallis ANOVA and post-hoc Conover-Iman-test and Bonferroni correction. Different letters indicate groups differ ($p < 0.05$).

VII Acknowledgements

Unlike my poor plants I did not suffer, shrivel and dry out during my PhD. This was by no small part made possible by the many wonderful people to whom I would like to express my heartfelt gratitude.

First and foremost, I would like to thank my supervisor Maria von Korff for giving me the opportunity to do my PhD in her group. Thank you for all your guidance and mentoring while also encouraging me to develop my own project.

Thanks to Rüdiger Simon for graciously agreeing to act as Co-supervisor for my thesis.

Thanks to all present members and alumni of the Korff group for the welcoming, supporting and friendly environment during our time together.

Thanks to our wonderful technicians Kerstin, Andrea, Thea and Rebekka that helped so much with this work sometimes solved the most unsolvable problems.
Thanks to Caren for forcefully preventing people from watering my plants.

Thanks to all Members of the GRK2064 for all the valuable discussions and scientific and non-scientific exchanges. Being part of a group of PhD Students with such diverse approaches on a common topic provided a very unique and stimulating environment to pursue my PhD.

Thanks to Anna for all your support and for keeping me sane.

Danke an meine Familie für die nimmermüde Unterstützung ohne die ich nie so weit gekommen wäre.