
# The homolog of Antirrhinum CENTRORADIALIS has pleiotropic effects on shoot and spike architecture in barley (*Hordeum vulgare* L.)

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# I. Table of Content

# II.Summary

Variation in the shoot architecture in grasses is controlled by the activity and size of the shoot apical meristem (SAM) and derived meristems, such as axillary meristems (AXMs), inflorescence meristems (IMs), spikelet (SMs) and floral meristems (FMs). The activity of these meristems is determined both by the plant's genetic program and by environmental factors. TERMINAL FLOWER 1 (TFL1) is a key regulator of SAM activity and IM determinacy in Arabidopsis and the cereal grasses rice and maize. Consequently, TFL1 has strong effects on yield traits, such as the number of inflorescences and the number of seeds per inflorescence. TFL1 antagonizes the function of its homolog, the floral inducer FLOWERING LOCUS T (FT), by competition for complex formation with FLOWERING LOCUS D (FD) and 14-3-3 proteins in the SAM. The barley homolog of TFL1, termed HvCEN (Antirrhinum CENTRORADIALIS), has been proposed as a flowering repressor that is important for successful environmental adaptation. However, the effects of HvCEN on reproductive developmental timing and shoot and inflorescence architecture and consequently seed yield have not been characterized so far. In addition, there is no information about interactions between *HvCEN* and *FT*-like genes in barley.

In the present project, I aim to 1) determine pleiotropic effects of HvCEN on the timing of reproductive development and the shoot and spike morphology of barley under different photoperiods; 2) identify transcriptional targets of HvCEN in the developing SAM under different photoperiods; and 3) investigate the genetic interactions between *HvCEN* and the *FT*-like genes *HvFT1* and *HvFT3.*

I characterized 23 independent *hvcen* mutants under different photoperiods to elucidate the functions of HvCEN in shoot and inflorescence development under outdoor and fully controlled long and short day conditions. All *hvcen* mutants were early flowering and showed a reduction in the number of spikelets per main spike and in tiller number at heading in the outdoor experiment. Microscopic dissection of developing SAM revealed that *hvcen* mutants accelerated spikelet initiation independent of photoperiod, but promoted floret development only under long days (LDs) compared to wild type plants, while the main inflorescences were aborted under short days (SDs) in the wild type and mutant plants. The acceleration in development was associated with a reduction in the final number of spikelet primordia and seeds as observed under outdoor conditions. Mutations in HvCEN thus increased the determinacy of the inflorescence but *hvcen* mutants did not form a terminal flower that is typical for Arabidopsis *tfl1* mutants. The reduction in number of axillary buds in the mutants was independent of the photoperiod, so that *hvcen* mutants generated fewer tillers under both photoperiods. The analysis of an *hvcen*/*hvft3* double mutant under SDs demonstrated that HvCEN interacts with HvFT3 to control spikelet initiation. Furthermore, an *hvcen hvelf3 (EARLY FLOWERING 3)* double mutant plant with high *HvFT1* expression levels under SDs suggested that HvCEN interacts with HvFT1 to control floral

development. In order to characterize the effects of HvCEN on molecular changes in the SAM, I conducted global transcriptome profiling in enriched developing SAMs and inflorescences of mutant and wild type plants. At the spikelet initiation stage HvCEN controlled transcripts involved in primary metabolic processes, chromatin modification, ribosome biogenesis and hormone signaling independent of the photoperiod. During early floral development, HvCEN induced transcriptional changes were mainly photoperiod dependent and included floral homeotic genes.

In conclusion, I could demonstrate that HvCEN delays spikelet initiation independent of photoperiod by interacting with HvFT3. In addition, HvCEN delays inflorescence development resulting in an increase in the number of spikelets per spike in a photoperiod dependent manner likely by interacting with HvFT1 and by controlling the expression of floral homeotic genes in the SAM.

# III. Zusammenfassung

Die Pflanzenarchitektur von Gräsern wird durch die Aktivität und Grösse des Sprossapikalmeristems, und der daraus entstehenden Seiten-, Infloreszenz-, Ährchen- und Blütenmeristeme kontrolliert. Die Aktivität dieser Meristeme wird durch das genetische Programm der Pflanze und durch Umweltfaktoren bestimmt. TERMINAL FLOWER 1 (TFL1) ist ein wichtiges Gen für die Spezifikation und Determination der Sprossapikal-, Seiten- und Infloreszenzmeristeme in der Modellpflanze *Arabidopsis thaliana* and den Getreidearten Reis und Mais. Folglich hat TFL1 einen grossen Einfluss auf Ertragsmerkmale, wie die Anzahl der Infloreszenzen und die Anzahl der Blüten pro Infloreszenz. TFL1 wirkt antagonistisch zu einem homologen Gen und Blühpromotor, FLOWERING LOCUS T (FT), durch die kompetitive Bindung mit FLOWERING LOCUS D (FD) and 14-3-3 Proteinen im Sprossapikalmeristem. Das Gerstenhomolog von TFL1, HvCEN (Antirrhinum CENTRORADIALIS) gilt als Blührepressor und als wichtig für die Anpassung von Gerste an verschiedene Umwelten. Der Effekt von HvCEN auf die reproductive Entwicklung, auf die Spross- und Infloreszenzarchitektur und Ertrag wurden jedoch noch nicht untersucht. Ausserdem, gibt es keine Information über die genetischen Interaktionen zwischen HvCEN und FT- ähnlichen Genen in Gerste.

Die Ziele der vorliegenden Arbeit waren 1) die Bestimmung von pleiotropen Effekten von HvCEN auf die zeitliche Koordinierung der Entwicklung und auf die Spross und Ährenmorphologie von Gerste unter verschiedenen Photoperioden; 2) die Identifikation von transkriptionellen Zielgenen von HvCEN im Sprossapikal- und Infloreszenzmeristem unter verschiedenen Photoperioden; und 3) Untersuchungen zu genetischen Interaktionen zwischen *HvCEN* und den FT-Genen *HvFT1* and *HvFT3.*

Im Rahmen dieser Arbeit habe ich 23 unabhängige *hvcen* Mutanten unter verschiedenen Photoperioden im Feld und unter kontrollierten Bedingungen untersucht, um die Funktion von HvCEN in der Entwicklung des Sprossapikal-und Infloreszenzmeristems unter Lang- und Kurztagbedingungen aufzuklären. Alle *hvcen* Mutanten zeigten eine frühe Blüte und eine Reduktion in der Anzal von Ährchen pro Hauptähre, sowie in der Anzahl von Seitentrieben zum Zeitpunkt der Blüte im Freilandversuch. Mikroskopische Untersuchungen des sich entwickelnden Sprossmeristems zeigten, dass die *hvcen* Mutanten früher Ährchenprimordien induzierten unabhängig von der Photoperiode, die Entwicklung der Blüten innerhalb der Ährchen jedoch nur unter Langtagbedingungen beschleunigt war im Vergleich zum Wildtyp. Unter Kurztagbedingungen wurde die Entwicklung des Apikalmeristems in der frühen Phase der Blütenentwicklung im Wildtyp und Mutanten abgebrochen, so dass sich keine Hauptähre entwickelte. Die beschleunigte reproduktive Entwicklung in den Mutanten führte zu einer Reduktion in der Anzahl der Ährchenprimordien und Körner pro Hauptähre. Die *hvcen* Mutanten zeichneten sich durch eine erhöhte Determination des Infloreszenzmeristems aus, bildeten aber keine

terminale Blüte aus wie das für Arabidopsis *tfl1* Mutanten beschrieben ist. Die Reduktion von Seitenermeristemen in den *hvcen* Mutanten war unabhängig von der Photoperiode, so dass *hvcen* Mutanten weniger Halme im Kurz- und Langtag bildeten. Der phänotypische Vergleich von *hvft3* Einzel- und *hvcen*/*hvft3* Doppelmutanten im Kurztag zeigte, dass der Effekt von HvCEN auf die Initiierung der Ährchenprimordien abhängig von einem funktionalen *HvFT3* Gen ist. Darüberhinaus, zeigte die Analyse einer *hvcen hvelf3 (EARLY FLOWERING 3)* Doppelmutante mit Expression von *HvFT1* im Kurztag, dass HvCEN und HvFT1 interagieren, um die Blütenentwicklung zu koordinieren. Um transkriptionelle Zielgene von HvCEN im Sprossapikalmeristem zu detektieren, habe ich eine globale Expressionsanalyse in sich entwickelndem Sprossapikal- und Infloreszenzmeristemen in zwei allelischen Mutanten und Wildtyp durchgeführt. Während der Transition zum reproduktiven Meristem kontrollierte HvCEN die Expression von Genen mit Funktionen im Primärmetabolismus, Chromatinmodifikation, Ribosome Biogenese und Hormonmetabolismus unabhängig von der Photoperiode. Während der frühen Blütenentwicklung regulierte HvCEN homöotische Gene mit einer Rolle in der Blütenbildung vorwiegend unter Langtagbedingungen.

Abschliessend konnte ich zeigen, dass HvCEN mit HvFT3 interagiert, um die Initiierung von Ährchenprimordien unter Lang- und Kurztagbedingungen zu verzögern. Ausserdem interagiert HvCEN mit HvFT1, um die Infloreszenzentwicklung im Langtag zu verzögern und folglich die Anzahl der Ährchen pro Ähre zu erhöhen. HvCEN beeinflusst die Expression homöotischer Blühgene und verzögert so möglicherweise die Ährenentwicklung und erhöht die Anzahl der Blüten und Körner pro Ähre.

# IV. Chapter 1 ---The effects of developmental timing on shoot and spike architecture in barley

## 1. Introduction

Barley (*Hordeum vulgare* L.), taxonomically belonging to the tribe Triticeae in the Poaceae (Gramineae) family (von Bothmer et al., 1995) is one of the most widely cultivated cereals in the world. It ranks after rice (*Oryza sativa*), wheat (*Triticeum aestivum* L.) and maize (*Zea mays* L.) as the fourth most important resource for eradicating hunger and securing food supply. In 2014, more than 49 million hectares were harvested worldwide, mainly from Europe, for livestock fodder and brewing industry (Food and Agriculture Organization of the United Nations; http://faostat.fao.org/)

In barley, the per-plant grain yield is largely determined by the number of spike-producing tillers (i.e. effective tillering) and spikelets per spike, and the mean grain weight. In the following sections, I will firstly summarize the development and genetic regulation of barley tillers and spikes. I will then introduce the flowering repressor *Hordeum vulgare CENTRORADIALIS* (*HvCEN*) which adapts barley to local environments.

## 2. Tiller formation and structure of spike and their genetic basis

### 2.1. Tiller formation in barley

Barley tillers develop from the outgrowth of AXMs/axillary buds from leaf axils at the basal nodes (Schmitz and Theres, 2005; Ward and Leyser, 2004). An axillary bud together with a node and a leaf constitutes a unit named phytomer, which is repeatedly formed from the base of the plant (Forster et al., 2007; Ward and Leyser, 2004). The first AXM is often formed during embryogenesis while the mature barley embryo usually has two axillary buds that are emerged from the axils of the coleoptile and of the first leaf primordium, respectively (Kirby and Appleyard, 1987). Similar to rice, more leaves and AXMs are generated in an ordered and coordinated pattern after germination in barley (Itoh et al., 2005; Oikawa and Kyozuka, 2009). In addition, the newly formed tillers have the potential to develop new axillary buds/tillers in a reiterative pattern (Kirby and Appleyard, 1987) (Figure 1). For instance, the axillary buds initiated from main shoot apex are called primary axillary buds and may develop into primary tillers. Likewise, each primary tiller generates phytomer units in that secondary axillary buds may grow out to form secondary tillers.

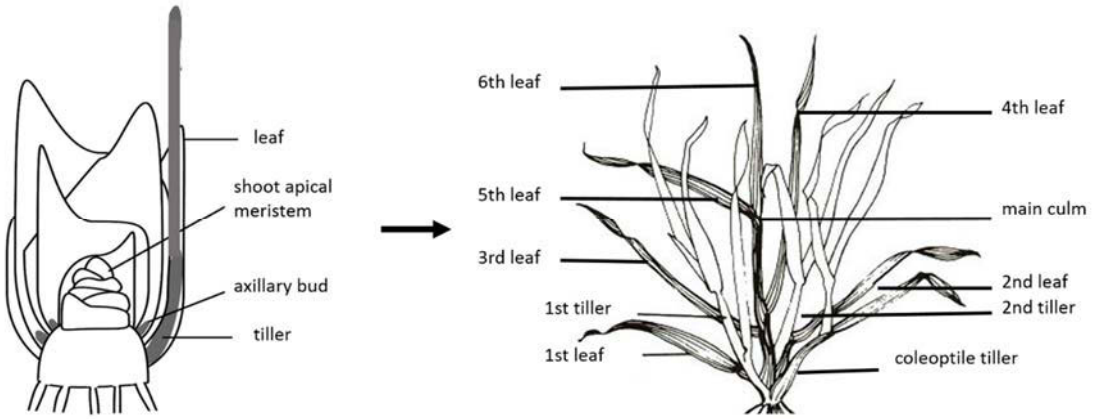



Figure 1 Tiller formation in barley adapted from Liller et al., 2015 and wheat tillering from http://forages.oregonstate.edu/regrowth/developmental-phases/vegetative-phase/tillering )

Tillering affects grain yield by competition and allocation of photoassimilates among tillers within an individual plant (Lauer and Simmons, 1988). The partitioning of photoassimilates strongly affects survival and grain morphologies of individual tiller in a plant. An axillary bud receives photoassimilates from the main stem or its parental tiller before emergence from the subtending leaf sheath until it establishes a sufficient leaf area (Lauer and Simmons, 1988). The removal of side tillers at an early stage leads to larger leaves in the main culm, thereby producing more spikelets and an increased grain number and weight (Alaoui et al., 1988; Jones and Kirby, 1977). This tiller-removal data suggests that side tillers compete for photoassimilates with main shoot apical meristem during early stages of SAM development (Jones and Kirby, 1977). The primary tillers develop earlier and thus have a higher chance of developing an increased leaf area, a higher sink capacity, an increased spikelet number and grain-filling ratio than the lateral tillers (Choi and Kwon, 1985). In addition, the main stems develop faster in low-tillering genotypes compared to those high-tillering counterparts (Kirby, 1973). As a consequence, the uniculm genotypes that routinely produce only one to several tillers displayed a taller stature, faster leaf appearance, and earlier flowering and maturation than the wild type genotypes. No more axillary buds are initiated after the formation of anther primordia on the main shoot apex (Kirby and Faris, 1972). Likewise, young tillers in rice undergo senescence when the main shoot apical meristems transit to reproductive growth, possibly due to rearrangement of nutrients with the necessary assimilates being moved from the young developing tillers to the internodes (Mahapatra and Behera, 2011). In barley, the yield in the main culm increased due to reduced side tillers under high density cultivation provided indirect evidence that side tillers and main culm compete for fixed carbon (Lauer, 1991).

How tillering affects per-plant grain yield is complicated and not easy to define due to the trade-off between tiller initiation/outgrowth/survival and other grain yield components (Kirby and Faris, 1972). Generally speaking, increased tiller number tends to result in higher number of spikes that potentially increase final yield. However, decreased tillering is also correlated with increased grain number and weight that otherwise enhances final grain yield (Kirby and Faris, 1972). Not all tillers survive and mature to produce seeds while some tillers remain dormant or senesce before producing seeds. The maturation of tillers per plant is greatly influenced by various endogenous and environmental cues (Kirby and Faris, 1972). The establishment of AXMs/axillary buds are mainly genetically controlled, however, the outgrowth of axillary buds is influenced genetically, environmentally and hormonally (Kebrom et al., 2013). For instance, spring barley sown in early spring generally produces increased number of total tillers and senescent tillers, however, those sown in late spring have decreased number of total tillers per plant but with an increased in tiller survival rate before heading (Lauer and Partridge, 1990). Tillering is also influenced by planting density in that per-plant tillers produced at low planting densities initiate earlier with higher tillering rate compared with those grown at high densities (Kirby and Faris, 1972). In addition, tillers under high planting densities senesce sooner and more rapidly relative to those under low densities (Kirby, 1967). Likewise, despite producing more tillers, high-tillering cultivars also have a higher mortality rate (Simmons et al., 1982), and show no increase in fertile tiller number per plant (Thorne, 1962; Kirby and Appleyard, 1987). Other environmental conditions, such as shading and nutrient availability, were also reported to affect bud outgrowth (Agusti and Greb, 2013). Environmental cues are found to influence shoot branching largely through phytohormones, such as auxin, cytokinins (CK), and strigolactone (SL) (Kebrom et al., 2013).

However, increasing total tiller number *per se* does not necessarily enhance yield due to the trade-off between individual yield components and cultivation density (Benbelkacem et al., 1984; Simmons et al., 1982). The total tiller number of two-row barley cultivar is negatively related to the final spike number per plant (Kirby, 1967). By contrast, the per-growing-unit yield of low-tillering *uniculm* genotypes in higher density did not increase due to increased temperatures associated with dense cultivation that led to the formation of abnormal spikes (Badra and Klinck, 1981; Donald, 1968, 1979; Kirby, 1973; McDonald, 1990).

## 2.2. Genetic control of tillering in barley

Mutants with altered tillering patterns have been identified and characterized, and helped uncover a series of promoters and suppressors of tillering (Dabbert et al., 2010). Low-tillering mutants have been used to identify the promoters of tillering. For instance, the *low number of tillers1* (*lnt1*) mutant with only 1 to 3 tillers is likely underlain by changes in *JUBEL2* (Dabbert et al., 2010). JUlBEL2 is a member

of BELL-family of homeodomain transcription factors and orthologous to Arabidopsis BELLRINGER(BLR) (Roeder et al., 2003; Müller et al., 2001; Dabbert et al., 2010; Smith and Hake, 2003; Byrne, 2003). JUBEL2 potentially promotes AXM and tiller development via its interaction with barley class I *KNOX* genes, such as a *SHOOT MERISTEMLESS* (*STM)*-like gene *Hooded*/*BKN3*, a mechanism similar to the interaction of *BLR* with a meristem identity gene *STM* in Arabidopsis (Müller et al., 2001; Cole et al., 2006; Rutjens et al., 2009; Long and Barton, 2000). Similar to the *lnt1* mutant, the *absent lower laterals1* (*als1*) and *uniculm4* (*cul4*) mutants typically develop only a few tillers, however, the molecular mechanisms of these two mutants remain less clear (Dabbert et al., 2010, 2009; Babb and Muehlbauer, 2003). In addition, the barley *uniculm2* (*cul2*) mutant is defective in axillary bud initiation, but the causative gene is not known so far (Okagaki et al., 2013; Babb and Muehlbauer, 2003). CUL2 regulates development of AXM by coordinating and integrating signaling pathways and stress response (Close, 2004; Okagaki et al., 2013). Some tillering mutants with a modest reduction in tiller number include the *uzu* mutant, a brassinosteroid (BR) insensitive mutant, and *intermedium-b* (*int-b*) mutant (Babb and Muehlbauer, 2003). The *Uzu* gene encodes a putative BR receptor *BRASSINOSTERD-INSENSITIVE1-like gene* (*HvBRI1*) and the phenotype of *uzu* mutant is caused by a nonsynonymous single-nucleotide substitution (Chono et al., 2003).

Many high-tillering mutants have been screened, however, the identities for the majority of the underlying causal genes are still poorly known. A barley *Gibberellin* (*GA*) *20-oxidase* gene (*Hv20ox2*) is suggested as a candidate for allelic high-tillering mutants *denso and semidwarf1* (*sdw1*) (Jia et al., 2009, 2011). Greatly decreased expression of *Hv20ox2* expression in the *denso* and *sdw1* mutants likely resulted in lower GA in the apical meristem that hence inhibits apical growth, plant height and promotes tillering (Jia et al., 2011). Another characterized high-tillering mutant is the *many noded dwarf6* (*mnd6*) mutant that is caused by a mutation in a member of the CYP78A family of cytochrome P450 enzymes (Babb and Muehlbauer, 2003; Druka et al., 2011; Mascher et al., 2014). Silencing the gene *P23K*, encoding a monocot-unique 23 kDa protein, resulted in lateral shoots from aerial nodes in association with downregulation of a cellulose synthase-like gene functioning in the (1,3;1,4)-β-D-glucan synthesis (Oikawa et al., 2009), indicating a link between branch/tiller development and cell wall polysaccharide synthesis.

The *densonidosum6* (*den6*), *granum-a* (*gra-a*), *grassy tillers* (*grassy*), *intermedium-m* (*int-m*), and *many noded dwarf1* (*mnd1*) mutants are high tillering mutants (Babb and Muehlbauer, 2003; Druka et al., 2011). The *gra-a* mutants show increased number of AXMs and axillary buds and form two shoot apical meristems occasionally (Babb and Muehlbauer, 2003). The molecular mechanisms of these tillering mutants remain poorly known, more efforts are needed to identify the underlying causative genes for

these mutants and other uncharacterized tillering mutants to better understand the genetic and molecular control of tillering in barley.

## 2.3. Structure of the barley spike

The inflorescence, called spike or ear in barley, is produced at the tip of the main culm or tillers whose development is defined by a series of characteristic changes. During vegetative to reproductive phase transition, the shoot apex elongates, concomitant with emergence of spikelet primordia that is characterized by the formation of double ridges alternately along the inflorescence axis (i.e. rachis) (Waddington et al., 1983). The final grain yield per spike is largely dependent on the number of spikelet primordia and final mature spikelets during development and spike growth. In temperate cereal grasses, the development of main shoot apical meristem has been divided into three key developmental phases based on its morphological characteristics, vegetative, early and late reproductive phase (Del Moral et al., 2002; González et al., 2002; Slafer and Rawson, 1994). With regard to the development of the most advanced spikelet primordia, a series of stages have been defined by Waddington (1983), known as Waddington scales. During the vegetative phase, leaves initiate surrounding the main shoot apical meristem until the emergence of double-ridge stage during which the spikelet primordia are formed (i.e. Waddington stage 2). After vegetative to reproductive phase transition, the most developed spikelet primordium develops into a floret (i.e. flower) primordium, being defined by visible stamen primordia (i.e. Waddington stage 3.5). Finally, the stem internodes elongate during the late reproductive phase, and no more spikelet primordia initiate after awn primordium stage (Waddington stage 4.5-5.0) while the initiated spikelet primordia continue to develop until fertilization or senescence (Digel et al., 2015; Waddington et al., 1983).

Triple spikelets are formed at each node of the rachis and each triplet develops along the rachis alternately. Normally, each spikelet develops a single floret consisting of a lemma, a palea, two lodicules, three stamens and a carpel. After threshing, in most barley cultivars the lemma and palea remain attached to the caryopsis. Barley is called as coarse grain, and its caryopsis is often referred to as a seed/grain, but not a kernel. Spikelet primordia initiated at the middle section develop more rapidly than those in the distal and proximal region of the spike/inflorescence. Depending on fertility of lateral spikelets, barley spikes are classified as six-row and two-row types with the two lateral spikelets being fertile and sterile, respectively (Forster et al., 2007; Sreenivasulu and Schnurbusch, 2012; Komatsuda et al., 2007) . Spikes of wild barley and other *Hordeum* species are two-rowed and the six-rowed spikes are thus derived from cultivated two-rowed type due to mutation in *SIX-ROWED SPIKE 1* (*VRS1*) or its upstream regulators such as *VRS2*, *VRS3*, and *VRS4* (Komatsuda et al., 2007;

Koppolu et al., 2013). Despite being less common in cultivated germplasms, a series of intermediate spike types have also been identified (Pourkheirandish and Komatsuda, 2007) (Figure 2).

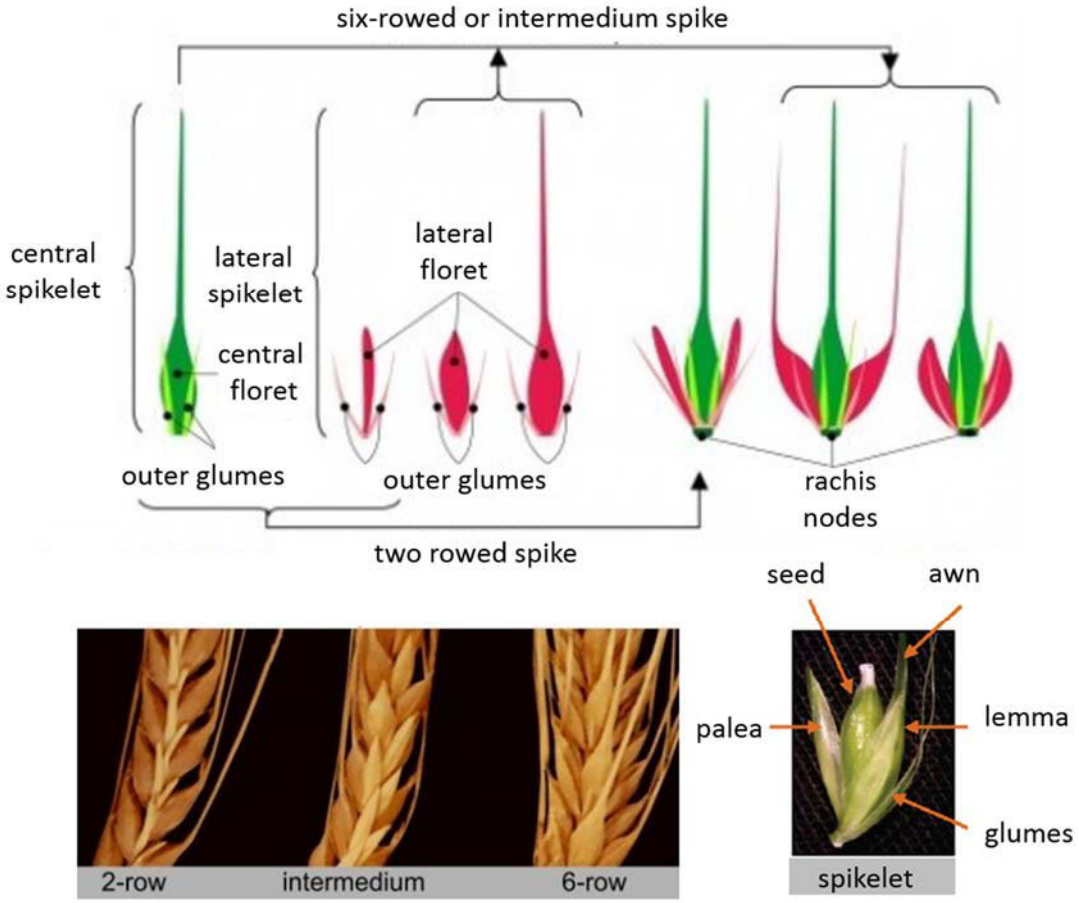



Figure 2: Components of two-rowed, six-rowed and intermedium spikes and a spikelet at grain filling stage (adapted from http://www.barleyhub.org/projects/row-genes and Abebe et al., 2009)

## 2.4. Genetic control of row-type in barley

Five major row-type genes have been identified and functionally characterized, including *VRS1*, *VRS2*, *VRS3*, *VRS4*, and *INTERMEDIUM-C* (*INT-C*). *VRS1* is a homeodomain-leucine zipper class I transcription factor that negatively regulates lateral spikelet fertility (Komatsuda et al., 2007). VRS2 shapes lateral fertility and inflorescence patterning via hormone-mediate gradients along spike and encodes for a *SHORT INTERNODES (SHI)* transcription factor (Youssef et al., 2016). VRS3, a histone demethylase, functions putatively upstream of *VRS1* and *INT-C*, thereby regulating lateral spikelet development (van Esse et al., 2017). VRS4 is a transcription factor, an ortholog of the maize RAMOSA2, that plays a role in lateral spikelet fertility and indeterminate triple spikelet meristems through regulation of VRS1 expression (Koppolu et al., 2013). INT-C, an ortholog of the maize domestication gene *TEOSINTE*

*BRANCHED 1* (*TB1*), modifies lateral spikelet development with VRS1 in a complementary manner (Ramsay et al., 2011).

The majority of the classical tillering and row-type genes affect both tiller number and the number of seeds per spike (Liller et al., 2015). For instance, the barley row type gene *INT-C* was found to control tillering during seedling stage (Ramsay et al., 2011). The low tillering mutant *cul2* also showed a spike phenotype: alteration of phyllotaxy and sometimes absence of the spikelets at the distal end of the inflorescence (Babb and Muehlbauer, 2003).

## 3. Tillering and inflorescence branching in other grass species

Barley is a powerful system to understand molecular mechanisms underlying variation in tillering and inflorescence morphologies in grasses. Likewise, insights from other grass species can be used to help uncover genetic basis of various valuable agronomic traits in barley. Cereal crops such as rice, maize, wheat and barley, and the model temperate grass Brachypodium (*Brachypodium distachyon*) are closely related, but the architectures of inflorescences differ considerably. Much variation in grass inflorescence architectures lies in the distinct modulation of branching patterns of successively emerging meristems before they terminate as short-branches, i.e., spikelets, the building blocks of grass inflorescences (Whipple, 2017). Maize and rice generate branched inflorescences, being indeterminate and determinate, respectively. Maize plants are monoecious producing two types of indeterminate inflorescences, i.e. the terminal branched male (i.e. tassel) and the lateral unbranched grain-bearing female (i.e. ear) inflorescences. Both tassel and ear inflorescences produce spikelet pair meristems that are subsequently differentiated into two paired single-floreted spikelets, a characteristic feature in maize and its close relatives (Andropogoneae) (Bortiri and Hake, 2007; Kellogg, 2007). In comparison, rice inflorescences are panicles that are formed by reiteratively formed primary and secondary branch meristems that produce lateral spikelet meristems and a terminal spikelet meristem. The rice spikelet consists of a pair of rudimentary glumes, a pair of sterile lemmas, and a terminal fertile floret that contains a pair of hulls (lemma and palea) and inner floral organs (Yoshida and Nagato, 2011). In a hypothesis, the sterile lemma are suggested as homologous to the lemma of two lateral florets (Yoshida and Nagato, 2011). A recent study could show the rice spikelet has the possibility to restore the phenotype called “three-florets spikelet” that may have existed in its ancestors by characterizing the *lateral florets 1* (*lf1*) mutant that developed lateral floret with proper inner floral organs (Zhang et al., 2017)

By contrast, barley and wheat produce unbranched inflorescences (Bonnett, 1935, 1936). Wheat inflorescences are determinate with the formation of one terminal spikelet meristem. While inflorescences in barley are indeterminate and produce triple spikelet meristems as depicted above,

with undifferentiated inflorescence tips. In addition, each spikelet meristem develops into one and several florets in barley and wheat, respectively.

A set of genes have been characterized that control tillering and inflorescence architectures in various grass species. BARREN STALK 1 (BA1) plays roles in the initiation and maintenance of axillary meristem in maize (Gallavotti et al., 2004). Maize BARREN INFLORESCENCE 2 (BIF2) is also required for the initiation and maintenance of meristems, including branch meristems, spikelet meristems and floret meristems (McSteen and Hake, 2001). LAX PANICLE 1 (LAX1) and LAX2 act together to control meristem initiation and maintenance in rice (Komatsu et al., 2001; Oikawa and Kyozuka, 2009; Tabuchi et al., 2011). *MONOCULM1* (*MOC1*) (also known as *SMALL PANICLE*), an ortholog of *LATERAL SUPPRESSOR* (*LAS*) in Arabidopsis, encodes a putative GRAS family nuclear protein which is mainly expressed in the axillary buds and initiates axillary buds and promotes their outgrowth in rice (Xu et al., 2012). TILLERING AND DWARF 1 (TAD1), and TILLER ENHANCER (TE) negatively regulate tillering by the degradation of MOC1 in rice (Lin et al., 2012; Xu et al., 2012). The best known maize TB1 inhibit tiller bud outgrowth, and is considered as the major domestication gene in maize (Doebley et al., 1995; Vann et al., 2015). Overexpression of *OsTB1* in rice reduced tillers and panicles whereas mutation in *OsTB1* increased them, indicating that this gene negatively regulates branching in rice (Choi et al., 2012). Phytohormone SL inhibits lateral branches by negative regulation of axillary bud growth (Umehara et al., 2008; Gomez-Roldan et al., 2008). *OsMADS57* inhibits *DWARF 14* (*D14*) , which is a negative tillering regulator responsive to SL (Arite et al., 2009; Guo et al., 2013). OsTB1 can interact with OsMADS57 to reduce the inhibition of D14 by OsMADS57 (Guo et al., 2013). Disruption of SL biosynthesis and signaling caused an increased number of tillers and a dwarf phenotype in rice (Beveridge and Kyozuka, 2010). Moreover, *D3* (Ishikawa et al., 2005), *D17* (Booker et al., 2004), *D10* (Arite et al., 2007), and *D27* (Lin et al., 2009) were also characterized to control of rice tillering through regulation of axillary buds outgrowth. Like D14, D3 is also involved in SL perception, whereas D10, D27 and D17 play roles in SL biosynthesis. Auxin and CK also mediate tillering and branching (Hayward et al., 2009; Leyser, 2003). Downregulation of *BRANCHED1* (*BRC1*), an Arabidopsis *TB1*-like gene, leads to branch outgrowth and it functions as downstream target of auxin and MORE AUXILIARY GROWTH (MAX) pathways to respond to endogenous and environmental cues to control bud outgrowth in Arabidopsis (Aguilar-Martinez et al., 2007). Reduced expression of *OsCKX2*, a cytokinin oxidase/dehydrogenase degrading CK, causes the accumulation of CK in inflorescence meristems resulting in enhanced grain yield by increasing the number of grains (Ashikari, 2005). Phytohormone BRs were reported to be involved in the regulation of rice tillering and DWARF AND LOW TILLERING (DLT) increase tillering by inhibition of BRs biosynthesis (Tong et al., 2009).

The decisions/fates/identities of meristems critically influence the final complexity and branching patterning of inflorescence architecture (Whipple, 2017). For instance, spikelet pair meristems in *ramosa1* (*ra1*), *ra2*, and *ra3* maize mutants were converted to branch meristems, resulting in a more branched tassel and abnormal ear branches (Vollbrecht et al., 2005; Bortiri et al., 2006; Satoh-Nagasawa et al., 2006). Likewise, spikelet meristems can be converted to branch meristem as shown in mutants with changes in orthologous genes maize *BRANCHED SILKLESS1 (BD1)* (Chuck, 2002), rice *FRIZZY PANICLE (FZP)* (Komatsu, 2003) and Brachypodium *MORE SPIKELETS1 (MOS1)* (Derbyshire and Byrne, 2013). These genes encode an ethylene-response transcription factor APETALA2 (AP2), and are conserved in specifying identity of spikelet meristems with mutant producing a highly branched inflorescences (Chuck, 2002; Colombo et al., 1998; Derbyshire and Byrne, 2013; Komatsu, 2003; Yi et al., 2005; Zhu et al., 2003). In addition, the number of florets within a spikelet differs greatly among species and is controlled by transitions of spikelet to floret meristems. *SUPERNUMERARY BRACT (SNB)* , an *INDETERMINATE SPIKELET 1 (IDS1)* -like gene, determines the number of florets in a spikelet by controlling the transition of a spikelet to a floret meristem (Lee and An, 2012; Lee et al., 2007).

In rice, SQUAMOSA PROMOTER BINDING PROTEIN-LIKE14 (OsSPL14) , also named as (IDEAL PLANT ARCHITECTURE1 (IPA)/ WEALTHY FARMER'S PANICLE (WFP) negatively controls tillering but promotes panicle branching by promoting inflorescence meristem and spikelet transition (Miura et al., 2010; Luo et al., 2012; Jiao et al., 2010; Wang et al., 2015). TaSPL14 was speculated to play a role in spikelet initiation in wheat (Feng et al., 2017). An *F-box* gene named *ABERRANT PANICLE ORGANIZATION 1 (APO1)*, an ortholog of Arabidopsis *UFO* (*UNUSUAL FLORAL ORGANS*), controls cell proliferation in the meristem and increase number of spikelets by suppressing the transition from inflorescence meristems to spikelet meristems in rice (Ikeda et al., 2005, 2007; Ikeda-Kawakatsu et al., 2009). Further evidence suggests that the rice ABERRANT PANICLE ORGANIZATION 2 (APO2)/RFL, the rice ortholog of Arabidopsis LEAFY (LFY), and APO1 function cooperatively in controlling the development of inflorescence and flower (Kyozuka et al., 1998; Ikeda-Kawakatsu et al., 2012).

The ABCDE model, defined as A,B,C,D and E five classes of homeotic genes has been proposed for floral organ specification (Rijpkema et al., 2010). In Arabidopsis, the A- and E-class genes specify the sepals in the outermost whorl, while the A, B and E-class genes specify petals in the second whorl, The third stamen whorl and forth carpel whorl are determined by the B + C + E and C +E -class genes, respectively (Smaczniak et al., 2012). In Arabidopsis, SEPALLATA1 (SEP1), SEP2, SEP3 and SEP4 are proposed as members of E class homeotic genes in floral organ identity. They function redundantly to help specify the identities of sepals, petals, stamens and carpels/ovules (Gary et al., 2004; Pelaz et al., 2000). AGAMOUS-LIKE 6 (AGL6) and AGL13, probably function like E class genes, playing a role in ovule generation (Schauer et al., 2009; Murai, 2013). The ABCDE model appears to be widely applicable in

eudicot like Arabidopsis but also in monocots like rice and wheat (Yoshida and Nagato, 2011; Ciaffi et al., 2011; Murai, 2013). The homologous organ of eudicot petals in grass species are lodicules, suggested by position criterion and gene expression data (Whipple et al., 2007). A typical grass floret such as that in rice and wheat has a palea, two lodicules, three stamens and a pistil in the whorl 1, 2, 3, and 4, respectively (Murai, 2013). Barley forms spikelet meristems in a similar way as wheat but different from 8-12 florets per spikelet formed in the center of the spike and 6-8 at the basal and distal spikelets of the spike in wheat, only one floret forms in one barley spikelet. The floral organs in barley also include a palea, lodicules, stamens and a pistil. They probably develop in a similar way as wheat counterparts developing in the four whorls.

## 4. A potential way to increase yield by altering flowering time to adapt barley to local environments

Shoot and inflorescence architectures can be affected by variation in the duration of reproductive development (Campoli and von Korff, 2014; Drosse et al., 2014). Therefore, flowering time regulators have a strong impact on the number of spike-bearing tillers and the number of seeds per spike. Flowering time regulators have also been proposed as important drivers for adaptation of barley to diverse environments (Nakamichi, 2015; Casao et al., 2011b). Barley *PRAEMATURUM (MAT)*/*EARLY MATURITY* (*EAM*) and the wheat ortholog *EARLINESS PER SE* (*EPS*) loci often control flowering time and life cycle independent of photoperiod and vernalization. Recent studies have uncovered the genetic identities of several of these *MAT/EAM*/*EPS* loci that are key internal oscillators in the circadian clock pathway (Pankin et al., 2014; Campoli et al., 2013; Faure et al., 2012; Zakhrabekova et al., 2012). For example, *EAM8 (MAT-A)* is underlain by an ortholog of the Arabidopsis circadian clock regulator *EARLY FLOWERING 3* (*HvELF3*) that causes a day-neutral early flowering phenotype (Faure et al., 2012; Zakhrabekova et al., 2012). Furthermore, the early maturity loci *EAM5* and *EAM10* encode circadian clock genes *PHYTOCHROME C (PHYC)* and *LUX ARHYTHMO (LUX)*, respectively (Pankin et al., 2014; Campoli et al., 2013). The mutations in *HvELF3*, *LUX-1* and *PHYC* led to a constitutive up-regulation of the major photoperiod response gene *PPD-H1* and the downstream *HvFT1* under both inductive long-day and non-inductive short-day conditions (Faure et al., 2012). As those in Arabidopsis, the changes in these mutants also greatly influence the expression of other clock oscillators and output genes. These studies suggested that circadian clock homologs have conserved function in flowering time control in barley and other plants.

In contrast, *MAT-C*/*EAM6*/*EPS6* has been identified as an ortholog of *CEN*) in *Antirrhinum* and *TFL1* in Arabidopsis, a well-documented modifier of inflorescence architecture (Bradley et al., 1996; Comadran et al., 2012; Shannon & Meeks-Wagner, 1991). A natural mutation leading to a substitution of Proline

with Alanine at the 135$^{th}$ amino acid of the HvCEN protein, was important in historical geographic range extension of barley cultivation (Comadran et al., 2012). It differentiates between winter and spring barley gene pools, indicating that different alleles perform better in certain environments. The ancestral winter allele of *HvCEN* accelerates flowering which caused an increase in yield in Mediterranean environments but a yield decrease in Scotland as compared to the derived spring allele (Comadran et al., 2012). However, it remains unclear how exactly HvCEN affects yield components in an environment-dependent manner in barley.

## 4.1. Antagonistic functions of HvCEN orthologs and their FT-like homologs in Arabidopsis and other species

Plant reproductive development is controlled by various environmental and endogenous stimuli to optimize plant adaptation and maximize reproductive success. In the model plant Arabidopsis, photoperiod, vernalization, gibberellins and autonomous pathways have been defined as main interconnected genetic pathways that coordinately regulate floral transition (Araki, 2001; Simpson and Dean, 2002; Blázquez et al., 2003; Mutasa-Göttgens and Hedden, 2009; Andrés and Coupland, 2012). These different pathways are converged on several floral integrator genes including *FT*, *LFY* and *SUPPRESSOR OF OVEREXPRESSION OF CO1 (SOC1)* (Moon et al., 2003; Corbesier and Coupland, 2006). One central integrator that has been extensively investigated is FT, the hypothetical "florigen" (Corbesier et al., 2007; Kobayashi and Weigel, 2007; Tamaki et al., 2007; Turck et al., 2008). *FT* is a member of phosphatidylethanolamine-binding protein (PEBP) family, whose homologs in human are often involved in several signaling pathways, such as the MAP kinase pathway and the NF-kappaB pathway via a PEBP Raf kinase inhibitor protein (RKIP) (Yeung et al., 2001; Corbit et al., 2003; Sedivy et al., 1999; Kardailsky, 1999; Kobayashi, 1999). *PEBP*s are reported to have multiple roles in modulation of in cell growth and differentiation (Hengst et al., 2001; Fu et al., 2003). Plant *PEBP*-related genes were initially identified as key regulator of inflorescence determinacy from *Antirrhinum majus* (Bradley et al., 1996), Arabidopsis (Bradley et al., 1997) and tomato (Pnueli et al., 1998). Their protein structures have also been determined in these two species (Banfield and Brady, 2000; Ahn et al., 2006). The PEBP family consists of six members including FT, TFL1, TWIN SISTER OF FT (TSF), Arabidopsis thaliana CENTRORADIALIS homologue (ATC), MOTHER OF FT AND TFL1 (MFT) and BROTHER OF FT AND TFL1 (BFT) in Arabidopsis (Bradley et al., 1997; Kardailsky, 1999; Kobayashi, 1999; Mimida et al., 2001; Yamaguchi et al., 2005; Yoo et al., 2004).

Despite being genetically identified as a key environmental adaptation contributor, the exact functional effects of HvCEN on barley vegetative and reproductive development thereby influencing the yield components remain largely unknown (Comadran et al., 2012). However, the functional roles

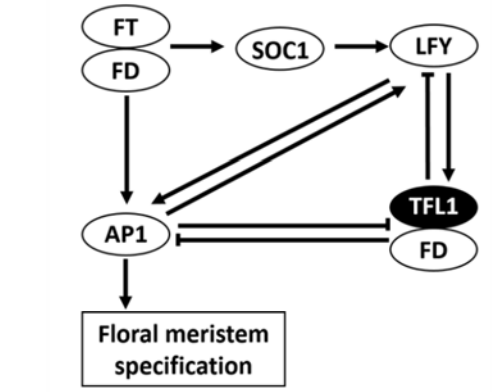
of its orthologs/homologs in Arabidopsis and other species have been extensively investigated that harbor diverse distinct functions in repressing phase transition from vegetative to reproductive or promoting flowering. For instance, Arabidopsis TFL1 represses flowering, whereas its closely related paralog FT promotes flowering (Ahn et al., 2006). Indeed, the functions of FT and TFL1-like (co)-orthologs are highly conserved across angiosperms with varying degree of rampant lineage-specific duplications and subsequent functional divergence, respectively. One of the FT homologs in poplar (*Populus deltoides*), termed FT2, accelerated transition from the juvenile to adult phase and functions as a promoter of seasonal flowering (Hsu et al., 2006). In sugar beet (*Beta vulgaris*), BvFT2 acts as flowering inducer, while BvFT1 acts as a flowering repressor. (Pin et al., 2010).

In flowering plants, FT/TFL1/CEN genes have experienced extensive lineage-specific expansion, with 19, 24 and 12 paralogs in rice, maize and barley, respectively (Danilevskaya et al., 2007; Halliwell et al., 2016). HEADING DATE 3A (HD3A), homologs of Arabidopsis florigen FT, functions as a mobile flowering signal in rice and promotes flowering under inductive short day condition (Tamaki et al., 2007; Kojima, 2002; Hayama et al., 2003). The mobile flowering signal in maize remains less clear, however, a *FT*-like gene *Zea CENTRORADIALIS 8* (*ZCN8*) complemented *ft1* mutants in Arabidopsis and induced flowering in transgenic resulted from flowering being delayed gene-silenced maize (Lazakis et al., 2011; Meng et al., 2011). HD3A orthologs in barley and wheat were initially identified as a major QTL in response to vernalization VERNALIZATION 3 (VRN3) and function as floral promoter correlating with early flowering in barley and wheat under long day conditions (Kikuchi et al., 2009; Yan et al., 2006). While a closely related paralog of VRN3 in barley (i.e. HvFT1), named HvFT3, promotes flowering under short days (Casao et al., 2011a).

Besides being a mobile flowering signal, *FT*-like genes have duplicated and diversified to perform different roles across the angiosperms. For example, the potato (*Solanum tuberosumandigena*) FT ortholog StSP6A induces tuberization under inductive short day condition (Navarro et al., 2011). The onion (*Allium cepa*) FT homologs regulate bulb formation (Lee et al., 2013). In addition, FT was reported to modulate shoot architecture by interaction with the TCP transcription factor BRC1 in the axillary meristem in Arabidopsis (Aguilar-Martinez et al., 2007; Niwa et al., 2013). Constitutive expression of *FT* resulted in a determinate inflorescence with a terminal flower analogous to phenotype of *tfl1* mutants (Hanano and Goto, 2011). The function of the majority of *FT*-like genes, however, is not well understood so far.

Unlike genes inducing flowering in the *FT* clade, *TFL1*-like genes are largely involved in suppressing flowering and promoting shoot indeterminacy (branching) that appears to be widely conserved across species. Ectopic expression of the *Citrus TFL1* homolog *CsTFL1* in Arabidopsis resulted in late flowering (Pillitteri et al., 2004). *SELF-PRUNING* (*SP*), the tomato (*Solanum lycopersicum*) homolog of *TFL1*,

established an indeterminate inflorescence producing inflorescence and fruits continuously, while the *sp* mutant plants developed a determinant inflorescence with shorter nodes between leaves and reduced fruit production (Pnueli et al., 1998; Jiang et al., 2013). In soybean (*Glycine max*), with the dominant *DETERMINATE STEM 1* (*DT1*) allele, plants grow continuously growth after flowering, i.e. indeterminate growth habit, while with *dt1* allele, growth is ceased after flowering, i.e. determinate habit (Liu et al., 2010; Tian et al., 2010). Likewise, the TFL1 homolog PvTFL1 in common bean (*Phaseolus vulgaris*) and PsTFL1a in pea (*Pisum sativum*) control indeterminate growth and shoot architecture (Kwak et al., 2012; Repinski et al., 2012; Foucher et al., 2003). Overexpression *Rice CEN-LIKE 1* (*RCN1*) and *RCN2*, rice TFL1 homologs, in Arabidopsis resulted in late flowering and an increase in branching. Likewise, the transgenic rice with *RCN1* or *RCN2* overexpression driven by the 35S promoter also showed a delayed transition to the reproductive phase and an increase in the number of panicle branches (Nakagawa et al., 2002). The repressive roles in flowering and constitutive expression of several *TFL1* homologs *ZCN*, *ZCN2*, *ZCN4* and *ZCN5* in maize led to a delay in flowering and alteration in inflorescence architecture to a bushy tassel with denser spikelets (Danilevskaya et al., 2010). Taken together, TFL1-like and FT-like proteins antagonistically control flowering, shoot branching, and determinacy of inflorescences.



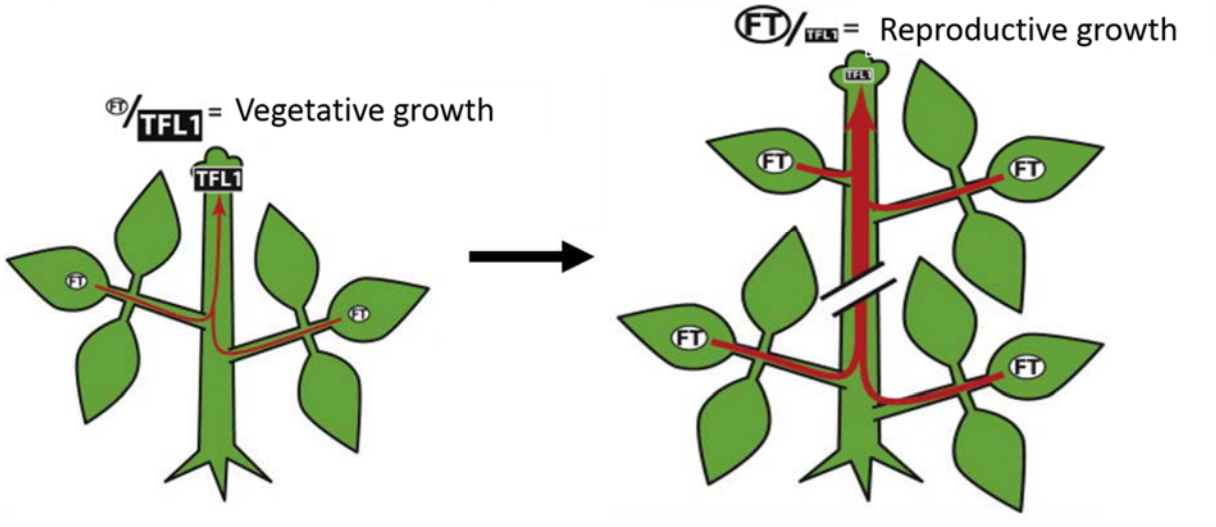



Figure 3 Contrasting roles of FT and TFL1 in growth regulation (adapted from McGarry and Ayre, 2012 and Jaeger et al., 2013)

## 4.2. The compositional and structural basis for the antagonistic function of FT/TFL1 proteins in flowering time control

However, the opposite effects of FT and TFL1 proteins in flowering time regulation may largely result from their compositional and structural differences. FT and TFL1 proteins are similar in length with approximately 175 amino acids, but exhibit only 55% identity. Indeed, the antagonistic functions of FT and TFL1 are determined by certain critical residue variants in their protein sequences. It was proposed that the substitution of Tyrosine with a Histidine at the position 85 in FT protein converts it from an inducer to a repressor of flowering, while the replacement of Histidine with Tyrosine at the position 88 in TFL1 resulted in weak FT like activities (Hanzawa et al., 2005). In addition, it was shown by Ho and Weigel (2014) that the modification of additional four specific residues in FT transmuted the protein into a repressor. Ectopic expression of the *TFL1* ortholog of orchid with a substitution of the His85 by Tyr caused the induction of flowering in Arabidopsis (Hou and Yang, 2009). A 14-amino-acid segment (residues 128–145) of TFL1 forms an external loop that makes a hydrogen bond with a residue near the entrance of a potential ligand-binding pocket. The external loop and the potential pocket both contributed to the opposing function of FT and TFL1 in flowering (Ahn et al., 2006). Taken together, FT and TFL1 are two closely related proteins the function of which is determined by a few amino acids in the external loop and the potential ligand binding pocket.

## 4.3. FT and TFL1 proteins function as long and short range mobile signals, respectively

*FT* is expressed in the leaf and its mature proteins are moved from leaf to the shoot apex for the vegetative to reproduction phase transition in shoot apical meristem (Liu et al., 2012). FT-INTERACTING PROTEIN 1 (FTIP1), an endoplasmic reticulum (ER) membrane protein, is needed for the movement of the mature FT protein from phloem companion cells to sieve elements (Liu et al., 2012). Flowering is delayed with loss-function mutation of FTIP1 which is partially due to altered FT trafficking to the shoot apex with FT being accumulated in phloem companion cells. Therefore, FT induces flowering through FTIP1-mediated translocation to the shoot apex (Liu et al., 2012). Specifically, FT enters the phloem flow stream and then moves from the phloem to the shoot apex due to its capability to dilate plasmodesmata microchannels during the process of cell-to-cell trafficking (Yoo et al., 2013). FT was documented to specifically bind to a membrane component phosphatidylcholine (PC) in vitro. Indeed, PC has been proposed to induce flowering by importing FT from the cytosol to the nucleus through the

nuclear membrane or by trafficking FT to FLOWERING LOCUS D (FD) through PC-containing PC vesicles (Nakamura et al., 2014).

By contrast, TFL1 functions as a short-distance mobile signal, with restricted activities within the shoot apical meristem (Conti and Bradley, 2007). While *TFL1* mRNA are transcribed broadly in young axillary meristem but later confined to the central region, TFL1 protein are widely found evenly throughout the shoot apex but not present in floral meristems. The diffused distribution of TFL1 proteins throughout the SAM possibly ensures the indeterminacy by suppressing expression of flowering genes (Conti and Bradley, 2007). In the *lfy* mutants, no such TFL1 movement is observed, suggesting that LFY facilitates TFL1 movement to outer regions of the meristem (Conti and Bradley, 2007). Nonfunctional TFL1 seems to account for the disruption of protein trafficking to protein storage vacuoles (Sohn et al., 2007). TFL1 is found in the cytoplasm and in the nucleus, therefore it is hypothesized that TFL1 shuttles FD protein from the nucleus to protein storage vacuoles to block FT-FD complex formation to negatively fine-tune flowering time and inflorescence meristem development (Hanano and Goto, 2011). To summarize, the antagonistic activities of FT and TFL1 proteins are likely achieved by their competition for binding with FD to form protein complex that promotes and suppresses flowering, respectively.

## 4.4. Putative targets regulated by FT and TFL1-like proteins in the shoot apical meristem

Contrary to *TFL1*, a group of well-characterized genes, including Arabidopsis *LFY*, *APETALA1* (*AP1*), and *CAULIFLOWER* (*CAL*), specify the identity of floral meristems (Irish and Sussex, 1990; Schultz and Haughn, 1991; Mandel et al., 1992; Weigel et al., 1992; Bowman et al., 1993; Gustafson-Brown et al., 1994). Overexpression of *LFY* or *AP1* converts vegetative shoots into reproductive flowers while the floral meristems are reverted to vegetative meristems in *lfy* and *ap1* mutants (Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995).

The expression domain of *HvCEN* is located at the center of subapical part in the SAM while the ones of *LFY*, *AP1*, and *CAL* are located in developing flowers (Bradley et al., 1997; Mandel et al., 1992; Ratcliffe et al., 1999). When TFL1 is mutated, the vegetative meristem converts into a floral meristem earlier which is associated with *LFY* and *AP1* and *CAL* ectopic expression (Weigel et al., 1992; Bowman et al., 1993; Gustafson-Brown et al., 1994; Bradley et al., 1997). Being consistent with this information, overexpression of *TFL1* repressed transition from vegetative to reproductive phase, correlated with a delayed upregulation of *LFY* and *AP1* (Ratcliffe et al., 1998).

FT and TFL1 are hypothesized as transcriptional co-regulators since they do not bind to DNA directly but they interact with the transcription factor FD. The interactions between FT/TFL1-like proteins and FD-like proteins have been demonstrated in other species. In rice SAM, HD3A interacts with 14-3-3 proteins, forming a complex that moves to bind the rice OsFD in the nucleus and finally producing a "florigen activation complex" (FAC) in the nucleus. The complex elevates expression of *OsMADS15*, homologous to Arabidopsis *AP1*, and induces flowering (Taoka et al., 2011). A potential binding site for 14-3-3 protein was found in *SP* in tomato (Pnueli et al., 2001). In soybean, overexpression of the *FT* homologs *GmFT2a* and *GmFT5a* accelerated flowering under the non-inductive long day condition and was accompanied by a significant upregulation of floral identity genes, such as *GmSOC1*, *GmAP1* and *GmLFY* (Nan et al., 2014). Yeast two-hybrid assays and bimolecular fluorescence complementation (BiFC) results further that these two proteins interact with GmFDL19, a soybean bZIP transcription factor homologous to Arabidopsis FD. Consistent with the elevated expression of *GmFT2a* and *GmFT5a*, overexpression of *GmFDL19* induces the expression of flowering identity genes and leads to early flowering. Furthermore, GmFDL19 bind to *cis*-elements in the promoter of *GmAP1a*, suggesting that GmFT2a and GmFT5a redundantly interact with the bZIP transcription factor GmFDL19 to induce the expression of floral identity genes in soybean (Nan et al., 2014). In maize, the most likely FT homolog is ZCN8 that was reported to interact with DELAYED FLOWERING 1 (DLF1), the maize FD ortholog, to induce flowering (Danilevskaya et al., 2007). The interaction between the maize TFL1 homolog ZCN2 and maize FD protein DLF1 leads to a repression of flowering (Danilevskaya et al., 2010).

In wheat, the HD3A ortholog TaFT1 (VRN3), integrates photoperiod and vernalization signals by interacting with the wheat FD-like protein TaFDL2, which is capable of binding the promotor of the *AP1* homolog *VRN1* to induce flowering (Li and Dubcovsky, 2008). In barley, HvFT1 interacts with FD-like bZIP protein HvFDL2 in the SAM to enhance the expression of *VRN1* (*AP1*) and *BARLEY MADS-BOX GENE 3(HvBM3)*/*FUL2* and *HvBM8*/*FUL3* and induce floral development (Chen and Dubcovsky, 2012; Hemming et al., 2008; Sasani et al., 2009; Schmitz et al., 2000). Mulki et al. (subm.) showed that HvFT3 specifically could promote spikelet initiation and accelerate the early reproductive phase under SD and LD conditions but play limited role in promoting inflorescence development and flowering through the upregulation of *AP1*-like genes *HvBM3*, *HvBM8* and *VRN-H1* in the leaves and at the shoot apices. The interaction of the FT-like proteins with the FD-like proteins are also observed in other species, such as chrysanthemum (*Chrysanthemum spp.*), rose (*Rosa spp.*) and kiwifruit (*Actinidia* spp.) (Higuchi et al., 2013; Randoux et al., 2014; Varkonyi-Gasic et al., 2013). In these species, the TFL1 homologs interact with the respective *FD* homologs as well to function as flowering repressors (Higuchi et al., 2013; Randoux et al., 2014; Varkonyi-Gasic et al., 2013).

Since no direct interaction between FT and TFL1 has been shown, speculation was made that TFL1 antagonizes the activities of FT through replacement of FT in the formation of the florigen activation complex thereby altering the effects of FT/florigen activation complex in flowering, inflorescence development and other processes (Wickland and Hanzawa, 2015). Indeed, manipulation of FT and TFL1 levels have been used to enhance agronomic or horticultural traits. The dosage of SINGLE FLOWER TRUSS (SFT) and SP, tomato orthologs of FT and TFL1, by optimization the ratio of SFT/SP (FT/TFL1) ,controlling determinacy and inflorescence branching, can increase yield in tomato (Krieger et al., 2010; Park et al., 2014, 2016; Jiang et al., 2013; Shalit et al., 2009). Consequently, FT and TFL1-like proteins likely compete for the binding of co-regulators, such as FD-like and 14-3-3-like proteins, thereby activating or prohibiting, respectively, putative downstream effector-targets genes that ultimately impact phase transition and morphologies (Kaneko-suzuki et al., 2018; Taoka et al., 2011; Pnueli et al., 2001; Ho and Weigel, 2014).

## 4.5. Selected barley *hvcen* mutants as a resource for investigations into the effects of HvCEN on developmental timing and correlated changes in yield components

Therefore, (effective) tillering and spike morphologies are important yield components that are greatly influenced by endogenous and environmental cues. Genes and phytohormones involved in meristem initiation and maintenance and determination of different meristem fates play critical roles in controlling tillering and inflorescence architectures thus yield in grasses including barley. FT and TFL1 are highly conserved across flowering plants and coordinately control flowering time, shoot branching and inflorescence determinacy. Determining the genetic and molecular relationships between flowering time and tillering, spike architecture would enable breeders to optimize and maximize yield components under different environments. HvCEN, a homolog of TFL1 represses flowering and contributes to environmental adaptation in barley (Comadran et al., 2012), however, its function in tillering and inflorescence architecture remains poorly known.

Barley natural and induced mutants with developmental and morphological phenotypes isolated by Swedish, Australian, and South American breeding programs represent a valuable resource to identify and or functionally characterize developmental mutants (Laurie et al., 1995; Lundqvist, 2009; Zakhrabekova et al., 2012). A rich collection of these mutants have been introgressed into Bowman, a spring cultivar. (Druka et al., 2011) that allows comparative analyses of allelic or functionally related mutants with same genetic background and minimized background mutations. The mutant collection comprises early maturity or *praematurum* mutants that are characterized by early flowering and seed maturation. The early maturity *eam6*/*mat*- mutants are controlled by *HvCEN* (Comadran et al., 2012) and a series of allelic *eam6*/*mat-c* mutants are available in the original spring barley backgrounds and

Bowman backcross lines. These mutants flower between 4-10 days earlier than their respective wild type progenitors. Resequencing of *HvCEN* in these allelic mutants found eight different allelic mutants carrying single-nucleotide substitutions that led to alterations in encoded amino acids. Two mutants have mutations at splice sites; two mutants carry one- and 12-base deletions, respectively; two mutants contain an introduction of a premature stop codon and an additional start codon in the coding sequences, respectively; while the final two mutants likely have a large deletion since the *HvCEN* locus could not be amplified (Comadran et al., 2012). These allelic mutants can be used to decipher the effect of HvCEN on shoot development and identify molecular networks underlying the mutant phenotypes. They serve as great material to probe its effects on yield-related traits, such as (effective) tillering, grain number per spike and their relationship with flowering time under different photoperiods. In addition, the *eam6/mat-c* mutants can be used to test the hypothesis of their antagonistic genetic interaction with barley *FT*-like genes. Finally, detailed phenotyping of main and axillary meristems accompanied by global transcriptome analyses in developing apices can reveal potential transcriptional targets of HvCEN in barley.

# 5. References

**Abebe, T., Wise, R.P., and Skadsen, R.W.** (2009). Comparative transcriptional profiling established the awn as the major photosynthetic organ of the barley spike while the lemma and the palea primarily protect the seed. Plant Genome J. **2**: 247.

**Aguilar-Martinez, J.A., Poza-Carrion, C., and Cubas, P.** (2007). Arabidopsis BRANCHED1 acts as an integrator of branching signals within axillary buds. Plant Cell. **19**: 458–472.

**Agusti, J. and Greb, T.** (2013). Going with the wind - Adaptive dynamics of plant secondary meristems. Mech. Dev. **130**: 34–44.

**Ahn, J.H., Miller, D., Winter, V.J., Banfield, M.J., Lee, J.H., Yoo, S.Y., Henz, S.R., Brady, R.L., and Weigel, D.** (2006). A divergent external loop confers antagonistic activity on floral regulators FT and TFL1. EMBO J. **25**: 605–14.

**Alaoui, A.C. El, Simmons, S.R., and Crookston, R.K.** (1988). Effects of tiller removal on spring barley. Crop Sci. **28**: 305–307.

**Andrés, F. and Coupland, G.** (2012). The genetic basis of flowering responses to seasonal cues. Nat. Rev. Genet. **13**: 627–639.

**Araki, T.** (2001). Transition from vegetative to reproductive phase. Curr. Opin. Plant Biol. **4**: 63–68.

**Arite, T., Iwata, H., Ohshima, K., Maekawa, M., Nakajima, M., Kojima, M., Sakakibara, H., and Kyozuka, J.** (2007). DWARF10, an RMS1/MAX4/DAD1 ortholog, controls lateral bud outgrowth in rice. Plant J. **51**: 1019–1029.

**Arite, T., Umehara, M., Ishikawa, S., Hanada, A., Maekawa, M., Yamaguchi, S., and Kyozuka, J.** (2009). D14, a strigolactone-Insensitive mutant of rice, shows an accelerated outgrowth of tillers. Plant Cell Physiol. **50**: 1416–1424.

**Ashikari, M.** (2005). Cytokinin oxidase regulates rice grain production. Science. **309**: 741–745.

**Babb, S. and Muehlbauer, G.** (2003). Genetic and morphological characterization of the barley uniculm2 (cul2) mutant. Theor. Appl. Genet. **106**: 846–857.

**Badra, A. and Klinck, H.R.** (1981). The influence of inter-plant competition on the morphological development of spikes of non-tillering barley (Hordeum vulgare L. emend Lam). Can. J. plant sci **61**: 829-835.

**Banfield, M.. and Brady, R..** (2000). The structure of Antirrhinum centroradialis protein (CEN) suggests a role as a kinase regulator1. J. Mol. Biol. **297**: 1159–1170.

**Benbelkacem, A., Mekni, M.S., and Rasmusson, D.C.** (1984). Breeding for high tiller number and yield in barley1. Crop Sci. **24**: 968–972.

**Beveridge, C.A. and Kyozuka, J.** (2010). New genes in the strigolactone-related shoot branching pathway. Curr. Opin. Plant Biol. **13**: 34–39.

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

**Bonnett, O.T.** (1935). The development of the barley spike. J. agric. Res **51**: 451-457.

**Bonnett, O.T.** (1936). The development of the wheat spike. J. Agric. Res. **53**: 445–451.

**Booker, J., Auldridge, M., Wills, S., McCarty, D., Klee, H., and Ottoline, L.** (2004). MAX3/CCD7 Is a carotenoid cleavage dioxygenase required for the synthesis of a novel plant signaling molecule. Curr. Biol. **14**: 1232–1238.

**Bortiri, E., Chuck, G., Vollbrecht, E., Rocheford, T., Martienssen, R., and Hake, S.** (2006). ramosa2 encodes a LATERAL ORGAN BOUNDARY domain protein that determines the fate of stem cells in branch meristems of maize, The Plant Cell. **18**: 574–585.

**Bortiri, E. and Hake, S.** (2007). Flowering and determinacy in maize. J. Exp. Bot. **58**: 909–916.

**von Bothmer, R. von, Jacobsen, N., Baden, C., Jørgensen, R.B., and Linde-Laursen, I.** (1995). An ecogeographical study of the genus Hordeum. Systematic and ecogeographic studies on crop genepools 7. Int. Plant Genet. Resour. Institute, Rome.

**Bowman, J.L., Alvarez, J., Weigel, D., Meyerowitz, E.M., and Smyth, D.R.** (1993). Control of flower development in Arabidopsis thaliana by APETALA1 and interacting genes. Development **119**: 721–743.

**Bradley, D., Carpenter, R., Copsey, L., Vincent, C., Rothstein, S., and Coen, E.** (1996). Control of inflorescence architecture in Antirrhinum. Nature **379**: 791–797.

**Bradley, D., Ratcliffe, O., Vincent, C., Carpenter, R., and Coen, E.** (1997). Inflorescence commitment and architecture in Arabidopsis. Science **275**: 80–83.

**Byrne, M.E.** (2003). Phyllotactic pattern and stem cell fate are determined by the Arabidopsis homeobox gene BELLRINGER. Development **130**: 3941–3950.

**Campoli, C. and von Korff, M.** (2014). Genetic control of reproductive development in temperate cereals. In Advances in botanical research **72**: 131–158.

**Campoli, C., Pankin, A., Drosse, B., Casao, C.M., Davis, S.J., and Von Korff, M.** (2013). HvLUX1 is a candidate gene underlying the early maturity 10 locus in barley: Phylogeny, diversity, and interactions with the circadian clock and photoperiodic pathways. New Phytol. **199**: 1045–1059.

**Casao, M.C., Igartua, E., Karsai, I., Lasa, J.M., Gracia, M.P., and Casas, A.M.** (2011a). Expression analysis of vernalization and day-length response genes in barley *(Hordeum vulgare* L.) indicates that VRNH2 is a repressor of PPDH2 (HvFT3) under long days. J. Exp. Bot. **62**: 1939–1949.

**Casao, M.C., Karsai, I., Igartua, E., Gracia, M.P., Veisz, O., and Casas, A.M.** (2011b). Adaptation of barley to mild winters: A role for PPDH2. BMC Plant Biol. **11**: 164.

**Chen, A. and Dubcovsky, J.** (2012). Wheat TILLING mutants show that the vernalization gene VRN1 down-regulates the flowering repressor VRN2 in leaves but is not essential for flowering. PLoS Genet. **8**.

**Choi, M.S., Woo, M.O., Koh, E.B., Lee, J., Ham, T.H., Seo, H.S., and Koh, H.J.** (2012). Teosinte Branched 1 modulates tillering in rice plants. Plant Cell Rep. **31**: 57–65.

**Choi, S. and Kwon, S.** (1985). Evaluation of varietal difference and environmental variation for some characters related to source and sink in the rice plants. Korean J. Crop Sci. (Korea R.) **30**: 460–470.

**Chono, M.** (2003). A Semidwarf Phenotype of Barley uzu Results from a Nucleotide Substitution in the Gene Encoding a Putative Brassinosteroid Receptor. Plant Physiol. **133**: 1209–1219.

**Chuck, G.** (2002). The Control of Spikelet Meristem Identity by the branched silkless1 Gene in Maize. Science. **298**: 1238–1241.

**Ciaffi, M., Paolacci, A.R., Tanzarella, O.A., and Porceddu, E.** (2011). Molecular aspects of flower development in grasses. Sex. Plant Reprod. **24**: 247–282.

**Close, T.J.** (2004). A new resource for cereal genomics: 22K barley GeneChip comes of age. Plant Physiol. **134**: 960–968.

**Cole, M., Nolte, C., and Werr, W.** (2006). Nuclear import of the transcription factor SHOOT MERISTEMLESS depends on heterodimerization with BLH proteins expressed in discrete sub-domains of the shoot apical meristem of Arabidopsis thaliana. Nucleic Acids Res. **34**: 1281–1292.

**Colombo, L., Marziani, G., Masiero, S., Wittich, P.E., Schmidt, R.J., Gorla, M.S., and Enrico Pè, M.** (1998). BRANCHED SILKLESS mediates the transition from spikelet to floral meristem during Zea mays ear development. Plant J. **16**: 355–363.

**Comadran, J. et al.** (2012). Natural variation in a homolog of Antirrhinum CENTRORADIALIS contributed to spring growth habit and environmental adaptation in cultivated barley. Nat. Genet. **44**: 1388–1392.

**Conti, L. and Bradley, D.** (2007). TERMINAL FLOWER1 is a mobile signal controlling Arabidopsis architecture. The Plant Cell

**19**: 767–778.

**Corbesier, L. and Coupland, G.** (2006). The quest for florigen: a review of recent progress. J. Exp. Bot. **57**: 3395–3403.

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

**Corbit, K.C., Trakul, N., Eves, E.M., Diaz, B., Marshall, M., and Rosner, M.R.** (2003). Activation of Raf-1 signaling by protein kinase C through a mechanism involving Raf kinase inhibitory protein. J. Biol. Chem. **278**: 13061—13068.

**Dabbert, T., Okagaki, R.J., Cho, S., Boddu, J., and Muehlbauer, G.J.** (2009). The genetics of barley low-tillering mutants: Absent lower laterals (als). Theor. Appl. Genet. **118**: 1351–1360.

**Dabbert, T., Okagaki, R.J., Cho, S., Heinen, S., Boddu, J., and Muehlbauer, G.J.** (2010). The genetics of barley low-tillering mutants: Low number of tillers-1 (lnt1). Theor. Appl. Genet. **121**: 705–715.

**Danilevskaya, O.N., Meng, X., and Ananiev, E. V** (2010). Concerted modification of flowering time and inflorescence architecture by ectopic expression of TFL1-like genes in maize. Plant Physiol. **153**: 238–251.

**Danilevskaya, O.N., Meng, X., Hou, Z., Ananiev, E. V., and Simmons, C.R.** (2007). A genomic and expression compendium of the expanded PEBP gene family from maize. Plant Physiol. **146**: 250–264.

**Derbyshire, P. and Byrne, M.E.** (2013). MORE SPIKELETS1 is required for spikelet fate in the inflorescence of brachypodium. Plant Physiol. **161**: 1291–1302.

**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**.**

**Doebley, J., Stec, A., and Gustus, C.** (1995). teosinte branched1 and the origin of maize: Evidence for epistasis and the evolution of dominance. Genetics **141**: 333–346.

**Donald, C.M.** (1979). A barley breeding programme based on an ideotype. J. Agric. Sci. **93**: 261.

**Donald, C.M.** (1968). The breeding of crop ideotypes. Euphytica **17**: 385–403.

**Drosse, B., Campoli, C., Mulki, A., and Korff, M. Von** (2014). Genetic control of reproductive development. Biotechnological approaches to barley improvement, Biotechnology in Agriculture and Forestry. **69.**

**Druka, A., Franckowiak, J., Lundqvist, U., Bonar, N., Alexander, J., Houston, K., Radovic, S., Shahinnia, F., Vendramin, V., Morgante, M., Stein, N., and Waugh, R.** (2011). Genetic dissection of barley morphology and development. Plant Physiol. **155**: 617–627.

**van Esse, G.W., Walla, A., Finke, A., Koornneef, M., Pecinka, A., and von Korff, M.** (2017). Six-rowed spike3 (VRS3) is a histone demethylase that controls lateral spikelet development in barley. Plant Physiol. **174**: 2397–2408.

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

**Feng, N. et al.** (2017). Transcriptome profiling of wheat inflorescence development from spikelet initiation to floral patterning identified stage-specific regulatory genes. Plant Physiol. **174**: 1779–1794.

**Forster, B.P., Franckowiak, J.D., Lundqvist, U., Lyon, J., Pitkethly, I., and Thomas, W.T.B.** (2007). The barley phytomer. Ann. Bot. **100**: 725–733.

**Foucher, F., Morin, J., Courtiade, J., Cadioux, S., Ellis, N., Banfield, M.J., and Rameau, C.** (2003). Late flowering. Society **15**: 2742–2754.

**Fu, Z., Smith, P.C., Zhang, L., Rubin, M.A., Dunn, R.L., Yao, Z., and Keller, E.T.** (2003). Effects of raf kinase inhibitor protein expression on suppression of prostate cancer metastasis. J. Natl. Cancer Inst. **95**: 878–89.

**Gallavotti, A., Zhao, Q., Kyozuka, J., Meeley, R.B., Ritter, M., Doebley, J.F., Pe`, M.E., and Schmidt, R.J.** (2004). The role of barren stalk1 in the architecture of maize. Nature **432**: 630–635.

**Gary, D., Pinyopich, A., Robles, P., Pelaz, S., and Yanofsky, M.F.** (2004). The SEP4 gene of Arabidopsis thaliana functions in floral organ and meristem identity. Can. Field-Naturalist **14**: 1935–1940.

**Gomez-Roldan, V. et al.** (2008). Strigolactone inhibition of shoot branching. Nature **455**: 189–194.

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

**Guo, S., Xu, Y., Liu, H., Mao, Z., Zhang, C., Ma, Y., Zhang, Q., Meng, Z., and Chong, K.** (2013). The interaction between OsMADS57 and OsTB1 modulates rice tillering via DWARF14. Nat. Commun. **4**: 1566.

**Gustafson-Brown, C., Savidge, B., and Yanofsky, M.F.** (1994). Regulation of the Arabidopsis floral homeotic gene APETALA1. Cell **76**: 131–143.

**Halliwell, J., Borrill, P., Gordon, A., Kowalczyk, R., Pagano, M.L., Saccomanno, B., Bentley, A.R., Uauy, C., and Cockram, J.** (2016). Systematic investigation of FLOWERING LOCUS T-like poaceae gene families identifies the short-day expressed flowering fathway gene, TaFT3 in Wheat *(Triticum aestivum* L.). Front. Plant Sci. **7**: 1–15.

**Hanano, S. and Goto, K.** (2011). Arabidopsis TERMINAL FLOWER1 is involved in the regulation of flowering time and inflorescence development through transcriptional repression. The Plant Cell**23**: 3172–3184.

**Hanzawa, Y., Money, T., and Bradley, D.** (2005). A single amino acid converts a repressor to an activator of flowering. Proc. Natl. Acad. Sci. **102**: 7748–7753.

**Hayama, R., Yokoi, S., Tamaki, S., Yano, M., and Shimamoto, K.** (2003). Adaptation of photoperiodic control pathways produces short-day flowering in rice. Nature **422**: 719–722.

**Hayward, A., Stirnberg, P., Beveridge, C., and Leyser, O.** (2009). Interactions between auxin and strigolactone in shoot branching control. Plant Physiol. **151**: 400–412.

**Hemming, M.N., Peacock, W.J., Dennis, E.S., and Trevaskis, B.** (2008). Low-temperature and daylength cues are integrated to regulate FLOWERING LOCUS T in barley. Plant Physiol. **147**: 355–366.

**Hengst, U., Albrecht, H., Hess, D., and Monard, D.** (2001). The phosphatidylethanolamine-binding protein is the prototype of a novel family of serine protease inhibitors. J. Biol. Chem. **276**: 535–40.

**Higuchi, Y., Narumi, T., Oda, A., Nakano, Y., Sumitomo, K., Fukai, S., and Hisamatsu, T.** (2013). The gated induction system of a systemic floral inhibitor, antiflorigen, determines obligate short-day flowering in chrysanthemums. Proc. Natl. Acad. Sci. **110**: 17137–17142.

**Ho, W.W.H. and Weigel, D.** (2014). Structural features determining flower-promoting activity of Arabidopsis FLOWERING LOCUS T. The Plant Cell **26**: 552–564.

**Hou, C.J. and Yang, C.H.** (2009). Functional analysis of FT and TFL1 orthologs from Orchid (Oncidium Gower Ramsey) that regulate the vegetative to reproductive transition. Plant Cell Physiol. **50**: 1544–1557.

**Hsu, C.-Y., Liu, Y., Luthe, D.S., and Yuceer, C.** (2006). Poplar FT2 shortens the juvenile phase and promotes seasonal flowering. The Plant Cell **18**: 1846–61.

**Ikeda-Kawakatsu, K., Maekawa, M., Izawa, T., Itoh, J.I., and Nagato, Y.** (2012). ABERRANT PANICLE ORGANIZATION 2/RFL, the rice ortholog of Arabidopsis LEAFY, suppresses the transition from inflorescence meristem to floral meristem through interaction with APO1. Plant J. **69**: 168–180.

**Ikeda-Kawakatsu, K., Yasuno, N., Oikawa, T., Iida, S., Nagato, Y., Maekawa, M., and Kyozuka, J.** (2009). Expression level of ABERRANT PANICLE ORGANIZATION1 determines rice inflorescence form through control of cell proliferation in the meristem. Plant Physiol. **150**: 736–747.

**Ikeda, K., Ito, M., Nagasawa, N., Kyozuka, J., and Nagato, Y.** (2007). Rice ABERRANT PANICLE ORGANIZATION 1, encoding an F-box protein, regulates meristem fate. Plant J. **51**: 1030–1040.

**Ikeda, K., Nagasawa, N., and Nagato, Y.** (2005). Aberrant panicle organization 1 temporally regulates meristem identity in rice. Dev. Biol. **282**: 349–360.

**Irish, V.F. and Sussex, I.M.** (1990). Function of the apetala-1 gene during Arabidopsis floral development. The Plant Cell **2**: 741–753.

**Ishikawa, S., Maekawa, M., Arite, T., Onishi, K., Takamure, I., and Kyozuka, J.** (2005). Suppression of tiller bud activity in tillering dwarf mutants of rice. Plant Cell Physiol. **46**: 79–86.

**Itoh, J.I., Nonomura, K.I., Ikeda, K., Yamaki, S., Inukai, Y., Yamagishi, H., Kitano, H., and Nagato, Y.** (2005). Rice plant development: From zygote to spikelet. Plant Cell Physiol. **46**: 23–47.

**Jaeger, K.E., Pullen, N., Lamzin, S., Morris, R.J., and Wigge, P.A.** (2013). Interlocking feedback loops govern the dynamic behavior of the floral transition in Arabidopsis. The Plant Cell **25**: 820–833.

**Jia, Q., Zhang, J., Westcott, S., Zhang, X., Bellgard, M., Lance, R., and Li, C.** (2009). GA-20 oxidase as a candidate for the semidwarf gene sdw1/denso in barley. Funct. Integr. Genomics **9**: 255–262.

**Jia, Q., Zhang, X., Westcott, S., Broughton, S., Cakir, M., Yang, J., Lance, R., and Li, C.** (2011). Expression level of a gibberellin 20-oxidase gene is associated with multiple agronomic and quality traits in barley. Theor. Appl. Genet. **122**: 1451–1460.

**Jiang, K., Liberatore, K.L., Park, S.J., Alvarez, J.P., and Lippman, Z.B.** (2013). Tomato yield heterosis is triggered by a dosage sensitivity of the florigen pathway that fine-tunes shoot architecture. PLoS Genet. **9**.

**Jiao, Y., Wang, Y., Xue, D., Wang, J., Yan, M., Liu, G., Dong, G., Zeng, D., Lu, Z., Zhu, X., Qian, Q., and Li, J.** (2010). Regulation of OsSPL14 by OsmiR156 defines ideal plant architecture in rice. Nat. Genet. **42**: 541–544.

**Jones, H.G. and Kirby, E.J.M.** (1977). Effects of manipulation of number of tillers and water supply on grain yield in barley. J. Agric. Sci. **88**: 391.

**Kaneko-Suzuki, M., Kurihara-Ishikawa, R., Okushita-Terakawa, C., Kojima, C., Nagano-Fujiwara, M., Ohki, I., Tsuji, H., Shimamoto, K., and Taoka, K.I.** (2018). TFL1-Like proteins in rice antagonize rice FT-like protein in inflorescence development by competition for complex formation with 14-3-3 and FD. Plant Cell Physiol. **59**: 458–468.

**Kardailsky, I.** (1999). Activation Tagging of the Floral Inducer FT. Science **286**: 1962–1965.

**Kebrom, T.H., Spielmeyer, W., and Finnegan, E.J.** (2013). Grasses provide new insights into regulation of shoot branching. Trends Plant Sci. **18**: 41–48.

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

**Kikuchi, R., Kawahigashi, H., Ando, T., Tonooka, T., and Handa, H.** (2009). Molecular and Functional Characterization of PEBP Genes in Barley Reveal the Diversification of Their Roles in Flowering. Plant Physiol. **149**.

**Kirby, E.J.M.** (1973). Effect of Temperature on Ear Abnormalities in Uniculm Barley. J. Exp. Bot. **24**: 935–947.

**Kirby, E.J.M.** (1967). The effect of plant density upon the growth and yield of barley. J. Agric. Sci. **68**: 317–324.

**Kirby, E.J.M. and Appleyard, M.** (1987). Development and structure of the wheat plant. In Wheat breeding (Springer), pp. 287–311.

**Kirby, E.J.M. and Faris, D.G.** (1972). The effect of plant density on tiller growth and morphology in barley. J. Agric. Sci. **78**: 281–288.

**Kobayashi, Y.** (1999). A Pair of Related Genes with Antagonistic Roles in Mediating Flowering Signals. Science **286**: 1960–1962.

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

**Kojima, S.** (2002). Hd3a, a Rice Ortholog of the Arabidopsis FT Gene, Promotes Transition to Flowering Downstream of Hd1 under Short-Day Conditions. Plant Cell Physiol. **43**: 1096–1105.

**Komatsu, M.** (2003). FRIZZY PANICLE is required to prevent the formation of axillary meristems and to establish floral meristem identity in rice spikelets. Development **130**: 3841–3850.

**Komatsu, M., Maekawa, M., Shimamoto, K., and Kyozuka, J.** (2001). The LAX1 and FRIZZY PANICLE 2 genes determine the inflorescence architecture of rice by controlling rachis-branch and spikelet development. Dev. Biol. **231**: 364–373.

**Komatsuda, T. et al.** (2007). Six-rowed barley originated from a mutation in a homeodomain-leucine zipper I-class homeobox gene. Proc. Natl. Acad. Sci. **104**: 1424–1429.

**Koppolu, R. et al.** (2013). Six-rowed spike4 (Vrs4) controls spikelet determinacy and row-type in barley. Proc. Natl. Acad. Sci. **110**: 13198–13203.

**Krieger, U., Lippman, Z.B., and Zamir, D.** (2010). The flowering gene SINGLE FLOWER TRUSS drives heterosis for yield in tomato. Nat. Genet. **42**: 459–463.

**Kwak, M., Toro, O., Debouck, D.G., and Gepts, P.** (2012). Multiple origins of the determinate growth habit in domesticated common bean (Phaseolus vulgaris). Ann. Bot. **110**: 1573–1580.

**Kyozuka, J., Konishi, S., Nemoto, K., Izawa, T., and Shimamoto, K.** (1998). Down-regulation of RFL, the FLO/LFY homolog of rice, accompanied with panicle branch initiation. Proc. Natl. Acad. Sci. **95**: 1979–82.

**Lauer, J.G.** (1991). Barley tiller response to plant density and ethephon. Agron. J. **83**: 968–973.

**Lauer, J.G. and Partridge, J.R.** (1990). Planting date and nitrogen rate effects on spring malting barley. Agron. J. **82**: 1083–1088.

**Lauer, J.G. and Simmons, S.R.** (1988). Photoassimilate partitioning by tillers and individual tiller leaves in field-grown spring barley. Crop Sci. **28**: 279–282.

**Laurie, D.A., Pratchett, N., Snape, J.W., and Bezant, J.H.** (1995). RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter× spring barley (Hordeum vulgare L.) cross. Genome **38**: 575–585.

**Lazakis, C.M., Coneva, V., and Colasanti, J.** (2011). ZCN8 encodes a potential orthologue of Arabidopsis FT florigen that integrates both endogenous and photoperiod flowering signals in maize. J. Exp. Bot. **62**: 4833–4842.

**Lee, D.Y. and An, G.** (2012). Two AP2 family genes, SUPERNUMERARY BRACT (SNB) and OsINDETERMINATE SPIKELET 1 (OsIDS1), synergistically control inflorescence architecture and floral meristem establishment in rice. Plant J. **69**: 445–461.

**Lee, D.Y., Lee, J., Moon, S., Park, S.Y., and An, G.** (2007). The rice heterochronic gene SUPERNUMERARY BRACT regulates the

transition from spikelet meristem to floral meristem. Plant J. **49**: 64–78.

**Lee, R., Baldwin, S., Kenel, F., McCallum, J., and Macknight, R.** (2013). FLOWERING LOCUS T genes control onion bulb formation and flowering. Nat. Commun. **4**: 1–9.

**Leyser, O.** (2003). Regulation of shoot branching by auxin. Trends Plant Sci. **8**: 541–545.

**Li, C. and Dubcovsky, J.** (2008). Wheat FT protein regulates VRN1 transcription through interactions with FDL2. Plant J. **55**: 543–554.

**Liller, C.B., Neuhaus, R., Von Korff, M., Koornneef, M., and Van Esse, W.** (2015). Mutations in barley row type genes have pleiotropic effects on shoot branching. PLoS One **10**: 1–20.

**Lin, H., Wang, R., Qian, Q., Yan, M., Meng, X., Fu, Z., Yan, C., Jiang, B., Su, Z., Li, J., and Wang, Y.** (2009). DWARF27, an iron-containing protein required for the biosynthesis of strigolactones, regulates rice tiller bud outgrowth. The Plant Cell **21**: 1512–1525.

**Lin, Q. et al.** (2012). Rice APC/CTE controls tillering by mediating the degradation of MONOCULM 1. Nat. Commun. **3**: 752.

**Liu, B. et al.** (2010). The soybean stem growth habit gene Dt1 is an ortholog of Arabidopsis TERMINAL FLOWER1. Plant Physiol. **153**: 198–210.

**Liu, L., Liu, C., Hou, X., Xi, W., Shen, L., Tao, Z., Wang, Y., and Yu, H.** (2012). FTIP1 is an essential regulator required for florigen transport. PLoS Biol. **10**.

**Long, J. and Barton, M.K.** (2000). Initiation of Axillary and Floral Meristems in Arabidopsis. Dev. Biol. **218**: 341–353.

**Lundqvist, U.** (2009). Eighty years of Scandinavian barley mutation genetics and breeding. Induc. plant Mutat. genomics era. Food Agric. Organ. United Nations, Rome: 39–43.

**Luo, L., Li, W., Miura, K., Ashikari, M., and Kyozuka, J.** (2012). Control of tiller growth of rice by osspl14 and strigolactones, which work in two independent pathways. Plant Cell Physiol. **53**: 1793–1801.

**Mahapatra, I.C. and Behera, U.K.** (2011). Rice (Oryza sativa)-based farming systems for livelihood improvement of Indian farmers. Indian J. Agron. **56**: 1–19.

**Mandel, A.M., Gustafson-Brown, C., Savidge, B., and Yanofsky, M.F.** (1992). Molecular characterization of the Arabidopsis floral homeotic gene APETALA1. Nature **360**: 273–277.

**Mandel, M.A. and Yanofsky, M.F.** (1995). A gene triggering flower formation in Arabidopsis. Nature **377**: 522–524.

**Mascher, M., Jost, M., Kuon, J.-E., Himmelbach, A., Aßfalg, A., Beier, S., Scholz, U., Graner, A., and Stein, N.** (2014). Mapping-by-sequencing accelerates forward genetics in barley. Genome Biol. **15**: R78.

**McDonald, O.** (1990). The growth and yield of uniculm and tillered barley over a range of sowing rates. Aust. J. Agric. Res. **41**: 449-461.

**McGarry, R.C. and Ayre, B.G.** (2012). Manipulating plant architecture with members of the CETS gene family. Plant Sci. **188–189**: 71–81.

**McSteen, P. and Hake, S.** (2001). Barren inflorescence2 regulates axillary meristem development in the maize inflorescence. Development **128**: 2881–2891.

**Meng, X., Muszynski, M.G., and Danilevskaya, O.N.** (2011). The FT-Like ZCN8 gene functions as a floral activator and is involved in photoperiod sensitivity in maize. The Plant Cell **23**: 942–960.

**Mimida, N., Goto, K., Kobayashi, Y., Araki, T., Ahn, J.H., Weigel, D., Murata, M., Motoyoshi, F., and Sakamoto, W.** (2001). Functional divergence of the *TFL1*-like gene family in *Arabidopsis* revealed by characterization of a novel homologue. Genes to Cells **6**: 327–336.

**Miura, K., Ikeda, M., Matsubara, A., Song, X.-J., Ito, M., Asano, K., Matsuoka, M., Kitano, H., and Ashikari, M.** (2010). OsSPL14 promotes panicle branching and higher grain productivity in rice. Nat. Genet. **42**: 545–549.

**Moon, J., Suh, S.S., Lee, H., Choi, K.R., Hong, C.B., Paek, N.C., Kim, S.G., and Lee, I.** (2003). The SOC1 MADS-box gene integrates vernalization and gibberellin signals for flowering in Arabidopsis. Plant J. **35**: 613–623.

**Del Moral, L.G., Miralles, D.J., and Slafer, G.** (2002). Initiation and appearance of vegetative and reproductive structures throughout barley development. Barley Sci. Recent Adv. from Mol. Biol. to Agron. yield Qual. GA Slafer, JL Molina-Cano, R Savin, JL Araus I Romagosa) pp: 243–267.

**Müller, J., Wang, Y., Franzen, R., Santi, L., Salamini, F., and Rohde, W.** (2001). In vitro interactions between barley TALE homeodomain proteins suggest a role for protein-protein associations in the regulation of Knox gene function. Plant J. **27**: 13–23.

**Murai, K.** (2013). Homeotic genes and the ABCDE model for floral organ formation in wheat. Plants **2**: 379–395.

**Mutasa-Göttgens, E. and Hedden, P.** (2009). Gibberellin as a factor in floral regulatory networks. J. Exp. Bot. **60**: 1979–1989.

**Nakagawa, M., Shimamoto, K., and Kyozuka, J.** (2002). Overexpression of RCN1 and RCN2, rice TERMINAL FLOWER 1/CENTRORADIALIS homologs, confers delay of phase transition and altered panicle morphology in rice. Plant J. **29***:* 743-750.

**Nakamichi, N.** (2015). Adaptation to the local environment by modifications of the photoperiod response in crops. Plant Cell Physiol. **56**: 594–604.

**Nakamura, Y., Andrés, F., Kanehara, K., Liu, Y., Dörmann, P., and Coupland, G.** (2014). Arabidopsis florigen FT binds to diurnally oscillating phospholipids that accelerate flowering. Nat. Commun. **5**: 4–8.

**Nan, H., Cao, D., Zhang, D., Li, Y., Lu, S., Tang, L., Yuan, X., Liu, B., and Kong, F.** (2014). GmFT2a and GmFT5a redundantly and differentially regulate flowering through interaction with and upregulation of the bZIP transcription factor GmFDL19 in soybean. PLoS One **9**.

**Navarro, C., Abelenda, J.A., Cruz-Oró, E., Cuéllar, C.A., Tamaki, S., Silva, J., Shimamoto, K., and Prat, S.** (2011). Control of flowering and storage organ formation in potato by FLOWERING LOCUS T. Nature **478**: 119–122.

**Niwa, M., Daimon, Y., Kurotani, K. -i., Higo, A., Pruneda-Paz, J.L., Breton, G., Mitsuda, N., Kay, S.A., Ohme-Takagi, M., Endo, M., and Araki, T.** (2013). BRANCHED1 interacts with FLOWERING LOCUS T to repress the floral transition of the axillary meristems in Arabidopsis. The Plant Cell **25**: 1228–1242.

**Oikawa, A., Nagai, K., Kato, K., and Kidou, S.** (2009). Gene silencing of barley P23k involved in secondary wall formation causes abnormal tiller formation and intercalary elongation. Breed. Sci. **59**: 664–670.

**Oikawa, T. and Kyozuka, J.** (2009). Two-step regulation of LAX PANICLE1 protein accumulation in axillary meristem formation in rice. The Plant Cell **21**: 1095–1108.

**Okagaki, R.J., Cho, S., Kruger, W.M., Xu, W.W., Heinen, S., and Muehlbauer, G.J.** (2013). The barley UNICULM2 gene resides in a centromeric region and may be associated with signaling and stress responses. Funct. Integr. Genomics **13**: 33–41.

**Pankin, A., Campoli, C., Dong, X., Kilian, B., Sharma, R., Himmelbach, A., Saini, R., Davis, S.J., Stein, N., Schneeberger, K., and von Korff, M.** (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.

**Park, S.J., Jiang, K., Tal, L., Yichie, Y., Gar, O., Zamir, D., Eshed, Y., and Lippman, Z.B.** (2014). Optimization of crop productivity in tomato using induced mutations in the florigen pathway. Nat. Genet. **46**: 1337–1342.

**Park, S.J., Lee, Y.K., Kang, M.S., and Bae, J.H.** (2016). Revisiting domestication to revitalize crop improvement: the florigen revolution. Plant Breed. Biotechnol. **4**: 387–397.

**Pelaz, S., Ditta, G.S., Baumann, E., Wisman, E., and Yanofsky, M.F.** (2000). B and C floral organ identity functions require SEPALLATA MADS-box genes. Nature **405**: 200–203.

**Pillitteri, L.J., Lovatt, C.J., and Walling, L.L.** (2004). Isolation and characterization of a TERMINAL FLOWER homolog and its correlation with juvenility in citrus. Plant Physiol. **135**: 1540–51.

**Pin, P.A., Benlloch, R., Bonnet, D., Wremerth-Weich, E., Kraft, T., Gielen, J.J.L., and Nilsson, O.** (2010). An antagonistic pair of FT homologs mediates the control of flowering time in sugar beet. Science **330**: 1397–400.

**Pnueli, L., Carmel-Goren, L., Hareven, D., Gutfinger, T., Alvarez, J., Ganal, M., Zamir, D., and Lifschitz, E.** (1998). The SELF-PRUNING gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of CEN and TFL1. Development **125**: 1979–1989.

**Pnueli, L., Gutfinger, T., Hareven, D., Ben-Naim, O., Ron, N., Adir, N., and Lifschitz, E.** (2001). Tomato SP-interacting proteins define a conserved signaling system that regulates shoot architecture and flowering. The Plant Cell **13**: 2687–2702.

**Pourkheirandish, M. and Komatsuda, T.** (2007). The importance of barley genetics and domestication in a global perspective. Ann. Bot. **100**: 999–1008.

**Ramsay, L. et al.** (2011). INTERMEDIUM-C, a modifier of lateral spikelet fertility in barley, is an ortholog of the maize domestication gene TEOSINTE BRANCHED 1. Nat. Genet. **43**: 169–172.

**Randoux, M., Davi??re, J.M., Jeauffre, J., Thouroude, T., Pierre, S., Toualbia, Y., Perrotte, J., Reynoird, J.P., Jammes, M.J., Hibrand-Saint Oyant, L., and Foucher, F.** (2014). RoKSN, a floral repressor, forms protein complexes with RoFD and RoFT to regulate vegetative and reproductive development in rose. New Phytol. **202**: 161–173.

**Ratcliffe, O.J., Amaya, I., Vincent, C.A., Rothstein, S., Carpenter, R., Coen, E.S., and Bradley, D.J.** (1998). A common mechanism controls the life cycle and architecture of plants. Development **125**: 1609–1615.

**Ratcliffe, O.J., Bradley, D.J., and Coen, E.S.** (1999). Separation of shoot and floral identity in Arabidopsis. Development **126**: 1109–1120.

**Repinski, S.L., Kwak, M., and Gepts, P.** (2012). The common bean growth habit gene PvTFL1y is a functional homolog of

Arabidopsis TFL1. Theor. Appl. Genet. **124**: 1539–1547.

**Rijpkema, A.S., Vandenbussche, M., Koes, R., Heijmans, K., and Gerats, T.** (2010). Variations on a theme: Changes in the floral ABCs in angiosperms. Semin. Cell Dev. Biol. **21**: 100–107.

**Roeder, A.H.K., Ferrandiz, C., and Yanofsky, M.F.** (2003). The role of the REPLUMLESS homeodomain protein in patterning the Arabidopsis fruit. Curr. Biol. **13**: 1630–1635.

**Rutjens, B., Bao, D., Van Eck-Stouten, E., Brand, M., Smeekens, S., and Proveniers, M.** (2009). Shoot apical meristem function in Arabidopsis requires the combined activities of three BEL1-like homeodomain proteins. Plant J. **58**: 641–654.

**Sasani, S., Hemming, M.N., Oliver, S.N., Greenup, A., Tavakkol-Afshari, R., Mahfoozi, S., Poustini, K., Sharifi, H.R., Dennis, E.S., Peacock, W.J., and Trevaskis, B.** (2009). The influence of vernalization and daylength on expression of flowering-time genes in the shoot apex and leaves of barley (Hordeum vulgare). J. Exp. Bot. **60**: 2169–2178.

**Satoh-Nagasawa, N., Nagasawa, N., Malcomber, S., Sakai, H., and Jackson, D.** (2006). A trehalose metabolic enzyme controls inflorescence architecture in maize. Nature **441**: 227–230.

**Schauer, S.E., Schlüter, P.M., Baskar, R., Gheyselinck, J., Bolaños, A., Curtis, M.D., and Grossniklaus, U.** (2009). Intronic regulatory elements determine the divergent expression patterns of agamous-like6 subfamily members in arabidopsis. Plant J. **59**: 987–1000.

**Schmitz, G. and Theres, K.** (2005). Shoot and inflorescence branching. Curr. Opin. Plant Biol. **8**: 506–511.

**Schmitz, J., Franzen, R., Ngyuen, T.H., Garcia-Maroto, F., Pozzi, C., Salamini, F., and Rohde, W.** (2000). Cloning, mapping and expression analysis of barley MADS-box genes. Plant Mol. Biol. **42**: 899–913.

**Schultz, E.A. and Haughn, G.W.** (1991). LEAFY, a homeotic gene that regulates inflorescence development in Arabidopsis. The Plant Cell **3**: 771–781.

**Shalit, A., Rozman, A., Goldshmidt, A., Alvarez, J.P., Bowman, J.L., Eshed, Y., and Lifschitz, E.** (2009). The flowering hormone florigen functions as a general systemic regulator of growth and termination. Proc. Natl. Acad. Sci

. **106**: 8392–8397.

**Shannon, S. and Ry Meeks-Wagner, O.** (1991). A mutation in the Arabidopsis TFL1 gene affects inflorescence meristem development. The Plant Cell **3**: 877–892.

**Simmons, S.R., Rasmusson, D.C., and Wiersma, J. V** (1982). Tillering in barley: genotype, row spacing, and seedling rate effects1. Crop Sci. **22**: 801–805.

**Simpson, G.G. and Dean, C.** (2002). Arabidopsis, the Rosetta stone of flowering time?. Science **296**: 285–289.

**Slafer, G.A. and Rawson, H.M.** (1994). Sensitivity of wheat phasic development to major environmental factors: a re-examination of some assumptions made by physiologists and modellers. Funct. Plant Biol. **21**: 393–426.

**Smaczniak, C., Immink, R.G.H., Angenent, G.C., and Kaufmann, K.** (2012). Developmental and evolutionary diversity of plant MADS-domain factors: insights from recent studies. Development **139**: 3081–3098.

**Smith, H.M. and Hake, S.** (2003). The interaction of two homeobox genes. BREVIPEDICELLUS PENNYWISE, Regul. internode patterning Arab. inflorescence. Plant Cell **15**: 1717–1727.

**Sohn, E.J., Rojas-Pierce, M., Pan, S., Carter, C., Serrano-Mislata, A., Madueño, F., Rojo, E., Surpin, M., and Raikhel, N. V** (2007). The shoot meristem identity gene TFL1 is involved in flower development and trafficking to the protein storage vacuole. Proc. Natl. Acad. Sci. **104**: 18801–18806.

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

**Tabuchi, H. et al.** (2011). LAX PANICLE2 of rice encodes a novel nuclear protein and regulates the formation of axillary meristems. The Plant Cell **23**: 3276–3287.

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

**Taoka, K. et al.** (2011). 14-3-3 proteins act as intracellular receptors for rice Hd3a florigen. Nature **476**: 332–335.

**Thorne, G.N.** (1962). Survival of tillers and distribution of dry matter between ear and shoot of barley varieties. Ann. Bot. **26**: 37–54.

**Tian, Z.X., Wang, X.B., Lee, R., Li, Y.H., Specht, J.E., Nelson, R.L., McClean, P.E., Qiu, L.J., and Ma, J.X.** (2010). Artificial selection for determinate growth habit in soybean. Proc. Natl. Acad. Sci. **107**: 8563–8568.

**Tong, H., Jin, Y., Liu, W., Li, F., Fang, J., Yin, Y., Qian, Q., Zhu, L., and Chu, C.** (2009). DWARF and LOW-TILLERING, a new member of the GRAS family, plays positive roles in brassinosteroid signaling in rice. Plant J. **58**: 803–816.

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

**Umehara, M., Hanada, A., Yoshida, S., Akiyama, K., Arite, T., Takeda-Kamiya, N., Magome, H., Kamiya, Y., Shirasu, K., Yoneyama, K., Kyozuka, J., and Yamaguchi, S.** (2008). Inhibition of shoot branching by new terpenoid plant hormones. Nature **455**: 195–200.

**Vann, L., Kono, T., Pyhäjärvi, T., Hufford, M.B., and Ross-Ibarra, J.** (2015). Natural variation in teosinte at the domestication locus *teosinte branched1* ( *tb1* ). PeerJ **3**: e900.

**Varkonyi-Gasic, E., Moss, S.M.A., Voogd, C., Wang, T., Putterill, J., and Hellens, R.P.** (2013). Homologs of FT, CEN and FD respond to developmental and environmental signals affecting growth and flowering in the perennial vine kiwifruit. New Phytol. **198**: 732–746.

**Vollbrecht, E., Springer, P.S., Goh, L., Buckler IV, E.S., and Martienssen, R.** (2005). Architecture of floral branch systems in maize and related grasses. Nature **436**: 1119–1126.

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

**Wang, L., Sun, S., Jin, J., Fu, D., Yang, X., Weng, X., Xu, C., Li, X., Xiao, J., and Zhang, Q.** (2015). Coordinated regulation of vegetative and reproductive branching in rice. Proc. Natl. Acad. Sci. **112**: 15504–15509.

**Ward, S.P. and Leyser, O.** (2004). Shoot branching. Curr. Opin. Plant Biol. **7**: 73–78.

**Weigel, D., Alvarez, J., Smyth, D.R., Yanofsky, M.F., and Meyerowitz, E.M.** (1992). LEAFY controls floral meristem identity in Arabidopsis. Cell **69**: 843–859.

**Weigel, D. and Nilsson, O.** (1995). A developmental switch sufficient for flower initiation in diverse plants. Nature **377**: 495–500.

**Whipple, C.J.** (2017). Grass inflorescence architecture and evolution: The origin of novel signaling centers. New Phytol. **216**: 367–372.

**Whipple, C.J., Zanis, M.J., Kellogg, E.A., and Schmidt, R.J.** (2007). Conservation of B class gene expression in the second whorl of a basal grass and outgroups links the origin of lodicules and petals. Proc. Natl. Acad. Sci. **104**: 1081–1086.

**Wickland, D.P. and Hanzawa, Y.** (2015). The FLOWERING LOCUS T/TERMINAL FLOWER 1 gene family: functional evolution and molecular mechanisms. Mol. Plant **8**: 983–997.

**Xu, C., Wang, Y., Yu, Y., Duan, J., Liao, Z., Xiong, G., Meng, X., Liu, G., Qian, Q., and Li, J.** (2012). Degradation of MONOCULM 1 by APC/CTAD1 regulates rice tillering. Nat. Commun. **3**: 750.

**Yamaguchi, A., Kobayashi, Y., Goto, K., Abe, M., and Araki, T.** (2005). TWIN SISTER of FT (TSF) acts as a floral pathway integrator redundantly with FT. Plant Cell Physiol. **46**: 1175–1189.

**Yan, L., Fu, D., Li, C., Blechl, A., Tranquilli, G., Bonafede, M., Sanchez, A., Valarik, M., Yasuda, S., and Dubcovsky, J.** (2006). The wheat and barley vernalization gene VRN3 is an orthologue of FT. Proc. Natl. Acad. Sci. **103**: 19581–19586.

**Yeung, K., Seitz, T., Li, S., Janosch, P., McFerran, B., Kaiser, C., Fee, F., Katsanakis, K.D., Rose, D.W., Mischak, H., Sedivy, J.M., and Kolch, W.** (1999). Suppression of Raf-1 kinase activity and MAP kinase signalling by RKIP. Nature **401**: 173–177.

**Yeung, K.C., Rose, D.W., Dhillon, a. S., Yaros, D., Gustafsson, M., Chatterjee, D., McFerran, B., Wyche, J., Kolch, W., and Sedivy, J.M.** (2001). Raf kinase inhibitor protein interacts with NFKB-inducing kinase and TAK1 and inhibits NF-K B activation. Mol. Cell. Biol. **21**: 7207–7217.

**Yi, G., Choi, J.-H., Jeong, E.-G., Chon, N.-S., Jena, K.K., Ku, Y.-C., Kim, D.-H., Eun, M.-Y., Jeon, J.-S., and Nam, M.-H.** (2005). Morphological and molecular characterization of a new frizzy panicle mutant, "fzp-9(t)", in rice (Oryza sativa L.). Hereditas **142**: 92–97.

**Yoo, S.C., Chen, C., Rojas, M., Daimon, Y., Ham, B.K., Araki, T., and Lucas, W.J.** (2013). Phloem long-distance delivery of FLOWERING LOCUS T (FT) to the apex. Plant J. **75**: 456–468.

**Yoo, S.Y., Kardailsky, I., Lee, J.S., Weigel, D., and Ahn, J.H.** (2004). Acceleration of flowering by overexpression of MFT (MOTHER OF FT AND TFL1). Mol. Cells **17**: 95–101.

**Yoshida, H. and Nagato, Y.** (2011). Flower development in rice. J. Exp. Bot. **62**: 4719–4730.

**Youssef, H.M. et al.** (2016). VRS2 regulates hormone-mediated inflorescence patterning in barley. Nat. Genet. **49**: 157–161.

**Zakhrabekova, S. et al.** (2012). Induced mutations in circadian clock regulator Mat-a facilitated short-season adaptation and range extension in cultivated barley. Proc. Natl. Acad. Sci. **109**: 4326–4331.

**Zhang, T. et al.** (2017). *LATERAL FLORET 1* induced the three-florets spikelet in rice. Proc. Natl. Acad. Sci. **114**: 9984-9989.

**Zhu, Q.-H., Hoque, M.S., Dennis, E.S., and Upadhyaya, N.M.** (2003). Ds tagging of BRANCHED FLORETLESS 1 (BFL1) that mediates the transition from spikelet to floret meristem in rice (Oryza sativa L). BMC Plant Biol. **3**: 6.

# V. Chapter 2 --- HvCEN has pleiotropic effects on shoot and spike architecture in barley

## Introduction

Plant architecture determines grain yield and is therefore a primary target of artificial selection of crop domestication and improvement. Plant architecture is largely determined by branching (tillering) patterns, plant height, leaf shape and arrangement, and inflorescence morphology. These traits are controlled by the combined activity of the SAM and AXMs (Teichmann and Muhr, 2015). A vegetative SAM gives rise to leaves and AXMs that form in the leaf axils (Turnbull, 2005). As plants shifts from vegetative to reproductive growth, the SAM undergoes a transition to the reproductive state and forms an inflorescence, flowers and eventually seeds. The compound inflorescence of grasses forms spikelets that are themselves a small inflorescence capable of producing multiple flowers or "florets" (Whipple, 2017). Grasses have a striking diversity in their inflorescence architectures that is determined by meristem determinacy/indeterminacy decisions, the acquisition of spikelet meristem identity and the determinacy of the spikelet meristem. In the model eudicot plant Arabidopsis thaliana, determinacy of the inflorescence meristems is controlled by the ratio of two gene products, TERMINAL FLOWER 1 and LEAFY, which repress and promote determinacy and floral development, respectively. Mutant collections in rice and maize have provided key insights into the molecular mechanisms regulating inflorescence meristem determinacy and spikelet identity (for review, see Bommert and Whipple, 2017). In rice, spikelet identity is promoted by FRIZZY PANICLE which contains an APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF) domain, by PANICLE PHYTOMER2/OSMADS34 (PAP2) a SEPALLATA-like MADS-box transcription factor and APETALA1/FRUITFULL (AP1/FUL)- like genes (Wu et al., 2017). Spikelet identify is regulated by ABERRANT PANICLE ORGANISATION 1 an F-box protein orthologous to UNUSUAL FLORAL ORGANS in Arabidopsis (Ikeda et al., 2007), *APO2* which encodes the rice LFY ortholog (Ikeda-Kawakatsu et al., 2012), a nuclear protein called TAWAWA1 (TAW1) that regulate transcription of the *SHORT VEGETATIVE PHASE*(*SVP*)-like MADS-box genes (Liu et al., 2013; Yoshida et al., 2013) and TERMINAL FLOWER 1/CENTRORADIALIS (Nakagawa et al., 2002). Spikelet meristem determination is likely controlled by a balance of factors that either promote or inhibit its identity (Bommert and Whipple, 2017).

The spikes of temperate cereals like barley and wheat display a raceme-like branchless shape and consists of spikelets produced on two opposite sides along the main axis (rachis). Individual spikelets contain one floret in barley or several florets in wheat, each producing one grain. Recent studies have shown that genetic factors controlling the timing of pre-anthesis development affect inflorescence

architecture in barley and wheat. In barley, the flowering time regulators PHOTOPERIOD1 (PPD-H1) and its downstream target HvFT1, homolog of Arabidopsis FT, repress the number of spikelets produced on the main inflorescence by affecting the rate and duration of spikelet initiation (Digel et al., 2015). Similarly, in wheat, PHOTOPERIOD-1 (PPD-1) was identified as a regulator of paired spikelet formation (Boden et al., 2015). Mutations in the *COMPOSITE SPIKE* gene, encoding an AP2/ERF transcription factor, affected spikelet determinacy in wheat as spikelet meristems reverted to inflorescence like meristems which formed branch-like structures (Poursarebani et al., 2015). Despite these recent advances on deciphering the molecular mechanisms underlying spikelet variations, little is known about molecular factors controlling determinacy decisions and therefore spikelet number and yield in barley or wheat. *FT*-like genes seem to have a major effect on inflorescence and spikelet meristem activity in barley and wheat. *FT*-like genes belong to phosphatidylethanolamine-binding proteins, whose homolog in human was characterized as a *Raf* kinase inhibitor protein (RKIP), mediating the RAF/MEK/ERK signal transduction pathway (Bradley et al., 1996, 1997; Sedivy et al., 1999; Ohshima et al., 1997). In plants, the *PEBP* family comprises proteins with antagonistic effects on reproductive development as they either promote or inhibit floral development. In Arabidopsis and rice, *FT* is expressed in the leaf vascular tissue and the FT protein is transported through the phloem to the SAM (Corbesier et al., 2007; Tamaki et al., 2007). The rice FT protein, HD3A then interacts with 14-3-3 proteins in the cytoplasm, and the resulting protein complex binds to OsFD1 in the nucleus to activate the expression of meristem identity genes (Taoka et al., 2011). TFL1 acts as a hypothetical competitor of FT in binding to FD and 14-3-3 proteins in the shoot apex to prevent the induction of flowering through transcriptional activation of floral identity genes by FT (Ahn et al., 2006; Hanano and Goto, 2011; McGarry and Ayre, 2012; Wigge et al., 2005; Abe, 2005). *TFL1* mRNA was detected at the center of the SAM, whereas its protein spreads into the whole meristem (Conti and Bradley, 2007; Wigge et al., 2005). An acceleration in flowering and the generation of a terminal flower were observed in both *tfl1* mutant and plants overexpressing *FT* (Bradley et al., 1997; Kardailsky, 1999; Kobayashi, 1999).

The function of TFL1 homologs in controlling of flowering time in inflorescence architecture are conserved between grasses and dicots, to some extent. The TFL1 homolog in Antirrhinum known as CENTRORADIALIS controls the determinacy of the inflorescence without affecting flowering time (Bradley et al., 1996). Similar to Arabidopsis, mutations in *SELF-PRUNING*, a homolog of *TFL1* in tomato (*Solanum lycopersicum*), caused an acceleration of flowering and a determinant and smaller inflorescence with reduced fruit production (Pnueli et al., 1998; Jiang et al., 2013). In pea (*Pisum sativum*), PsTFL1c/LATE FLOWERING (LF) delays flowering, whereas PsTFL1a/DETERMINATE (DET) maintains the indeterminate inflorescence meristem (Foucher et al., 2003). In ryegrass (*Lolium perenne*), LpTFL1 represses flowering and controls axillary meristem identity (Jensen et al., 2001).

RCN1 and RCN2*,* rice homologs of TFL1, delay flowering and change the panicle architecture (Nakagawa et al., 2002). Likewise, ectopic expression of the *TFL1* homologs *ZCN2*, *ZCN4*, *ZCN5* in maize lead to delayed flowering and a bushy tassel with denser spikelets (Danilevskaya et al., 2010).

Barley has six different FT-like homologs, of which only HvFT1 and HvFT3 were functionally characterized (Halliwell et al., 2016; Casao et al., 2011b; Yan et al., 2006; Schmitz et al., 2000; Hemming et al., 2008; Sasani et al., 2009; Chen and Dubcovsky, 2012). High expression levels of *HvFT1* in the leaf are correlated with a strong acceleration of floral development, while *HvFT3* only induces the initiation of spikelet primordia but does not affect later floral development (Digel et al., 2015, Mulki et al., subm.). HvCEN, the barley homolog of Antirrhinum CEN and Arabidopsis TFL1, was identified as a contributor to successful environmental adaptation in a large germplasm screen (Comadran et al., 2012). A natural Pro135Ala substitution in the HvCEN protein is enriched in spring barley genotypes and delays flowering time (Comadran et al., 2012). The authors could also show that induced mutants for *HvCEN* flowered a few days earlier under natural long-day conditions. However, a thorough functional characterization of the HvCEN has not yet been conducted. I therefore analyzed a large collection of *hvcen* mutant lines to: 1) identify pleiotropic effects of HvCEN on shoot and spike morphology, including vegetative and reproductive traits and the timing of different developmental stages of the shoot apical meristem, inflorescence and spikelet meristems; 2) decipher transcriptional targets of HvCEN in the developing shoot apical meristem under different photoperiods and 3) investigate the genetic interactions between *HvCEN* and the *FT*-like genes *HvFT1* and *HvFT3*.

In this study, I demonstrate that HvCEN has pleiotropic effects on several shoot traits, as it delays reproductive development and flowering, promotes axillary bud initiation/tillering, and increases the number of spikelet primordia and plant height. Mutations in *HvCEN* shortened the vegetative phase under both LDs and SDs but accelerated floret development only under LDs. These photoperiod specific effects of *HvCEN* might be controlled by the interaction with different HvFT proteins during development. During early reproductive development HvCEN interacted with HvFT3 which is expressed under both photoperiods. During later floral development HvCEN interacted with HvFT1 which is only expressed under LDs. Global transcriptome analysis in developing shoot apical meristems showed that *HvCEN* affected the expression of genes involved in primary metabolic processes, chromatin modification, ribosome biogenesis, hormone signaling, and stress response under both photoperiods, while it controlled floral homeotic genes mostly under LDs.

# Results

## 1. HvCEN controls shoot and spike architecture in barley

To characterize the effect of different *HvCEN* mutations on shoot development, 23 allelic *hvcen* mutants in six different spring barley backgrounds, including *mat-c.16*, *mat-c.19*, *mat-c.32*, *mat-c.770*, *mat-c.907*, *mat-c.913*, *mat-c.93*, *mat-c.94*, *mat-c.943* in the background of Bonus, *BW507* and *BW508* in the background of Bowman, *mat-c.400* in Foma, *mat-c.1096*, *mat-c.1107*, *mat-c.1108*, *mat-c.1109*, *mat-c.1111*, *mat-c.1114*, mat-*c.1115* in Frida, *mat-c.745* in Kristina, and *mat-c.1102*, *mat-c.1118* and *mat-c.1120* in Semira, and the parental lines were grown under outdoor conditions over two consecutive years and flowering time, grains per spike, plant height, tiller number and different seed parameters were measured. All the *hvcen* mutants flowered significantly earlier and produced fewer grains per main spike than the respective wild type genotypes (Figure 1A, C). In addition, all *hvcen* mutants, except for *mat-c.19* and *mat-c.913*, produced fewer tillers at flowering time compared to the corresponding parental genotypes (Figure 1B). Likewise, culms of most *hvcen* mutants, excluding *mat-c.19*, *mat-c.32* and *mat-c.93*, were shorter compared to the respective wild type genotypes (Figure 1D). Although these effects of HvCEN were consistent across all backgrounds, minor differences between *hvcen* mutants in the same background were observed. For example, *mat-c.19* and *mat-c.913*, both in cv. Bonus were, in contrast to the other *mat-c* mutants not affected in their tiller number. Similarly, *mat-c.19, mat-c.913* and *mat-c.32* all in cv. Bonus and all mutants in cv. Frida background were not affected in plant height. I observed no consistent differences in seed parameters, including seeds length, width, area and thousand kernel weight (TKW) in the mutants versus wild type (Supplemental Figure 1).

As I observed some mutation specific effects, I tested for a correlation between the type of mutation and the severity of the phenotype by calculating the degree of amino acid conservation across taxa for individual mutations. However, no correlation was observed between the nature of the mutations and the severity of the phenotype (Supplemental table 1, 2). Among the eight *hvcen* mutants in the Bonus background *mat-c.16*, *mat-c.19* are putative deletion mutants while mutations in *mat-c.770* and *mat-c.94* by introduced stop codons resulting in truncated proteins. I did also not observe a clear effect difference between amino acid mutations and deletions of the whole protein in the phenotypic comparison.

## 2. Mutations in *HvCEN* reduce the number of axillary meristems and spikelet primordia

To determine how HvCEN and photoperiod function together to influence the duration of different pre-anthesis phases, I scored the development of the main shoot apices (MSA) in three selected *hvcen* mutants (*mat-c.907, mat-c.94, and mat-c.943*) and Bonus under LDs and SDs (Figure 3A, B). Overall, *hvcen* mutants initiated spikelet primordia (W2.0) on average 4.2 days earlier under LDs and 5.9 days earlier under SDs compared to their wildtype counterparts, respectively. However, *hvcen* mutants exhibited photoperiod specific patterns of growth and inflorescence development. Particularly, under LDs stem elongation and inflorescence development were greatly accelerated in *hvcen* mutants starting from the stamen primordium stage (W3.5) compared with the wildtype (Bonus) (Figure 3A). The *hvcen* mutant plants flowered (W9.0-10.0) 18 days earlier than Bonus under LDs (Figure 3A). However, under SDs inflorescence development did not proceed beyond stage when stylar canal closing (W5.5) in the mutants and carpel primordium stage (W4.5) in Bonus (data not shown). In addition, *hvcen* mutants produced a lower number of spikelet primordia (Figure 3C, D). Specifically, under LDs, the number of spikelet primordia increased until W4.0 in the mutants with a maximum of on average 25 spikelet primordia while in wild type plants spikelets developed until W5.0 and produced a maximum of approximately 40 spikelets (Figure 3C). However, under SDs, the mutants produced fewer spikelet primordia only before the lemma primordia stage (W3.0) compared to the wild type. After this stage no differences in spikelet numbers were observed between the mutants and wildtype. Furthermore, *hvcen* mutants produced fewer axillary buds under both LDs and SDs, but a clear difference was observed earlier under SDs (after spikelet initiation, W2.0) than LDs (starting from awn primordia stage, W5.0) (Figure 3E, F). Correlated to accelerated development of MSA and reduced axillary buds, the mutants produced fewer visible leaves after spikelet initiation (W2.0) and eventually produced fewer leaves in total on the main shoot (Figure 4F, E). Furthermore, the mutants produced shorter leaves under LDs and SDs (Figure 4A, B). Specifically, the longest leaf of the mutants was clearly shorter under LDs and SDs. The effects of the *hvcen* mutations on leaf width was less pronounced than on leaf length (Figure 4C, D). No significant differences were observed in the mutants compared to wildtype except for *mat-c.943* with wider leaves. Because the number of tillers and the number and size of leaves on the main culm were reduced the total plant biomass was lower in the mutants compared to wild type.

Taken together, mutations in the three *hvcen (mat-c)* mutants caused an advanced initiation of spikelet primordia under LDs and SDs, while floret development was only accelerated under LDs. Under SDs, genetic effects on number of spikelet primordia were observed only during the spikelet initiation phase, but under LDs genotypes differed during spikelet and floret initiation and development. In addition,

HvCEN affected the total leaf area per plants by reducing the number of tillers and leaves on the main culm and leaf size.

## 3. HvCEN interacts with HvFT1 and HvFT3 to control reproductive development under LDs and SDs

In Arabidopsis, the HvCEN homolog TFL1 acts antagonistically to FT in the meristem and the relative abundance of FT and TFL1 proteins controls floral development and shoot architecture in Arabidopsis and other crop plants (McGarry and Ayre, 2012). Thus, I assumed that the effects of HvCEN on development are likely dependent on *FT*-like genes in barley. Barley has several *FT*-like genes (Halliwell et al., 2016), however, only *HvFT1* and *HvFT3* have been functionally characterized and integrated into flowering pathways. *HvFT1* is only transcribed under LDs and its protein promotes spikelet initiation and inflorescence/floret development (Yan et al., 2006, Digel et al. 2015). In contrast, *HvFT3* is expressed under SDs and LDs and specifically accelerates the timing of spikelet initiation but has no effects on inflorescence/floret development (Mulki et al. subm.). To test whether the effects of HvCEN on spikelet initiation are dependent on HvFT3, I scored development of single and double *hvcen hvft3* mutants under SDs. I observed that only in the presence of a functional *HvFT3* the *hvcen* allele advanced spikelet initiation, and decreased the number of spikelet primordia before floret differentiation (W3.0-W3.5) and axillary buds (Figure 5D-F), while the double *hvcen hvft3* mutant did not differ in development, the number of spikelet primordia and axillary buds from the *HvCEN hvft3* line (Figure 5A-C). Therefore, I inferred that the photoperiod independent effects of HvCEN are dependent on HvFT3.

Next, I tested if the LD specific effects could be explained by the LD specific expression of HvFT1 or the absence of HvFT1 under SDs. For this purpose, I crossed the *hvcen* mutant with a mutant line carrying a non-functional HvELF3 protein. ELF3 is a circadian clock gene that modulates light signal transduction downstream of phytochromes and mediates the circadian gating of light perception and responses (Hicks et al., 1996; Zagotta et al., 1996; Liu et al., 2001). *hvelf3* mutant plants are characterize by photoperiod independent early flowering and HvFT1 expression under LDs and SDs (Faure et al. 2012). I confirmed that expression levels of HvFT1 were comparable between LDs and SDs in the *hvelf3* mutant, while *HvFT1* was only expressed under LDs in the wild type (Figure 7). MSA development was examined in double and single *hvelf3 hvcen* mutants under SD condition. The results suggested that the timing of spikelet initiation was also controlled by the interaction of hvelf3 or HvFT1 with HvCEN since hvcen initiate spikelet primordia earlier than HvCEN with approximately three days and six days in background of *hvelf3* and *HvELF3*, respectively (Figure 6A, D). The *hvelf3* plants developed significantly faster than plants with the wild type *HvELF3* allele irrespective of the *HvCEN* allele. More

interestingly, variation at HvCEN affected floral development under SDs in the background of the *hvelf3* mutant allele. This suggested that under conditions where HvFT1 is expressed, either under LDs or in the clock mutant background, HvCEN interacted with HvFT1 to modulate floral development. The mutation in *hvcen* reduced the number of spikelets per MSA and axillary buds in the background of *hvelf3* as it did in the *HvELF3* background under LDs (Figure 6B, 6C, 6E, 6F). This genotype and photoperiod specific expression patterns suggested that the photoperiod dependency of *hvcen* on inflorescence/floret development and spikelet number were likely regulated via *HvFT1*. However, mutations in the clock gene *HvELF3* modify the expression of large number of genes, and I cannot rule out that the *hvelf3* specific effect of *hvcen* might be caused by genes other than HvFT1.

## 4. Global transcriptome profiling in developing shoot apical meristems revealed differentially expressed transcripts in two allelic *hvcen* mutants

To further understand how HvCEN regulates the development of MSA in a photoperiod-dependent manner, I performed genome-wide transcriptome profiling in developing MSA in two allelic *hvcen* mutants (*mat-c.907* and *mat-c.943*) and the wild type (cv. Bonus). I focused on three development stages during which genotypes exhibited phenotypic differences under LDs and SDs (Figure 3, 4F), including the vegetative stage (W1.0), the spikelet initiation stage (W2.0) and the stamen primordium stage (W3.5). This approach allowed me to identify photoperiod dependent and independent targets of HvCEN. Transcriptome analysis revealed the expression of 24703 and 25037 transcripts, 62.2% and 63.0% of the total number of annotated transcripts in barley (Mascher et al., 2017), at levels greater than 5 counts in at least 2 libraries under LDs and SDs, respectively. Principle component analysis on all expressed genes clustered the samples according to the developmental stage under LDs and SDs and separated wild type and mutant samples at all stages under SDs, but not LDs (Supplemental figure 2). To determine differentially expressed transcripts (DETs), I performed individual pairwise comparisons between each mutant (two allelic mutants) and wildtype background Bonus at each developmental stage (three stages) within each photoperiod treatment (LDs and SDs), yielding 12 total sets of DETs. A large number of DETs were identified at the spikelet initiation stage (W2.0), with 3177 DETs in *mat-c.907*, 2849 DETs in *mat-c.943* under LDs and 5200 DETs in *mat-c.907*, 5585 DETs in *mat-c.943* under SDs. Much fewer DETs were obtained at W1.0 and W3.5. At W1.0, 50 DETs in *mat-c.907*, 419 DETs in *mat-c.943* under LDs and 14 DETs in *mat-c.907*, 39 DETs in *mat-c.943* under SDs were found. At the stamen primordia stage (W3.5), 503 DETs in *mat-c.907* and 477 DETs in *mat-c.943* under LDs and 281 DETs in *mat-c.907*, 489 DETs in *mat-c.943* under SDs were identified (Figure 8A, B). The higher number of DETs at W2.0 is correlated to the higher expression of *HvCEN* at W2.0 compared to the other two stages (Figure 11A). By comparing DETs found in the two allelic mutants, I found that a large proportion of DETs exhibited mutant specific expression profiles. Under LDs, 17 (34%), 1251

(39.4%), 274 (54.5%) DETs were *mat-c.907* specific and 386 (92.1%), 950 (33%), 248 (52.0%) DETs were *mat-c.943* specific at W1.0, W2.0 and W3.5, respectively. Under SDs, 7 (50%), 890 (17.1%), 164 (58.4%) DETs were *mat-c.907* specific and 32 (82.1%), 1275 (22.8%) and 372 (76.1%) DETs were *mat-c.943* specific at W1.0, W2.0 and W3.5, respectively (Figure 8A, B).

Variant calling in the two focal mutants compared to the wild type Bonus, revealed many transcripts carrying mutations (Table 1). In *mat-c.907* and *mat-c.943*, 12 and 78 transcripts contain mutations compared to Bonus. Among them, only one transcript (HvCEN) carried mutations at different positions in the two mutants. Each mutant contains mutant specific mutations that are likely the reason for the mutant specific DETs. Therefore, to minimize noise likely caused by other background mutations, I focused in the further analyses on transcripts that were differentially regulated in both *hvcen* allelic mutants, with 33, 1926, 229 DETs under LDs and 7, 4310 and 117 DETs under SDs at the three stages (Figure 8A, B). In total, I found 5308 DETs that were observed in both mutants in at least one developmental stage and photoperiod condition. Hierarchical cluster analysis demonstrated that all samples at W1.0 and W3.0 grouped together all three genotypes and separated according to photoperiod. In contrast, for samples at W2.0 mutants and wild type fell in separate clusters (Figure 9A). This result is consistent with the principle component analysis (PCA) on those DETs (Figure 9B). The first principle component separates the developmental stages, while the second principle component explains the difference between LDs and SDs.

The 5308 DETs could be grouped in four major clusters according to their expression pattern (Figure 9A). Cluster one comprised all DETs that were upregulated over development and downregulated in the mutants compared to wild type at W2.0 under both photoperiods. Cluster two contained DETs downregulated in the mutants at W1.0, W2.0 and W3.5 under LDs and at the W2.0 and W3.5 under SDs compared to the wild type. Cluster three comprised all genes that were downregulated over development, showed a higher expression under SDs compared to LDs and were specifically upregulated at W2.0 in the mutants compared to wild type under LDs and SDs. Cluster four contained all DETs that were downregulated between W1.0 and W2.0 in the wildtype, but not the mutant genotypes, and showed a higher expression in LDs compared to SDs at W3.5 in all three genotypes (Figure 9C). Taken together, HvCEN had the strongest effect on gene expression at spikelet initiation, specifically under SDs. In addition, the majority of transcripts showed a photoperiod and stage specific regulation in the mutant versus the wild type plants.

In order to explain the photoperiod dependent phenotype (Figure 2), I compared DETs found under LD and those under SDs. For further analysis, I focused on transcripts that were only regulated either in LDs or SDs in both mutants (Figure 8C, Supplemental figure 3A). At W1.0, 28 LD-specific (7 upregulated, 21 downregulated), 4 SD-specific (2 upregulated, 2 downregulated) and 2 photoperiod independent

DETs (1 upregulated, 1 down regulated) were found. At the spikelet initiation stage (W2.0), 550 LD-specific (217 upregulated, 333 downregulated), 2545 SD-specific (1542 upregulated, 1002 downregulated and 1 with different expression pattern between mutants), and 1124 photoperiod independent DETs (675 upregulated, 446 downregulated, 3 with different expression pattern between mutants) were identified. Furthermore, at the stamen primordia stage (W3.5), 179 LD- specific, 83 SD-specific and 22 photoperiod independent DETs were observed. Only a very small number of transcripts were commonly regulated across all stages, namely five under LDs and two under SDs (Supplemental Figure 3B). This suggested that the effects of *hvcen* on the global MSA transcriptome were stage specific. In addition, the majority of DETs were photoperiod specific, at W2.0 approximately 26.6% of the DETs, while at W3.5 92% of the DETs were photoperiod specific (Figure 8C).

In the following, I will present and discuss transcripts that were regulated at W2.0 under LDs and SDs as spikelet initiation was accelerated in the mutants under both photoperiod. In addition, we will analyze photoperiod dependent transcripts at the stamen primordium stage (W3.5) which correlated with the photoperiod dependent effect of HvCEN on inflorescence development.

## 4.1. Transcript regulated at the spikelet initiation stage correlate with an early transition to reproductive growth in the *hvcen* mutants

Since, spikelet initiation was accelerated in the mutants compared to wild type under both photoperiods, I first focused my further analysis on the 1124 photoperiod independent DETs at spikelet initiation. Transcripts regulated at W2.0 display very similar expression patterns over development in mutant and wild type plants. For a large number of transcripts, we observed a clear up or downregulation between the vegetative and the spikelet initiation stage in the wild type and no or little regulation in the mutants, suggesting that HvCEN controlled the W2.0 specific regulation of a large number of transcripts. However, as the tissue collected at the different stages was not identical, crown tissue (removed visible axillary buds) at W1.0, shoot apical meristem with leaf primordia at W2.0 and inflorescence tissue at W3.5, we conducted a comparison of genotypes at the each developmental stage separately. Gene ontology enrichment analysis suggested that HvCEN, as a transcription co-regulator, affected metabolic and biosynthetic processes and gene expression. The 1124 DETs were mainly enriched for transcripts involved in primary and protein metabolic and biosynthetic processes, in gene expression and ribosome biogenesis, response to cytokinin, cell proliferation and had molecular functions in nucleic acid binding, protein binding, and as structural constituent of ribosome at intracellular organelles, cytosol and ribosomes (Table 2).

### 4.1.1. DETs with roles in chromatin modification

Among the 1124 DETs at W2.0, I identified genes with roles in chromatin modification and consequent regulation of development and organ initiation (selected genes in Table 3). I observed, for instance, the upregulation of two homologs of Arabidopsis MULTICOPY SUPRESSOR OF IRA1 (AtMSI1, *HORVU5Hr1G084160* (Figure 10A), *HORVU5Hr1G093230*) which promote floral transition in Arabidopsis by inducing the expression of *CONSTANS (CO)* and *SOC1* (Bouveret et al., 2006; Steinbach and Hennig, 2014). In addition, the expression of homologs of *PROTEIN ARGININE METHYLTRANSFERASES* (*AtPRMT5*, *HORVU6Hr1G019540*, and *AtPRMT10*, *HORVU7Hr1G020620*) was increased in mutants compared to wild type. In Arabidopsis, *PRMT* genes promote flowering by mediating the epigenetic silencing of *FLOWERING LOCUS C* (*AtFLC*) and by affecting pre-mRNA splicing (Bouveret et al., 2006, Schmitz et al. 2008, Deng et al. 2010). A putative target of epigenetic factors and repressor of flowering, a homolog of *FLC*, *HvODDSOC2* (*HORVU3Hr1G095240*), was downregulated in the mutants. I found two transcripts (*HORVU1Hr1G071960*, *HORVU7Hr1G012850*), encoding histone proteins H2A and H1-3, that were upregulated in the mutants. A putative histone methyltransferase (*HORVU6Hr1G011950*), a homolog of *ARABIDOPSIS TRITHORAX-RELATED PROTEIN 6 (ATXR6)* was found upregulated in the mutants. Three putative S-adenosylmethionine synthase genes (*HORVU6Hr1G063490*, *HORVU6Hr1G063540*, *HORVU6Hr1G063570*), homologs of Arabidopsis *S-ADENOSYLMETHIONINE SYNTHETASE 3 (AtSAMS3)*, were transcriptionally upregulated in the mutants. In rice, these cause methylation alterations of DNAs and histones and lead to late flowering (Li et al. 2011). Taken together, HvCEN controlled the expression of genes with putative functions in chromatin structure and chromatin accessibility.

### 4.1.2. DETs with roles in ribosome biogenesis

Among the upregulated genes were also 87 ribosomal proteins (selected genes in Table 3), including ribosomal proteins with functions in leaf development, vascular patterning and adaxial cell fate (Table 3), such as two homologs of PIGGYBACK 2 (PGY2, *HORVU0Hr1G006020* (Figure 10B), *HORVU3Hr1G001140*), a homolog of PGY3 (*HORVU5Hr1G092630*) and a RIBOSOMAL PROTEIN S13 (RPS13, *HORVU2Hr1G037940*). In addition, ribosomal proteins with functions in gametogenesis and apical-basal gynoecium patterning and cell division, as for example, barley homologs of NUCLEAR FUSION DEFECTIVE (NFD1, *HORVU1Hr1G085550*), BREAST BASIC CONSERVED 1 (BBC1, *HORVU7Hr1G067060*) and RPS6 (*HORVU2Hr1G029890*) were differentially regulated. Further, a nucleolar GTPase *NUCLEOSTEMIN-LIKE 1 (AtNSN1)* like transcript (*HORVU2Hr1G016650*, Figure 10A), which plays a role in the maintenance of inflorescence meristem identity and floral organ development by modulating ribosome biogenesis in Arabidopsis (Wang et al., 2012; Jeon et al., 2015), was upregulated in the mutants. In addition, homologs of genes involved in cell division were upregulated

in the mutants, including, for example, homologs of *CELL DIVISION CONTROL 6* (*CDC6*, *HORVU3Hr1G084800*, Figure 10B), *PROLIFERATING CELL NUCLEAR ANTIGEN 2* (*PCNA2*, *HORVU6Hr1G088120 HORVU0Hr1G031140*) and *REPLICON PROTEIN A2* (*HORVU6Hr1G094080*). We also observed the upregulation of chaperone homologs with potential roles in proliferating tissues, such as a Chaperone htpG family protein (*HORVU7Hr1G117000*) or *GAMETOPHYTIC FACTOR 2* (*GFA2*, *HORVU7Hr1G009230*, Figure 10B) involved in regulation of meristem size and organization.

### 4.1.3. DETs related to hormone signaling

Furthermore, we found DETs with putative roles in various hormone signaling pathways, suggesting that HvCEN regulates flowering also through changing hormone levels and responses (selected genes in Table 3). For example, I observed the upregulation of a barley 26S proteasome non-ATPase regulatory subunit 8 homolog A (*RPN12a*, *HORVU4Hr1G002140*, Figure 10C), which regulates cytokinin responses by upregulating type A *ARRs*, negative regulators of cytokinin signaling (Ryu et al., 2009). In addition, barley homologs of type *A and B RESPONSE REGULATORS* (*ARR2*, *HORVU5Hr1G097560.5*; *ARR3*, *HORVU2Hr1G077000*, *HORVU3Hr1G108540*; *ARR6*, *HORVU2Hr1G120490*, Figure 10C) that act as regulators in the two-component cytokinin signaling pathway were upregulated. In contrast, barley homologs of histidine kinases and putative cytokinin receptors, *HISTIDINE KINASE 3* and *HISTIDINE KINASE 4* (*AHK3*, *HORVU3Hr1G094870*, Figure 10C; *AHK4*, *HORVU6Hr1G077070*) and a cytokinin riboside 5'-monophosphate phosphoribohydrolase *LONELY GUY 3 (LOG3, HORVU4Hr1G005660)* were downregulated in the mutants compared to wild type plants indicating an altered cytokinin response in the mutants. In addition to cytokinin, DETs involved in the synthesis and response to other hormones were affected, such as auxin, abscisic acid (ABA) and gibberellin (Table 3). For instance, a chloroplast localized subunit of casein kinase 4 (*HORVU5Hr1G097400*) was upregulated, which acts as a subunit in ABA mediated suppression of flowering time and lateral root development and seed germination. The knockout mutant delayed flowering, suggesting that the gene acts as a flowering promotor (Wang et al., 2016). In addition, auxin response genes (*HORVU6Hr1G091260*) and auxin efflux carries (*HORVU6Hr1G094970*) were downregulated, which indicated that auxin transport and auxin distribution rather than auxin biosynthesis were altered.

### 4.1.4. DETs involved in primary metabolic process/cellular respiration

Many barley homologs of genes involved in cellular respiration, including glycolysis, pyruvate metabolism and citrate cycle showed differential expression between mutant and wild type plants (selected genes in Table 3). For example, transcripts with roles in glycolysis and carbohydrate metabolism were upregulated in the mutant compared to wild type, i.e. *CELL WALL INVERTASE* 2 (*HORVU2Hr1G073210*, Figure 10D), a Raffinose synthase family protein (*HORVU7Hr1G027930*), and a

TREHALOSE-6-PHOSPHATASE (*TPS1*, *HORVU1Hr1G013450*). Further proteins with roles in glycolysis, such as a Pyruvate kinase family protein (*HORVU3Hr1G039200*), three homologs of fructose-bisphosphate aldolase 2 (*HORVU3Hr1G002780*, Figure 10D), *HORVU3Hr1G088540*, *HORVU3Hr1G088570*) and three homologs of ATP-dependent 6-phosphofructokinases (*PFK2*, *HORVU2Hr1G081670*; *PFK3*, *HORVU3Hr1G070300*; *PFK7*, *HORVU6Hr1G070270*, Figure 10D) were upregulated in the mutant versus wild type plants. The mutants were characterized by a faster apical growth and/or a reduction of source tissue in the mutants as the mutant plants were characterized by smaller and fewer leaves than wild type plants. The upregulation of genes involved in cellular respiration might be a consequence of changes in the source sink balance and a stronger energy demand in the fast developing MSA of the mutant plants compared to the wild type.

## 4.2. The majority of transcripts regulated at the stamen primordium stage are photoperiod specific

Spikelet initiation was advanced in the mutants under both LDs and SDs, but inflorescences did only develop and set seeds under LDs, while the MSAs were aborted under SDs. Under LDs, inflorescence development was faster in the mutants than wild type. I, therefore, focused my further analysis on the photoperiod dependent DETs at the stamen primordium phase, which included 179 DETs specific under LDs and 84 DETs specific under SDs.

### 4.2.1. HvCEN controls floral homeotic genes under LDs

Among the 179 LD specific DETs at W3.5, 50 DETs were upregulated and 129 DETs downregulated in the mutants compared to wild type. Among the transcripts that were upregulated to a higher extent in the mutants compared to wild type were transcription factors that act in a combinatorial manner to achieve floral patterning in Arabidopsis (Coen and Meyerowitz, 1991). These are designated as class A, B, C and E genes and, except for the class A gene *AP2*, encode members of the MIKC type of MADS-box transcription factors. The mutations in *HvCEN* caused an upregulation of five *SEP*-like genes (*SEP1*, *HORVU7Hr1G025700*, Figure 11E; *HORVU5Hr1G095710,* Figure 12, FDR<0.05; *SEP2*, *HORVU4Hr1G067680* Figure 11A; *SEP3, HORVU7Hr1G054220* Figure 12, FDR<0.05; *HORVU5Hr1G076400*, Figure 12, FDR<0.05) at the stamen primordium stage. In addition, a *PISTILLATA (PI)-like (HORVU1Hr1G063620,* Figure 12, FDR<0.05*), an AP1*-like gene (*HvBM8, HORVU2Hr1G063800,* Figure 12, FDR<0.05) and an *AP2*-like gene (*HORVU2Hr1G113880,* FDR<0.05*)* were upregulated in a LD-dependent manner. An *APO1*-like transcript (*HORVU7Hr1G108970*) was downregulated in the mutants compared to wild type. Interestingly, APO1 protein positively controls spikelet number by suppressing the precocious conversion of inflorescence meristems to spikelet meristems in rice (Ikeda et al. 2007). Additional DETs with roles in in inflorescence and flower development included barley

homologs of *SOC1* (*HORVU1Hr1G051660*), *AGL6* (*HORVU6Hr1G066140*), *FLOWERING PROMOTING FACTOR 1* (*FPF1*, *HORVU2Hr1G007350*), and *HvCEN* (*HORVU2Hr1G072750*) which were upregulated in the mutants under LDs. We also observed the downregulation of three barley homologs of NUCLEAR FACTOR-YA subunits (NF-YA) (*HORVU2Hr1G032130*; *HORVU4Hr1G075830*, Figure 11B; and *HORVU5Hr1G007890*) in the mutant at W2.0 and W3.5 under LDs, but only at W2.0 under SDs. *AtNF-YA* is repressed by members of the miR169 family to induce flowering under stress (Zhao et al., 2009). In addition, in Arabidopsis downregulation of *AtNF-YA* resulted in a reduction of the expression of *FLC* to promote flowering (Xu et al., 2014). Furthermore, a barley ortholog of PHYTOCLOCK1/LUX (*HORVU3Hr1G114970*), a component of the evening complex of the circadian clock was downregulated in the mutants at W3.5 under LDs. The barley and Arabidopsis LUX represses FT-like genes *lux* mutants are characterized by day neutral, early flowering (Campoli et al., 2013; Hazen et al., 2005). In addition, barley homologs of a *SWEET* sucrose transporter (*HORVU5Hr1G076770*, Figure 11A), of a glycogen synthase (*HORVU2Hr1G106410*) and a trehalose-6-phosphate phosphatase (*HORVU6Hr1G074960*) were upregulated specifically at W3.5 in the mutants compared to the wild type. Likewise, a barley homolog of a SHAGGY-related kinase (*HORVU3Hr1G034440*) required for the establishment of tissue patterning and cell fate determination and a KNOTTED1-like homeobox gene (*HORVU7Hr1G114650*) involved in meristem differentiation were upregulated in the mutants at W3.5 under LDs. Interestingly, also a barley homolog of *HIGH CHLOROPHYLL FLUORESCENCE (HCF173, HORVU7Hr1G096250*) controlling the initiation of translation of the psbA mRNA in Arabidopsis thaliana, was upregulated under LDs at W3.5 in the mutants.

I detected WRKY DNA-binding proteins (WRKY13, *HORVU2Hr1G093350,* Figure 11B; *HORVU6Hr1G061940*). In addition to alleviating stress, the WRKY proteins play important roles in plant developmental progression. In Arabidopsis, disruption of WRKY12 caused a delay in flowering, while loss of WRKY13 function promoted flowering under SDs through interactions with the GA pathway (Li et al., 2016). Furthermore, four barley homologs of type A Response Regulators (*HORVU2Hr1G077000*, Figure 11B; *HORVU2Hr1G120490; HORVU3Hr1G108540; HORVU5Hr1G043090*) were downregulated in the mutants at W3.5 under LDs. *ARR-A* are induced by cytokinin and function as negative regulators in cytokinin signaling (Choi and Hwang, 2007). The LD specific expression patterns of genes involved in the development of spikelets and flowers correlated with the differential development of mutants and wild type under LDs but not SDs.

### 4.2.2. Transcripts involved in stress response and transport processes are regulated in an SD-specific manner.

Under SD, 83 DETs were differentially regulated at W3.5, among them 58 were downregulated and 25 upregulated in the mutants compared to wild type (selected genes in Table 4). Among the upregulated

genes, we identified a number of stress related genes involved in detoxification such as a stress responsive A/B Barrel Domain protein (*HORVU0Hr1G011450*, Figure 11C), an *Aldehyde dehydrogenase family 3 like gene* (*HORVU2Hr1G092530*), a Cytochrome P450 superfamily like protein (CYP71A22, *HORVU2Hr1G006910*), a homolog of *ACYL-COA-BINDING PROTEIN 6* (*AtACBP6*, *HORVU7Hr1G008320*, Figure 11C), and homologs of Arabidopsis *COBALAMIN-INDEPENDENT METHIONINE SYNTHASE* and *METHIONINE SYNTHESIS 1* (*AtCIMS*, *HORVU4Hr1G002270*, *HORVU5Hr1G006780*). Downregulated genes with functions in stress response included for example, a homolog of *MAP KINASE SUBSTRATE 1 (AtMKS1, HORVU0Hr1G004060)*, a regulator of plant defense (Fiil and Petersen, 2011) and a *SALT INDUCED SERINE RICH PROTEIN (SIS, HORVU2Hr1G065000)*. A barley homolog of *OXIDATIVE STRESS 3 (AtOXS3, HORVU1Hr1G075580,* Figure 11D) conferring tolerance to heavy metals and oxidative stress, and two homologs of heavy metal transport/detoxification superfamily proteins (*HORVU4Hr1G0720601*, *HORVU2Hr1G011070*) showed a lower expression in the mutants than wild type at W3.5 (Blanvillain et al., 2009). In addition, genes involved in hormone response and signaling were downregulated in the mutants. These comprised, for example, *EIN3-BINDING F BOX PROTEIN 1 (AtEBF1, HORVU7Hr1G090240)*, a repressor of ethylene action (Ding et al., 2014) and two homologs of Ethylene-responsive transcription factor 4 (*HORVU3Hr1G078150*; *HORVU0Hr1G007050*, Figure 11D). Further, a homolog of *CYP707A1 (HORVU0Hr1G016780)* involved in ABA catabolism (Okamoto et al., 2006) and a homolog of *AtWKRY46 (HORVU5Hr1G056130)* which induces BR-regulated growth and represses drought response genes (Chen et al. 2017, Ding et al., 2014) were upregulated in the wildtype, but not in the mutant plants at W3.5 under SDs. Furthermore, we recorded the mutant specific upregulation of transcripts with roles in transport, such as an orthologue of *PLASMA MEMBERANE INTRINSIC PROTEIN 3 (AtPIP3, HORVU5Hr1G125600,* Figure 11C), forming water channels, and an orthologue of *CYCLIC NUCLEOTIDE-GATED ION CHANNEL14 (AtCNGC2, HORVU5Hr1G096440)*, building nonselective cation channels (Li et al., 2016).

Finally, proteins with roles in starch and sugar metabolism, such as a trehalose-6-phosphate phosphatase (*HORVU5Hr1G058300*, Figure 11D), a sucrose synthase 4 (*HORVU7Hr1G033230*) and a UDP-glucose 4-epimerase (*HORVU7Hr1G053260*) showed a stronger upregulation in the wild type than the mutants.

Taken together, an acceleration in the timing of spikelet initiation under SDs was accompanied by changes in the expression of genes implicated in abiotic stress responses and in transport.

## 4.3. Transcripts commonly regulated under both LDs and SDs.

Among the 22 genes differentially regulated under both LD and SD conditions (selected genes in Table 4), we identified two barley homolog of *SEP1* (*HORVU7Hr1G025700*, Figure 11E) that was only

expressed at W3.5 and expression levels were higher in both mutants compared to wild type under LDs and SDs. The downregulated genes include a *HORVU2Hr1G035160* (Figure 11E), a homolog of *LOB DOMAIN-CONTAINING PROTEIN 37 (AtLBD37)*. It is well known that the N status can be sensed by plants to regulate their development, physiology, and metabolism. Members of LBD [lateral organs boundaries (LOB) domain] family are negative regulators of N availability signals, repressing many known N-responsive genes, including key genes required for *NO3*– uptake and assimilation (Rubin et al., 2009). In addition, genes involved in nitrogen transport were downregulated such as a protein of the NRT1/PTR family 5.10 (*HORVU3Hr1G082580*) and an ortholog of GLUTAMINE DUMPER 4 (AtGDU4, *HORVU7Hr1G092710*, Figure 11E) involved in amino acid export by activating non-selective amino acid facilitators in Arabidopsis (Pratelli et al., 2010; Léran et al., 2015). In summary, only a handful of transcripts were regulated in the mutants under both photoperiods and these had roles in nitrogen response and included a floral regulator, *SEP1*. The majority of floral homeotic genes were regulated by HvCEN only under LDs.

Taken together, a large proportion of DETs were identified at W2.0 compared to W3.5, correlated with *HvCEN* higher expression at W2.0 under LDs and SDs. HvCEN affect transition from vegetative to reproductive growth and spikelet initiation (W2.0) likely through processes including chromatin modification, ribosome biogenesis, hormone signaling, and primary metabolic process/cellular respiration independent of photoperiod. Corresponding to the difference of the development between LDs and SDs and more developed MSAs in mutants before abortion under SDs, I observed candidate targets of HvCEN at stamen primordia stage (W3.5) in photoperiod dependent and independent manners. HvCEN affect inflorescence development at W3.5 likely through upregulation of *floral homeotic* genes under LDs. SD-specific candidate downstream targets of HvCEN at W3.5 were found mostly in stress related processes, including detoxification and stress response.

# Discussion

## 1.1. *HvCEN* genetically interact with *HvFT1* and *HvFT3* to control reproductive development in barley

HvCEN was originally identified as a contributor to successful environmental adaptation in spring versus winter sown barleys (Comadran et al., 2012). The authors could show that a natural Pro135Ala amino acid substitution was enriched in spring barley and caused a delay in flowering time. However, the effects of HvCEN on spike development and correlated molecular changes in the inflorescence were not investigated. In the present work, I analyzed natural and induced *hvcen* mutants to dissect the effects of HvCEN on pre-anthesis development and yield components under different photoperiods and identify molecular targets of HvCEN in the shoot apical meristem.

I dissected the different stages of SAM development to identify stage and photoperiod specific effects of HvCEN on developmental timing and processes that affect plant and spike architecture and consequently final seed number. I could show that mutations in HvCEN accelerate the timing of spikelet initiation under LDs and SDs while inflorescence development was only affected under LDs. HvCEN revealed therefore stage and photoperiod dependent effects on the development of the MSA. The repressive effect of HvCEN on the vegetative and reproductive development is in accordance with previous studies in Arabidopsis. These demonstrated that the HvCEN homolog TFL1 repressed the transition from a vegetative to reproductive and from an inflorescence meristem to floret meristem (Ratcliffe et al., 1998; Hanzawa et al., 2005; Hanano and Goto, 2011). Likewise, the vegetative stages were extended by overexpressing the *HvCEN* homologs *RCN1* and *RCN2* in rice (Nakagawa et al., 2002) and Antirrhinum *CEN* in tobacco (*Nicotiana tabacum cv Samsun*) (Amaya et al., 1999). Furthermore, a recent study in tomato has demonstrated that the LD specific upregulation of *SELF PRUNING 5G* (*SP5G*), a homolog of *HvCEN*, causes late flowering in the wild tomato relative *L. galapagense* under LDs (Soyk et al., 2017). Consequently, the function of CEN-like proteins on reproductive development is likely conserved even between the distant plant lineages of eudicots and monocots.

Spikelet initiation occurred under LDs and SDs and was delayed under both photoperiods by HvCEN. However, floral development proceeded only under LDs. Previous studies have already indicated that spikelet initiation occurs under LDs and SDs, while floral development requires LDs in spring barley genotypes (Digel et al., 2015). The authors suggested that floral development, but not spikelet initiation was dependent on the expression of *HvFT1 and TaFT* in barley and wheat, respectively (Digel et al., 2015; Pearce et al., 2013). In Arabidopsis, *FT* is expressed in the leaf and translocated as a protein to the shoot apical meristem, while *TFL1* is expressed in the shoot apical meristem itself. In Arabidopsis,

TFL1 acts antagonistically to the FT protein to repress floral transition and development (Ruiz-García et al., 1997; Abe, 2005; Jaeger et al., 2013; Taoka et al., 2011). In the shoot apical meristem, FT and TFL1 likely compete for the formation of a functional protein complex with the bZIP transcription factor FD and 14-3-3 proteins (Hanano and Goto, 2011; McGarry and Ayre, 2012). Barley has six different FT-like homologs, of which only HvFT1 and HvFT3 were functionally characterized (Halliwell et al., 2016; Casao et al., 2011; Yan et al., 2006, Mulki et al. subm.). While HvFT1 primarily accelerates floral development under long days (Hemming et al., 2008; Sasani et al., 2009), HvFT3 controls spikelet initiation under long and short days (Mulki et al., subm.). I therefore postulated that under LDs and SDs, HvCEN acts antagonistically to HvFT3 to control spikelet initiation, while its effect on floral development under LDs suggested interactions with HvFT1.

Indeed, the double mutant *hvcen hvft3* did not differ in the timing of spikelet initiation under SDs from the wild type *HvCEN hvft3*, suggesting that the repressive effect of *HvCEN* on the timing of spikelet initiation depends on a functional *HvFT3* gene. This is in line with a recent study on the function of HvFT3, that demonstrated that HvFT3 specifically controls spikelet initiation but does not affect further floral development and acts under SDs and LDs (Mulki et al. subm). In contrast to *HvFT3*, *HvFT1* is only expressed under LDs and *HvFT1* expression is crucial for floral development and flowering (Digel et al., 2015). Spring barley genotypes initiate spikelet primordia, but never flower under SDs (Digel et al., 2015). Consequently, we tested if the LD specific effect of HvCEN on floral development was dependent on the LD specific *HvFT1* expression. For this purpose I analyzed an *HvCEN hvelf3* single and *hvcen hvelf3* double mutant that both expressed *HvFT1* under SDs to similar levels as seen under LDs. The strong delay in inflorescence development and flowering time in the *HvCEN hvelf3* single mutant as compared to the *hvcen hvelf3* double mutant indicated that the repressive effect of HvCEN on floral development depended on *HvFT1* expression and successful floral development.

This suggested that *HvCEN* interacted with different *FT*-like genes in the shoot apical meristem to control different phases of development, with HvFT3 to control spikelet initiation and with HvFT1 to control floral development. The photoperiod specific effects of HvCEN are therefore caused by the photoperiod specific expression of its likely competitors HvFT3 and HvFT1.

## 1.2. HvCEN controls yield component traits

Variation at HvCEN affected the number of axillary meristems, tillers and spikes per plant. The effects of HvCEN on axillary meristem development and tiller number may be indirect by affecting the determinacy of the SAM and apical dominance or direct by controlling the development of axillary meristems. Arabidopsis plants overexpressing TFL1 exhibit dramatic changes in shoot architecture including basal branching (Hanano and Goto, 2011). In addition, in the ryegrass the *HvCEN* homolog

*LpTFL1* is mainly expressed in AXMs and was therefore proposed as a repressor of axillary meristem development (Jensen et al., 2001). Transgenic Arabidopsis lines overexpressing *LpTFL1* under long photoperiods showed late flowering and increased shoot branching (Jensen et al., 2001). Promotor of LpTFL1 drived GUS expression in Arabidopsis has shown effective activity in the AXMs in the axils of the rosette leaves during the vegetative stage. Likewise, the expression of *CEN*-like (*CET2*/*CET4)* genes in tobacco are limited to the AXMs and excluded from the SAM (Amaya et al., 1999). The expression domain of HvCEN within the barley main shoot apex still needs to be investigated. However, the phenotypic analysis suggested that HvCEN acts in the SAM and the AXMs.

The most prominent phenotypic effect of *TFL1*-like genes is on inflorescence determinacy and branching. In Arabidopsis, *tfl1* mutants do not only flower earlier, but also display a phenotype of a terminal flower at the tip of the inflorescence (Bradley et al. 1997; Shannon and Ry Meeks-Wagner 1991). In addition, the modification of *TFL1*-like genes leads to differences in rice panicle branching (Nakagawa et al., 2002), in maize cob architecture (Danilevskaya et al., 2010), and tomato inflorescence branching (Pnueli et al., 1998). I found, however, that the inflorescence meristem in *hvcen* mutants did not form a terminal spikelet. Many species are characterized by several *TFL1* or *CEN* like genes which might have evolutionarily diverged in their expressions and functions. Particularly, the number of copies for othologs of the genes between species are different (Esumi et al., 2009; Mimida et al., 2012, 2013), thus resulting in divergences in the regulating networks of meristem identity control . In snapdragon (*Antirrhinum spp*.) *CEN*, the homolog of *TFL1*, is only transcribed in the inflorescence meristem, and mutations of this gene lead to formation of terminal flowers without affecting the flowering time (Bradley et al. 1996). In pea (Pisum sativum), the two TFL1 homologs control flowering time and meristem determinacy, respectively (Foucher et al., 2003). In specific, LATE FLOWERING (LF) functions only as an inhibitor of flowering, whereas DETERMINATE (DET) plays a role in maintenance of the inflorescence meristem indeterminacy. In barley, I identified next to *HvCEN* two additional *TFL1* homologs, which differ in their expression patterns and intensity (Supplemental figure 4). Phylogenetic analysis (Supplemental figure 5) suggested that *TFL1*-like genes have experienced a series of separate duplications in eudicots and in monocots, respectively. At least three duplication events have occurred that gave rise to three barley paralogs - one likely occurred at the base of the monocots producing two major clades of *HvCEN*/*HvTFL1*-like genes in monocots. The second one appeared to occur at the base of Poaceae with poor support, probably due to incomplete sampling or missing or losses of sequences. The third duplication was unique to Triticeae and produced two copies of *TFL1*-like genes, *HvTFL1-2* and *HvCEN.* While *HvCEN* was clearly expressed in the early developing MSA, expression levels of *HvTFL1-1* and *HvTFL1-2* were very low in the same tissue (Supplemental table 4, Supplemental figure 4).

An in silico expression analysis of the three *TFL1* homologs demonstrated that *HvCEN*, *HvTFL1-1* had high expression levels in the embryo and root, while *HvTFL1-1* and *HvTFL1-2* were strongly expressed in developing tillers (Supplemental Figure 4). The analysis of the evolutionary relationships and expression domains of *TFL1*-like genes suggested that these might have divergent but also partly redundant functions in barley. *HvTFL1-1* on chromosome 5H and *HvCEN* on chromosome 2H coincide with QTL hotspots for yield related traits and are relevant for population differentiation and environmental adaptation in wild barley (Xu et al., 2018; Fang et al., 2014). Future studies need to further dissect the specific functions of HvCEN and HvTFL1-1 in barley.

While *hvcen* mutants did not form a terminal spikelet (Supplemental figure 6), their MSA was characterized by a stronger determinacy as seen in the lower number of induced spikelet primordia. The functional allele of *HvCEN* extended the period of spikelet primordia initiation and thereby increased the number of spikelets on the MSA. The wild type reached the maximal number of spikelets at awn primordium stage as observed in previous studies (Kirby and Appleyard, 1987; Alqudah and Schnurbusch, 2014; Riggs and Kirby, 1978; Kernich et al., 1997; Waddington et al., 1983), while the *hvcen* (*mat-c*) mutants stopped to produce further spikelets at the pistil primordium stage under inductive long day conditions. Overexpression of the *TFL1*-like genes *ZCN1-ZCN6* in maize maintains the indeterminacy of the vegetative meristems resulting in modified inflorescence architecture (Danilevskaya et al., 2010). In addition, the dosage of SFT and SP tomato orthologs of FT and TFL1 controls determinacy and inflorescence branching (Krieger et al., 2010; Park et al., 2014, 2016; Jiang et al., 2013). Further, it has been shown before that variation in the developmental timing of the early reproductive phase has strong effects on the number of spikelet primordia on the MSA (Digel et al., 2015; Ejaz and von Korff, 2017; Campoli and von Korff, 2014). For instance, in the presence of a wild type *PPD-H1* allele the development of the MSA is accelerated and this is associated with a reduction in number of spikelet primordia and final grain number per main spike (Digel et al., 2015). In wheat, Alvarez et al. (2016) observed an acceleration of flowering time and an associated reduction in spikelet number in an *elf3* mutant. Both, PPD-H1 and HvELF3 likely affect developmental timing and spikelet number by inducing or repressing *HvFT1,* respectively. There are only a few studies that have directly modified *HvFT1* to study its effect on MSA development. Overexpression of *HvFT1* leads to premature flower formation already in tissue culture while *HvFT1* knock-outs lead to non-flowering plants and in both cases no viable seeds are formed (Lv et al., 2014). However, the effects of the natural mutation at PPD-H1 and mutations in HvCEN on pre-anthesis development are comparable, a delay in inflorescence development and a reduction of spikelet primordia. While variation at PPD-H1 significantly reduces *HvFT1* expression levels, mutations in *HvCEN* likely modify the HvFT1 activity in the meristem. The similar phenotypic variation caused by mutation in *PPD-H1* and *HvCEN* further suggested that HvCEN interact with HvFT1 in flowering time and spike architecture control.

The *hvcen* mutant formed fewer spikelet primordia, but a relatively higher number of these developed into fertile florets as compared to the *HvCEN* in the background of *hvelf3* (Supplemental figure 7). The plant carrying wild type HvCEN could not sustain the development of one quarter of the formed spikelet primordia possibly because of resource limitations. On the other hand, a mutation in *hvcen* might have promoted the development of flowers. It has been postulated before that HvFT1 promotes floral development and floret fertility in barley (Digel et al., 2015). A stronger activity of HvFT1 in the *hvcen* mutants might have improved floral development, possible by inducing floral homeotic genes as further discussed below.

## 2.1. HvCEN regulates a large number of genes at spikelet initiation

To further unravel the molecular function of HvCEN in the MSA, I surveyed changes in the global transcriptome in wild type and mutant plants at key developmental transitions, spikelet initiation and beginning of floral development. I observed a high number of differentially expressed transcripts in particular at the spikelet initiation stage with a large number of genes that were commonly regulated under LDs and SDs conditions. This coincided with the similar effect of HvCEN on accelerating the timing of spikelet initiation under LDs and SDs, while later effects on reproductive development were photoperiod specific.

Overall, DETs associated with metabolism and biosynthesis; ribosome biogenesis; response to cytokinin; cell proliferation; nucleic acid and protein binding, and histone methyltransferase activity were highly enriched at spikelet initiation, suggesting that epigenetic regulation and coordinated changes in many different biological processes controlled the advancement of spikelet initiation in the *hvcen* mutant lines. A number of epigenetic genes putatively upstream of floral regulators were differentially regulated at spikelet initiation. These included, for example, MSI1, which is part of the evolutionarily conserved Polycomb group (PcG) chromatin-remodeling complex and controls spatial and temporal expression of several homeotic genes that regulate plant development and organ identity (Chanvivattana, 2004; Hennig et al., 2005; Derkacheva et al., 2013; Steinbach and Hennig, 2014). Similarly *PRMT*-like genes were upregulated in the mutant and these control the epigenetic silencing of the floral repressor *FLC* and flowering time in Arabidopsis (Niu et al., 2007; Schmitz et al., 2008; Wang et al., 2007; Pei et al., 2007). The epigenetic control of developmental decisions in Arabidopsis and rice has been described (Berr et al., 2011; He, 2012; Ietswaart et al., 2012). In addition, work in rice has revealed a number of epigenetic modifiers that control chromatin state, floral regulators and consequently flowering time such as *OsEMF2B* (Conrad et al., 2014), *SAMS* genes (Li et al., 2011), and Trithorax genes (Choi et al., 2014). However, little is known about the epigenetic control of developmental transitions in barley and wheat. In our study, the upregulation of *PRMT*-like genes was correlated with a downregulation of a putative repressor of flowering and homolog of *FLC*,

*HvODDSOC2*, suggesting that its expression might also be controlled epigenetically. It was so far shown that *HvODDSOC2* is controlled by *VRN1* and overexpression of *HvODDSOC2* delayed flowering and reduced spike, stem, and leaf length in barley plants (Greenup et al., 2010). Furthermore, we observed a strong downregulation of histone variants (histone H2A 7*, HORVU1Hr1G071960*, histone H1-3, *HORVU7Hr1G012850*), that play critical roles in chromatin compaction and therefore chromatin accessibility in higher eukaryotes (for review, see Hergeth and Schneider, 2015). Further, we recorded a strong upregulation of *ATXR6* (*HORVU6Hr1G011950*). Trithorax group proteins are chromatin-remodeling factors that were first discovered as a key regulator of downstream homeobox genes during early developmental stages of the fruit fly (*Drosophila melanogaster*) (Ingham, 1998). In Arabidopsis and rice, Trithorax proteins (ATX) regulate flowering time and floral organ identity (Alvarez-Venegas et al., 2003; Choi et al., 2014). *HvCEN* dependent regulation of epigenetic modifiers in the MSA of barley specifically at spikelet initiation suggested that the transition from vegetative to reproductive meristem requires strong reprogramming of transcriptional networks by epigenetic modifiers. These modifiers and their role for developmental transitions in barley await further functional characterization.

The transition to reproductive growth is accompanied by strong subsequent growth of the inflorescence. Accordingly we observed the upregulation of genes involved in cell cycle regulation, hormone signaling and many ribosomal proteins that are thought to control cellular growth (Bhavsar et al., 2010; Naora and Naora, 1999). The upregulation of type A response regulators and the downregulation of type B response regulator, cytokinin receptor histidine kinases *AHK3* and *AHK4* and cytokinin riboside 5'-monophosphate phosphoribohydrolase *LOG3* suggested altered cytokinin levels or distribution in the mutants. Lower cytokinin concentrations and signaling activity decrease meristem size and activity and affects inflorescence architecture. For example, the *LOG* genes catalyze the final step of cytokinin biosynthesis and the triple *log3 log4 log7* mutants developed a semidwarf phenotype and formed reduced flowers than the wild type suggesting that the activity of inflorescence meristem is reduced (Kuroha et al., 2009). In rice, mutations in *LOG* resulte in shoot meristem premature termination (Kurakawa et al., 2007). Similarly, the *ahk* triple mutants display a smaller inflorescence meristem with premature termination, leading to a simplified inflorescence consisting of a few flowers (Nishimura et al., 2004). These phenotypes caused by cytokinin biosynthesis and signaling genes are reminiscent of the effect of *hvcen* on the inflorescence meristem, which was also reduced in size and initiated fewer spikelets.

Similarly, ribosomal proteins have been implicated in cellular growth but the discrete phenotypes of ribosomal protein mutants have also led to the suggestion that ribosomes play a regulatory role in development (Szakonyi and Byrne, 2011b, 2011a). Changes in embryo morphogenesis, inflorescence

development, the transition to flowering, leaf shape, and plant stature are likely displayed in ribosomal mutants (Van Lijsebettens et al., 1994; Ito et al., 2000; Rosado et al., 2010; Stirnberg et al., 2012; Szakonyi and Byrne, 2011a, 2011b). Several of the ribosomal proteins upregulated in the mutants were identified as important regulators of leaf development, vascular patterning and phase change, such as PGY-like genes or an RPS13-like gene (Ito et al., 2000). The morphology modifications in ribosomal protein mutants might be resulted from changes in their capacity to ensure sufficient level of protein synthesis for proliferative cell divisions, or for the translation of genes required for organ patterning. This might involve genes that determine the distribution of auxin in the developing leaf because differential concentrations of localized auxin are essential for leaf margin initiation and vascular patterning (Nishimura et al., 2005; Zhou et al., 2010). The upregulation of ribosomal proteins in the mutants was specific for the spikelet initiation stage suggesting that the transition from a vegetative to a reproductive meristem requires a strong increase in translational efficiency and de novo protein synthesis.

The faster inflorescence growth in the mutants might have also caused an increased demand for carbohydrates and other nutrients. This was suggested by the observed upregulation of genes involved in carbon metabolism, glycolysis, cellular respiration and tricarboxylic acid cycle. An upregulation of transcripts involved in cellular respiration was also observed in the rapidly developing shoot apices of barley (Digel et al., 2015). Further, Ghiglione et al. (2008) have demonstrated that fast growing barley inflorescences show strongly reduced soluble carbohydrate levels as compared to slowly developing spikes and suggested that these fast growing tissues suffer from carbohydrate starvation. However, the faster inflorescence growth in mutants did not result in a higher but rather in reduced floret abortion, suggesting that the upregulation of cellular respiration genes was important to maintain the energy supply to the developing organ.

## 2.2. HvCEN is a repressor of floral homeotic genes during inflorescence development

Floral development was photoperiod dependent and differential regulation of transcripts at the stamen primordium stage was also strongly affected by photoperiod. The majority of transcripts were regulated by HvCEN only under LDs. In Arabidopsis, floral development is controlled by combinatory of floral patterning required transcription factors which are designated as class A, B, and C genes (Coen and Meyerowitz, 1991). They all encode members of the MIKC type of MADS-box transcription factors, except for the class A gene *AP2*. Class A (AP1 and AP2) alone specify sepals, A *and B (AP3 and PI)* together define petals, B and C (AG) determine stamens, and C alone specify carpels (Coen and Meyerowitz, 1991). Class D and E genes are involved in the expanded ABCDE model. Class D genes

promote ovule development and class E, or *SEP*, genes, acting as cofactors to all the other four class genes (for review, see Theißen, 2001). Although the floral morphologies strongly differ between eudicot and monocot grasses, some of the floral genes identified in rice and maize correspond to the A, B, C genes (Thompson and Hake, 2009).

The mutations in *HvCEN* caused an upregulation of five *SEP*-like genes (two of them were observed at cut-off FDR 0.05) at the stamen primordium stage. Grasses have diversified *SEP*-like genes, with five *SEP*-like members, *OsMADS1*, *OsMADS5*, *OsMADS7*, *OsMADS8* , and *OsMADS34* in rice (Malcomber and Kellogg, 2005; Zahn et al., 2005; Arora et al., 2007). OsMADS1 (LEAFY HULL STERILE1) is essential for specification of the lemma/palea identity and the inner floral organ meristems (Kobayashi et al., 2010; Prasad et al., 2001, 2005; Malcomber et al., 2004; Agrawal et al., 2005; Chen et al., 2006). By knocking down both *OsMADS7* and *OsMADS8,* the plants flowered late, produced palea/lemma-like organs resulted from homeotic transformations of lodicules, stamens, and carpels and lose floral determinacy. Homeotic transformation of all floral organs except the lemma into leaf-like organs was observed when simultaneously reduced the expression of *OsMADS1*, *OsMADS5*, *OsMADS7*, and *OsMADS8*, the four rice *SEP*-like genes (Cui et al., 2010). OsMADS34 (also called PANICLE PHYTOMER2) is important for controlling inflorescences and spikelets development in rice (Gao et al., 2010; Kobayashi et al., 2010). Furthermore, by analysis of *osmads34 osmads1* double mutants, OsMADS34 and OsMADS1 are suggested to act redundantly in specify the identities of lemma/palea, lodicules, stamens, and carpel (Gao et al., 2010). All five grass *SEP*-like genes (three at FDR<0.01 and two at FDR<0.05) were upregulated in the *hvcen* mutants under LDs suggesting that HvCEN controls the development of all floral organs by modifying class E gene expression.

In addition, we observed increased expression levels of a class B gene (PI, OsMADS4, FDR<0.05). I did not record any differential regulation of *AP1*, *AP2* or *AP3*-like genes (FDR cut-off 0.01), but one *AP1*-like gene (HvBM8, FDR<0.05) and one *AP2*-like gene (FDR<0.05) between genotypes at the stamen primordium stage.

The expression analysis suggest that the E and B class genes in barley are potential targets of HvCEN under LDs. Among the floral homeotic genes controlled by HvCEN, two barley homologs of *SEP1* (*OsMADS5 (FDR<0.01), OsMADS34 (FDR<0.05)*) were upregulated under LDs and SDs, while all other *SEP*- and *PI*-like genes were controlled by HvCEN only under LDs. In addition, many *HvCEN* independent floral patterning genes displayed higher expression levels under LDs than SDs. This suggest that the upregulation of most floral patterning genes is photoperiod dependent in barley. The upregulation of these genes under LDs is likely essential for maintenance and promotion of MSA development in barley. As barley clock mutants that express *HvFT1* under SDs are not impaired in spike development under

SDs (Faure et al., 2012; Pankin et al., 2014), I speculate that *HvFT1* dependent changes in the MSA are important to induce floral development.

# Conclusion

HvCEN has pleiotropic effects on the timing of SAM development, shoot and inflorescence architectures. The tillers, spikelets and seed sets per spike are increased by HvCEN through extending the vegetative and reproductive phase. HvCEN delays the transition to reproductive phase independently of photoperiod while its repression in floral development is LD dependent. The timing of the transition to reproductive phase and inflorescence development are determined, at least partially, by interactions of HvCEN with HvFT3 and HvFT1, respectively. HvCEN likely plays roles in the regulation of genes involved in chromatin modification, ribosome biogenesis, hormone signaling and cellular respiration independent of photoperiod during spikelet initiation, while represses floral homeotic genes in a LD dependent manner to inhibits inflorescence development.

# Material and methods

## 1. The *hvcen* (*praematurum-c, mat-c*) mutants

All mutants and parental lines were obtained from the Nordic Gene Bank (NordGen; http://www.nordgen.org/). The 21 allelic *mat-c* mutants were originally generated using different mutagens in various barley spring cultivars: Bonus, Foma, Frida, Kristina and Semira (Franckowiak and Lundqvist, 2012, Comadran 2012, Matyszczak, 2014, Supplemental table1). The putative effects of the different mutations were evaluated by computing the taxonomic level of conservation of the substituted amino acid including premature stop codons, changes in splice sites, amino acid replacement, frameshift and whole gene deletions. The mutation in *mat-c.770* introduced a stop codon, resulting in a truncated protein. Mutations in *mat-c.94*, *mat-c.1111* and *mat-c.1114* caused splice site changes. Amino-acid substitution at conserved sites were detected in *mat-c.32, mat-c.770*, *mat-c.907*, *mat-c.943*, *mat-c.913* and *mat-c.93* (Supplemental table 2). The *mat-c.1109* genotype carries a 1bp deletion which resulted in a frame shift, while the *mat-c.1118* is characterized by 12bp deletion resulting in a truncated protein. The *mat-c.16, mat-c.19*, *mat-c.1096*, *mat-c.1107*, *mat-c.1102*, *mat-c.1108*, *mat-c.1120* mutants are putative deletion mutants as *HvCEN* could not be amplified in these genotypes. In addition, I analysed two backcross derived introgression lines *BW508*, with an introgression of *mat-c.19* and *BW507* with an introgression of *mat-b.7* in the background of Bowman (Druka et al., 2011). *BW507* contains a large deletion of HvCEN since no amplicons of this gene were found. The effect of a single amino acid substitution in the point mutants were evaluated by PROVEAN (**Pro**tein **V**ariation **E**ffect **An**alyzer, http://provean.jcvi.org), a software that computationally predicts the influence of single amino acid substitution, in-frame insertions, deletions, and multiple amino acid substitutions from any organisms on the protein biological function (Supplemental table 2). The pairwise sequence alignment-based score (PROVEN score) measures the change in sequence similarity of a query sequence to a protein sequence homolog before and after the introducing an amino acid variation to the query sequence (Choi et al. 2012).

## 2. The *hvelf3 hvcen* and *hvft3 hvcen* double mutants

To determine if *HvCEN* interacts with *HvFT1* and *HvFT3*, I produced *hvelf3 hvcen* (with *HvFT1* expression under SDs) and *hvft3 hvcen* double mutants. The *hvelf3 hvcen* double mutants were generated by crossing the *hvcen* mutant in Bonus (*mat-c.907*) with the *hvelf3* mutant in Bonus (*mat-a.8,* NGB110008). Three F2 progenies verified as homozygous *hvelf3 hvcen* double and two as *hvelf3 HvCEN* single mutants were propagated, respectively. F4 plants from these selected five lines were grown and dissected in a controlled climate chamber under SD conditions (8h/16h, light/dark, 20°C/18°C).

To obtain *hvft3 hvcen* double mutant, the *hvcen* mutant (*mat-c.907*) in the Bonus background was crossed to an introgressed line carrying a natural mutation in *hvft3* in the background of Golden Promise. This introgression line was an F3 progeny derived from crosses between the winter barley Igri and the spring cultivar Golden Promise, carrying a recessive *hvft3* allele and a functional *HvFT3* gene, respectively. Specifically, the introgression line carried a non-functional *hvft3* allele from Igri and the natural mutation at *Ppd-H1*, a deletion in the first regulatory intron of *VRN1* and a deletion of the *VRN2* locus from Golden Promise. This introgression line shows a reduced photoperiod response and does not require vernalization. By genotyping, three *F2* lines carrying homozygous *hvft3 hvcen* double mutations and two *hvft3 HvCEN* progenies were identified and propagated.

## 3. Plant growth conditions and phenotyping in outdoor experiment

To assess the effect of *HvCEN* on reproductive development, the heading date, the number of tillers and spikelets per main spike and plant height of all mutants and their parental lines (Supplemental table 1) were scored under outdoor conditions in two consecutive years. The numbers of *mat-c* mutants and wild type parental plants grown in each year are in supplemental table 1. They were sown in 96-well trays in mid-February in 2014 and early-March in 2015, germinated in the greenhouse and then transferred outside (MPIPZ Cologne, Germany). After five weeks in 2014, three weeks in 2015, plants were transferred to 12 L pots with one plant per pot during late-March, each filled with a custom-made peat and clay soil mixture (EinheitsErde® ED73 Osmocote, Einheitserdewerke Werkverband e.V., Sinntal-Altengronau, Germany) containing a long term fertilizer. The pots were arranged in 22 rows with a distance of 1 m between rows where each row contained 54 pots with a distance of 10 cm. To avoid edge effects, the plot was surrounded by border pots containing cv. Morex barley plants. The plot was irrigated by a sprinkling robot and treated with additional fertilizer or pesticides when necessary. Heading date, determined by the emergence of awn from the main shoot, and tiller number at this stage were recorded and the number of grains per main spike and plant height was measured (soil to base of topmost spike) at full maturity, two weeks before harvest.

## 4. Plant growth conditions and phenotyping in a climate chamber

In parallel, I conducted phenotyping in the environment-controlled growth chambers using *hvcen* mutants from Bonus background. Specifically, three Bonus background *hvcen* mutants (*mat-c.907*, *mat-c.94* and *mat-c.943*) and Bonus were grown in 96-well trays using “Mini Tray” (Einheitserde®) as soil. To synchronize germination, the trays were stratified in the dark at 4°C for three days followed by growth under LDs (16h, 22°C day; 8h, 18°C night) or SDs (8h, 22°C day; 16h, 18°C night). The developmental stage of the main shoot apex (MSA) of *hvcen* mutants and their respective genetic backgrounds was determined using the Waddington quantitative scale of shoot apex development, that is based on the progression of the most advanced floret

primordium and carpel of the inflorescence (Waddington et al., 1983). Six individual plants were phenotyped for their MSA development for the selected accessions in the background of Bonus and leaf primordia number was scored during the dissection. Leaf size and visible leaf number on the main culm were scored in 20-well trays as described by Digel et.al (2016).

Additionally, the *hvelf3 hvcen*, the *hvft3 hvcen* double mutants and their control or parental lines were scored for differences in pre-anthesis development, number of spikelet primordia per main spike and number of axillary buds by dissecting the lines every two/four days (*hvelf3 hvcen)* or every week (*hvft3 hvcen)* in 96-well trays under SDs.

## 5. RNA isolation and sample preparation for RNA sequencing

Total mRNA was isolated from tissues from plants grown under long day (LD) condition (16h, 22°C day; 8h, 18°C night) and short day (SD) condition (8h, 22°C day; 16h, 18°C night). Main shoot apex (MSA) enriched tissues were harvested and pooled for three distinct developmental stages, Waddington stage 1.0, 2.0, and shoot apex tissues were harvested at Waddington stage 3.5. Noticeably, the double ridge stage (W2.0) specifies a reproductive MSA during which the first spikelet primordia on the shoot apex emerge (Waddington et al., 1983), while the stamen primordium stage (W3.5) marks the stem elongation and differentiation of the first floral organ primordia. The samples were harvested 2 hours before dark under both LD and SD conditions. Developmental stages of the MSA were assessed by dissecting three plants per genotype before sampling. To enrich shoot apex specific mRNA, leaves surrounding the MSA were removed manually using a microsurgical stab knife (5-mm blade at 15° [SSC#72-1551]). The enriched MSA tissue was cut from the base of the MSA and included leaf primordia covering the MSA. The older the MSAs were, the less residual leaf primordia the samples contained. At least 10 MSA were pooled for each of the three biological replicates per time point. All tissue harvested for RNA extraction were frozen immediately in liquid nitrogen and stored at -80°C. Total mRNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and further purified using an RNA easy Micro Kit (Qiagen). The residual DNA was removed using a DNA-free kit (Ambion) and the quality of the RNA was assessed using a bioanalyzer (Agilent 2100 Bioanalyzer). The Illumina cDNA libraries were prepared according to the TruSeq RNA sample preparation (version 2; Illumina). A cBot (Illumina) was used for clonal sequence amplification, and generation of sequence clusters. Single-end sequencing was performed using a HiSeq 3000 (Illumina) platform by multiplexing 8 libraries resulting in ~ 18 million reads per library. The requested single end read length was 100 bp for LD samples and 150bp for SD samples. The initial quality control of the raw reads was performed using the FastQC software (version0.10.1; http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/).

## 6. Transcriptional profiling and variant calling

The obtained RNA sequencing reads were mapped to a barley High Confidence (HC) transcripts reference (Mascher et al., 2017) using Salmon in quasi-mapping-based mode. When building the quasi-mapping-based index, an auxiliary k-mer hash over k-mers of length 31 was used. U (unstranded single end read) was chosen as library type to quantify the reads of each library. The expected number of reads (NumReads) that have originated from each transcript given the structure of the uniquely mapping and multi-mapping reads and the relative abundance estimates for each transcript and transcripts Per Million (TPM) values were extracted using Salmon (Patro et al., 2015). Transcripts with expression levels greater than 5 NumReads in at least two libraries under LDs or SDs were retained. Tables with expected NumReads (raw counts) and expression levels (normalized counts per million, cpm) are provided in a supplemental table (Supplemental table 4). To identify differentially expressed transcripts (DETs), pairwise comparisons, including *mat-c.907* vs. Bonus, *mat-c.943* vs. Bonus at each stage and photoperiod condition were done using the R bioconductor package Limma-vroom with a Benjamin & Hochberg adjustment for multiple testing (false discovery rate, FDR) (Ritchie et al., 2015). The comparisons between the stages and between the photoperiods were not conducted to avoid false positive DETs due to differences in the sample types and potential effects of diurnal gene expression differences between photoperiods. The FDR value of 0.01 was used as initial cut-off value for the selection of DETs. In the end, the DETs were extracted per mutant per developmental stage per photoperiod. To avoid the potential effects by other mutations in the two *hvcen* mutants, I focus on the DETs regulated in both mutants compared with wildtype. To visualize the number of genotype or stage or photoperiod dependent DETs, venn diagrams were drawn using the R package VennDiagram (Chen and Boutros, 2011). The DETs that were observed in both mutants were considered as candidate DETs regulated by HvCEN under each condition. Totally, 5308 DETs regulated at least at one stage in the two *hvcen* mutant compared to wildtype under LDs or SDs were obtained. To examine the expression pattern of all the DETs (5308), hierarchical cluster analysis (using Pearson correlation coefficients), principle component analysis (PCA) and Z-score plots of the DETs were done in R. To determinate photoperiod dependent and independent DETs, the DETs were observed in two mutants under one photoperiod and also in one of the mutants under the other photoperiod were excluded. Go annotation of the HC-transcripts was done using Blast2Go local blast (e value cut-off 1 X $10^{-5}$) (Conesa et al., 2005).To assess the effect of HvCEN on biological process, molecular function and cell component, the overrepresentation analysis of particular GO terms was performed based on the Fisher's Exact Test (significant cut-off 0.05) using Blast2Go 5.0 (https://www.blast2go.com/). The repetitive go categories were removed and the representative ones were retained manually.

The obtained RNA sequencing reads were mapped to a barley High Confidence (HC) CDS reference (Mascher et al., 2017) using BWA-MEM (version 0.7.15;(Li, 2013)). To ensure a high mapping rate, I used 3 as the

mismatch penalty. To evaluate mappings, I applied PicardTools (version 1.1.00; http://picard.sourceforge.net) CollectAlignmentSummaryMetrics on resulting SAM files. To determine whether the number of reads mapped with good mapping quality scores (MAPQ > 1), I used SAMtools (version 1.1.3; (Li et al., 2009)). Higher MAPQ suggesting a higher mapping quality because MAPQ indicate the confidence of the alignments and uniqueness of the mapping position in the reference. To maximize the number of mapped reads, I exclude read alignments with a MAPQ smaller than 1 when converted SAM to BAM format using SAMtools. To reduce the number of false-positive SNP calls, I used PicardTools MarkDuplicates to remove duplicates and GATK (version 3.1-1; (McKenna et al., 2010)) IndelRealigner to do INDEL realignment. Resulting alignments were subjected to variant calling with GATK Unifiedgenotyper using 30.0 as a minimum confidence threshold for calling and 10.0 for emitting of called SNPs and 1 for ploidy. Filtered variants with a depth of coverage >= 100, a quality of the assigned genotype >= 98 and a value of Phred-scaled likelihood >= 2000 were taken into consideration. Mutation types include SNP and InDEL. The number of mutations and number of mutated transcripts were summarized in table 1.

## 7. Leaf sample preparation for *HvFT1* expression

*HvFT1* expression levels were examined in the *hvelf3* mutant under both LDs and SDs, to confirm that expression levels are comparable between LDs and SDs in this mutant line. RNA was isolated from leaf tissue harvested from plants grown under LD (16h, 22°C day; 8h, 18°C night) and SD (8h, 22°C day; 16h, 18°C night) conditions. Under LD condition, the second youngest leaves on the main shoot of Bonus (*HvCEN HvELF3*) and *mat-c.907* (*hvcen HvELF3*) at W3.5 were harvested at two hours before dark. Under SD condition, the second youngest leaves of three *hvcen hvelf3*, two *HvCEN hvelf3*, *mat-a.8* (*HvCEN hvelf3 -3*), *mat-c.907* and Bonus were harvested at seven hours after the beginning of the dark period at the four- to five-leaf stage. Total RNA extraction, first-strand cDNA synthesis and quantitative real-time polymerase chain reaction (qRT-PCR) were performed as described in Campoli et al. (2012). The primer for *HvFT1* expression is shown in supplemental table 3. Two technical replicates were used for each cDNA sample and starting amounts for each data point were calculated based on the titration curve for each target gene and the reference (*HvActin*) gene using the LightCycler 480 Software (Roche; version 1.5).

## 8. Phylogenetic Analysis

I used HvCEN protein sequence as a query to retrieve its homologs in monocots and eudicots with blastp (E-value cutoff 1 X $10^{-10}$) from Phytozome 12.1.6 ( https://phytozome.jgi.doe.gov/pz/portal.html, Goodstein et al., 2012). The protein-coding nucleotide sequences of best blastp hits were then extracted for the focal taxa and aligned with translation alignment option with MAFFT program implemented in Geneious 6.1.8 (http://www.geneious.com, Kearse et al., 2012). The evolutionary history of HvCEN and its homologous

nucleotides was inferred using RAxML (Stamatakis, 2014) with 1000 bootstrap replicates and visualized using Dendroscope 3.5.9 (Huson et al., 2012). The ortholog of TFL1 in *Amborella* (evm_27_model_AmTr_v1_0_scaffold00114_23, *A. Trichopoda*) was used as the outgroup for rooting.

# Figures

## Figure 1

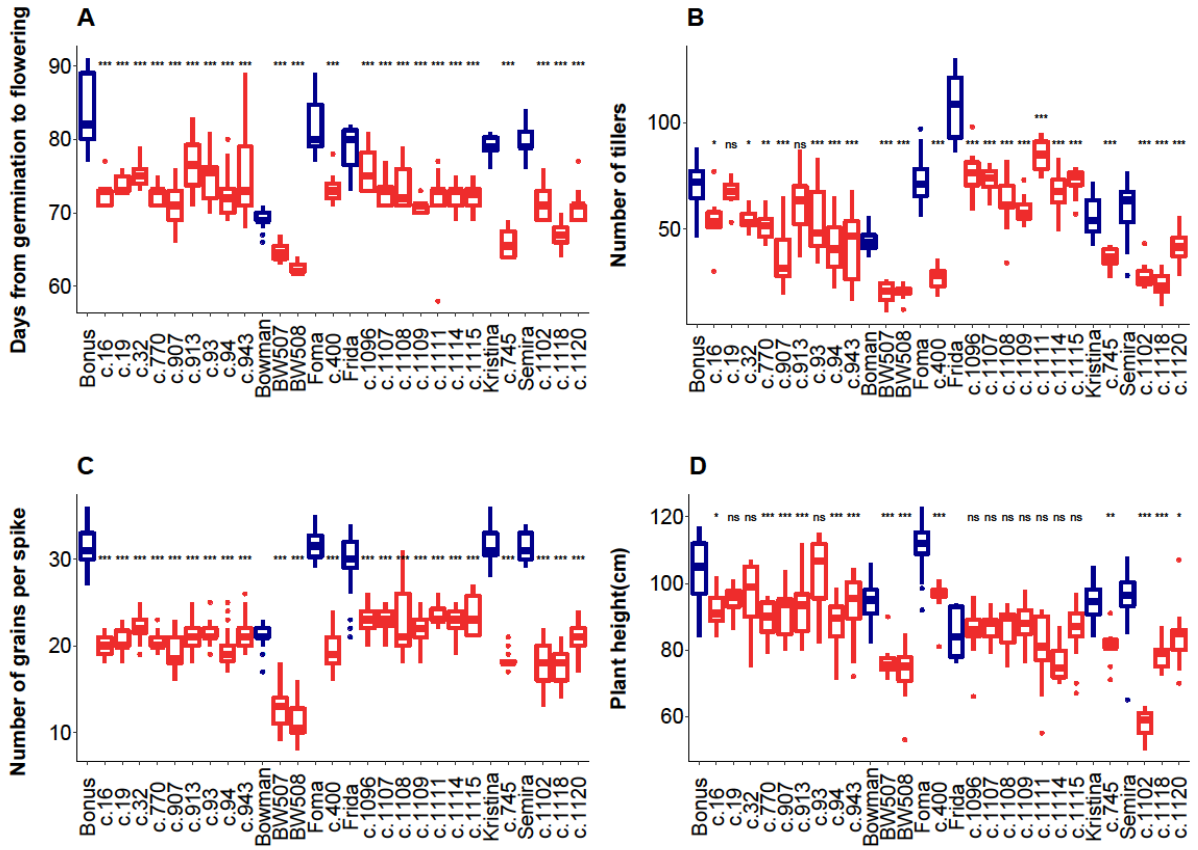



**Figure 1. Phenotypes of *hvcen* (*mat-c*) mutants trialed under outdoor conditions over two consecutive years.** A) Flowering time, B) tiller numbers at flowering time, C) grain number per main spike, D) plant height of *hvcen* mutants (in red) and the genetic background cultivars cv. Bonus, Bowman, Foma, Frida, Kristina, Semira (in blue) under outdoor conditions (number of replicates, in table 1) averaged over two years. Significant differences between the mutants and their wild type parents were calculated using student t test, p<0.001 "***", P<0.01 "**", P<0.05 "*", not significant: "ns".

Figure 2

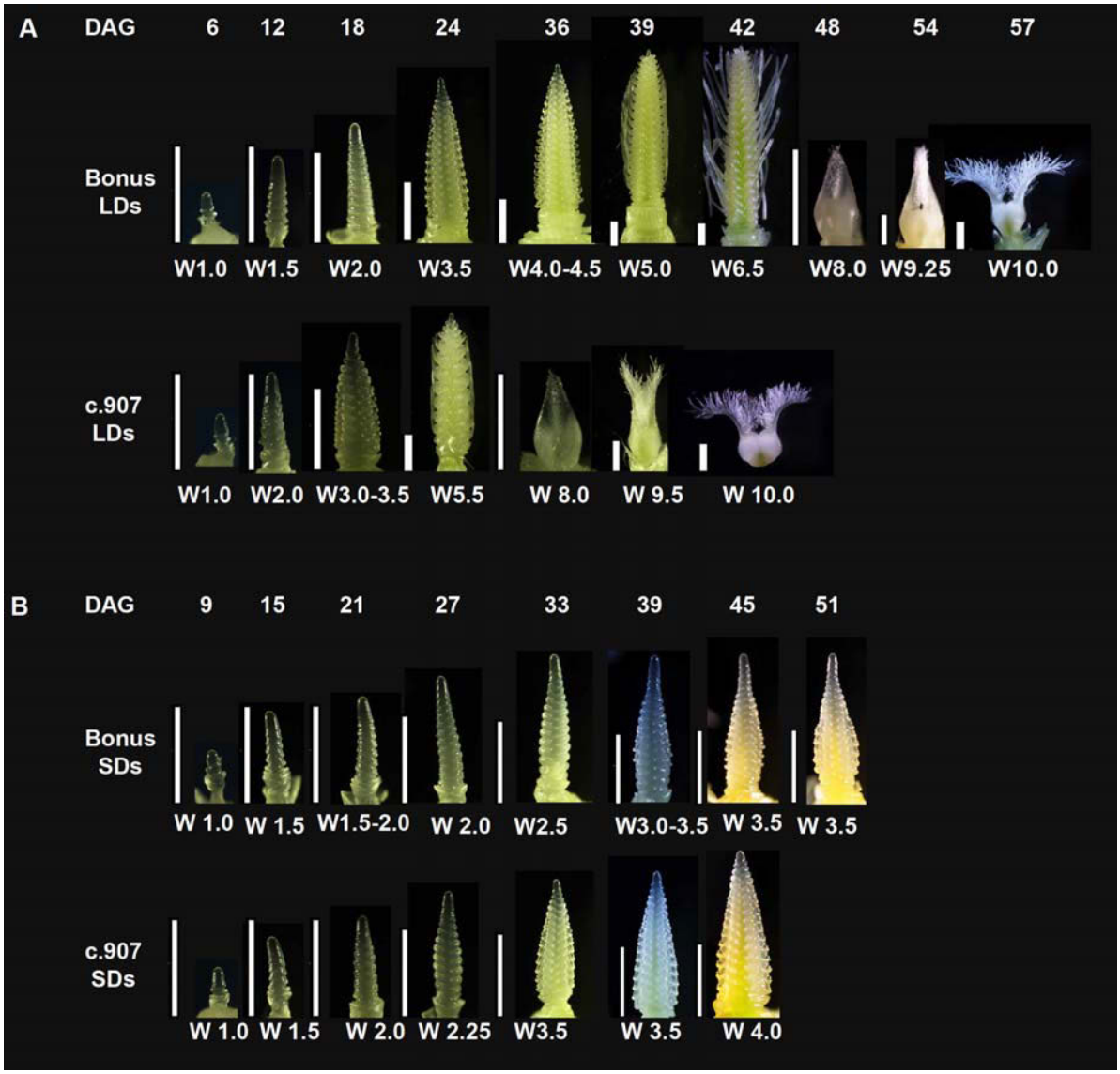



**Figure 2. Representative microscopic pictures of MSA from Bonus and *mat-c.907* under A) LDs and B) SDs.** LDs: long day condition (16h/8h, light/dark); SDs: short day condition (8h/16h, light/dark); DAG: days after germination; W: Waddington stage; white bar: 1mm.

Figure 3

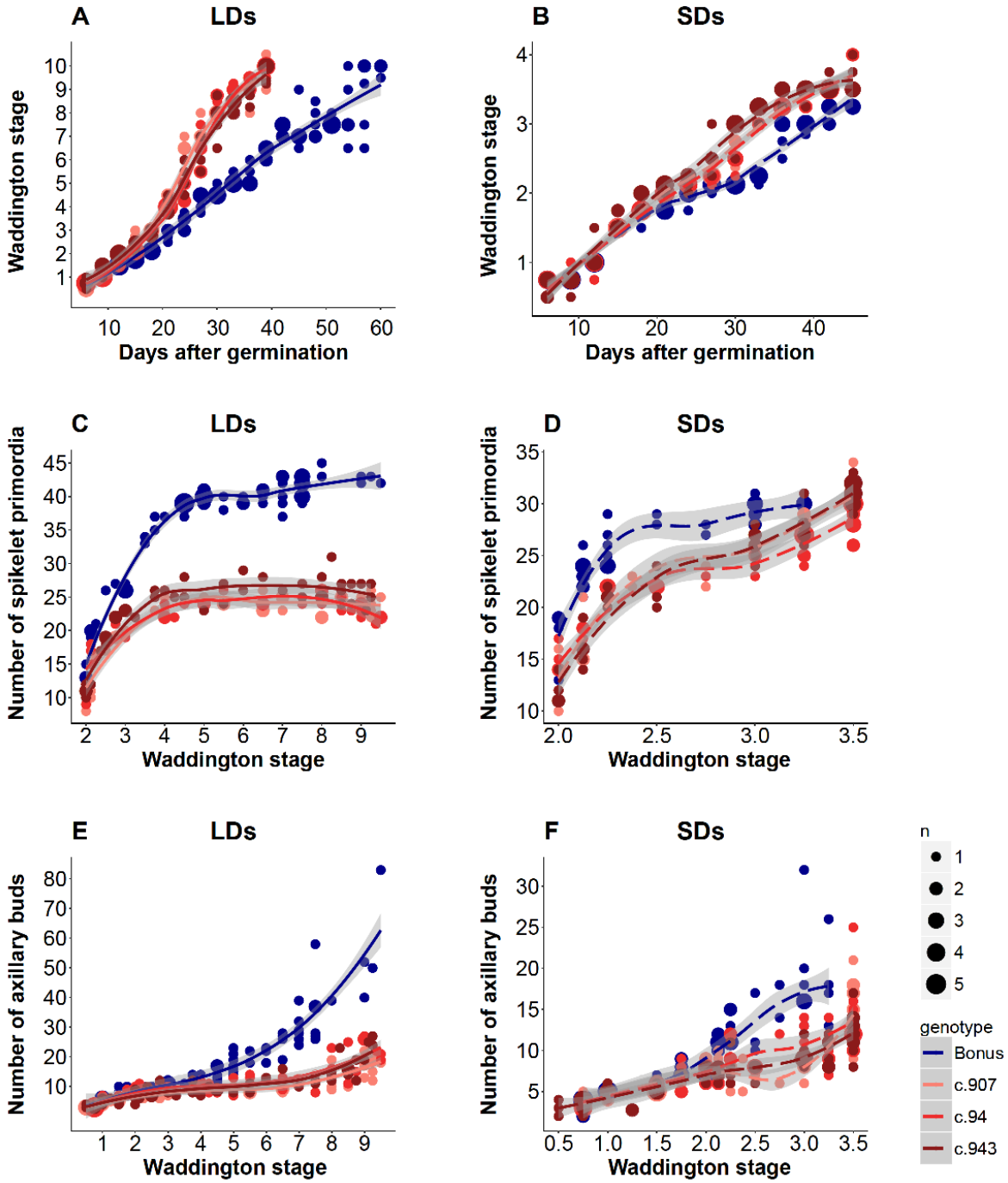



**Figure 3. Microscopic phenotypes of *hvcen* mutants (*mat-c.907, mat-c.94, mat-c.943*) and Bonus under LDs and SDs.** Development of the MSA, spikelet primordium number and axillary bud number at different Waddington stages under LDs (A, C, E) and SDs (B, D F). Five or six plants per genotype were dissected at each time point under LDs (16 h light/8 h night) and SDs (8h/16h, light/dark). Statistical differences (P<0.05) were calculated using a polynomial regression model at 95% confidence interval (Loess smooth line).

Figure 4

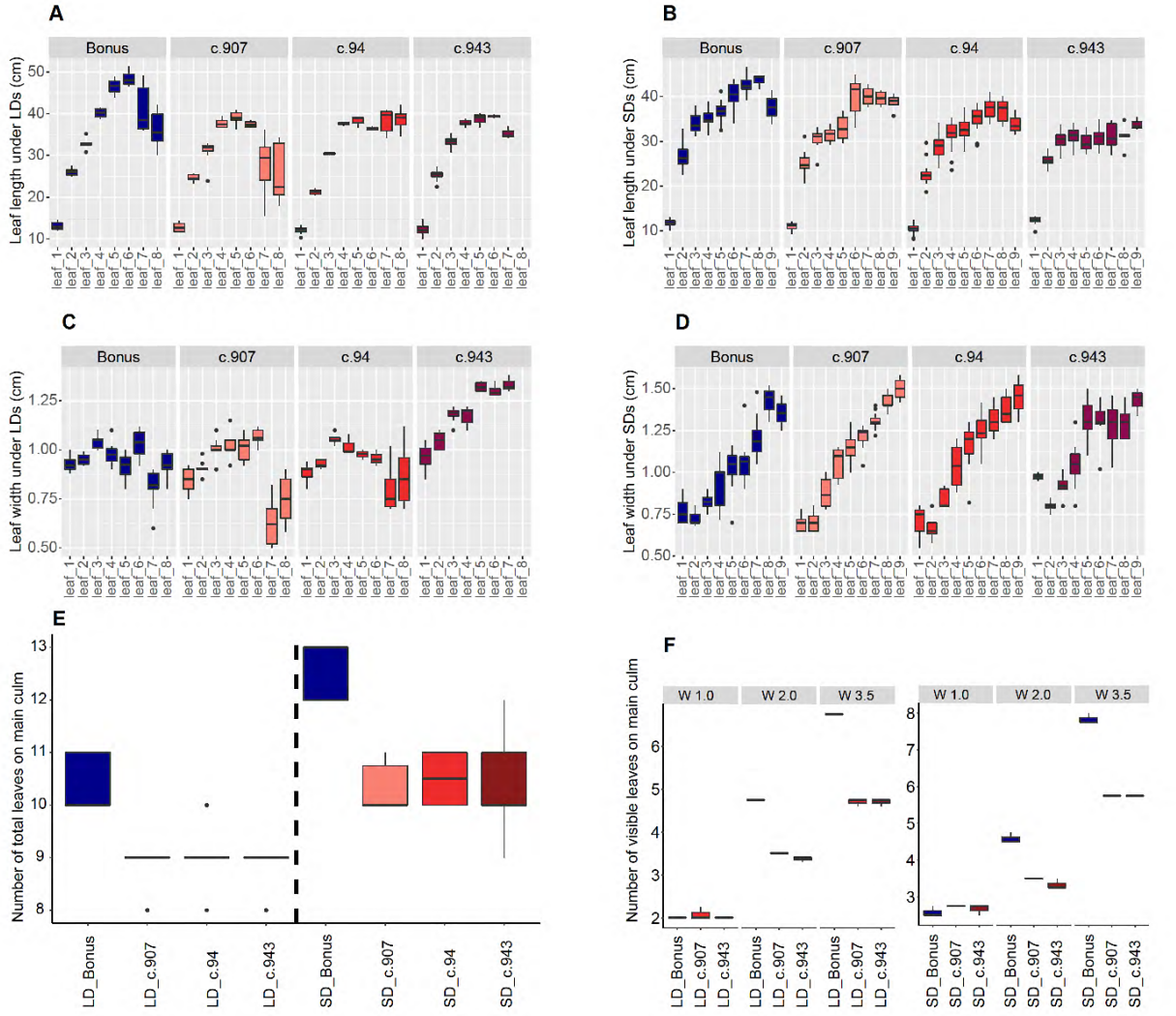



**Figure 4. Leaf length, width and number of leaves on main culm of *hvcen* mutants (*mat-c.907, mat-c.94, mat-c.943*) and Bonus under LDs and SDs.** A-B) leaf length; C, D) leaf width; E) total leaf or leaf primordium number on the main culm; F) number of visible leaves on main culm at different Waddington stages of *hvcen* mutants and wild type under LDs (16h/8h light/dark); and SDs (8h/16h, light/dark). The total number of leaves or leaf primordia on main culm was from the MSA dissection experiments.

Figure 5

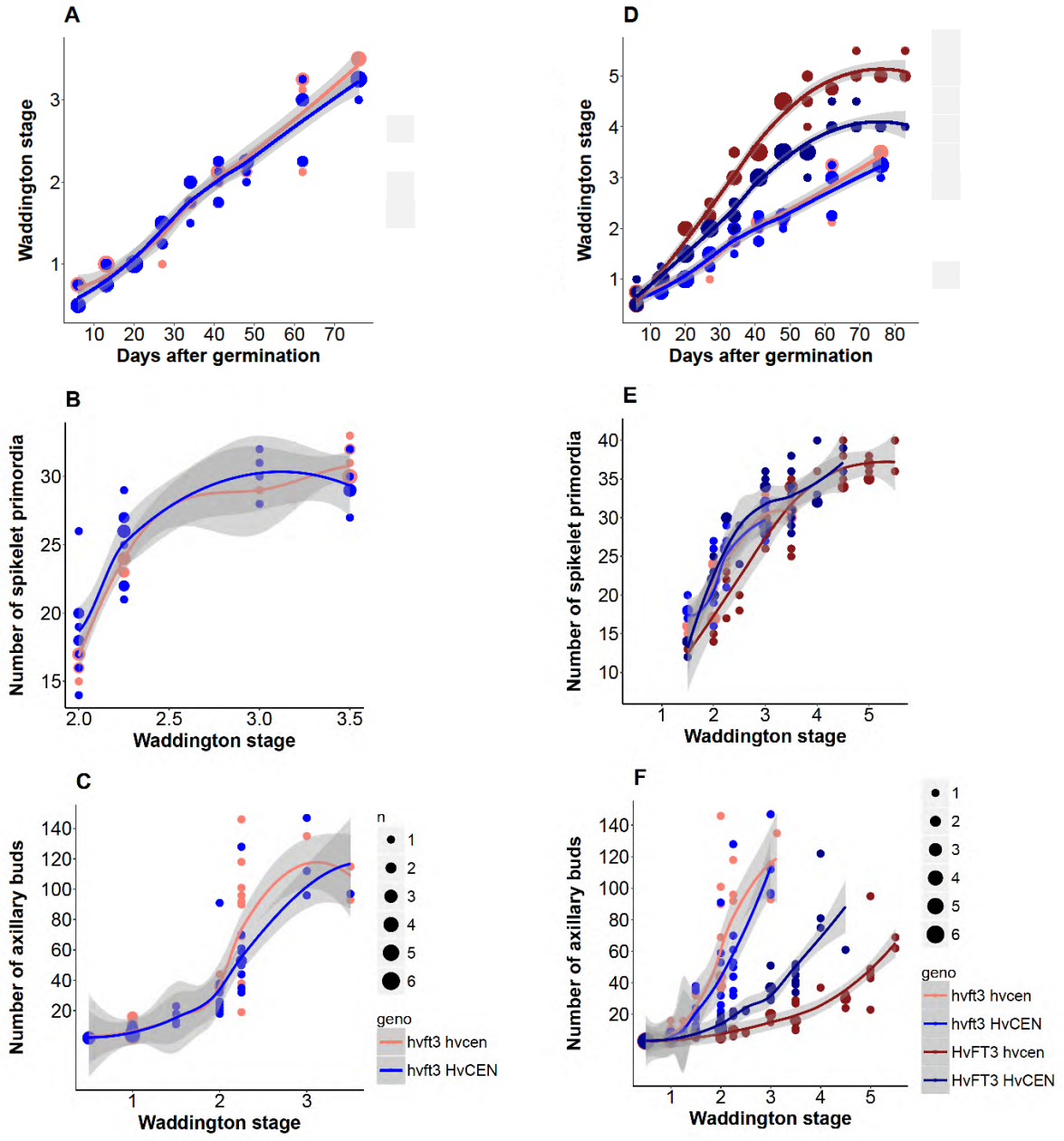



**Figure 5. Microscopic phenotypes of *hvft3 hvcen*, *hvft3 HvCEN* under SDs.** Development of the MSA, spikelet primordium number and number of axillary buds of plants A, B, C) in *hvft3* background and C, D, E) and in HvFT3 or *hvft3* background at different Waddington stages under SDs (8h/16h, light/dark). Three to six plants per genotype were dissected at each time point. Statistical differences (P<0.05) were calculated using a polynomial regression model at 95% confidence interval (Loess smooth line).

Figure 6

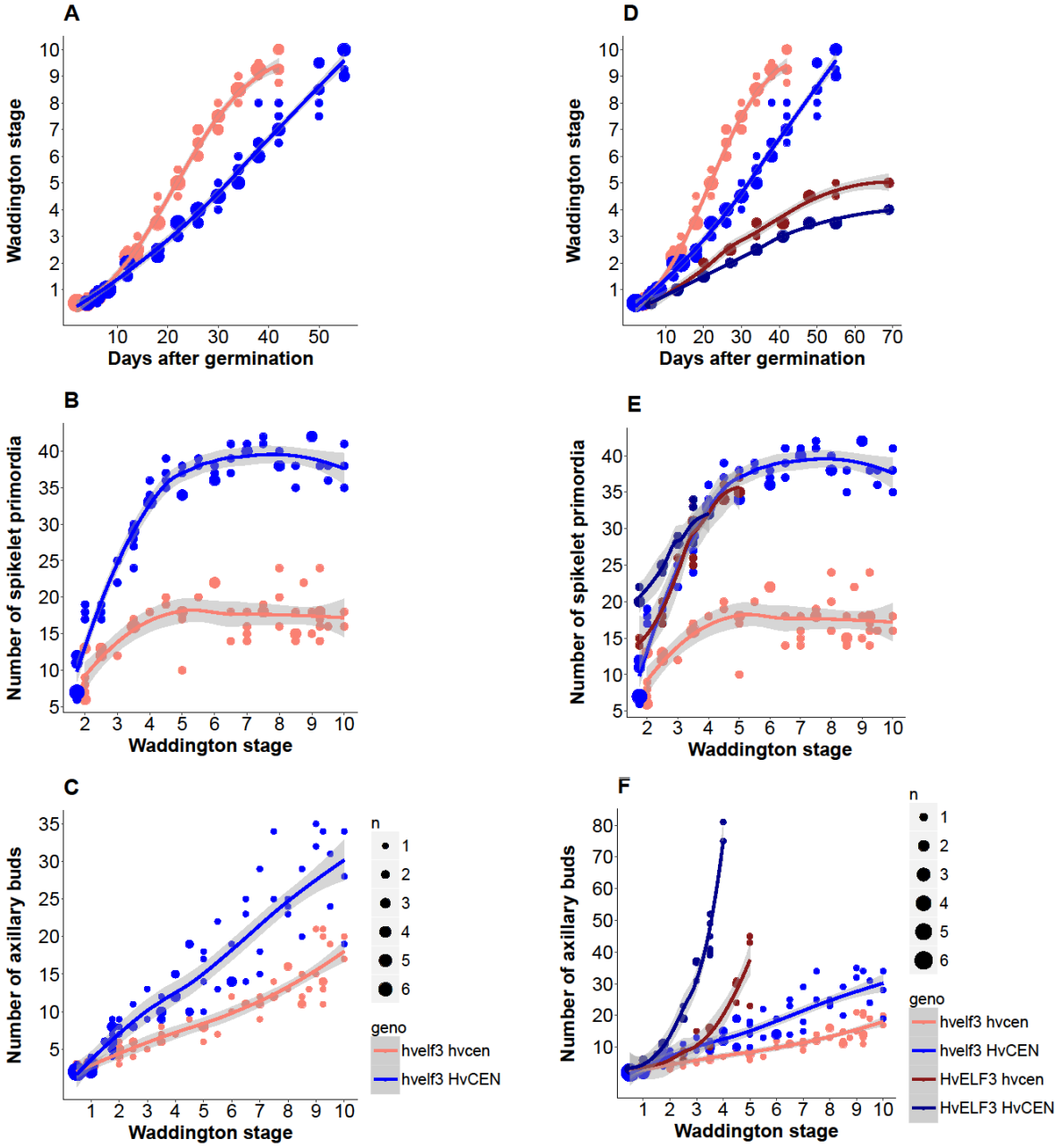



**Figure 6. Microscopic phenotypes of *hvelf3 hvcen*, *hvelf3 HvCEN* under SDs.** Development of the MSA, spikelet primordia number at different Waddington stages and number of axillary buds of plants A, B, C) in hvelf3 background and C, D, E) HvELF3 or *hvelf3* background at different Waddington stages under SDs (8h/16h, light/dark). Three to six plants per genotype were dissected at each time point. Statistical differences (P<0.05) were calculated using a polynomial regression model at 95% confidence interval (Loess smooth line).

Figure 7

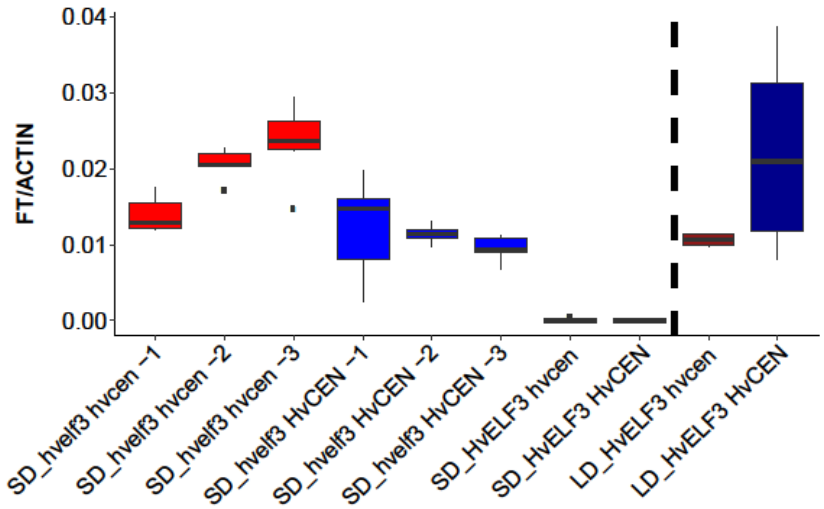



**Figure 7. *HvFT1* expression in leaves.** *hvelf3 hvcen-1,2,3* and *hvelf3 HvCEN-1,2* are progenies of lines selected from the cross (*mat-c.907* X *mat-a.8*); *hvelf3 HvCEN-3* is *mat-a.8*; *HvELF3 hvcen* is *mat-c.907*; *HvELF3 HvCEN* is Bonus. LD: long day (16h/8h, light/dark), SD: (8h/16h, dark/light).

Figure 8

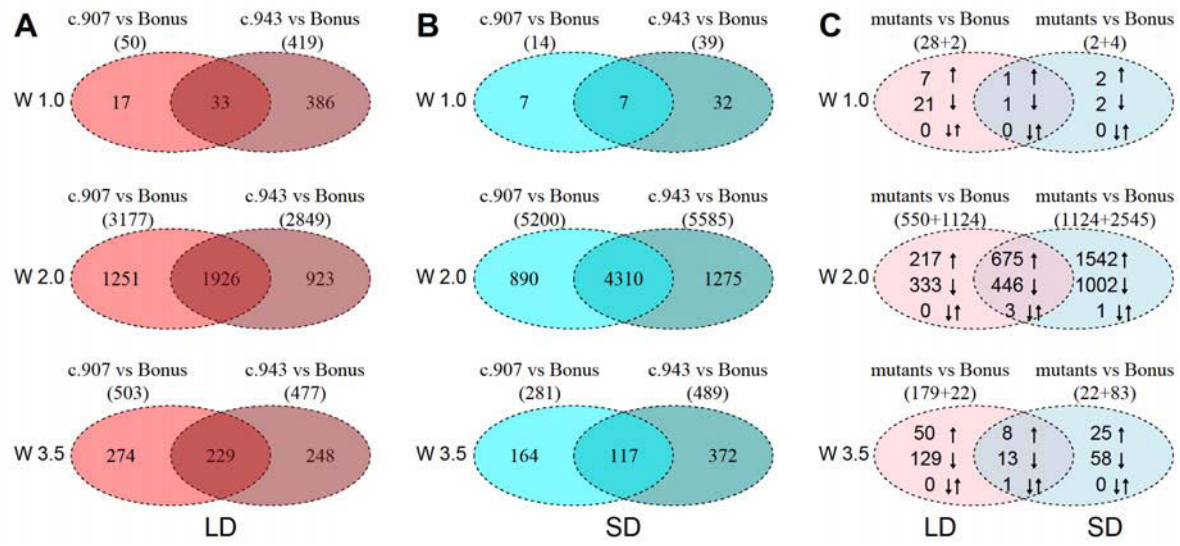



**Figure 8. Number of differentially regulated transcripts (DETs) between *hvcen* mutants (*mat-c.907*, *mat-c.943*) and wild type Bonus.** DETs in *hvcen* mutants (*mat-c.907* and *mat-c.943*) compared with wild type Bonus under A) LD (16h light/8h dark) and B) SD (8h light/ 16h dark) conditions at Waddington stage 1.0, 2.0 and 3.5; C) DETs specifically regulated under LDs and/or SDs in both mutants.

Figure 9

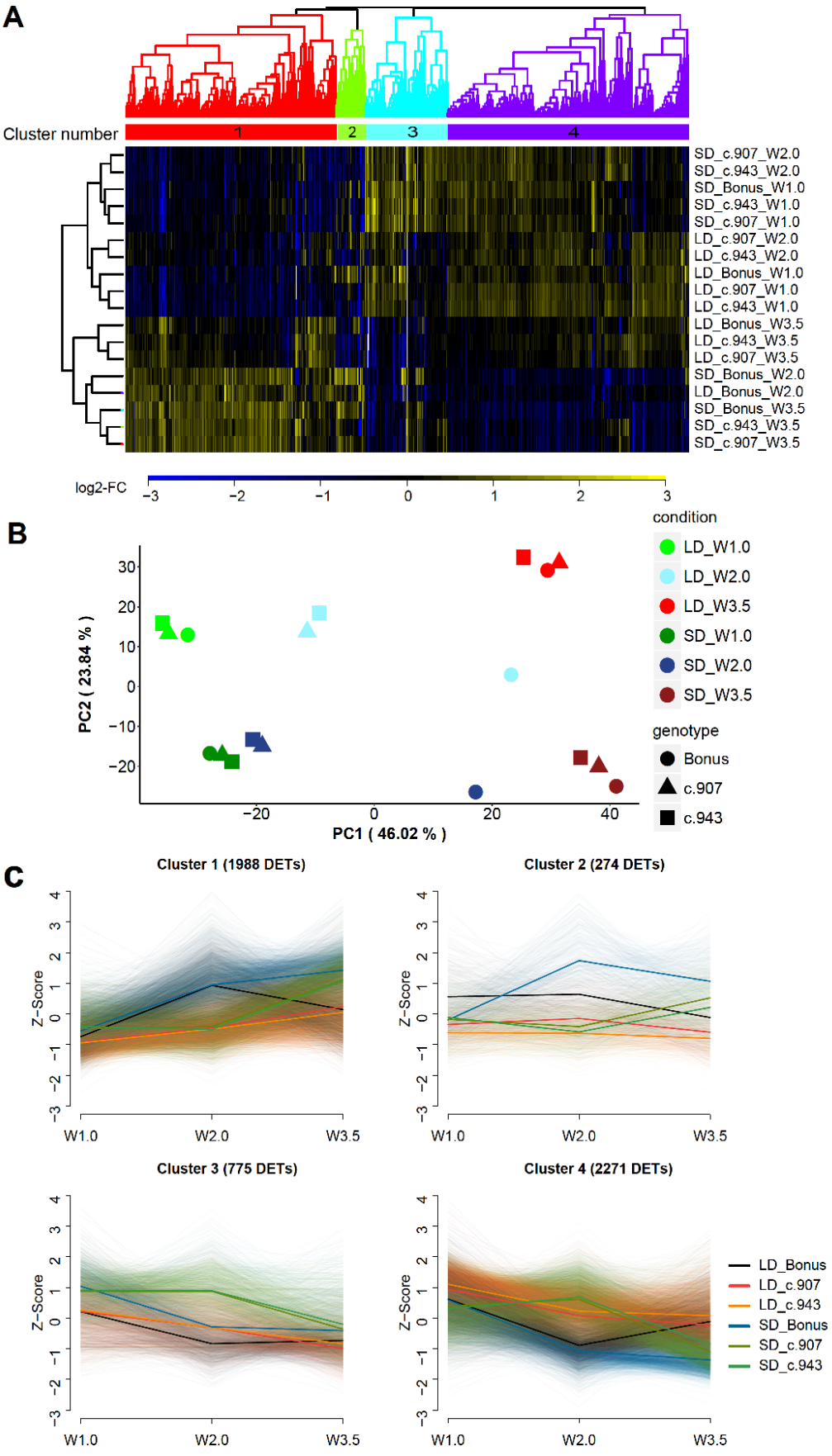

A
Cluster number
1
2
3
4
SD_c.907_W2.0
SD_c.943_W2.0
SD_Bonus_W1.0
SD_c.943_W1.0
SD_c.907_W1.0
LD_c.907_W2.0
LD_c.943_W2.0
LD_Bonus_W1.0
LD_c.907_W1.0
LD_c.943_W1.0
LD_Bonus_W3.5
LD_c.943_W3.5
LD_c.907_W3.5
SD_Bonus_W2.0
LD_Bonus_W2.0
SD_Bonus_W3.5
SD_c.943_W3.5
SD_c.907_W3.5
log2-FC
−3
−2
−1
0
1
2
3
B
condition
LD_W1.0
LD_W2.0
LD_W3.5
SD_W1.0
SD_W2.0
SD_W3.5
genotype
Bonus
c.907
c.943
PC2 ( 23.84 % )
PC1 ( 46.02 % )
C
Cluster 1 (1988 DETs)
Cluster 2 (274 DETs)
Cluster 3 (775 DETs)
Cluster 4 (2271 DETs)
Z-Score
W1.0
W2.0
W3.5
LD_Bonus
LD_c.907
LD_c.943
SD_Bonus
SD_c.907
SD_c.943

**Figure 9. Co-expression clusters of DETs regulated during shoot apex development and DETs regulated between two *hvcen* mutants (*mat-c.907* and *mat-c.943*) and wildtype (Bonus) in at least one photoperiod condition (LD/SDs).** A) Heatmap of co-expression clusters for 5308 DETs. Colors represent log2-fold changes (log2-FC) in expression levels relative to the mean transcript abundance across the tested conditions, i.e., apex (enriched) samples of *mat-c.907*, *mat-c.943* and Bonus when plants were grown under LD and SD conditions and harvested at different developmental stages (Waddington stage 1.0, 2.0, 3.5). LD, long day; SD, short day; W, Waddington stage. B) PCA of the 5038 DETs; C) Co-expression clusters of DETs during shoot apex development. There are 1988 DETs in cluster 1, 227 DETs in cluster 2, 775 DETs in cluster 3 and 2271 DETs in cluster 4. The expression levels for individual transcripts (light colors) and the mean expression level across all transcripts within each cluster (bright color) were plotted. The co-expression plots are shown as the mean centered and scaled transcript levels (Z-score).

Figure 10

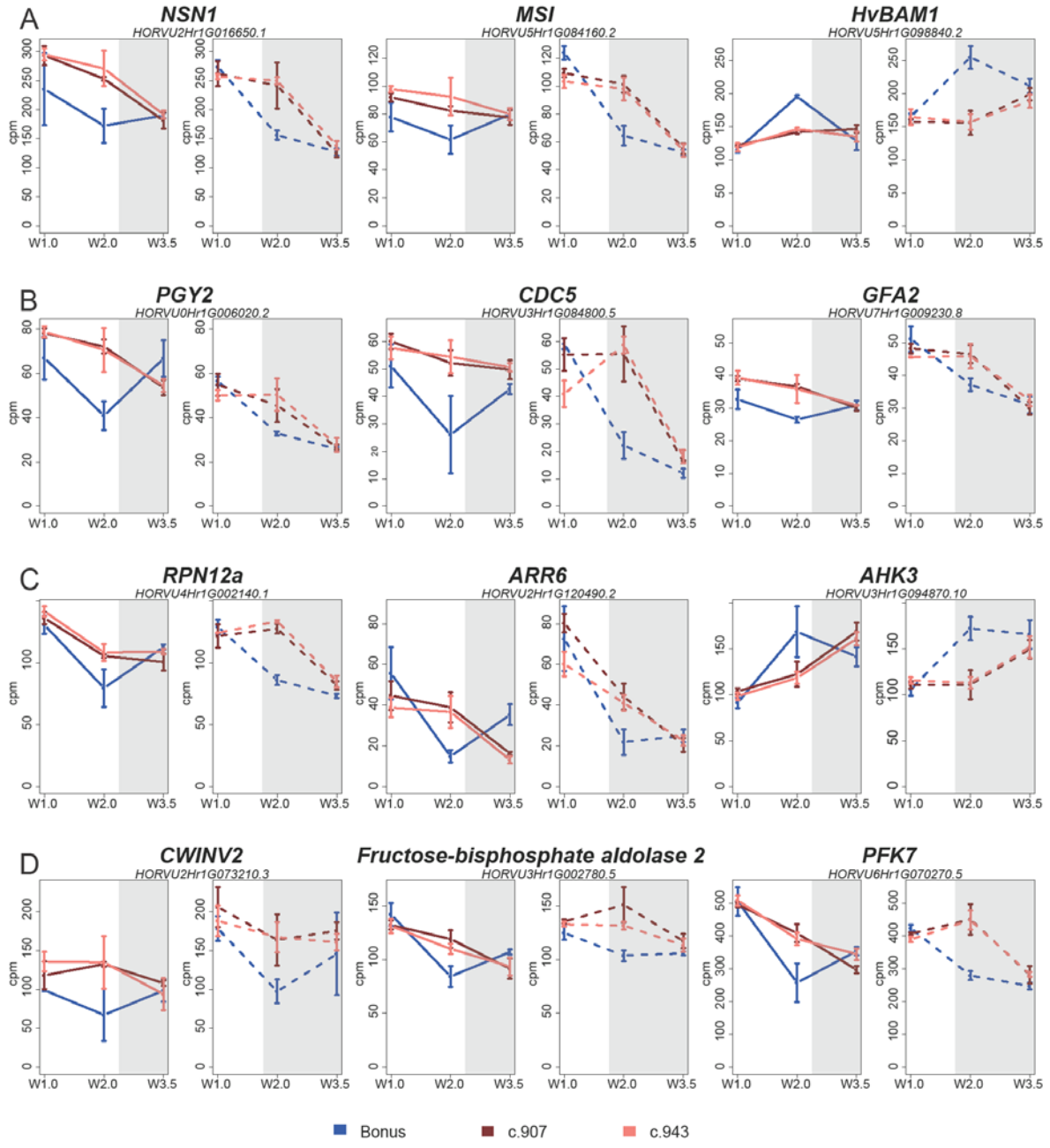



**Figure 10. Expression profiles of selected DETs between the *hvcen* mutants (*mat-c.907*, *mat-c.943*) and wildtype (Bonus) at W2.0** related to A) chromatin modification, regulation of development and organ initiation; B) ribosomal proteins, leaf development, leaf patterning; C) regulation of hormone level and hormone response D) cellular respiration, sink strength, carbohydrate metabolism, glycolytic processes under both LDs and SDs. LDs and SDs are shown by white and grey colors. White: light period, gray: dark period, W: Waddington stage, cpm: normalized counts per million. Transcripts with FDR<0.01 were considered as DETs.

Figure 11

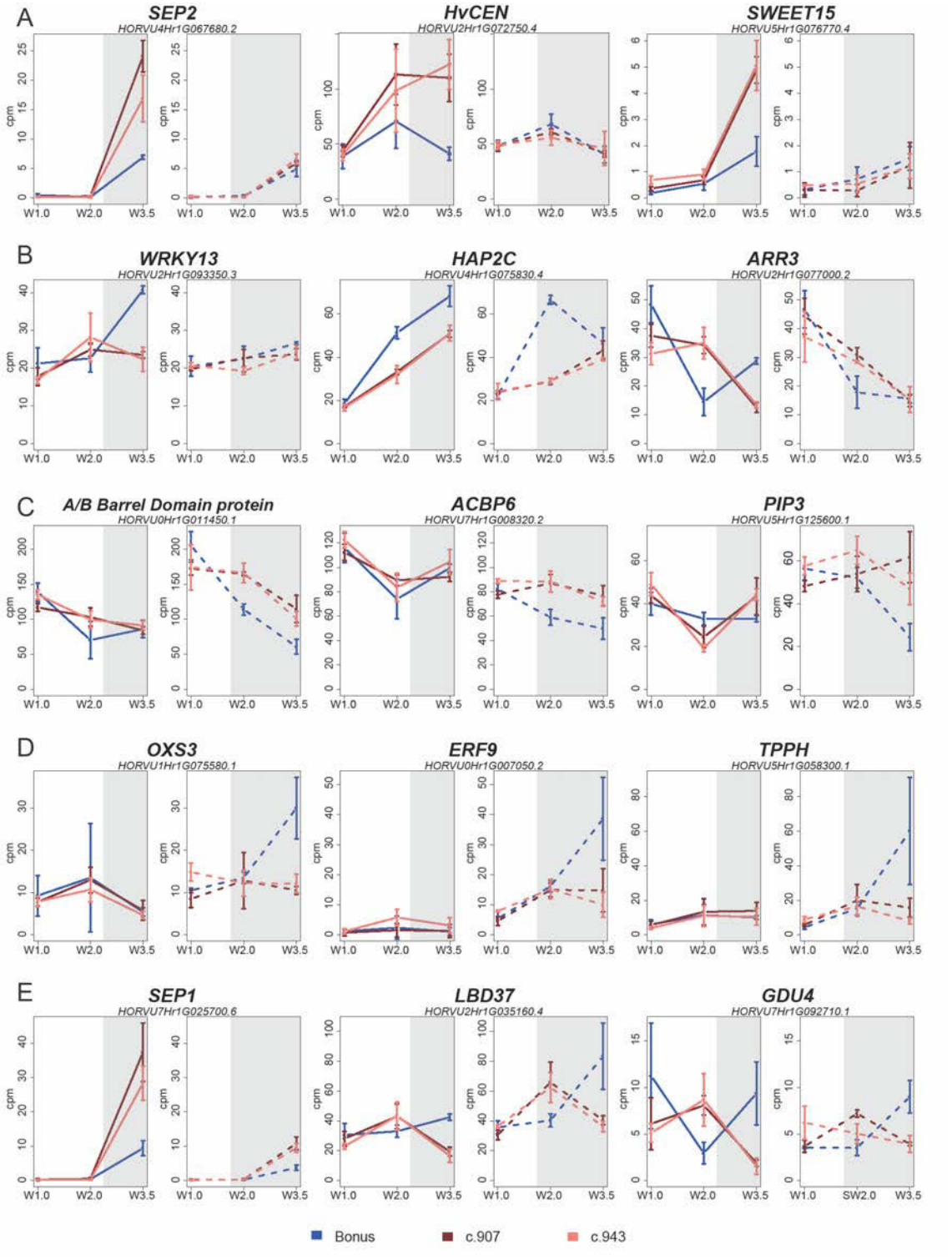



**Figure 11. Expression profiles of selected DETs between the *hvcen* mutants (*mat-c.907*, *mat-c.943*) and wildtype (Bonus) at W3.5** specifically A) upregulated or B) downregulated under LDs; C) upregulated or D) downregulated under SDs; and E) differentially regulated under both LDs and SDs. LDs and SDs are shown by white and grey colors. White: light period, gray: dark period, W: Waddington stage, cpm: normalized counts per million. Transcripts with FDR<0.01 were considered as DETs.

Figure 12

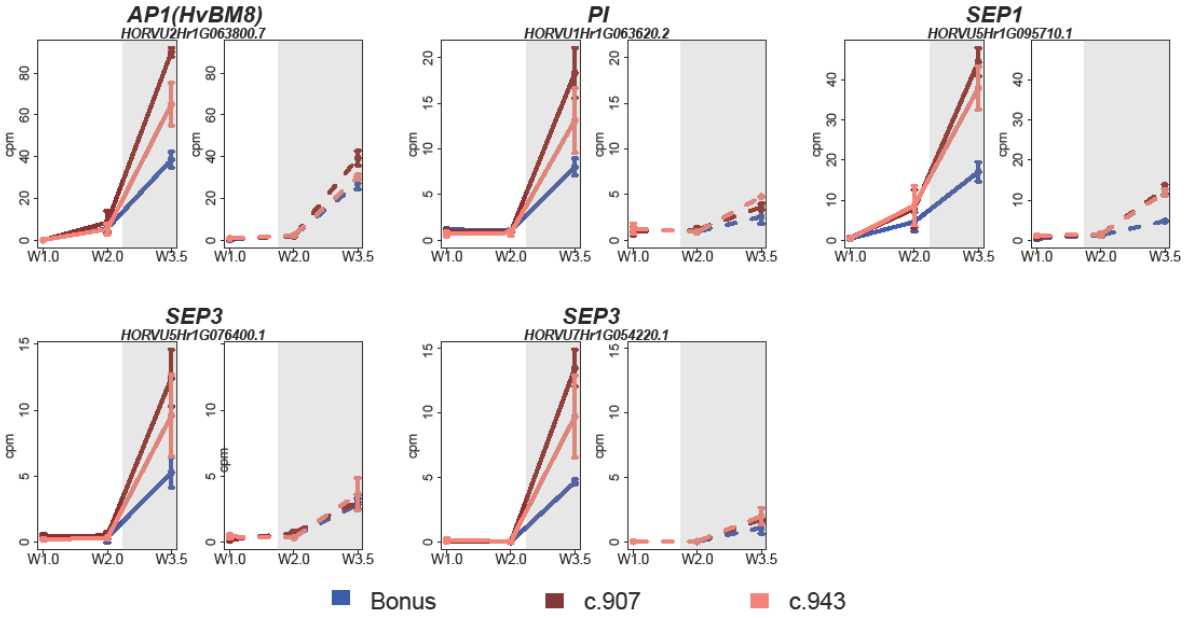



**Figure 12. Expression profiles of other floral homeotic transcription factors that were differentially regulated between the *hvcen* mutants (*mat-c.907*, *mat-c.943*) and wildtype (Bonus) at FDR<0.05**. *HvBM8, PI* and two *SEP3* were regulated only under LDs, *SEP1* was differentially regulated under LDs and SDs. LDs and SDs are shown by white and grey colors. White: light period, gray: dark period, W: Waddington stage, cpm: normalized counts per million.

# Tables

## Table 1

Table 1 Number of transcript mutated in the two mutants compared to wild type

| number of mutations per transcript | c.907 vs. Bonus[1] | c.943 vs. Bonus[2] | c.907&c.943 vs Bonus[3] |
|---|---|---|---|
| 1 | 12 | 51 | 1 (HvCEN) |
| 2 | 0 | 13 | 0 |
| 4 | 0 | 4 | 0 |
| 5 | 0 | 4 | 0 |
| 6 | 0 | 2 | 0 |
| 8 | 0 | 1 | 0 |
| 10 | 0 | 2 | 0 |
| 15 | 0 | 1 | 0 |
| total number of transcripts[4] | 12 | 78 | 1 |
| total number of mutations | 12 | 168 | 0 |

1. Number of transcripts in *mat-c.907* carrying mutation compared with the wild type Bonus
2. Number of transcripts in *mat-c.943* carrying mutation compared with the wild type Bonus
3. Number of transcripts in *mat-c.943* and *mat-c.907* carrying mutations on the same transcripts compared with the wild type Bonus
4. Mutation types include SNP and InDEL.

## Table 2

Table 2 Go enrichments of the 1124 DETs observed at W2.0 under both LDs and SDs

| GO accessions | Term | FDR | Query item | Query total | Ref item | Ref total |
|---|---|---|---|---|---|---|
| **Biological process** | | | | | | |
| GO:0009987 | cellular process | 5.91E-18 | 702 | 15828 | 276 | 11785 |
| GO:0008152 | metabolic process | 6.77E-08 | 655 | 15823 | 323 | 11790 |
| GO:0071704 | organic substance metabolic process | 4.57E-15 | 601 | 13200 | 377 | 14413 |
| GO:0044237 | cellular metabolic process | 1.79E-15 | 592 | 12892 | 386 | 14721 |
| GO:0044238 | primary metabolic process | 2.8E-17 | 577 | 12250 | 401 | 15363 |
| GO:0043170 | macromolecule metabolic process | 1.47E-10 | 454 | 9725 | 524 | 17888 |
| GO:0006807 | nitrogen compound metabolic process | 1.8E-29 | 413 | 6802 | 565 | 20811 |
| GO:0009058 | biosynthetic process | 1.52E-15 | 345 | 6337 | 633 | 21276 |
| GO:0044249 | cellular biosynthetic process | 6.26E-21 | 337 | 5661 | 641 | 21952 |
| GO:0019538 | protein metabolic process | 2.82E-12 | 298 | 5522 | 680 | 22091 |
| GO:0010467 | gene expression | 9.49E-23 | 281 | 4245 | 697 | 23368 |
| GO:0044763 | single-organism cellular process | 0.00045 | 278 | 6168 | 700 | 21445 |
| GO:0006725 | cellular aromatic compound metabolic process | 3.12E-07 | 272 | 5496 | 706 | 22117 |
| GO:0046483 | heterocycle metabolic process | 9.78E-09 | 272 | 5292 | 706 | 22321 |
| GO:1901360 | organic cyclic compound metabolic process | 1.98E-06 | 272 | 5609 | 706 | 22004 |
| GO:0071840 | cellular component organization or biogenesis | 2.35E-19 | 235 | 3479 | 743 | 24134 |
| GO:1901564 | organonitrogen compound metabolic process | 5.22E-32 | 233 | 2730 | 745 | 24883 |
| GO:0043603 | cellular amide metabolic process | 3.37E-27 | 148 | 1417 | 830 | 26196 |
| GO:0044281 | small molecule metabolic process | 4.65E-08 | 140 | 2280 | 838 | 25333 |
| GO:0042254 | ribosome biogenesis | 4.2E-32 | 88 | 443 | 890 | 27170 |
| GO:0034660 | ncRNA metabolic process | 4.72E-20 | 71 | 468 | 907 | 27145 |
| GO:1901135 | carbohydrate derivative metabolic process | 0.00195 | 58 | 921 | 920 | 26692 |
| GO:0048519 | negative regulation of biological process | 0.0289 | 57 | 1029 | 921 | 26584 |
| GO:0048518 | positive regulation of biological process | 0.00863 | 56 | 944 | 922 | 26669 |
| GO:0016072 | rRNA metabolic process | 4.42E-19 | 51 | 247 | 927 | 27366 |
| GO:0033036 | macromolecule localization | 0.0107 | 51 | 845 | 927 | 26768 |
| GO:0009605 | response to external stimulus | 0.027 | 49 | 846 | 929 | 26767 |
| GO:0006091 | generation of precursor metabolites and energy | 0.00193 | 44 | 631 | 934 | 26982 |
| GO:0051641 | cellular localization | 0.00944 | 44 | 690 | 934 | 26923 |
| GO:0018193 | peptidyl-amino acid modification | 0.000127 | 41 | 497 | 937 | 27116 |
| GO:1901657 | glycosyl compound metabolic process | 0.000876 | 40 | 529 | 938 | 27084 |
| GO:0051186 | cofactor metabolic process | 7.53E-05 | 38 | 432 | 940 | 27181 |
| GO:0072521 | purine-containing compound metabolic process | 0.000199 | 37 | 435 | 941 | 27178 |
| GO:0006457 | protein folding | 4.67E-08 | 34 | 256 | 944 | 27357 |
| GO:0048229 | gametophyte development | 0.00119 | 27 | 298 | 951 | 27315 |
| GO:0002181 | cytoplasmic translation | 1.22E-12 | 25 | 78 | 953 | 27535 |
| GO:0044724 | single-organism carbohydrate catabolic process | 1.34E-09 | 25 | 114 | 953 | 27499 |

| GO accessions | Term | FDR | Query item | Query total | Ref item | Ref total |
|---|---|---|---|---|---|---|
| GO:0045892 | negative regulation of transcription, DNA-templated | 0.001839 | 25 | 272 | 953 | 27341 |
| GO:0006605 | protein targeting | 6.81E-05 | 24 | 201 | 954 | 27412 |
| GO:0040029 | regulation of gene expression, epigenetic | 0.0346 | 22 | 292 | 956 | 27321 |
| GO:0072524 | pyridine-containing compound metabolic process | 2.61E-07 | 22 | 119 | 956 | 27494 |
| GO:0006090 | pyruvate metabolic process | 5.76E-09 | 21 | 83 | 957 | 27530 |
| GO:0006260 | DNA replication | 0.00544 | 21 | 226 | 957 | 27387 |
| GO:0009132 | nucleoside diphosphate metabolic process | 1.04E-08 | 20 | 77 | 958 | 27536 |
| GO:0009735 | response to cytokinin | 0.000593 | 19 | 157 | 959 | 27456 |
| GO:0046686 | response to cadmium ion | 0.000219 | 19 | 144 | 959 | 27469 |
| GO:0044272 | sulfur compound biosynthetic process | 0.000155 | 17 | 113 | 961 | 27500 |
| GO:0002831 | regulation of response to biotic stimulus | 2.76E-05 | 14 | 64 | 964 | 27549 |
| GO:0006914 | autophagy | 3.22E-05 | 14 | 65 | 964 | 27548 |
| GO:0008283 | cell proliferation | 0.0104 | 13 | 109 | 965 | 27504 |
| GO:0045787 | positive regulation of cell cycle | 1.12E-05 | 13 | 49 | 965 | 27564 |
| GO:0006839 | mitochondrial transport | 0.0056 | 11 | 73 | 967 | 27540 |
| GO:0016236 | macroautophagy | 0.00322 | 9 | 44 | 969 | 27569 |
| GO:0002697 | regulation of immune effector process | 0.000536 | 8 | 24 | 970 | 27589 |
| GO:0010026 | trichome differentiation | 0.0287 | 8 | 52 | 970 | 27561 |
| GO:0061077 | chaperone-mediated protein folding | 0.0235 | 7 | 38 | 971 | 27575 |
| GO:0006108 | malate metabolic process | 0.00779 | 6 | 20 | 972 | 27593 |
| GO:0006730 | one-carbon metabolic process | 0.0208 | 6 | 26 | 972 | 27587 |
| **Molecular function** | | | | | | |
| GO:0005488 | binding | 4.51E-07 | 607 | 14571 | 371 | 13042 |
| GO:0097159 | organic cyclic compound binding | 2.59E-06 | 410 | 9225 | 568 | 18388 |
| GO:1901363 | heterocyclic compound binding | 2.13E-06 | 410 | 9210 | 568 | 18403 |
| GO:0003676 | nucleic acid binding | 3.6E-10 | 237 | 4282 | 741 | 23331 |
| GO:0036094 | small molecule binding | 0.000954 | 221 | 4771 | 757 | 22842 |
| GO:1901265 | nucleoside phosphate binding | 0.002466 | 211 | 4597 | 767 | 23016 |
| GO:0043168 | anion binding | 0.033882 | 184 | 4180 | 794 | 23433 |
| GO:0005515 | protein binding | 1.73E-07 | 182 | 3287 | 796 | 24326 |
| GO:0097367 | carbohydrate derivative binding | 0.032252 | 161 | 3583 | 817 | 24030 |
| GO:0003723 | RNA binding | 7.2E-25 | 143 | 1416 | 835 | 26197 |
| GO:0005198 | structural molecule activity | 9.16E-19 | 97 | 879 | 881 | 26734 |
| GO:0003735 | structural constituent of ribosome | 4.47E-22 | 94 | 735 | 884 | 26878 |
| GO:0016874 | ligase activity | 0.000534 | 34 | 404 | 944 | 27209 |
| GO:0008135 | translation factor activity, RNA binding | 1.14E-09 | 30 | 168 | 948 | 27445 |
| GO:0005525 | GTP binding | 0.000199 | 28 | 280 | 950 | 27333 |
| GO:0019001 | guanyl nucleotide binding | 0.000209 | 28 | 281 | 950 | 27332 |
| GO:0016853 | isomerase activity | 0.037708 | 23 | 314 | 955 | 27299 |
| GO:0051082 | unfolded protein binding | 4.67E-08 | 21 | 96 | 957 | 27517 |
| GO:0003924 | GTPase activity | 0.000175 | 20 | 155 | 958 | 27458 |
| GO:0004812 | aminoacyl-tRNA ligase activity | 0.032506 | 10 | 82 | 968 | 27531 |
| GO:0016417 | S-acyltransferase activity | 0.000576 | 9 | 33 | 969 | 27580 |

| GO accessions | Term | FDR | Query item | Query total | Ref item | Ref total |
|---|---|---|---|---|---|---|
| GO:0042054 | histone methyltransferase activity | 0.038875 | 7 | 43 | 971 | 27570 |
| GO:0016615 | malate dehydrogenase activity | 0.007788 | 6 | 20 | 972 | 27593 |
| **Cell component** | | | | | | |
| GO:0005622 | intracellular | 1.85E-29 | 776 | 16982 | 202 | 10631 |
| GO:0005623 | cell | 1.23E-21 | 812 | 18947 | 166 | 8666 |
| GO:0005829 | cytosol | 4.92E-37 | 198 | 1894 | 780 | 25719 |
| GO:0005852 | eukaryotic translation initiation factor 3 complex | 0.001042 | 7 | 19 | 971 | 27594 |
| GO:0009570 | chloroplast stroma | 0.016448 | 36 | 545 | 942 | 27068 |
| GO:0031974 | membrane-enclosed lumen | 3.26E-21 | 140 | 1508 | 838 | 26105 |
| GO:0031975 | envelope | 3.69E-06 | 69 | 941 | 909 | 26672 |
| GO:0032991 | macromolecular complex | 3.76E-31 | 278 | 3672 | 700 | 23941 |
| GO:0032993 | protein-DNA complex | 0.019348 | 20 | 238 | 958 | 27375 |
| GO:0043226 | organelle | 5.37E-17 | 675 | 15126 | 303 | 12487 |
| GO:0044424 | intracellular part | 6.56E-27 | 763 | 16799 | 215 | 10814 |
| GO:0044444 | cytoplasmic part | 6.29E-11 | 524 | 11580 | 454 | 16033 |
| GO:0044464 | cell part | 4E-22 | 812 | 18896 | 166 | 8717 |
| GO:0070013 | intracellular organelle lumen | 3.26E-21 | 140 | 1508 | 838 | 26105 |
| GO:1902494 | catalytic complex | 0.000214 | 71 | 1110 | 907 | 26503 |

**Table 2.** Go enrichment of the 1124 DETs observed in the two focal *hvcen* mutants compared with wild type (Bonus) at W2.0. The Go annotation and enrichment analysis were done using Blast2Go (Götz et al., 2008). E-value $1X10^{-5}$ was used as cut-off for blast. The redundant Go accessions were removed manually.

## Table 3

Table 3: Selected* transcripts differentially regulated in the mutant MSA at W2.0 under LDs and SDs (FDR<0.01)

| Transcript[1] | Regulation[2] | Description[3] | Arabidopsis Gene Model[4] | Arabidopsis Gene Identifier |
|---|---|---|---|---|
| **Chromatin modification, consequent regulation of development and organ initiation** | | | | |
| HORVU5Hr1G084160.2 | LD&SD_up | WD-40 repeat family protein | AT5G58230.1 | MSI1, MEE70, ATMSI1 |
| HORVU5Hr1G093230.3 | LD&SD_up | Histone-binding protein RBBP7 | AT5G58230.1 | MSI1, MEE70, ATMSI1 |
| HORVU6Hr1G019540.3 | LD&SD_up | Protein arginine N-methyltransferase 5 | AT4G31120.1 | SKB1, ATPRMT5 |
| HORVU7Hr1G020620.2 | LD&SD_up | protein arginine methyltransferase 10 | AT1G04870.2 | PRMT10, ATPRMT10 |
| HORVU6Hr1G063490.2 | LD&SD_up | S-adenosylmethionine synthase 3 | AT3G17390.1 | MTO3, SAMS3, MAT4 |
| HORVU6Hr1G063540.3 | LD&SD_up | S-adenosylmethionine synthase 1 | AT3G17390.1 | MTO3, SAMS3, MAT4 |
| HORVU6Hr1G063570.1 | LD&SD_up | S-adenosylmethionine synthase 2 | AT3G17390.1 | MTO3, SAMS3, MAT4 |
| HORVU1Hr1G071960.1 | LD&SD_up | histone H2A 7 | NA | NA |
| HORVU7Hr1G012850.1 | LD&SD_up | histone H1-3 | AT2G18050.1 | HIS1-3 |
| HORVU6Hr1G011950.3 | LD&SD_up | Histone-lysine N-methyltransferase 2C | AT5G24330.1 | ATXR6, SDG34 |
| HORVU5Hr1G088420.1 | LD&SD_down | Histone-lysine N-methyltransferase 2C | AT1G05830.2 | ATX2 |
| HORVU2Hr1G016650.1 | LD&SD_up | Guanine nucleotide-binding protein-like 3 homolog | AT3G07050.1 | NSN1 |
| HORVU3Hr1G095240.6 | LD&SD_down | MADS-box transcription factor family protein | | ODDSOC2 |
| HORVU5Hr1G098840.2 | LD&SD_down | Leucine-rich receptor-like protein kinase family protein | AT5G65700.2 | BAM1 |
| HORVU2Hr1G082470.1 | LD&SD_down | FAR1-related sequence 6 | AT1G52520.1 | FRS6 |
| HORVU7Hr1G099010.1 | LD&SD_down | Kelch repeat-containing F-box family protein | AT5G57360.1 | ZTL, LKP1, ADO1, FKL2 |
| HORVU7Hr1G107550.5 | LD&SD_down | myb-like transcription factor family protein | AT5G52660.2 | |
| HORVU3Hr1G097200.1 | LD&SD_down | auxin response factor 2 | AT5G62000.3 | ARF2, ARF1-BP, HSS, ORE14 |
| HORVU5Hr1G045180.1 | LD&SD_down | ABSCISIC ACID-INSENSITIVE 5-like protein 2 | AT3G56850.1 | AREB3, DPBF3 |
| HORVU6Hr1G081080.12 | LD&SD_down | Nuclear transcription factor Y subunit A-5 | AT1G54160.1 | NFYA5, NF-YA5 |
| HORVU5Hr1G095630.3 | LD&SD_down | MADS-box transcription factor 14 | AT1G69120.1 | AP1 ,AGL7, VRN1 |
| HORVU0Hr1G018300.1 | LD&SD_down | Chaperone protein dnaJ 3 | AT3G44110.1 | ATJ3, ATJ |
| HORVU1Hr1G073230.1 | LD&SD_down | Coronatine-insensitive protein homolog 1b | AT2G39940.1 | COI1 |
| HORVU3Hr1G085210.2 | LD&SD_down | Coronatine-insensitive protein homolog 1a | AT2G39940.1 | COI1 |
| HORVU2Hr1G090980.8 | LD&SD_down | Starch synthase 2, chloroplastic/amyloplastic | AT1G32900.1 | |
| HORVU2Hr1G103330.2 | LD&SD_down | Transcription factor GTE7 | AT1G73150.1 | GTE3 |
| HORVU5Hr1G046490.15 | LD&SD_down | vacuolar sorting receptor homolog 1 | AT3G52850.1 | VSR1, BP-80, ATELP, BP80, BP80B, ATELP1, ATVSR1, GFS1, VSR1;1, BP80-1;1 |
| HORVU7Hr1G023990.4 | LD&SD_down | Ring E3 ubiquitin ligase | AT1G78420.2 | |
| HORVU4Hr1G007370.1 | LD&SD_up | 14-3-3-like protein GF14-F | AT5G38480.1 | GRF3, RCI1 |
| HORVU1Hr1G015590.1 | LD&SD_up | Glutamate--cysteine ligase B, chloroplastic | AT4G23100.3 | GSH1 |
| HORVU6Hr1G054520.3 | LD&SD_up | glyceraldehyde-3-phosphate dehydrogenase C2 | AT3G04120.1 | GAPC, GAPC-1, GAPC1 |
| **Ribosomal proteins, leaf development, vascular patterning, adaxial cell fate** | | | | |
| HORVU3Hr1G001140.5 | LD&SD_up | Ribosomal protein L6 family | AT1G33140.1 | PGY2 |
| HORVU0Hr1G006020.2 | LD&SD_up | Ribosomal protein L6 family | AT1G33140.1 | PGY2 |
| HORVU5Hr1G092630.1 | LD&SD_up | Ribosomal protein L6 family | AT3G25520.1 | ATL5, PGY3, OLI5, RPL5A |
| HORVU2Hr1G037940.1 | LD&SD_up | 40S ribosomal protein S13-1 | AT4G00100.1 | ATRPS13A, RPS13, PFL2, RPS13A |
| HORVU1Hr1G085550.2 | LD&SD_up | 50S ribosomal protein L21 | AT4G30930.1 | NFD1 |
| HORVU7Hr1G067060.1 | LD&SD_up | 60S ribosomal protein L13-1 | AT3G49010.3 | ATBBC1, BBC1, RSU2 |
| HORVU2Hr1G029890.1 | LD&SD_up | 40S ribosomal protein S6 | AT5G10360.1 | EMB3010, RPS6B |
| HORVU3Hr1G084800.5 | LD&SD_up | cell division control 6 | AT1G07270.1 | |

| Transcript[1] | Regulation[2] | Description[3] | Arabidopsis Gene Model[4] | Arabidopsis Gene Identifier |
|---|---|---|---|---|
| HORVU6Hr1G088120.3 | LD&SD_up | proliferating cell nuclear antigen 2 | AT2G29570.1 | PCNA2, ATPCNA2 |
| HORVU0Hr1G031140.1 | LD&SD_up | proliferating cell nuclear antigen 2 | NA | NA |
| HORVU6Hr1G094080.1 | LD&SD_up | Replication protein A 32 kDa subunit A | AT2G24490.2 | RPA2, ATRPA2, ROR1, ATRPA32A, RPA32A |
| HORVU7Hr1G117000.1 | LD&SD_up | Chaperone protein htpG family protein | AT4G24190.2 | SHD, AtHsp90.7, AtHsp90-7 |
| HORVU7Hr1G009230.8 | LD&SD_up | Chaperone protein DnaJ | AT5G48030.1 | GFA2 |
| **Flowering, regulation of hormone level and hormone response** | | | | |
| HORVU4Hr1G002140.1 | LD&SD_up | 26S proteasome non-ATPase regulatory subunit 8 homolog A | AT1G64520.1 | RPN12a |
| HORVU5Hr1G097560.5 | LD&SD_up | Two-component response regulator ARR2 | AT4G37180.1 | ARR2 |
| HORVU2Hr1G077000.2 | LD&SD_up | Two-component response regulator ORR1 | AT1G59940.1 | ARR3 |
| HORVU3Hr1G108540.2 | LD&SD_up | Two-component response regulator ARR8 | AT2G41310.1 | ATRR3, ARR8, RR3 |
| HORVU2Hr1G120490.2 | LD&SD_up | Two-component response regulator ORR6 | AT5G62920.1 | ARR6 |
| HORVU6Hr1G028680.1 | LD&SD_down | Two-component response regulator ARR12 | AT2G25180.1 | ARR12, RR12 |
| HORVU3Hr1G094870.10 | LD&SD_down | histidine kinase 3 | AT1G27320.1 | AHK3, HK3 |
| HORVU6Hr1G077070.5 | LD&SD_down | histidine kinase 3 | AT2G01830.1 | WOL, CRE1, WOL1, AHK4, ATCRE1 |
| HORVU4Hr1G005660.6 | LD&SD_down | Cytokinin riboside 5'-monophosphate phosphoribohydrolase | AT2G37210.1 | LOG3 |
| HORVU5Hr1G097400.2 | LD&SD_up | Protein kinase superfamily protein | AT2G23070.1 | |
| HORVU3Hr1G097200.1 | LD&SD_down | auxin response factor 2 | AT5G62000.3 | ARF2, ARF1-BP, HSS, ORE14 |
| HORVU4Hr1G013210.2 | LD&SD_down | Gibberellin-regulated family protein | AT2G39540.1 | |
| HORVU4Hr1G047240.2 | LD&SD_down | Dormancy/auxin associated family protein | AT1G56220.4 | |
| HORVU6Hr1G091260.4 | LD&SD_down | Auxin-responsive protein IAA10 | AT2G33310.3 | IAA13 |
| HORVU6Hr1G094970.8 | LD&SD_down | Auxin efflux carrier family protein | AT5G65980.1 | |
| HORVU5Hr1G045180.1 | LD&SD_down | ABSCISIC ACID-INSENSITIVE 5-like protein 2 | AT3G56850.1 | AREB3, DPBF3 |
| HORVU7Hr1G019510.2 | LD&SD_up | Dormancy/auxin associated family protein | AT1G56220.4 | |
| **Cellular respiration, sink strength, carbohydrate metabolism** | | | | |
| HORVU2Hr1G073210.3 | LD&SD_up | Beta-fructofuranosidase, insoluble isoenzyme 2 | AT3G52600.1 | AtcwINV2, CWINV2 |
| HORVU7Hr1G027930.7 | LD&SD_up | Raffinose synthase family protein | AT5G20250.4 | DIN10 |
| HORVU1Hr1G013450.2 | LD&SD_up | Alpha,alpha-trehalose-phosphate synthase [UDP-forming] 1 | AT1G78580.1 | ATTPS1, TPS1 |
| pyruvate metabolism | | | | |
| HORVU6Hr1G003770.1 | LD&SD_up | Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex | AT3G13930.1 | |
| HORVU7Hr1G001330.9 | LD&SD_up | Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex | AT3G13930.1 | |
| **glycolytic process** | | | | |
| HORVU1Hr1G006860.7 | LD&SD_up | Glucose-6-phosphate isomerase | AT5G42740.1 | |
| HORVU3Hr1G039200.3 | LD&SD_up | Pyruvate kinase family protein | AT5G56350.1 | |
| HORVU3Hr1G002780.5 | LD&SD_up | fructose-bisphosphate aldolase 2 | AT2G01140.1 | |
| HORVU3Hr1G088540.1 | LD&SD_up | fructose-bisphosphate aldolase 2 | AT2G36460.1 | |
| HORVU3Hr1G088570.1 | LD&SD_up | fructose-bisphosphate aldolase 2 | AT2G36460.1 | |

Tables

| Transcript[1] | Regulation[2] | Description[3] | Arabidopsis Gene Model[4] | Arabidopsis Gene Identifier |
|---|---|---|---|---|
| HORVU2Hr1G081670.4 | LD&SD_down | ATP-dependent 6-phosphofructokinase 2 | AT5G47810.1 | PFK2 |
| HORVU3Hr1G070300.2 | LD&SD_up | ATP-dependent 6-phosphofructokinase 3 | AT4G26270.1 | PFK3 |
| HORVU6Hr1G070270.5 | LD&SD_up | ATP-dependent 6-phosphofructokinase 7 | AT1G76550.1 | |
| HORVU5Hr1G041120.1 | LD&SD_up | Pyruvate kinase family protein | AT3G52990.1 | |
| HORVU5Hr1G058950.3 | LD&SD_up | enolase 1 | AT1G74030.1 | ENO1 |
| HORVU6Hr1G031830.4 | LD&SD_up | phosphoglycerate kinase | AT1G79550.2 | PGK |
| HORVU7Hr1G040550.1 | LD&SD_up | Pyrophosphate--fructose 6-phosphate 1-phosphotransferase | AT1G12000.1 | |
| HORVU4Hr1G019570.1 | LD_down, SD_up | fructose-bisphosphate aldolase 2 | AT4G38970.1 | FBA2 |
| **tricarboxylic acid cycle** | | | | |
| HORVU0Hr1G013850.1 | LD&SD_up | Succinate dehydrogenase [ubiquinone] flavor protein subunit, mitochondrial | AT5G66760.1 | SDH1-1 |
| HORVU2Hr1G006250.1 | LD&SD_up | Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex | AT5G55070.1 | |
| HORVU2Hr1G013170.1 | LD&SD_up | 2-oxoglutarate dehydrogenase, E1 component | AT3G55410.1 | |
| HORVU4Hr1G011850.1 | LD&SD_up | Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex | AT5G55070.1 | |
| HORVU3Hr1G073020.2 | LD&SD_down | phosphoenolpyruvate carboxylase 3 | AT3G14940.1 | ATPPC3 |
| HORVU1Hr1G050110.1 | LD&SD_up | Abscisic acid receptor PYR1 | AT4G17870.1 | PYR1, RCAR11 |

[1]Transcript identifier from barley (Mascher et al., 2017)

[2]Expression levels of mutants (*mat-c.907* and *mat-c.943*) compared with wildtype (Bonus) under LDs and SDs

[3]Annotation of *Arabidopsis* gene model (TAIR 10, from Digel et al. (2015))

[4]*Arabidopsi*s gene model (Best BLASTx hit from Digel et al. (2015))

## Table 4

Table 4: Selected DETs independent/dependent of photoperiod at W3.5 (FDR<0.01)

| Transcript[1] | Regulation[2] | Description[3] | Arabidopsis Gene Model[4] | Arabidopsis Gene Identifier |
|---|---|---|---|---|
| **DETs upregulated in mutants under LDs** | | | | |
| HORVU1Hr1G051660.8 | LD_up | MADS-box transcription factor 56 | AT2G45660.1 | AGL20, SOC1, ATSOC1 |
| HORVU4Hr1G067680.2 | LD_up | MADS-box transcription factor 1 | AT3G02310.1 | SEP2, AGL4 |
| HORVU6Hr1G066140.9 | LD_up | MADS-box transcription factor 6 | AT2G45650.1 | AGL-6 |
| HORVU7Hr1G054220.1 | LD_up | MADS-box transcription factor 7 | AT1G24260.1 | SEP3, AGL9 |
| HORVU2Hr1G007350.1 | LD_up | flowering promoting factor 1 | AT5G24860.1 | FPF1, ATFPF1 |
| HORVU2Hr1G072750.4 | LD_up | Protein TERMINAL FLOWER 1 | AT2G27550.1 | ATC |
| HORVU5Hr1G076770.4 | LD_up | Sugar transporter SWEET | AT5G13170.1 | SAG29, SWEET15, AtSWEET15 |
| HORVU2Hr1G106410.9 | LD_up | Glycogen synthase 1 | AT1G11720.2 | SS3 |
| HORVU6Hr1G074960.2 | LD_up | trehalose-6-phosphate phosphatase | AT1G35910.1 | TPPD |
| HORVU3Hr1G034440.2 | LD_up | Protein kinase superfamily protein | AT5G26751.1 | ATSK11, SK 11 |
| HORVU7Hr1G114650.5 | LD_up | Homeobox protein knotted-1-like 11 | AT5G25220.2 | KNAT3 |
| HORVU7Hr1G096250.1 | LD_up | high chlorophyll fluorescence phenotype 173 | AT1G16720.1 | HCF173 |
| **DETs downregulated in mutants under LDs** | | | | |
| HORVU2Hr1G093350.3 | LD_down | WRKY DNA-binding protein 13 | AT2G44745.1 | WRKY13 |
| HORVU6Hr1G061940.2 | LD_down | WRKY DNA-binding protein 13 | AT2G44745.1 | WRKY13 |
| HORVU3Hr1G114970.1 | LD_down | Two-component response regulator ARR1 | AT3G46640.2 | PCL1 |
| HORVU7Hr1G108970.1 | LD_down | Aberrant panicle organization 1 protein | AT1G30950.1 | UFO |
| HORVU2Hr1G032130.6 | LD_down | Nuclear transcription factor Y subunit A-5 | AT1G54160.1 | NFYA5, NF-YA5 |
| HORVU4Hr1G075830.4 | LD_down | Nuclear transcription factor Y subunit A-3 | AT1G72830.1 | HAP2C, ATHAP2C, NF-YA3 |
| HORVU5Hr1G007890.11 | LD_down | Nuclear transcription factor Y subunit A-10 | AT3G05690.1 | UNE8, ATHAP2B, HAP2B, NF-YA2 |
| HORVU2Hr1G077000.2 | LD_down | Two-component response regulator ORR1 | AT1G59940.1 | ARR3 |
| HORVU2Hr1G120490.2 | LD_down | Two-component response regulator ORR6 | AT5G62920.1 | ARR6 |
| HORVU3Hr1G108540.2 | LD_down | Two-component response regulator ARR8 | AT2G41310.1 | ATRR3, ARR8, RR3 |
| HORVU5Hr1G043090.1 | LD_down | Two-component response regulator ARR8 | AT2G41310.1 | ATRR3, ARR8, RR3 |
| **DETs upregulated in mutants under SDs** | | | | |
| HORVU0Hr1G011450.1 | SD_up | Stress responsive A/B Barrel Domain | AT5G22580.1 | |
| HORVU2Hr1G092530.12 | SD_up | Aldehyde dehydrogenase family 3 member F1 | AT4G36250.1 | ALDH3F1 |
| HORVU2Hr1G006910.10 | SD_up | Cytochrome P450 superfamily protein | AT3G48310.1 | CYP71A22 |
| HORVU7Hr1G008320.2 | SD_up | acyl-CoA-binding protein 6 | AT1G31812.1 | ACBP6, ACBP |
| HORVU4Hr1G002270.3 | SD_up | 5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase 2 | AT5G17920.2 | ATCIMS |
| HORVU5Hr1G006780.7 | SD_up | 5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase 1 | AT5G17920.2 | ATCIMS |
| HORVU7Hr1G040380.5 | SD_up | Chlorophyll a-b binding protein, chloroplastic | AT2G34420.1 | LHB1B2, LHCB1.5 |
| HORVU5Hr1G125600.1 | SD_up | Aquaporin-like superfamily protein | AT4G35100.2 | PIP3 |
| HORVU5Hr1G096440.1 | SD_up | cyclic nucleotide-gated channel 14 | AT5G15410.2 | DND1, ATCNGC2, CNGC2 |
| **DETs downregulation in mutants under SDs** | | | | |
| HORVU0Hr1G004060.1 | SD_down | VQ motif family protein | AT3G18690.1 | MKS1 |

Tables

| Transcript[1] | Regulation[2] | Description[3] | Arabidopsis Gene Model[4] | Arabidopsis Gene Identifier |
|---|---|---|---|---|
| HORVU2Hr1G065000.1 | SD_down | Encodes a protein involved in salt tolerance, names SIS (Salt Induced Serine rich). | AT5G02020.1 | SIS |
| HORVU1Hr1G075580.1 | SD_down | oxidative stress 3 | AT5G56550.1 | OXS3, ATOXS3 |
| HORVU4Hr1G072060.1 | SD_down | Heavy metal transport/detoxification superfamily protein | AT5G03380.2 | |
| HORVU2Hr1G011070.4 | SD_down | Heavy metal transport/detoxification superfamily protein | | NA |
| HORVU7Hr1G090240.1 | SD_down | F-box family protein | AT2G25490.1 | EBF1, FBL6 |
| HORVU3Hr1G078150.2 | SD_down | Ethylene-responsive transcription factor 4 | NA | NA |
| HORVU0Hr1G007050.2 | SD_down | Ethylene-responsive transcription factor 4 | AT5G44210.1 | ERF9, ATERF9, ATERF-9 |
| HORVU0Hr1G016780.1 | SD_down | Cytochrome P450 superfamily protein | AT4G19230.1 | CYP707A1 |
| HORVU5Hr1G056130.2 | SD_down | WRKY family transcription factor | AT2G46400.1 | WRKY46, ATWRKY46 |
| HORVU5Hr1G058300.1 | SD_down | trehalose-6-phosphate phosphatase | AT4G39770.1 | TPPH |
| HORVU7Hr1G033230.8 | SD_down | sucrose synthase 4 | AT3G43190.1 | SUS4, ATSUS4 |
| HORVU7Hr1G053260.3 | SD_down | UDP-glucose 4-epimerase | AT3G23820.1 | GAE6 |
| **DETs independent of photoperiod** | | | | |
| HORVU7Hr1G025700.6 | LD&SD_up | MADS-box transcription factor 5 | AT5G15800.1 | AtSEP1 |
| HORVU2Hr1G035160.4 | LD&SD_Down | LOB domain-containing protein 37 | AT5G67420.1 | AtLBD37 |
| HORVU3Hr1G082580.2 | LD&SD_Down | Protein NRT1/ PTR FAMILY 5.10 | AT1G22540.1 | |
| HORVU7Hr1G092710.1 | LD&SD_Down | glutamine dumper 4 | AT2G24762.1 | AtGDU4 |

[1]Transcript identifier from barley (Mascher et al., 2017)

[2]Expression levels of mutants (*mat-c.907* and *mat-c.943*) compared with wildtype (Bonus) under LDs and SDs

[3]Annotation of *Arabidopsis* gene model (TAIR 10, from Digel et al. (2015))

[4]*Arabidopsi*s gene model (Best BLASTx hit from Digel et al. (2015))

## Table 5

Table 5 Selected* DETs involved in floral development and identity at W3.5 at less stringent cut-off (FDR<0.05)

| Transcript[1] | Regulation[2] | Description[3] | Arabidopsis Gene Model[4] | Arabidopsis Gene Identifier | Rice Gene Identifier |
|---|---|---|---|---|---|
| HORVU5Hr1G095710.1 | LD&SD_up | K-box region and MADS-box transcription factor family protein | AT5G15800.1 | SEP1, AGL2 | OsMADS56 |
| HORVU7Hr1G025700.6 | LD&SD_up | K-box region and MADS-box transcription factor family protein | AT5G15800.1 | SEP1, AGL2 | OsMADS4 |
| HORVU4Hr1G067680.2 | LD_up | K-box region and MADS-box transcription factor family protein | AT3G02310.1 | SEP2, AGL4 | OsMADS34 |
| HORVU5Hr1G076400.1 | LD_up | K-box region and MADS-box transcription factor family protein | AT1G24260.1 | SEP3, AGL9 | NA |
| HORVU7Hr1G054220.1 | LD_up | K-box region and MADS-box transcription factor family protein | AT1G24260.1 | SEP3, AGL9 | OsMADS22 |
| HORVU1Hr1G063620.2 | LD_up | K-box region and MADS-box transcription factor family protein | AT5G20240.1 | PI | OsMADS1 |
| HORVU2Hr1G063800.7 | LD_up | K-box region and MADS-box transcription factor family protein | AT1G69120.1 | AP1, AGL7 | OsMADS8 |
| HORVU1Hr1G051660.8 | LD_up | AGAMOUS-like 20 | AT2G45660.1 | AGL20, SOC1, ATSOC1 | OsMADS15 |
| HORVU6Hr1G066140.9 | LD_up | AGAMOUS-like 6 | AT2G45650.1 | AGL6 | OsMADS5 |
| HORVU7Hr1G036130.1 | LD_down | K-box region and MADS-box transcription factor family protein | AT2G22540.1 | SVP, AGL22 | OsMADS7 |

[1]Transcript identifier from barley (Mascher et al., 2017)

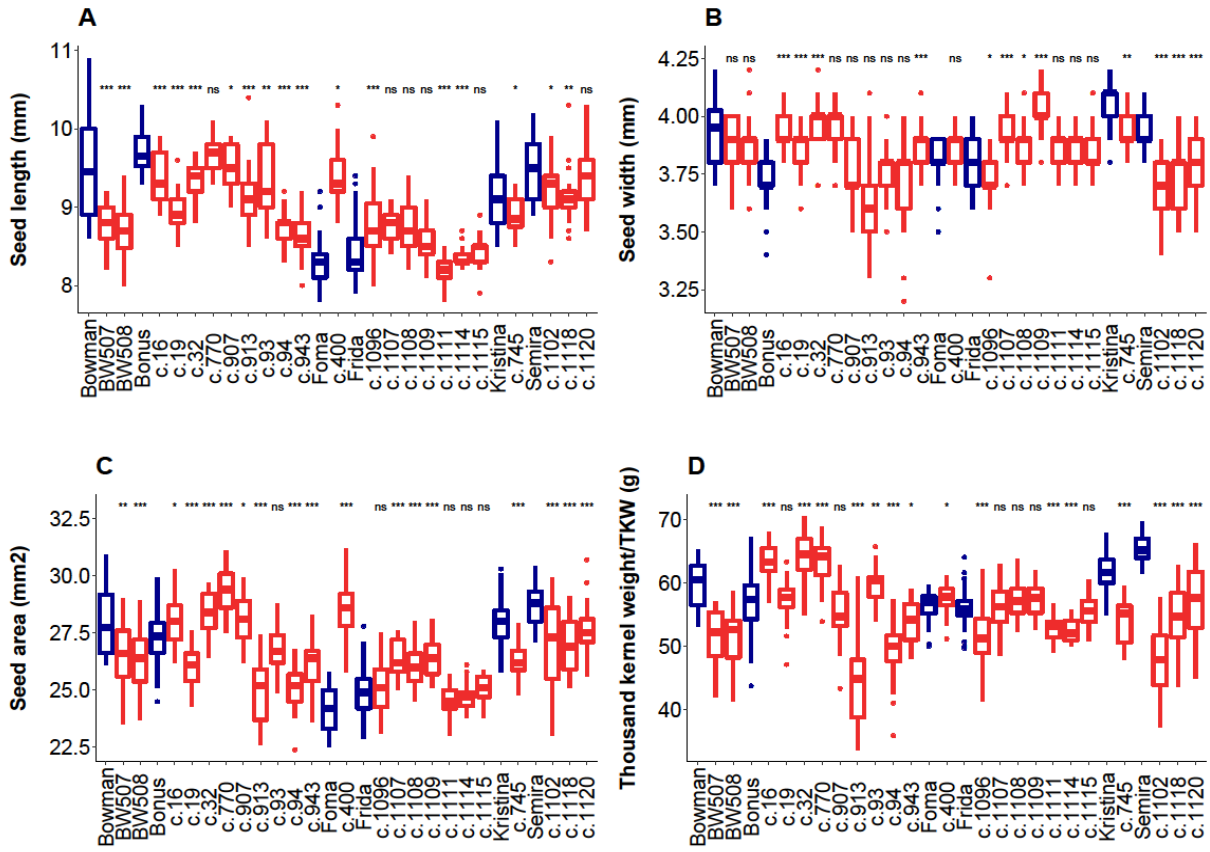
[2]Expression levels of mutants (*mat-c.907* and *mat-c.943*) compared with wildtype (Bonus) under LDs and SDs

[3]Annotation of *Arabidopsis* gene model (TAIR 10, from Digel et al. (2015))

[4]*Arabidopsi*s gene model (Best BLASTx hit from Digel et al. (2015))

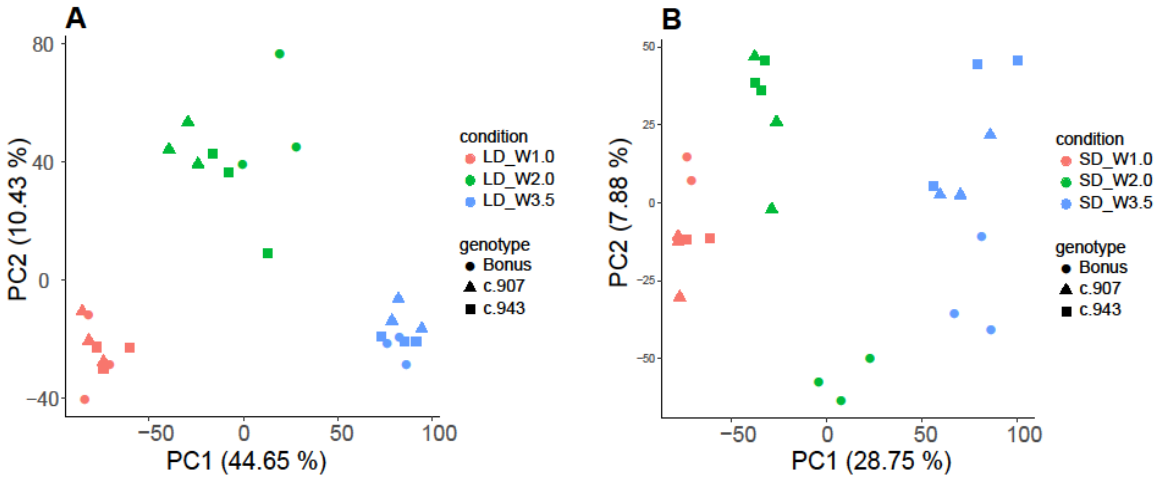
# Supplemental figures

## Supplemental figure 1



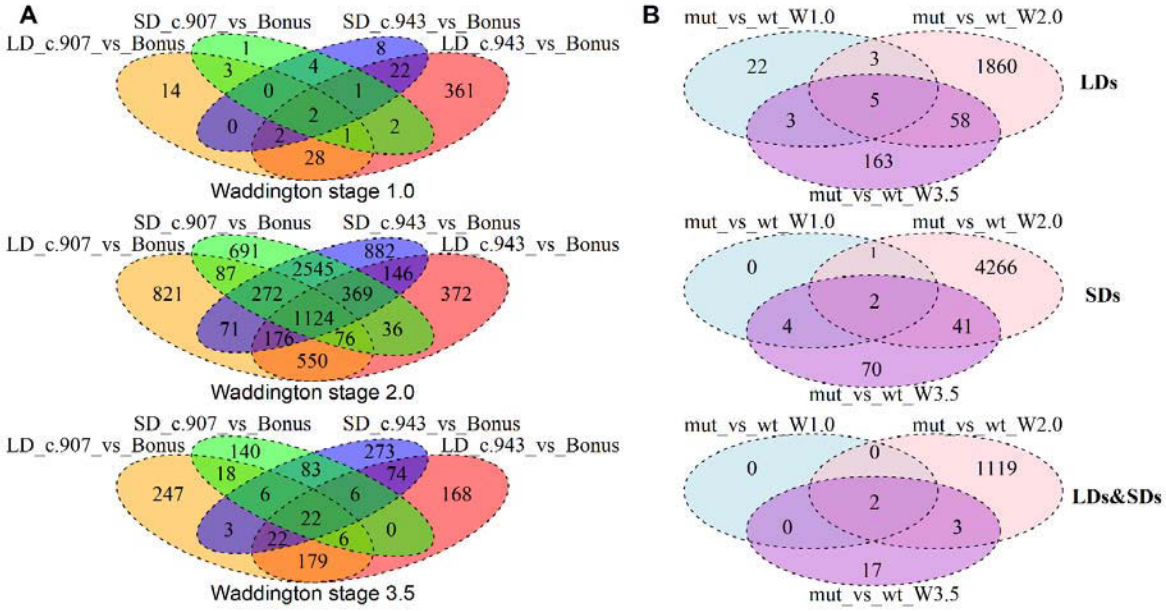

**Supplemental figure 1. Seed parameters of *hvcen* (*mat-c*) mutants trialed under outdoor condition.** A) Seed length, B) seed width, C) seed area and D) thousand kernel weight (TKW) in field condition in one year, 5 plants per genotype and 5 representative spikes per plant were analyzed. Significant differences between the mutants and their wild type parents were calculated using student t test, p<0.001 "***", P<0.01 "**", P<0.05 "*", not significant: "ns".

## Supplemental figure 2



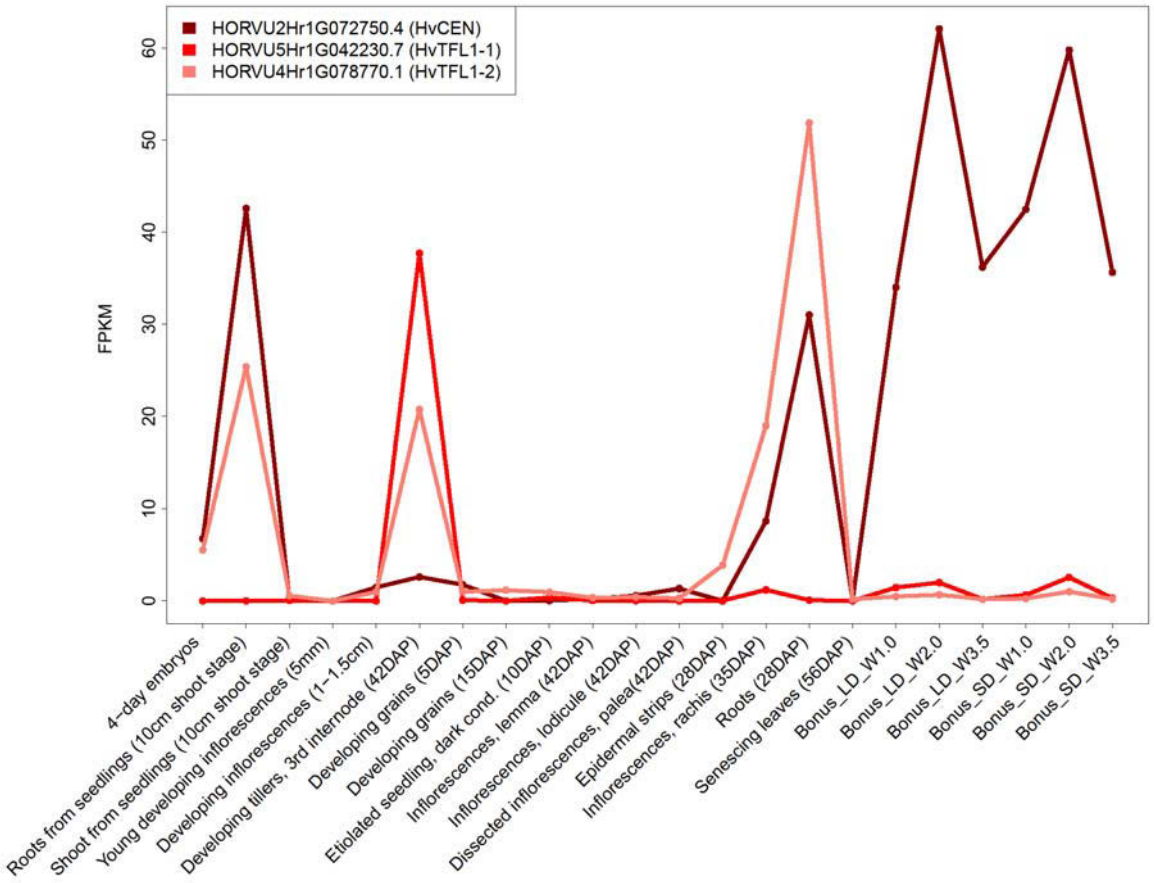

**Supplemental figure 2. PCA of normalized expression profiles for all expressed genes under LDs and SDs**. The first two PCs account for ~40% of the overall variation under A) LDs, 31% of the overall variation under B) SDs. Transcripts with number of read >=5 were considered as expressed genes.

## Supplemental figure 3



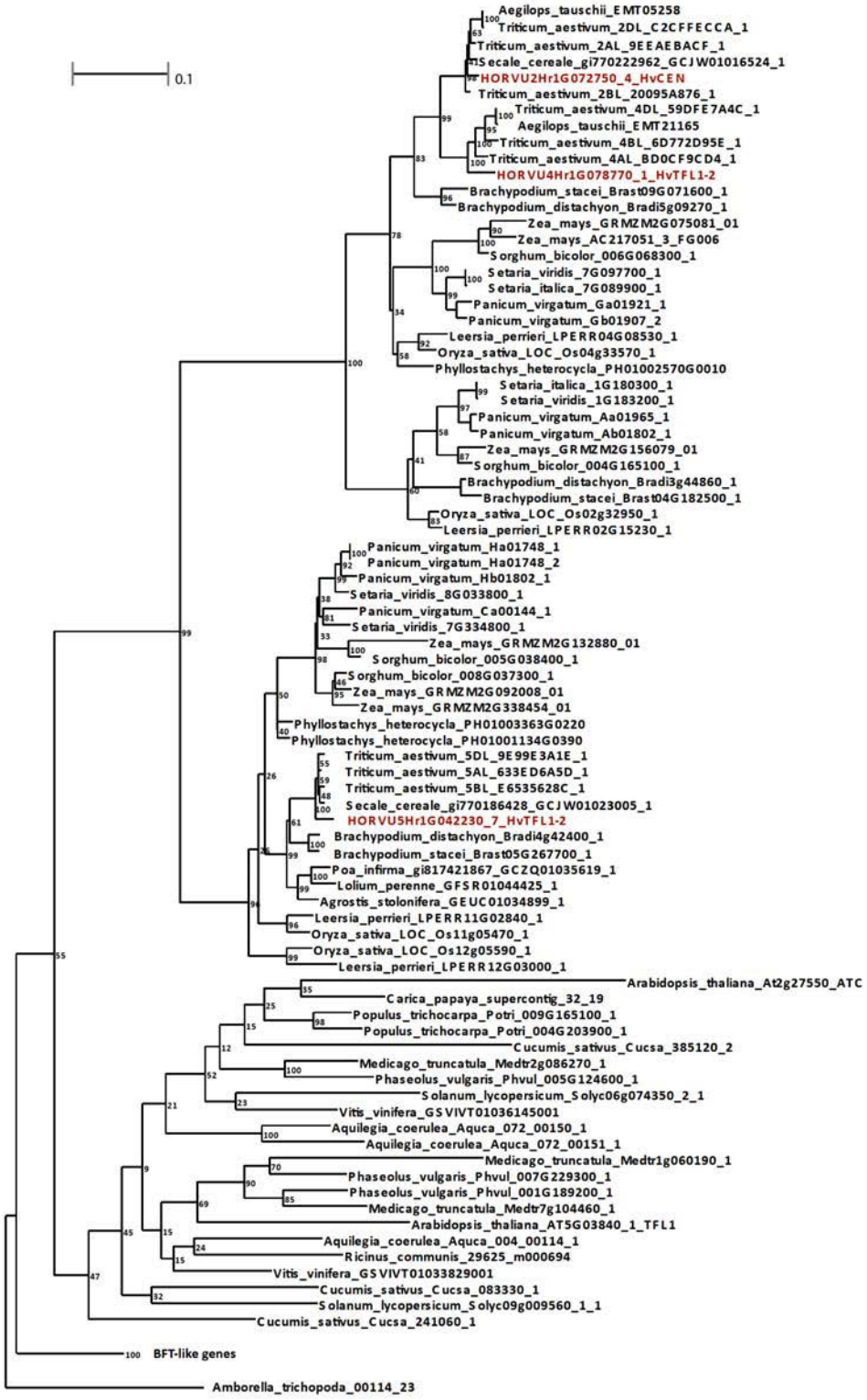

**Supplemental figure 3.** A) DETs in each mutant (*mat-c.907* or *mat-c.943*) compared with wildtype (Bonus) under each photoperiod (LDs, SDs) at W1.0, W2.0 and W3.5; B) DETs were found in both mutants at 3 Waddington stages under LDs, SDs, and both LDs and SDs.

## Supplemental figure 4



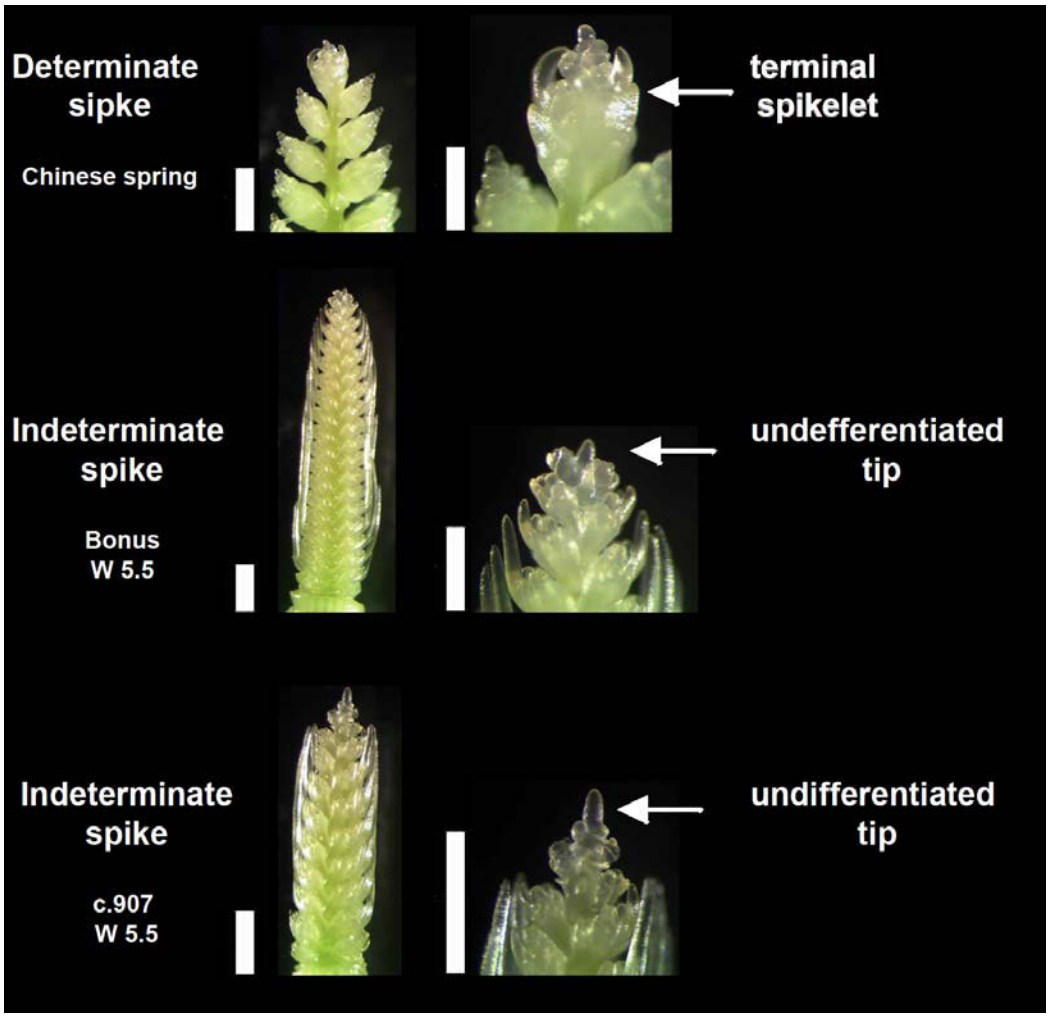

**Supplemental figure 4.** Expression of *HvCEN* and its two paralogs *HvTFL1-1* and *HvTFL1-2* in different tissues. The expression data was obtained from the Barley Genome Explorer (https://apex.ipk-gatersleben.de/apex/f?p=284:10::::::) and the expression profiles of Bonus in this study.

## Supplemental figure 5



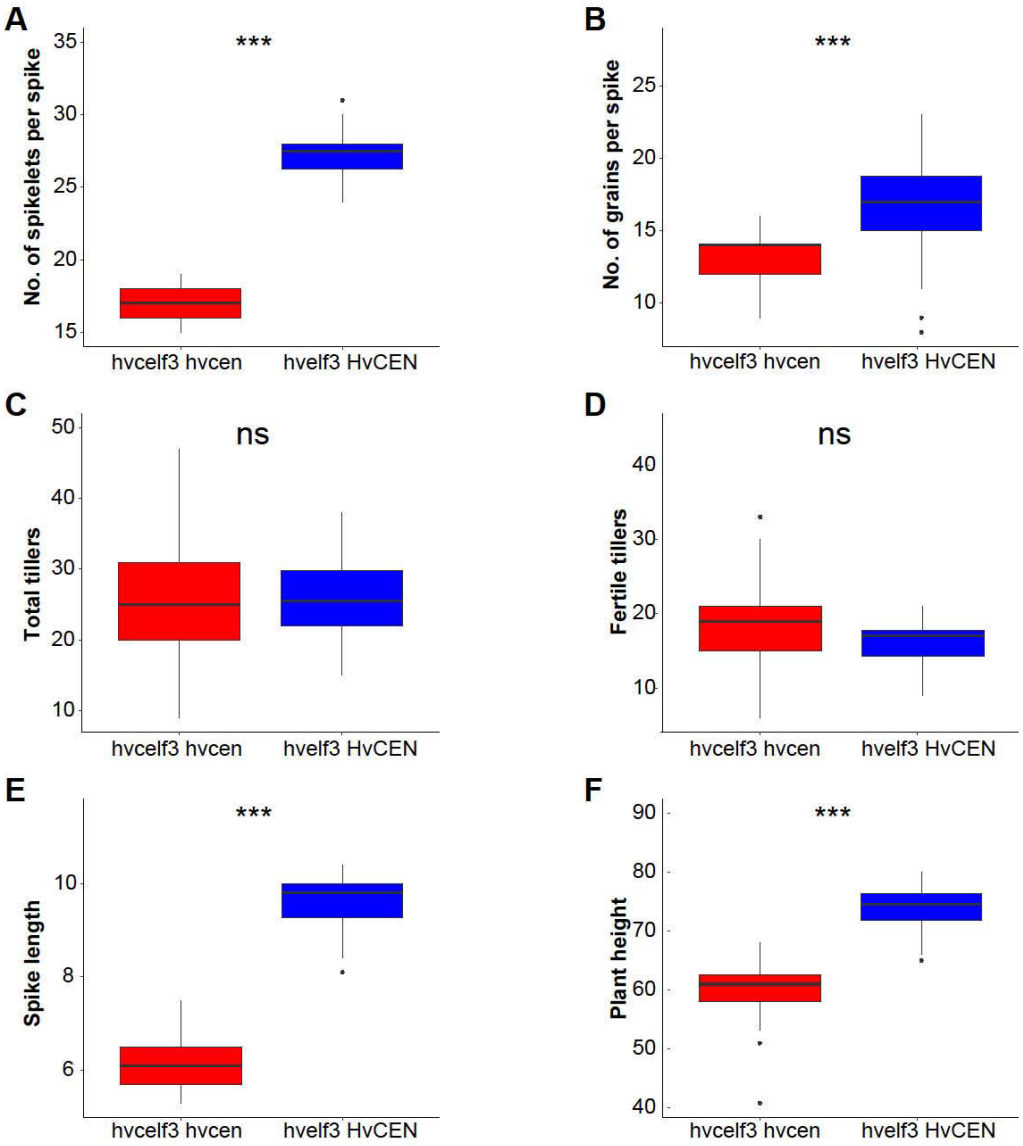

**Supplemental figure 5.** Phylogenetic tree of TFL1 homologs in different species using the TFL1 homolog in *Amborella trichopoda* as outgroup.

## Supplemental figure 6




**Supplemental figure 6.** Determinate spike with a terminal spikelet in wheat (chinese spring) and indeterminate spike without terminal spikelet in barley (*mat-c.907* and Bonus).

## Supplemental figure 7




**Supplemental figure 7. Phenotypes of double mutant *hvelf3 hvcen* and *hvelf3 HvCEN***. Plants were moved to LDs after 2 weeks' growth under SDs. Significant differences between the mutants and their wild type parents were calculated using student t test, p<0.001 "***",not significant: "ns". Number of replicates >=14. LD: long day (16h/8h, light/dark), SD: (8h/16h, dark/light).

# Supplemental tables

## Supplemental table 1

Supplemental table 1 *hvcen* mutants carrying different type of mutations of *HvCEN*

| name | mutation | NGB number | mutagens | number of replicates in year2014 / 2015 |
|---|---|---|---|---|
| Semira(WT) | - | - | - | 16 / 16 |
| *mat-c.1118* | 12bp deletion | 119566 | X-rays | 5 / 16 |
| *mat-c.1102* | large deletion | 117633 | neutrons | 6 / 16 |
| *mat-c.1120* | large deletion | 119568 | neutrons | 6 / 16 |
| Foma(WT) | - | - | - | 16 / 14 |
| *mat-c.400* | R139W | 110400 | ethylene imine | 0 / 9 |
| Bonus(WT) | - | - | - | 16 / 15 |
| *mat-c.16* | large deletion | 110016 | neutrons | 0 / 8 |
| *mat-c.19* | large deletion | 110019 | neutrons | 0 / 10 |
| *mat-c.32* | P113L | 110032 | ethylene imine | 0 / 8 |
| *mat-c.770* | STOP | 110770 | sodium azide | 0 / 8 |
| *mat-c.907* | G116D | 116862 | sodium azide | 16 / 8 |
| *mat-c.943* | P52S | 117474 | sodium azide | 16 / 8 |
| *mat-c.94* | splice site | 110094 | ethyl methaanesulfonate | 16 / 8 |
| *mat-c.913* | D71N | 117444 | sodium azide | 16 / 8 |
| *mat-c.93* | S78N | 110093 | ethyl methaanesulfonate | 15 / 10 |
| Frida | - | - | - | 0 / 8 |
| *mat-c.1096* | large deletion | 117627 | X-rays | 0 / 12 |
| *mat-c.1107* | large deletion | 119555 | X-rays | 0 / 8 |
| *mat-c.1108* | large deletion | 119556 | X-rays | 0 / 10 |
| *mat-c.1109* | 1bp deletion | 119557 | X-rays | 0 / 8 |
| *mat-c.1111* | Splice site | 119559 | sodium azide | 0 / 9 |
| *mat-c.1114* | Splice site | 119562 | sodium azide | 0 / 8 |
| *mat-c.1115* | R83W | 119563 | sodium azide | 0 / 8 |
| Kristina | - | | | 0 / 8 |
| *mat-c.745* | P52T | 110745 | Iso-propyl methanesulfanate | 0 / 8 |
| Bowman | _ | _ | | 0 / 24 |
| *BW507* | large deletion | GSHO2282 | mat-c.19 introgressed into Bowman | 0 / 8 |
| *BW508* | large deletion | GSHO2283 | mat-b.7 introgressed into Bowman | 0 / 10 |

## Supplemental table 2

Supplemental table 2 : Effects of amino acid substitutions in the *mat-c* (*hvcen*) mutants

| name | mutation | background | PROVEAN SCORE | mutagens |
|---|---|---|---|---|
| *mat-c.943* | P52S | Bonus | -6.417 | putative 14-3-3 protein interaction site |
| *mat-c.913* | D71N | Bonus | -4.217 | putative ligand binding pocket |
| *mat-c.93* | S78N | Bonus | -2.62 | putative ligand binding pocket |
| *mat-c.32* | P113L | Bonus | -9.575 | putative ligand binding pocket |
| *mat-c.907* | G116D | Bonus | -6.719 | putative ligand binding pocket |
| *mat-400* | R139W | Foma | -7.081 | putative external loop |
| *mat-1115* | R83W | Frida | -6.816 | putative ligand binding pocket |
| *mat-c.745* | P52T | Kristina | -6.458 | putative 14-3-3 protein interaction site |

The PROVEAN score indicates the extent of conservation where a score lower than -2.5 is identified as conserved amino acids were substituted.

## Supplemental table 3

Supplemental Table 3 Primers used in qRT-PCR and genotyping

| Gene | Forward primer 5’ -> 3’ | Reverse primer 5’ -> 3’ | Reference |
|---|---|---|---|
| *HvActin* (qRT-PCR) | CGTGTTGGATTCTGGTGATG | AGCCACATATGCGAGCTTCT | Campoli et al. 2012 |
| *HvFT1* (qRT-PCR) | GGTAGACCCAGATGCTCCAA | TCGTAGCACATCACCTCCTG | Digel et al. 2015 |
| *HvFT3* winter (genotyping) | GTCCTCCTCCAGTATATGTC | CTACTCCCCTTGAGAACTTTC | Kikuchi et al. 2009 |
| *HvFT3* spring (genotyping) | AAGGCTGTTAATTGGTAGTCCTCC | CTGCACATTATTTGTGATGCAA | Kikuchi et al. 2009 |
| *HvCEN* (genotyping) | TCCCATGGACATAAAACTTGCC | GCCATGCATGCAAGAGAAGA | This study |

## Supplemental table 4

Supplemental table 4 Normalized expression value (cpm), log2FC, FDR and annotation of all expressed transcripts

https://www.dropbox.com/s/tydqf86d4kaqxhb/Supplemental%20table%204.xlsx?dl=0

# References

**Abe, M.** (2005). FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. Science. **309**: 1052–1056.

**Abebe, T., Wise, R.P., and Skadsen, R.W.** (2009). Comparative transcriptional profiling established the awn as the major photosynthetic organ of the barley spike while the lemma and the palea primarily protect the seed. Plant Genome J. **2**: 247.

**Agrawal, G.K., Abe, K., Yamazaki, M., Miyao, A., and Hirochika, H.** (2005). Conservation of the E-function for loral organ identity in rice revealed by the analysis of tissue culture-induced loss-of-function mutants of the OsMADS1 gene. Plant Mol. Biol. **59**: 125–135.

**Aguilar-Martinez, J.A., Poza-Carrion, C., and Cubas, P.** (2007). Arabidopsis BRANCHED1 acts as an integrator of branching signals within axillary buds. The Plant Cell **19**: 458–472.

**Agusti, J. and Greb, T.** (2013). Going with the wind - Adaptive dynamics of plant secondary meristems. Mech. Dev. **130**: 34–44.

**Ahn, J.H., Miller, D., Winter, V.J., Banfield, M.J., Lee, J.H., Yoo, S.Y., Henz, S.R., Brady, R.L., and Weigel, D.** (2006). A divergent external loop confers antagonistic activity on floral regulators FT and TFL1. EMBO J. **25**: 605–14.

**Alaoui, A.C. El, Simmons, S.R., and Crookston, R.K.** (1988). Effects of tiller removal on spring barley. Crop Sci. **28**: 305–307.

**Alqudah, A.M. and Schnurbusch, T.** (2014). Awn primordium to tipping is the most decisive developmental phase for spikelet survival in barley. Funct. Plant Biol. **41**: 424–436.

**Alvarez-Venegas, R., Pien, S., Sadder, M., Witmer, X., Grossniklaus, U., and Avramova, Z.** (2003). ATX-1, an Arabidopsis homolog of trithorax, activates flower homeotic genes. Curr. Biol. **13**: 627–637.

**Amaya, I., Ratcliffe, O.J., and Bradley, D.J.** (1999). Expression of CENTRORADIALIS (CEN) and CEN-like genes in tobacco reveals a conserved mechanism controlling phase change in diverse species. The Plant Cell **11**: 1405–1418.

**Andrés, F. and Coupland, G.** (2012). The genetic basis of flowering responses to seasonal cues. Nat. Rev. Genet. **13**: 627–639.

**Araki, T.** (2001). Transition from vegetative to reproductive phase. Curr. Opin. Plant Biol. **4**: 63–68.

**Arite, T., Iwata, H., Ohshima, K., Maekawa, M., Nakajima, M., Kojima, M., Sakakibara, H., and Kyozuka, J.** (2007). DWARF10, an RMS1/MAX4/DAD1 ortholog, controls lateral bud outgrowth in rice. Plant J. **51**: 1019–1029.

**Arite, T., Umehara, M., Ishikawa, S., Hanada, A., Maekawa, M., Yamaguchi, S., and Kyozuka, J.** (2009). D14, a strigolactone-Insensitive mutant of rice, shows an accelerated outgrowth of tillers. Plant Cell Physiol. **50**: 1416–1424.

**Ashikari, M.** (2005). Cytokinin oxidase regulates rice grain production. Science. **309**: 741–745.

**Babb, S. and Muehlbauer, G.** (2003). Genetic and morphological characterization of the barley uniculm2 (cul2) mutant. Theor. Appl. Genet. **106**: 846–857.

**Badra, A. and Klinck, H.R.** (1981). The influence of inter-plant competition on the morphological development of spikes of non-tillering barley (Hordeum vulgare L. emend Lam). Can. J. plant sci **61**: 829-835.

**Banfield, M.. and Brady, R..** (2000). The structure of Antirrhinum centroradialis protein (CEN) suggests a role as a kinase regulator1. J. Mol. Biol. **297**: 1159–1170.

**Benbelkacem, A., Mekni, M.S., and Rasmusson, D.C.** (1984). Breeding for high tiller number and yield in barley1. Crop Sci. **24**: 968–972.

**Berr, A., Shafiq, S., and Shen, W.-H.** (2011). Histone modifications in transcriptional activation during plant development. Biochim. Biophys. Acta - Gene Regul. Mech. **1809**: 567–576.

**Beveridge, C.A. and Kyozuka, J.** (2010). New genes in the strigolactone-related shoot branching pathway. Curr. Opin. Plant Biol. **13**: 34–39.

**Bhavsar, R.B., Makley, L.N., and Tsonis, P.A.** (2010). The other lives of ribosomal proteins. Hum Genomics **4**: 327–344.

**Blanvillain, R., Kim, J.H., Wu, S., Lima, A., and Ow, D.W.** (2009). OXIDATIVE STRESS 3 is a chromatin-associated factor involved in tolerance to heavy metals and oxidative stress. Plant J. **57**: 654–665.

**Blázquez, M.A., Ahn, J.H., and Weigel, D.** (2003). A thermosensory pathway controlling flowering time in Arabidopsis thaliana.

Nat. Genet. **33**: 168–171.

**Boden, S.A., Cavanagh, C., Cullis, B.R., Ramm, K., Greenwood, J., Jean Finnegan, E., Trevaskis, B., and Swain, S.M.** (2015). Ppd-1 is a key regulator of inflorescence architecture and paired spikelet development in wheat. Nat. Plants **1**: 1–6.

**Bommert, P. and Whipple, C.** (2017). Grass inflorescence architecture and meristem determinacy. Semin. Cell Dev. Biol. Academic Press. **79**: 37-47

**Bonnett, O.T. (1935).** The development of the barley spike. J. Agric. Res. **51**: 451-457.

**Bonnett, O.T. (1936).** The development of the wheat spike. J. Agric. Res. **53**: 445–451.

**Booker, J., Auldridge, M., Wills, S., McCarty, D., Klee, H., and Ottoline, L.** (2004). MAX3/CCD7 Is a Carotenoid cleavage dioxygenase required for the synthesis of a novel plant signaling molecule. Curr. Biol. **14**: 1232–1238.

**Bortiri, E., Chuck, G., Vollbrecht, E., Rocheford, T., Martienssen, R., and Hake, S.** (2006). ramosa2 encodes a LATERAL ORGAN BOUNDARY domain protein that determines the fate of stem cells in branch meristems of maize. The Plant Cell **18**: 574-585.

**Bortiri, E. and Hake, S.** (2007). Flowering and determinacy in maize. J. Exp. Bot. **58**: 909–916.

**von Bothmer, R. von, Jacobsen, N., Baden, C., Jørgensen, R.B., and Linde-Laursen, I.** (1995). An ecogeographical study of the genus Hordeum. Systematic and ecogeographic studies on crop genepools 7. Int. Plant Genet. Resour. Institute, Rome.

**Bouveret, R., Schönrock, N., Gruissem, W., and Hennig, L.** (2006). Regulation of flowering time by Arabidopsis MSI1. Development **133**: 1693-1702.

**Bowman, J.L., Alvarez, J., Weigel, D., Meyerowitz, E.M., and Smyth, D.R.** (1993). Control of flower development in Arabidopsis thaliana by APETALA1 and interacting genes. Development **119**: 721–743.

**Bradley, D., Carpenter, R., Copsey, L., Vincent, C., Rothstein, S., and Coen, E.** (1996). Control of inflorescence architecture in Antirrhinum. Nature **379**: 791–797.

**Bradley, D., Ratcliffe, O., Vincent, C., Carpenter, R., and Coen, E.** (1997). Inflorescence commitment and architecture in arabidopsis. Science. **275**: 80–83.

**Byrne, M.E.** (2003). Phyllotactic pattern and stem cell fate are determined by the Arabidopsis homeobox gene BELLRINGER. Development **130**: 3941–3950.

**Campoli, C. and von Korff, M.** (2014). Genetic control of reproductive development in temperate cereals. In Advances in botanical research **72**: 131–158.

**Campoli, C., Pankin, A., Drosse, B., Casao, C.M., Davis, S.J., and Von Korff, M.** (2013). HvLUX1 is a candidate gene underlying the early maturity 10 locus in barley: Phylogeny, diversity, and interactions with the circadian clock and photoperiodic pathways. New Phytol. **199**: 1045–1059.

**Casao, M.C., Igartua, E., Karsai, I., Lasa, J.M., Gracia, M.P., and Casas, A.M.** (2011a). Expression analysis of vernalization and day-length response genes in barley (Hordeum vulgare L.) indicates that VRNH2 is a repressor of PPDH2 (HvFT3) under long days. J. Exp. Bot. **62**: 1939–1949.

**Casao, M.C., Karsai, I., Igartua, E., Gracia, M.P., Veisz, O., and Casas, A.M.** (2011b). Adaptation of barley to mild winters: A role for PPDH2. BMC Plant Biol. **11**: 164.

**Chanvivattana, Y.** (2004). Interaction of Polycomb-group proteins controlling flowering in Arabidopsis. Development **131**: 5263–5276.

**Chen, A. and Dubcovsky, J.** (2012). Wheat TILLING mutants show that the vernalization gene VRN1 down-regulates the flowering repressor VRN2 in leaves but Is not essential for flowering. PLoS Genet. **8**.

**Chen, Z.X., Wu, J.G., Ding, W.N., Chen, H.M., Wu, P., and Shi, C.H.** (2006). Morphogenesis and molecular basis on naked seed rice, a novel homeotic mutation of OsMADS1 regulating transcript level of AP3 homologue in rice. Planta **223**: 882–890.

**Choi, J. and Hwang, I.** (2007). Cytokinin: perception, signal transduction, and role in plant growth and development. J. Plant Biol. **50**: 98–108.

**Choi, M.S., Woo, M.O., Koh, E.B., Lee, J., Ham, T.H., Seo, H.S., and Koh, H.J.** (2012). Teosinte Branched 1 modulates tillering in rice plants. Plant Cell Rep. **31**: 57–65.

**Choi, S. and Kwon, S.** (1985). Evaluation of varietal difference and environmental variation for some characters related to source and sink in the rice plants. Korean J. Crop Sci. (Korea R.) **30**: 460–470.

**Choi, S.C., Lee, S., Kim, S.-R., Lee, Y.-S., Liu, C., Cao, X., and An, G.** (2014). Trithorax group protein Oryza sativa trithorax1 controls flowering time in rice via interaction with early heading date3. Plant Physiol. **164**: 1326–1337.

**Chono, M.** (2003). A semidwarf phenotype of barley uzu results from a nucleotide substitution in the gene encoding a putative brassinosteroid receptor. Plant Physiol. **133**: 1209–1219.

**Chuck, G.** (2002). The control of spikelet meristem identity by the branched silkless1 gene in maize. Science. **298**: 1238–1241.

**Ciaffi, M., Paolacci, A.R., Tanzarella, O.A., and Porceddu, E.** (2011). Molecular aspects of flower development in grasses. Sex. Plant Reprod. **24**: 247–282.

**Close, T.J.** (2004). A new resource for cereal genomics: 22K barley GeneChip comes of age. Plant Physiol. **134**: 960–968.

**Coen, E.S. and Meyerowitz, E.M.** (1991). The war of the whorls: genetic interactions controlling flower development. Nature **353**: 31–37.

**Cole, M., Nolte, C., and Werr, W.** (2006). Nuclear import of the transcription factor SHOOT MERISTEMLESS depends on heterodimerization with BLH proteins expressed in discrete sub-domains of the shoot apical meristem of Arabidopsis thaliana. Nucleic Acids Res. **34**: 1281–1292.

**Colombo, L., Marziani, G., Masiero, S., Wittich, P.E., Schmidt, R.J., Gorla, M.S., and Enrico Pè, M.** (1998). BRANCHED SILKLESS mediates the transition from spikelet to floral meristem during Zea mays ear development. Plant J. **16**: 355–363.

**Comadran, J. et al.** (2012). Natural variation in a homolog of Antirrhinum CENTRORADIALIS contributed to spring growth habit and environmental adaptation in cultivated barley. Nat. Genet. **44**: 1388–1392.

**Conesa, A., Götz, S., García-Gómez, J.M., Terol, J., Talón, M., and Robles, M.** (2005). Blast2GO: A universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics **21**: 3674–3676.

**Conrad, L.J., Khanday, I., Johnson, C., Guiderdoni, E., An, G., Vijayraghavan, U., and Sundaresan, V.** (2014). The polycomb group gene EMF2B is essential for maintenance of floral meristem determinacy in rice. Plant J. **80**: 883–894.

**Conti, L. and Bradley, D.** (2007). TERMINAL FLOWER1 is a mobile signal controlling Arabidopsis architecture. The Plant Cell **19**: 767–778.

**Corbesier, L. and Coupland, G.** (2006). The quest for florigen: a review of recent progress. J. Exp. Bot. **57**: 3395–3403.

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

**Corbit, K.C., Trakul, N., Eves, E.M., Diaz, B., Marshall, M., and Rosner, M.R.** (2003). Activation of Raf-1 signaling by protein kinase C through a mechanism involving Raf kinase inhibitory protein. J. Biol. Chem. **278**: 13061—13068.

**Cui, R., Han, J., Zhao, S., Su, K., Wu, F., Du, X., Xu, Q., Chong, K., Theißen, G., and Meng, Z.** (2010). Functional conservation and diversification of class e floral homeotic genes in rice (Oryza sativa). Plant J. **61**: 767–781.

**Dabbert, T., Okagaki, R.J., Cho, S., Boddu, J., and Muehlbauer, G.J.** (2009). The genetics of barley low-tillering mutants: Absent lower laterals (als). Theor. Appl. Genet. **118**: 1351–1360.

**Dabbert, T., Okagaki, R.J., Cho, S., Heinen, S., Boddu, J., and Muehlbauer, G.J.** (2010). The genetics of barley low-tillering mutants: Low number of tillers-1 (lnt1). Theor. Appl. Genet. **121**: 705–715.

**Danilevskaya, O.N., Meng, X., and Ananiev, E. V** (2010). Concerted modification of flowering time and inflorescence architecture by ectopic expression of TFL1-like genes in maize. Plant Physiol. **153**: 238–251.

**Danilevskaya, O.N., Meng, X., Hou, Z., Ananiev, E. V., and Simmons, C.R.** (2007). A Genomic and Expression Compendium of the Expanded PEBP Gene Family from Maize. Plant Physiol. **146**: 250–264.

**Derbyshire, P. and Byrne, M.E.** (2013). MORE SPIKELETS1 is required for spikelet fate in the inflorescence of brachypodium. Plant Physiol. **161**: 1291–1302.

**Derkacheva, M., Steinbach, Y., Wildhaber, T., Mozgová, I., Mahrez, W., Nanni, P., Bischof, S., Gruissem, W., and Hennig, L.** (2013). Arabidopsis MSI1 connects LHP1 to PRC2 complexes. EMBO J. **32**: 2073–2085.

**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.

**Ding, Z.J., Yan, J.Y., Xu, X.Y., Yu, D.Q., Li, G.X., Zhang, S.Q., and Zheng, S.J.** (2014). Transcription factor WRKY46 regulates osmotic stress responses and stomatal movement independently in Arabidopsis. Plant J. **79**: 13–27.

**Doebley, J., Stec, A., and Gustus, C.** (1995). teosinte branched1 and the origin of maize: Evidence for epistasis and the evolution of dominance. Genetics **141**: 333–346.

**Donald, C.M.** (1979). A barley breeding programme based on an ideotype. J. Agric. Sci. **93**: 261.

**Donald, C.M.** (1968). The breeding of crop ideotypes. Euphytica **17**: 385–403.

**Drosse, B., Campoli, C., Mulki, A., and Korff, M. Von** (2014). Genetic control of reproductive development. Biotechnological approaches to barley improvement, Biotechnology in Agriculture and Forestry. **69.**

**Druka, A., Franckowiak, J., Lundqvist, U., Bonar, N., Alexander, J., Houston, K., Radovic, S., Shahinnia, F., Vendramin, V., Morgante, M., Stein, N., and Waugh, R.** (2011). Genetic dissection of barley morphology and development. Plant Physiol. **155**: 617–627.

**Ejaz, M. and von Korff, M.** (2017). The genetic control of reproductive development under high ambient temperature. Plant Physiol. **173**: 294–306.

**van Esse, G.W., Walla, A., Finke, A., Koornneef, M., Pecinka, A., and von Korff, M.** (2017). Six-rowed spike3 (VRS3) is a histone demethylase that controls lateral spikelet development in barley. Plant Physiol. **174**: 2397–2408.

**Esumi, T., Hagihara, C., Kitamura, Y., Yamane, H., and Tao, R.** (2009). Identification of an FT ortholog in Japanese apricot (Prunus mume Sieb. et Zucc.). J. Hortic. Sci. Biotechnol. **84**: 149–154.

**Fang, Z., Gonzales, A.M., Clegg, M.T., Smith, K.P., Muehlbauer, G.J., Steffenson, B.J., and Morrell, P.L.** (2014). Two genomic regions contribute disproportionately to geographic differentiation in wild barley. G3: Genes, Genomes, Genetics **4**: 1193–1203.

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

**Feng, N. et al.** (2017). Transcriptome profiling of wheat inflorescence development from spikelet initiation to floral patterning identified stage-specific regulatory genes. Plant Physiol. **174**: 1779–1794.

**Fiil, B.K. and Petersen, M.** (2011). Constitutive expression of MKS1 confers susceptibility to Botrytis cinerea infection independent of PAD3 expression. Plant Signal. Behav. **6**: 1425–1427.

**Forster, B.P., Franckowiak, J.D., Lundqvist, U., Lyon, J., Pitkethly, I., and Thomas, W.T.B.** (2007). The barley phytomer. Ann. Bot. **100**: 725–733.

**Foucher, F., Morin, J., Courtiade, J., Cadioux, S., Ellis, N., Banfield, M.J., and Rameau, C.** (2003). DETERMINATE and LATE FLOWERING are two TERMINAL FLOWER1/CENTRORADIALIS homologs that control two distinct phases of flowering initiation and development in pea. The Plant Cell **15**: 2742-2754.

**Fu, Z., Smith, P.C., Zhang, L., Rubin, M.A., Dunn, R.L., Yao, Z., and Keller, E.T.** (2003). Effects of raf kinase inhibitor protein expression on suppression of prostate cancer metastasis. J. Natl. Cancer Inst. **95**: 878–89.

**Gallavotti, A., Zhao, Q., Kyozuka, J., Meeley, R.B., Ritter, M., Doebley, J.F., Pe`, M.E., and Schmidt, R.J.** (2004). The role of barren stalk1 in the architecture of maize. Nature **432**: 630–635.

**Gao, X., Liang, W., Yin, C., Ji, S., Wang, H., Su, X., Guo, C., Kong, H., Xue, H., and Zhang, D.** (2010). The SEPALLATA-like gene OsMADS34 is required for rice inflorescence and spikelet development. Plant Physiol. **153**: 728–740.

**Gary, D., Pinyopich, A., Robles, P., Pelaz, S., and Yanofsky, M.F.** (2004). The SEP4 gene of Arabidopsis thaliana functions in floral organ and meristem identity. Can. Field-Naturalist **14**: 1935–1940.

**Gomez-Roldan, V. et al.** (2008). Strigolactone inhibition of shoot branching. Nature **455**: 189–194.

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

**Goodstein, D.M., Shu, S., Howson, R., Neupane, R., Hayes, R.D., Fazo, J., Mitros, T., Dirks, W., Hellsten, U., Putnam, N., and Rokhsar, D.S.** (2012). Phytozome: A comparative platform for green plant genomics. Nucleic Acids Res. **40**: 1178–1186.

**Götz, S., García-Gómez, J.M., Terol, J., Williams, T.D., Nagaraj, S.H., Nueda, M.J., Robles, M., Talón, M., Dopazo, J., and Conesa, A.** (2008). High-throughput functional annotation and data mining with the Blast2GO suite. Nucleic Acids Res. **36**: 3420–3435.

**Greenup, A.G., Sasani, S., Oliver, S.N., Talbot, M.J., Dennis, E.S., Hemming, M.N., and Trevaskis, B.** (2010). ODDSOC2 is a MADS Box floral repressor that is down-regulated by vernalization in temperate cereals. Plant Physiol. **153**: 1062–1073.

**Guo, S., Xu, Y., Liu, H., Mao, Z., Zhang, C., Ma, Y., Zhang, Q., Meng, Z., and Chong, K.** (2013). The interaction between OsMADS57 and OsTB1 modulates rice tillering via DWARF14. Nat. Commun. **4**: 1566.

**Gustafson-Brown, C., Savidge, B., and Yanofsky, M.F.** (1994). Regulation of the Arabidopsis floral homeotic gene APETALA1. Cell **76**: 131–143.

**Halliwell, J., Borrill, P., Gordon, A., Kowalczyk, R., Pagano, M.L., Saccomanno, B., Bentley, A.R., Uauy, C., and Cockram, J.** (2016). Systematic investigation of FLOWERING LOCUS T-Like Poaceae gene families identifies the short-day expressed flowering pathway gene, TaFT3 in wheat (Triticum aestivum L.). Front. Plant Sci. **7**: 1–15.

**Hanano, S. and Goto, K.** (2011). Arabidopsis TERMINAL FLOWER1 is involved in the regulation of flowering time and inflorescence development through transcriptional repression. The Plant Cell **23**: 3172–3184.

**Hanzawa, Y., Money, T., and Bradley, D.** (2005). A single amino acid converts a repressor to an activator of flowering. Proc. Natl. Acad. Sci. **102**: 7748–7753.

**Hayama, R., Yokoi, S., Tamaki, S., Yano, M., and Shimamoto, K.** (2003). Adaptation of photoperiodic control pathways produces short-day flowering in rice. Nature **422**: 719–722.

**Hayward, A., Stirnberg, P., Beveridge, C., and Leyser, O.** (2009). Interactions between auxin and strigolactone in shoot branching control. Plant Physiol. **151**: 400–412.

**Hazen, S.P., Schultz, T.F., Pruneda-Paz, J.L., Borevitz, J.O., Ecker, J.R., and Kay, S.A.** (2005). LUX ARRHYTHMO encodes a Myb domain protein essential for circadian rhythms. Proc. Natl. Acad. Sci. **102**: 10387–92.

**He, Y.** (2012). Chromatin regulation of flowering. Trends Plant Sci. **17**: 556–562.

**Hemming, M.N., Peacock, W.J., Dennis, E.S., and Trevaskis, B.** (2008). Low-temperature and daylength cues are integrated to regulate FLOWERING LOCUS T in barley. Plant Physiol. **147**: 355–366.

**Hengst, U., Albrecht, H., Hess, D., and Monard, D.** (2001). The phosphatidylethanolamine-binding protein is the prototype of a novel family of serine protease inhibitors. J. Biol. Chem. **276**: 535–40.

**Hennig, L., Bouveret, R., and Gruissem, W.** (2005). MSI1-like proteins: An escort service for chromatin assembly and remodeling complexes. Trends Cell Biol. **15**: 295–302.

**Hergeth, S.P. and Schneider, R.** (2015). The H1 linker histones: multifunctional proteins beyond the nucleosomal core particle. EMBO Rep. **16**: 1439–1453.

**Hicks, K.A., Millar, A.J., Carre, I.A., Somers, D.E., Straume, M., Meeks-Wagner, R., and Kay, S.A.** (1996). Conditional circadian dysfuncion of the Arabidopsis early-flowering 3 mutant. Science. **274**: 790–792.

**Higuchi, Y., Narumi, T., Oda, A., Nakano, Y., Sumitomo, K., Fukai, S., and Hisamatsu, T.** (2013). The gated induction system of a systemic floral inhibitor, antiflorigen, determines obligate short-day flowering in chrysanthemums. Proc. Natl. Acad. Sci. **110**: 17137–17142.

**Ho, W.W.H. and Weigel, D.** (2014). Structural features determining flower-promoting activity of Arabidopsis FLOWERING LOCUS T. The Plant Cell **26:** 552-564

**Hou, C.J. and Yang, C.H.** (2009). Functional analysis of FT and TFL1 orthologs from Orchid (Oncidium Gower Ramsey) that regulate the vegetative to reproductive transition. Plant Cell Physiol. **50**: 1544–1557.

**Hsu, C.-Y., Liu, Y., Luthe, D.S., and Yuceer, C.** (2006). Poplar FT2 shortens the juvenile phase and promotes seasonal flowering. The Plant Cell **18**: 1846–61.

**Ietswaart, R., Wu, Z., and Dean, C.** (2012). Flowering time control: Another window to the connection between antisense RNA and chromatin. Trends Genet. **28**: 445–453.

**Ikeda-Kawakatsu, K., Maekawa, M., Izawa, T., Itoh, J.I., and Nagato, Y.** (2012). ABERRANT PANICLE ORGANIZATION 2/RFL, the rice ortholog of Arabidopsis LEAFY, suppresses the transition from inflorescence meristem to floral meristem through interaction with APO1. Plant J. **69**: 168–180.

**Ikeda-Kawakatsu, K., Yasuno, N., Oikawa, T., Iida, S., Nagato, Y., Maekawa, M., and Kyozuka, J.** (2009). Expression level of ABERRANT PANICLE ORGANIZATION1 determines rice inflorescence form through control of cell proliferation in the meristem. Plant Physiol. **150**: 736–747.

**Ikeda, K., Ito, M., Nagasawa, N., Kyozuka, J., and Nagato, Y.** (2007). Rice ABERRANT PANICLE ORGANIZATION 1, encoding an F-box protein, regulates meristem fate. Plant J. **51**: 1030–1040.

**Ikeda, K., Nagasawa, N., and Nagato, Y.** (2005). Aberrant panicle organization 1 temporally regulates meristem identity in

rice. Dev. Biol. **282**: 349–360.

**Ingham, P.W.** (1998). Trithorax and the regulation of homeotic gene expression in Drosophila: a historical perspective. Int. J. Dev. Biol. **42**: 423–429.

**Irish, V.F. and Sussex, I.M.** (1990). Function of the apetala-1 gene during Arabidopsis floral development. The Plant Cell **2**: 741–753.

**Ishikawa, S., Maekawa, M., Arite, T., Onishi, K., Takamure, I., and Kyozuka, J.** (2005). Suppression of tiller bud activity in tillering dwarf mutants of rice. Plant Cell Physiol. **46**: 79–86.

**Ito, T., Kim, G.T., and Shinozaki, K.** (2000). Disruption of an Arabidopsis cytoplasmic ribosomal protein S13-homologous gene by transposon-mediated mutagenesis causes aberrant growth and development. Plant J. **22**: 257–264.

**Itoh, J.I., Nonomura, K.I., Ikeda, K., Yamaki, S., Inukai, Y., Yamagishi, H., Kitano, H., and Nagato, Y.** (2005). Rice plant development: From zygote to spikelet. Plant Cell Physiol. **46**: 23–47.

**Jaeger, K.E., Pullen, N., Lamzin, S., Morris, R.J., and Wigge, P.A.** (2013). Interlocking feedback loops govern the dynamic behavior of the floral transition in Arabidopsis. The Plant Cell **25**: 820–833.

**Jensen, C.S., Salchert, K., and Nielsen, K.K.** (2001). A TERMINAL FLOWER1-like gene from perennial ryegrass involved in floral transition and axillary meristem identity. Plant Physiol. **125**: 1517–1528.

**Jeon, Y., Park, Y.J., Cho, H.K., Jung, H.J., Ahn, T.K., Kang, H., and Pai, H.S.** (2015). The nucleolar GTPase nucleostemin-like 1 plays a role in plant growth and senescence by modulating ribosome biogenesis. J. Exp. Bot. **66**: 6297–6310.

**Jia, Q., Zhang, J., Westcott, S., Zhang, X., Bellgard, M., Lance, R., and Li, C.** (2009). GA-20 oxidase as a candidate for the semidwarf gene sdw1/denso in barley. Funct. Integr. Genomics **9**: 255–262.

**Jia, Q., Zhang, X., Westcott, S., Broughton, S., Cakir, M., Yang, J., Lance, R., and Li, C.** (2011). Expression level of a gibberellin 20-oxidase gene is associated with multiple agronomic and quality traits in barley. Theor. Appl. Genet. **122**: 1451–1460.

**Jiang, K., Liberatore, K.L., Park, S.J., Alvarez, J.P., and Lippman, Z.B.** (2013). Tomato yield heterosis is triggered by a dosage sensitivity of the florigen pathway that fine-tunes shoot architecture. PLoS Genet. **9**.

**Jiao, Y., Wang, Y., Xue, D., Wang, J., Yan, M., Liu, G., Dong, G., Zeng, D., Lu, Z., Zhu, X., Qian, Q., and Li, J.** (2010). Regulation of OsSPL14 by OsmiR156 defines ideal plant architecture in rice. Nat. Genet. **42**: 541–544.

**Jones, H.G. and Kirby, E.J.M.** (1977). Effects of manipulation of number of tillers and water supply on grain yield in barley. J. Agric. Sci. **88**: 391.

**Kaneko-suzuki, M., Kurihara-ishikawa, R., Okushita-terakawa, C., Kojima, C., Nagano-fujiwara, M., Ohki, I., Tsuji, H., Shimamoto, K., and Taoka, K.** (2018). TFL1-Like proteins in rice antagonize rice FT-like protein in inflorescence development by competition for complex formation with 14-3-3 and FD. Plant and Cell Physiol. **59**: 458–468.

**Kardailsky, I.** (1999). Activation tagging of the floral inducer FT. Science. **286**: 1962–1965.

**Kearse, M. et al.** (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics **28**: 1647–1649.

**Kebrom, T.H., Spielmeyer, W., and Finnegan, E.J.** (2013). Grasses provide new insights into regulation of shoot branching. Trends Plant Sci. **18**: 41–48.

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

**Kernich, G.C., Halloran, G.M., and Flood, R.G.** (1997). Variation in duration of pre-anthesis phases of development in barley (Hordeum vulgare). Aust. J. Agric. Res. **48**: 59–66.

**Kikuchi, R., Kawahigashi, H., Ando, T., Tonooka, T., and Handa, H.** (2009). Molecular and functional characterization of PEBP genes in barley reveal the diversification of their roles in flowering. Plant Physiol. **149**.

**Kirby, E.J.M.** (1973). Effect of temperature on ear abnormalities in uniculm barley. J. Exp. Bot. **24**: 935–947.

**Kirby, E.J.M.** (1967). The effect of plant density upon the growth and yield of barley. J. Agric. Sci. **68**: 317–324.

**Kirby, E.J.M. and Appleyard, M.** (1987). Development and structure of the wheat plant. In Wheat breeding (Springer), pp. 287–311.

**Kirby, E.J.M. and Faris, D.G.** (1972). The effect of plant density on tiller growth and morphology in barley. J. Agric. Sci. **78**: 281–288.

**Kobayashi, K., Maekawa, M., Miyao, A., Hirochika, H., and Kyozuka, J.** (2010). PANICLE PHYTOMER2 (PAP2), encoding a SEPALLATA subfamily MADS-box protein, positively controls spikelet meristem identity in rice. Plant Cell Physiol. **51**: 47–57.

**Kobayashi, Y.** (1999). A pair of related genes with antagonistic roles in mediating flowering signals. Science. **286**: 1960–1962.

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

**Kojima, S.** (2002). Hd3a, a Rice ortholog of the Arabidopsis FT gene, promotes transition to flowering downstream of Hd1 under short-day conditions. Plant Cell Physiol. **43**: 1096–1105.

**Komatsu, M.** (2003). FRIZZY PANICLE is required to prevent the formation of axillary meristems and to establish floral meristem identity in rice spikelets. Development **130**: 3841–3850.

**Komatsu, M., Maekawa, M., Shimamoto, K., and Kyozuka, J.** (2001). The LAX1 and FRIZZY PANICLE 2 genes determine the inflorescence architecture of rice by controlling rachis-branch and spikelet development. Dev. Biol. **231**: 364–373.

**Komatsuda, T. et al.** (2007). Six-rowed barley originated from a mutation in a homeodomain-leucine zipper I-class homeobox gene. Proc. Natl. Acad. Sci. **104**: 1424–1429.

**Koppolu, R. et al.** (2013). Six-rowed spike4 (Vrs4) controls spikelet determinacy and row-type in barley. Proc. Natl. Acad. Sci. **110**: 13198–13203.

**Krieger, U., Lippman, Z.B., and Zamir, D.** (2010). The flowering gene SINGLE FLOWER TRUSS drives heterosis for yield in tomato. Nat. Genet. **42**: 459–463.

**Kurakawa, T., Ueda, N., Maekawa, M., Kobayashi, K., Kojima, M., Nagato, Y., Sakakibara, H., and Kyozuka, J.** (2007). Direct control of shoot meristem activity by a cytokinin-activating enzyme. Nature **445**: 652–655.

**Kuroha, T., Tokunaga, H., Kojima, M., Ueda, N., Ishida, T., Nagawa, S., Fukuda, H., Sugimoto, K., and Sakakibara, H.** (2009). Functional analyses of LONELY GUY cytokinin-activating enzymes reveal the importance of the direct activation pathway in Arabidopsis. The Plant Cell **21**: 3152–3169.

**Kwak, M., Toro, O., Debouck, D.G., and Gepts, P.** (2012). Multiple origins of the determinate growth habit in domesticated common bean (Phaseolus vulgaris). Ann. Bot. **110**: 1573–1580.

**Kyozuka, J., Konishi, S., Nemoto, K., Izawa, T., and Shimamoto, K.** (1998). Down-regulation of RFL, the FLO/LFY homolog of rice, accompanied with panicle branch initiation. Proc. Natl. Acad. Sci. **95**: 1979–82.

**Lauer, J.G.** (1991). Barley tiller response to plant density and ethephon. Agron. J. **83**: 968–973.

**Lauer, J.G. and Partridge, J.R.** (1990). Planting date and nitrogen rate effects on spring malting barley. Agron. J. **82**: 1083–1088.

**Lauer, J.G. and Simmons, S.R.** (1988). Photoassimilate partitioning by tillers and individual tiller leaves in field-grown spring barley. Crop Sci. **28**: 279–282.

**Laurie, D.A., Pratchett, N., Snape, J.W., and Bezant, J.H.** (1995). RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter× spring barley (Hordeum vulgare L.) cross. Genome **38**: 575–585.

**Lazakis, C.M., Coneva, V., and Colasanti, J.** (2011). ZCN8 encodes a potential orthologue of Arabidopsis FT florigen that integrates both endogenous and photoperiod flowering signals in maize. J. Exp. Bot. **62**: 4833–4842.

**Lee, D.Y. and An, G.** (2012). Two AP2 family genes, SUPERNUMERARY BRACT (SNB) and OsINDETERMINATE SPIKELET 1 (OsIDS1), synergistically control inflorescence architecture and floral meristem establishment in rice. Plant J. **69**: 445–461.

**Lee, D.Y., Lee, J., Moon, S., Park, S.Y., and An, G.** (2007). The rice heterochronic gene SUPERNUMERARY BRACT regulates the transition from spikelet meristem to floral meristem. Plant J. **49**: 64–78.

**Lee, R., Baldwin, S., Kenel, F., McCallum, J., and Macknight, R.** (2013). FLOWERING LOCUS T genes control onion bulb formation and flowering. Nat. Commun. **4**: 1–9.

**Léran, S., Garg, B., Boursiac, Y., Corratgé-Faillie, C., Brachet, C., Tillard, P., Gojon, A., and Lacombe, B.** (2015). AtNPF5.5, a nitrate transporter affecting nitrogen accumulation in Arabidopsis embryo. Sci. Rep. **5**: 7962.

**Leyser, O.** (2003). Regulation of shoot branching by auxin. Trends Plant Sci. **8**: 541–545.

**Li, C. and Dubcovsky, J.** (2008). Wheat FT protein regulates VRN1 transcription through interactions with FDL2. Plant J. **55**: 543–554.

**Li, W., Han, Y., Tao, F., and Chong, K.** (2011). Knockdown of SAMS genes encoding S-adenosyl-l-methionine synthetases causes methylation alterations of DNAs and histones and leads to late flowering in rice. J. Plant Physiol. **168**: 1837–1843.

**Li, W., Wang, H., and Yu, D.** (2016). Arabidopsis WRKY transcription factors WRKY12 and WRKY13 oppositely regulate flowering under short-day conditions. Mol. Plant **9**: 1492–1503.

**Van Lijsebettens, M., Vanderhaeghen, R., De Block, M., Bauw, G., Villarroel, R., and Van Montagu, M.** (1994). An S18 ribosomal protein gene copy at the Arabidopsis PFL locus affects plant development by its specific expression in meristems. EMBO J. **13**: 3378–3388.

**Liller, C.B., Neuhaus, R., Von Korff, M., Koornneef, M., and Van Esse, W.** (2015). Mutations in barley row type genes have pleiotropic effects on shoot branching. PLoS One **10**: 1–20.

**Lin, H., Wang, R., Qian, Q., Yan, M., Meng, X., Fu, Z., Yan, C., Jiang, B., Su, Z., Li, J., and Wang, Y.** (2009). DWARF27, an iron-containing protein required for the biosynthesis of strigolactones, regulates rice tiller bud outgrowth. The Plant Cell **21**: 1512–1525.

**Lin, Q. et al.** (2012). Rice APC/CTE controls tillering by mediating the degradation of MONOCULM 1. Nat. Commun. **3**: 752.

**Liu, B. et al.** (2010). The soybean stem growth habit gene Dt1 Is an ortholog of Arabidopsis TERMINAL FLOWER1. Plant Physiol. **153**: 198–210.

**Liu, C., Teo, Z.W.N., Bi, Y., Song, S., Xi, W., Yang, X., Yin, Z., and Yu, H.** (2013). A conserved genetic pathway determines inflorescence architecture in Arabidopsis and rice. Dev. Cell **24**: 612–622.

**Liu, L., Liu, C., Hou, X., Xi, W., Shen, L., Tao, Z., Wang, Y., and Yu, H.** (2012). FTIP1 is an essential regulator required for florigen transport. PLoS Biol. **10**.

**Liu, X.L., Covington, M.F., Fankhauser, C., Chory, J., and D. Ry Wagner** (2001). ELF3 encodes a circadian clock-regulated nuclear protein that functions in an Arabidopsis PHYB signal transduction pathway. The Plant Cell **13**: 1293–1304.

**Long, J. and Barton, M.K.** (2000). Initiation of axillary and floral meristems in Arabidopsis. Dev. Biol. **218**: 341–353.

**Lundqvist, U.** (2009). Eighty years of Scandinavian barley mutation genetics and breeding. Induc. plant Mutat. genomics era. Food Agric. Organ. United Nations, Rome: 39–43.

**Luo, L., Li, W., Miura, K., Ashikari, M., and Kyozuka, J.** (2012). Control of tiller growth of rice by osspl14 and strigolactones, which work in two independent pathways. Plant Cell Physiol. **53**: 1793–1801.

**Lv, B., Nitcher, R., Han, X., Wang, S., Ni, F., Li, K., Pearce, S., Wu, J., Dubcovsky, J., and Fu, D.** (2014). Characterization of Flowering Locus T1 (FT1) gene in Brachypodium and wheat. PLoS One **9**.

**Mahapatra, I.C. and Behera, U.K.** (2011). Rice (Oryza sativa)-based farming systems for livelihood improvement of Indian farmers. Indian J. Agron. **56**: 1–19.

**Malcomber, S.T., Kellogg, E.A., The, S., Cell, P., Jul, N., Malcomber, S.T., and Kellogg, E.A.** (2004). Heterogeneous expression patterns and separate roles of the SEPALLATA Gene LEAFY HULL STERILE1 in grasses. The Plant Cell **16**: 1692-1706

**Mandel, A.M., Gustafson-Brown, C., Savidge, B., and Yanofsky, M.F.** (1992). Molecular characterization of the Arabidopsis floral homeotic gene APETALA1. Nature **360**: 273–277.

**Mandel, M.A. and Yanofsky, M.F.** (1995). A gene triggering flower formation in Arabidopsis. Nature **377**: 522–524.

**Mascher, M. et al.** (2017). A chromosome conformation capture ordered sequence of the barley genome. Nature **544**: 427–433.

**Mascher, M., Jost, M., Kuon, J.-E., Himmelbach, A., Aßfalg, A., Beier, S., Scholz, U., Graner, A., and Stein, N.** (2014). Mapping-by-sequencing accelerates forward genetics in barley. Genome Biol. **15**: R78.

**McDonald, O.** (1990). The growth and yield of uniculm and tillered barley over a range of sowing rates. Aust. J. Agric. Res. **v. 41**.

**McGarry, R.C. and Ayre, B.G.** (2012). Manipulating plant architecture with members of the CETS gene family. Plant Sci. **188**: 71–81.

**McSteen, P. and Hake, S.** (2001). Barren inflorescence2 regulates axillary meristem development in the maize inflorescence. Development **128**: 2881–2891.

**Meng, X., Muszynski, M.G., and Danilevskaya, O.N.** (2011). The FT-Like ZCN8 gene functions as a floral activator and is involved in photoperiod sensitivity in maize. The Plant Cell **23**: 942–960.

**Mimida, N., Goto, K., Kobayashi, Y., Araki, T., Ahn, J.H., Weigel, D., Murata, M., Motoyoshi, F., and Sakamoto, W.** (2001). Functional divergence of the *TFL1*-like gene family in *Arabidopsis* revealed by characterization of a novel homologue. Genes to Cells **6**: 327–336.

**Mimida, N., Komori, S., Suzuki, A., and Wada, M.** (2013). Functions of the apple TFL1/FT orthologs in phase transition. Sci. Hortic. (Amsterdam). **156**: 106–112.

**Mimida, N., Li, J., Zhang, C., Moriya, S., Moriya-Tanaka, Y., Iwanami, H., Honda, C., Oshino, H., Takagishi, K., Suzuki, A., Komori, S., and Wada, M.** (2012). Divergence of TERMINAL FLOWER1 -like genes in Rosaceae. Biol. Plant. **56**: 465–472.

**Miura, K., Ikeda, M., Matsubara, A., Song, X.-J., Ito, M., Asano, K., Matsuoka, M., Kitano, H., and Ashikari, M.** (2010). OsSPL14 promotes panicle branching and higher grain productivity in rice. Nat. Genet. **42**: 545–549.

**Moon, J., Suh, S.S., Lee, H., Choi, K.R., Hong, C.B., Paek, N.C., Kim, S.G., and Lee, I.** (2003). The SOC1 MADS-box gene integrates vernalization and gibberellin signals for flowering in Arabidopsis. Plant J. **35**: 613–623.

**Del Moral, L.G., Miralles, D.J., and Slafer, G.** (2002). Initiation and appearance of vegetative and reproductive structures throughout barley development. Barley Sci. Recent Adv. from Mol. Biol. to Agron. yield Qual. GA Slafer, JL Molina-Cano, R Savin, JL Araus I Romagosa): 243–267.

**Müller, J., Wang, Y., Franzen, R., Santi, L., Salamini, F., and Rohde, W.** (2001). In vitro interactions between barley TALE homeodomain proteins suggest a role for protein-protein associations in the regulation of Knox gene function. Plant J. **27**: 13–23.

**Murai, K.** (2013). Homeotic genes and the ABCDE model for floral organ formation in wheat. Plants **2**: 379–395.

**Mutasa-Göttgens, E. and Hedden, P.** (2009). Gibberellin as a factor in floral regulatory networks. J. Exp. Bot. **60**: 1979–1989.

**Nakagawa, M., Shimamoto, K., and Kyozuka, J.** (2002). Overexpression of *RCN1* and *RCN2* , rice *TERMINAL FLOWER 1/CENTRORADIALIS* homologs, confers delay of phase transition and altered panicle morphology in rice. Plant J. **29**: 743–750.

**Nakamichi, N.** (2015). Adaptation to the local environment by modifications of the photoperiod response in crops. Plant Cell Physiol. **56**: 594–604.

**Nakamura, Y., Andrés, F., Kanehara, K., Liu, Y., Dörmann, P., and Coupland, G.** (2014). Arabidopsis florigen FT binds to diurnally oscillating phospholipids that accelerate flowering. Nat. Commun. **5**: 4–8.

**Nan, H., Cao, D., Zhang, D., Li, Y., Lu, S., Tang, L., Yuan, X., Liu, B., and Kong, F.** (2014). GmFT2a and GmFT5a redundantly and differentially regulate flowering through interaction with and upregulation of the bZIP transcription factor GmFDL19 in soybean. PLoS One **9**.

**Naora, H. and Naora, H.** (1999). Involvement of ribosomal proteins in regulating cell growth and apoptosis: Translational modulation or recruitment for extraribosomal activity? Immunol. Cell Biol. **77**: 197–205.

**Navarro, C., Abelenda, J.A., Cruz-Oró, E., Cuéllar, C.A., Tamaki, S., Silva, J., Shimamoto, K., and Prat, S.** (2011). Control of flowering and storage organ formation in potato by FLOWERING LOCUS T. Nature **478**: 119–122.

**Nishimura, C., Ohashi, Y., Sato, S., Kato, T., Tabata, S., and Ueguchia, C.** (2004). Histidine kinase homologs that act as cytokinin receptors possess overlapping functions in the regulation of shoot and root growth in Arabidopsis. The Plant Cell **16**: 1365–1377.

**Nishimura, T., Wada, T., Yamamoto, K.T., and Okada, K.** (2005). The Arabidopsis STV1 protein, responsible for translation reinitiation, is required for auxin-mediated gynoecium patterning. The Plant Cell **17**: 2940–2953.

**Niu, L., Lu, F., Pei, Y., Liu, C., and Cao, X.** (2007). Regulation of flowering time by the protein arginine methyltransferase AtPRMT10. EMBO Rep. **8**: 1190–1195.

**Niwa, M., Daimon, Y., Kurotani, K. -i., Higo, A., Pruneda-Paz, J.L., Breton, G., Mitsuda, N., Kay, S.A., Ohme-Takagi, M., Endo, M., and Araki, T.** (2013). BRANCHED1 interacts with FLOWERING LOCUS T to repress the floral transition of the axillary meristems in Arabidopsis. The Plant Cell **25**: 1228–1242.

**Ohshima, S., Murata, M., Sakamoto, W., Ogura, Y., and Motoyoshi, F.** (1997). Cloning and molecular analysis of the Arabidopsis gene Terminal Flower 1. Mol. Gen. Genet. **254**: 186–94.

**Oikawa, A., Nagai, K., Kato, K., and Kidou, S.** (2009). Gene silencing of barley P23k involved in secondary wall formation causes abnormal tiller formation and intercalary elongation. Breed. Sci. **59**: 664–670.

**Oikawa, T. and Kyozuka, J.** (2009). Two-step regulation of LAX PANICLE1 protein accumulation in axillary meristem formation in rice.The Plant Cell **21**: 1095–1108.

**Okagaki, R.J., Cho, S., Kruger, W.M., Xu, W.W., Heinen, S., and Muehlbauer, G.J.** (2013). The barley UNICULM2 gene resides in a centromeric region and may be associated with signaling and stress responses. Funct. Integr. Genomics **13**: 33–41.

**Okamoto, M., Kuwahara, A., Seo, M., Kushiro, T., Asami, T., Hirai, N., Kamiya, Y., Koshiba, T., and Nambara, E.** (2006). CYP707A1 and CYP707A2, which encode abscisic acid 8'-hydroxylases, are indispensable for proper control of seed dormancy and germination in Arabidopsis. Plant Physiol. **141**: 97-107.

**Pankin, A., Campoli, C., Dong, X., Kilian, B., Sharma, R., Himmelbach, A., Saini, R., Davis, S.J., Stein, N., Schneeberger, K., and von Korff, M.** (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.

**Park, S.J., Jiang, K., Tal, L., Yichie, Y., Gar, O., Zamir, D., Eshed, Y., and Lippman, Z.B.** (2014). Optimization of crop productivity in tomato using induced mutations in the florigen pathway. Nat. Genet. **46**: 1337–1342.

**Park, S.J., Lee, Y.K., Kang, M.S., and Bae, J.H.** (2016). Revisiting domestication to revitalize crop improvement: The florigen revolution. Plant Breed. Biotechnol. **4**: 387–397.

**Pearce, S., Vanzetti, L.S., and Dubcovsky, J.** (2013). Exogenous gibberellins induce wheat spike development under short days only in the presence of VERNALIZATION1. Plant Physiol. **163**: 1433–1445.

**Pei, Y., Niu, L., Lu, F., Liu, C., Zhai, J., Kong, X., and Cao, X.** (2007). Mutations in the type II protein arginine methyltransferase AtPRMT5 result in pleiotropic developmental defects in Arabidopsis. Plant Physiol. **144**: 1913–1923.

**Pelaz, S., Ditta, G.S., Baumann, E., Wisman, E., and Yanofsky, M.F.** (2000). B and C floral organ identity functions require SEPALLATA MADS-box genes. Nature **405**: 200–203.

**Pillitteri, L.J., Lovatt, C.J., and Walling, L.L.** (2004). Isolation and characterization of a TERMINAL FLOWER homolog and its correlation with juvenility in citrus. Plant Physiol. **135**: 1540–51.

**Pin, P.A., Benlloch, R., Bonnet, D., Wremerth-Weich, E., Kraft, T., Gielen, J.J.L., and Nilsson, O.** (2010). An antagonistic pair of FT homologs mediates the control of flowering time in sugar beet. Science. **330**: 1397–400.

**Pnueli, L., Carmel-Goren, L., Hareven, D., Gutfinger, T., Alvarez, J., Ganal, M., Zamir, D., and Lifschitz, E.** (1998). The SELF-PRUNING gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of CEN and TFL1. Development **125**: 1979–1989.

**Pnueli, L., Gutfinger, T., Hareven, D., Ben-Naim, O., Ron, N., Adir, N., and Lifschitz, E.** (2001). Tomato SP-interacting proteins define a conserved signaling system that regulates shoot architecture and flowering. The Plant Cell **13**: 2687–2702.

**Pourkheirandish, M. and Komatsuda, T.** (2007). The importance of barley genetics and domestication in a global perspective. Ann. Bot. **100**: 999–1008.

**Poursarebani, N. et al.** (2015). The genetic basis of composite spike form in barley and 'miracle-wheat'. Genetics **201**: 155–165.

**Prasad, K., Parameswaran, S., and Vijayraghavan, U.** (2005). OsMADS1, a rice MADS-box factor, controls differentiation of specific cell types in the lemma and palea and is an early-acting regulator of inner floral organs. Plant J. **43**: 915–928.

**Prasad, K., Sriram, P., Santhosh Kumar, C., Kushalappa, K., and Vijayraghavan, U.** (2001). Ectopic expression of rice OsMADS1 reveals a role in specifying the lemma and palea, grass floral organs analogous to sepals. Dev. Genes Evol. **211**: 281–290.

**Pratelli, R., Voll, L.M., Horst, R.J., Frommer, W.B., and Pilot, G.** (2010). Stimulation of nonselective amino acid export by glutamine dumper proteins. Plant Physiol. **152**.

**Ramsay, L. et al.** (2011). INTERMEDIUM-C, a modifier of lateral spikelet fertility in barley, is an ortholog of the maize domestication gene TEOSINTE BRANCHED 1. Nat. Genet. **43**: 169–172.

**Randoux, M., Davi??re, J.M., Jeauffre, J., Thouroude, T., Pierre, S., Toualbia, Y., Perrotte, J., Reynoird, J.P., Jammes, M.J., Hibrand-Saint Oyant, L., and Foucher, F.** (2014). RoKSN, a floral repressor, forms protein complexes with RoFD and RoFT to regulate vegetative and reproductive development in rose. New Phytol. **202**: 161–173.

**Ratcliffe, O.J., Amaya, I., Vincent, C.A., Rothstein, S., Carpenter, R., Coen, E.S., and Bradley, D.J.** (1998). A common mechanism controls the life cycle and architecture of plants. Development **125**: 1609–1615.

**Ratcliffe, O.J., Bradley, D.J., and Coen, E.S.** (1999). Separation of shoot and floral identity in Arabidopsis. Development **126**: 1109–1120.

**Repinski, S.L., Kwak, M., and Gepts, P.** (2012). The common bean growth habit gene PvTFL1y is a functional homolog of Arabidopsis TFL1. Theor. Appl. Genet. **124**: 1539–1547.

**Riggs, T.J. and Kirby, E.J.M.** (1978). Developmental consequences of two-row and six-row ear type in spring barley: 1. Genetical analysis and comparison of mature plant characters. J. Agric. Sci. **91**: 199–205.

**Rijpkema, A.S., Vandenbussche, M., Koes, R., Heijmans, K., and Gerats, T.** (2010). Variations on a theme: Changes in the floral ABCs in angiosperms. Semin. Cell Dev. Biol. **21**: 100–107.

**Roeder, A.H.K., Ferrandiz, C., and Yanofsky, M.F.** (2003). The role of the REPLUMLESS homeodomain protein in patterning the Arabidopsis fruit. Curr. Biol. **13**: 1630–1635.

**Rosado, A., Sohn, E.J., Drakakaki, G., Pan, S., Swidergal, A., Xiong, Y., Kang, B.-H., Bressan, R.A., and Raikhel, N. V.** (2010). Auxin-mediated ribosomal biogenesis regulates vacuolar trafficking in Arabidopsis. The Plant Cell **22**: 143–158.

**Ruiz-García, L., Madueno, F., Wilkinson, M., Haughn, G., Salinas, J., and Martínez-Zapater, J.M.** (1997). Different roles of flowering-time genes in the activation of floral initiation genes in Arabidopsis. The Plant Cell **9**: 1921–1934.

**Rutjens, B., Bao, D., Van Eck-Stouten, E., Brand, M., Smeekens, S., and Proveniers, M.** (2009). Shoot apical meristem function in Arabidopsis requires the combined activities of three BEL1-like homeodomain proteins. Plant J. **58**: 641–654.

**Ryu, M.Y., Cho, S.K., and Kim, W.T.** (2009). RNAi suppression of RPN12a decreases the expression of type-A ARRs, negative regulators of cytokinin signaling pathway, in Arabidopsis. Mol. Cells **28**: 375–382.

**Sasani, S., Hemming, M.N., Oliver, S.N., Greenup, A., Tavakkol-Afshari, R., Mahfoozi, S., Poustini, K., Sharifi, H.R., Dennis, E.S., Peacock, W.J., and Trevaskis, B.** (2009). The influence of vernalization and daylength on expression of flowering-time genes in the shoot apex and leaves of barley (Hordeum vulgare). J. Exp. Bot. **60**: 2169–2178.

**Satoh-Nagasawa, N., Nagasawa, N., Malcomber, S., Sakai, H., and Jackson, D.** (2006). A trehalose metabolic enzyme controls inflorescence architecture in maize. Nature **441**: 227–230.

**Schauer, S.E., Schlüter, P.M., Baskar, R., Gheyselinck, J., Bolaños, A., Curtis, M.D., and Grossniklaus, U.** (2009). Intronic regulatory elements determine the divergent expression patterns of agamous-like6 subfamily members in arabidopsis. Plant J. **59**: 987–1000.

**Schmitz, G. and Theres, K.** (2005). Shoot and inflorescence branching. Curr. Opin. Plant Biol. **8**: 506–511.

**Schmitz, J., Franzen, R., Ngyuen, T.H., Garcia-Maroto, F., Pozzi, C., Salamini, F., and Rohde, W.** (2000). Cloning, mapping and expression analysis of barley MADS-box genes. Plant Mol. Biol. **42**: 899–913.

**Schmitz, R.J., Sung, S., and Amasino, R.M.** (2008). Histone arginine methylation is required for vernalization-induced epigenetic silencing of FLC in winter-annual Arabidopsis thaliana. Proc. Natl. Acad. Sci. **105**: 411–416.

**Schultz, E.A. and Haughn, G.W.** (1991). LEAFY, a Homeotic gene that regulates inflorescence development in Arabidopsis. The Plant Cell **3**: 771–781.

**Sedivy, J.M., Kolch, W., Yeung, K., Seitz, T., Li, S., Janosch, P., McFerran, B., Kaiser, C., Fee, F., Katsanakis, K.D., Rose, D.W., and Mischak, H.** (1999). Suppression of Raf-1 kinase activity and MAP kinase signalling by RKIP. Nature **401**: 173–177.

**Shalit, A., Rozman, A., Goldshmidt, A., Alvarez, J.P., Bowman, J.L., Eshed, Y., and Lifschitz, E.** (2009). The flowering hormone florigen functions as a general systemic regulator of growth and termination. Proc. Natl. Acad. Sci. **106**: 8392–8397.

**Shannon, S. and Ry Meeks-Wagner, O.** (1991). A mutation in the Arabidopsis TFL1 gene affects inflorescence meristem development. The Plant Cell **3**: 877–892.

**Simmons, S.R., Rasmusson, D.C., and Wiersma, J. V** (1982). Tillering in barley: genotype, row spacing, and seedling rate effects1. Crop Sci. **22**: 801–805.

**Simpson, G.G. and Dean, C.** (2002). Arabidopsis, the Rosetta stone of flowering time?. Science. **296**: 285–289.

**Slafer, G.A. and Rawson, H.M.** (1994). Sensitivity of wheat phasic development to major environmental factors: a re-examination of some assumptions made by physiologists and modellers. Funct. Plant Biol. **21**: 393–426.

**Smaczniak, C., Immink, R.G.H., Angenent, G.C., and Kaufmann, K.** (2012). Developmental and evolutionary diversity of plant MADS-domain factors: insights from recent studies. Development **139**: 3081–3098.

**Smith, H.M. and Hake, S.** (2003). The interaction of two homeobox genes, BREVIPEDICELLUS and PENNYWISE, regulates internode patterning in the Arabidopsis inflorescence. The Plant Cell **15**: 1717–1727.

**Sohn, E.J., Rojas-Pierce, M., Pan, S., Carter, C., Serrano-Mislata, A., Madueño, F., Rojo, E., Surpin, M., and Raikhel, N. V** (2007). The shoot meristem identity gene TFL1 is involved in flower development and trafficking to the protein storage vacuole. Proc. Natl. Acad. Sci. **104**: 18801–18806.

**Soyk, S., Müller, N.A., Park, S.J., Schmalenbach, I., Jiang, K., Hayama, R., Zhang, L., Van Eck, J., Jiménez-Gómez, J.M., and**

**Lippman, Z.B.** (2017). Variation in the flowering gene SELF PRUNING 5G promotes day-neutrality and early yield in tomato. Nat. Genet. **49**: 162–168.

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

**Stamatakis, A.** (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics **30**: 1312–1313.

**Steinbach, Y. and Hennig, L.** (2014). Arabidopsis MSI1 functions in photoperiodic flowering time control. Front. Plant Sci. **5**: 77.

**Stirnberg, P., Liu, J.P., Ward, S., Kendall, S.L., and Leyser, O.** (2012). Mutation of the cytosolic ribosomal protein-encoding RPS10B gene affects shoot meristematic function in Arabidopsis. BMC Plant Biol. **12**: 1–20.

**Szakonyi, D. and Byrne, M.E.** (2011a). Involvement of ribosomal protein RPL27a in meristem activity and organ development. Plant Signal. Behav. **6**: 712–714.

**Szakonyi, D. and Byrne, M.E.** (2011b). Ribosomal protein L27a is required for growth and patterning in Arabidopsis thaliana. Plant J. **65**: 269–281.

**Tabuchi, H. et al.** (2011). LAX PANICLE2 of Rice Encodes a Novel Nuclear Protein and Regulates the Formation of Axillary Meristems. The Plant Cell **23**: 3276–3287.

**Tamaki, S., Matsuo, S., Wong, H.L., Yokoi, S., and Shimamoto, K.** (2007). Hd3a Protein Is a Mobile Flowering Signal in Rice. Science. **316**: 1033–1036.

**Taoka, K. et al.** (2011). 14-3-3 proteins act as intracellular receptors for rice Hd3a florigen. Nature **476**: 332–335.

**Teichmann, T. and Muhr, M.** (2015). Shaping plant architecture. Front. Plant Sci. **6**: 1–18.

**Theißen, G.** (2001). Development of floral organ identity: Stories from the MADS house. Curr. Opin. Plant Biol. **4**: 75–85.

**Thompson, B.E. and Hake, S.** (2009). Translational Biology: From Arabidopsis Flowers to Grass Inflorescence Architecture. Plant Physiol. **149**: 38–45.

**Thorne, G.N.** (1962). Survival of Tillers and Distribution of Dry Matter between Ear and Shoot of Barley Varieties. Ann. Bot. **26**: 37–54.

**Tian, Z.X., Wang, X.B., Lee, R., Li, Y.H., Specht, J.E., Nelson, R.L., McClean, P.E., Qiu, L.J., and Ma, J.X.** (2010). Artificial selection for determinate growth habit in soybean. Proc. Natl. Acad. Sci. **107**: 8563–8568.

**Tong, H., Jin, Y., Liu, W., Li, F., Fang, J., Yin, Y., Qian, Q., Zhu, L., and Chu, C.** (2009). DWARF and LOW-TILLERING, a new member of the GRAS family, plays positive roles in brassinosteroid signaling in rice. Plant J. **58**: 803–816.

**Turck, F., Fornara, F., and Coupland, G.** (2008). Regulation and Identity of Florigen: FLOWERING LOCUS T Moves Center Stage. Annu. Rev. Plant Biol. **59**: 573–594.

**Turnbull, C.G.N.** (2005). Plant architecture and its manipulation. Oxford: Blackwell Publishers Ltd.

**Umehara, M., Hanada, A., Yoshida, S., Akiyama, K., Arite, T., Takeda-Kamiya, N., Magome, H., Kamiya, Y., Shirasu, K., Yoneyama, K., Kyozuka, J., and Yamaguchi, S.** (2008). Inhibition of shoot branching by new terpenoid plant hormones. Nature **455**: 195–200.

**Vann, L., Kono, T., Pyhäjärvi, T., Hufford, M.B., and Ross-Ibarra, J.** (2015). Natural variation in teosinte at the domestication locus *teosinte branched1* ( *tb1* ). PeerJ **3**: e900.

**Varkonyi-Gasic, E., Moss, S.M.A., Voogd, C., Wang, T., Putterill, J., and Hellens, R.P.** (2013). Homologs of FT, CEN and FD respond to developmental and environmental signals affecting growth and flowering in the perennial vine kiwifruit. New Phytol. **198**: 732–746.

**Vollbrecht, E., Springer, P.S., Goh, L., Buckler IV, E.S., and Martienssen, R.** (2005). Architecture of floral branch systems in maize and related grasses. Nature **436**: 1119–1126.

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

**Wang, L., Sun, S., Jin, J., Fu, D., Yang, X., Weng, X., Xu, C., Li, X., Xiao, J., and Zhang, Q.** (2015). Coordinated regulation of vegetative and reproductive branching in rice. Proc. Natl. Acad. Sci. **112**: 15504–15509.

**Wang, W.-S., Zhu, J., Zhang, K.-X., Lü, Y.-T., and Xu, H.-H.** (2016). A mutation of casein kinase 2 α4 subunit affects multiple

developmental processes in Arabidopsis. Plant Cell Rep. **35**: 1071–1080.

**Wang, X., Gingrich, D.K., Deng, Y., and Hong, Z.** (2012). A nucleostemin-like GTPase required for normal apical and floral meristem development in Arabidopsis. Mol. Biol. Cell **23**: 1446–56.

**Wang, X., Zhang, Y., Ma, Q., Zhang, Z., Xue, Y., Bao, S., and Chong, K.** (2007). SKB1-mediated symmetric dimethylation of histone H4R3 controls flowering time in Arabidopsis. EMBO J. **26**: 1934–1941.

**Ward, S.P. and Leyser, O.** (2004). Shoot branching. Curr. Opin. Plant Biol. **7**: 73–78.

**Weigel, D., Alvarez, J., Smyth, D.R., Yanofsky, M.F., and Meyerowitz, E.M.** (1992). LEAFY controls floral meristem identity in Arabidopsis. Cell **69**: 843–859.

**Weigel, D. and Nilsson, O.** (1995). A developmental switch sufficient for flower initiation in diverse plants. Nature **377**: 495–500.

**Whipple, C.J.** (2017). Grass inflorescence architecture and evolution: The origin of novel signaling centers. New Phytol.: 367–372.

**Whipple, C.J., Zanis, M.J., Kellogg, E.A., and Schmidt, R.J.** (2007). Conservation of B class gene expression in the second whorl of a basal grass and outgroups links the origin of lodicules and petals. Proc. Natl. Acad. Sci. **104**: 1081–1086.

**Wickland, D.P. and Hanzawa, Y.** (2015). The FLOWERING LOCUS T/TERMINAL FLOWER 1 Gene Family: Functional Evolution and Molecular Mechanisms. Mol. Plant **8**: 983–997.

**Wigge, P.A., Kim, M.C., Jaeger, K.E., Busch, W., Schmid, M., Lohmann, J.U., and Weigel, D.** (2005). Integration of Spatial and Temporal Information During Floral Induction in Arabidopsis. Science. **309**: 1056–1059.

**Wu, D., Liang, W., Zhu, W., Chen, M., Ferrandiz, C., Burton, R.A., Dreni, L., and Zhang, D.** (2017). Loss of LOFSEP transcription factor function converts Spikelet to Leaf-like Structures in Rice. Plant Physiol.: pp.00704.2017.

**Xu, C., Wang, Y., Yu, Y., Duan, J., Liao, Z., Xiong, G., Meng, X., Liu, G., Qian, Q., and Li, J.** (2012). Degradation of MONOCULM 1 by APC/CTAD1 regulates rice tillering. Nat. Commun. **3**: 750.

**Xu, M.Y., Zhang, L., Li, W.W., Hu, X.L., Wang, M.-B., Fan, Y.L., Zhang, C.Y., Wang, L., MD, M., and D, W.** (2014). Stress-induced early flowering is mediated by miR169 in Arabidopsis thaliana. J. Exp. Bot. **65**: 89–101.

**Xu, X., Sharma, R., Tondelli, A., Russell, J., Comadran, J., Schnaithmann, F., Pillen, K., Kilian, B., Cattivelli, L., Thomas, W.T.B., and Flavell, A.J.** (2018). Genome-Wide Association Analysis of Grain Yield-Associated Traits in a Pan-European Barley Cultivar Collection. Plant Genome **0**: 0.

**Yamaguchi, A., Kobayashi, Y., Goto, K., Abe, M., and Araki, T.** (2005). TWIN SISTER of FT (TSF) acts as a floral pathway integrator redundantly with FT. Plant Cell Physiol. **46**: 1175–1189.

**Yan, L., Fu, D., Li, C., Blechl, A., Tranquilli, G., Bonafede, M., Sanchez, A., Valarik, M., Yasuda, S., and Dubcovsky, J.** (2006). The wheat and barley vernalization gene VRN3 is an orthologue of FT. Proc. Natl. Acad. Sci. **103**: 19581–19586.

**Yeung, K.C., Rose, D.W., Dhillon, a. S., Yaros, D., Gustafsson, M., Chatterjee, D., McFerran, B., Wyche, J., Kolch, W., and Sedivy, J.M.** (2001). Raf kinase inhibitor protein interacts with NFKB-inducing kinase and TAK1 and inhibits NF-K B activation. Mol. Cell. Biol. **21**: 7207–7217.

**Yi, G., Choi, J.-H., Jeong, E.-G., Chon, N.-S., Jena, K.K., Ku, Y.-C., Kim, D.-H., Eun, M.-Y., Jeon, J.-S., and Nam, M.-H.** (2005). Morphological and molecular characterization of a new frizzy panicle mutant, "fzp-9(t)", in rice (Oryza sativa L.). Hereditas **142**: 92–97.

**Yoo, S.C., Chen, C., Rojas, M., Daimon, Y., Ham, B.K., Araki, T., and Lucas, W.J.** (2013). Phloem long-distance delivery of FLOWERING LOCUS T (FT) to the apex. Plant J. **75**: 456–468.

**Yoo, S.Y., Kardailsky, I., Lee, J.S., Weigel, D., and Ahn, J.H.** (2004). Acceleration of flowering by overexpression of MFT (MOTHER OF FT AND TFL1). Mol. Cells **17**: 95–101.

**Yoshida, A. et al.** (2013). TAWAWA1, a regulator of rice inflorescence architecture, functions through the suppression of meristem phase transition. Proc. Natl. Acad. Sci. **110**: 767–772.

**Yoshida, H. and Nagato, Y.** (2011). Flower development in rice. J. Exp. Bot. **62**: 4719–4730.

**Youssef, H.M. et al.** (2016). VRS2 regulates hormone-mediated inflorescence patterning in barley. Nat. Genet. **49**: 157–161.

**Zagotta, M.T., Hicks, K.A., Jacobs, C.I., Young, J.C., Hangarter, R.P., and Meeks‐Wagner, D.R.** (1996). The Arabidopsis ELF3 gene regulates vegetative photomorphogenesis and the photoperiodic induction of flowering. Plant J. **10**: 691–702.

**Zakhrabekova, S. et al.** (2012). Induced mutations in circadian clock regulator Mat-a facilitated short-season adaptation and range extension in cultivated barley. Proc. Natl. Acad. Sci. **109**: 4326–4331.

**Zhang, T. et al.** (2017). *LATERAL FLORET 1* induced the three-florets spikelet in rice. Proc. Natl. Acad. Sci. **114**: 201700504.

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

**Zhou, F., Roy, B., and von Arnim, A.G.** (2010). Translation reinitiation and development are compromised in similar ways by mutations in translation initiation factor eIF3h and the ribosomal protein RPL24. BMC Plant Biol. **10**: 193.

**Zhu, Q.-H., Hoque, M.S., Dennis, E.S., and Upadhyaya, N.M.** (2003). Ds tagging of BRANCHED FLORETLESS 1 (BFL1) that mediates the transition from spikelet to floret meristem in rice (Oryza sativa L). BMC Plant Biol. **3**: 6.

# VI. Abbreviations

| Abbreviation | Explanation |
|---|---|
| ABA | abscisic acid |
| ACBP6 | ACYL-COA-BINDING PROTEIN 6 |
| AP1 | APETALA1 |
| AGL6/13 | AGAMOUS-LIKE 6/13 |
| AHK3/4 | HISTIDINE KINASE 3/4 |
| als1 | absent lower laterals1 |
| FUL | FRUITFULL |
| AP2/ERF | APETALA2/ETHYLENE RESPONSE FACTOR |
| APO1 | ABERRANT PANICLE ORGANIZATION 1 |
| ARR-A/B | type A and B RESPONSE REGULATORS |
| ATC | Arabidopsis thaliana CENTRORADIALIS homologue |
| ATXR6 | ARABIDOPSIS TRITHORAX-RELATED PROTEIN 6 |
| AtMSI1 | ARABIDOPSIS MULTICOPY SUPRESSOR OF IRA1 |
| AtPRMT5/10 | PROTEIN ARGININE METHYLTRANSFERASES 5/10 |
| AXM | axillary meristem |
| BA1 | BARREN STALK 1 |
| BBC1 | BREAST BASIC CONSERVED 1 |
| BD1 | BRANCHED SILKLESS1 |
| BFT | BROTHER OF FT AND TFL 1 |
| BIF2 | BARREN INFLORESCENCE 2 |
| BiFC | bimolecular fluorescence complementation |
| BLR | BELLRINGER |
| BRC1 | BRANCHED 1 |
| BRs | Brassinosteroids |
| CAL | CAULIFLOWER |
| CDC6 | CELL DIVISION CONTROL 6 |
| CEN | CENTRORADIALIS |
| CIMS | COBALAMIN-INDEPENDENT METHIONINE SYNTHASE and METHIONINE SYNTHESIS 1 |
| CK | cytokinins |
| CNGC2 | CYCLIC NUCLEOTIDE-GATED ION CHANNEL14 |
| CO | CONSTANS |
| cul2 | uniculm2 |
| cul4 | uniculm4 |
| D14 | DWARF 14 |
| den6 | densonidosum6 |
| DET | DETERMINATE |
| DETs | differentially expressed transcripts |
| DLF1 | DELAYED FLOWERING 1 |
| DLT | DWARF AND LOW TILLERING |
| DT1 | DETERMINATE STEM 1 |
| EAM | EARLY MATURITY |
| EBF1 | EIN3-BINDING F BOX PROTEIN 1 |

| Abbreviation | Explanation |
|---|---|
| ELF3 | EARLY FLOWERING 3 |
| EPS | EARLINESS PER SE |
| ER | endoplasmic reticulum |
| FD | FLOWERING LOCUS D |
| FLC | FLOWERING LOCUS C |
| FT | FLOWERING LOCUS T |
| FTIP1 | FT-INTERACTING PROTEIN 1 |
| FZP | FRIZZY PANICLE |
| GDU4 | GLUTAMINE DUMPER 4 |
| GFA2 | GAMETOPHYTIC FACTOR 2 |
| gra-a | granum-a |
| grassy | grassy tillers |
| HCF173 | HIGH CHLOROPHYLL FLUORESCENCE173 |
| HD3A | HEADING DATE 3A |
| Hv20ox2 | Barley Gibberellin (GA) 20-oxidase gene |
| HvBM3 | BARLEY MADS-BOX GENE 3 |
| HvBRI1 | Barley BRASSINOSTERD-INSENSITIVE1-like gene |
| IDS1 | INDETERMINATE SPIKELET1 |
| int-b | intermedium-b |
| INT-C | Intermedium-c |
| int-m | intermedium-m |
| IPA1 | IDEAL PLANT ARCHITECTURE1 |
| LAS | LATERAL SUPPRESSOR |
| LAX1 | LAX PANICLE 1 |
| LBD37 | LOB domain containing protein 37 |
| LF | LATE FLOWERING |
| lf1 | lateral florets 1 |
| LFY | LEAFY |
| lnt1 | low number of tillers1 |
| LOB | lateral organs boundaries |
| LUX | LUX ARHYTHMO |
| MAT | PRAEMATURUM |
| MFT | MOTHER OF FT AND TFL1 |
| MKS1 | MAP KINASE SUBSTRATE 1 |
| mnd1 | many noded dwarf1 |
| mnd6 | many noded dwarf6 |
| MOC1 | MONOCULM1 |
| MOS1 | MORE SPIKELETS1 |
| MSA | main shoot apical meristem |
| NFD1 | NUCLEAR FUSION DEFECTIVE |
| NF-YA | NUCLEAR FACTOR-YA subunits |
| NSN1 | NUCLEOSTEMIN-LIKE 1 |
| OsSPL14 | SQUAMOSA PROMOTER BINDING PROTEIN-LIKE14 |
| OXS3 | OXIDATIVE STRESS 3 |
| PAP2 | PANICLE PHYTOMER2/OSMADS34 |

| Abbreviation | Explanation |
|---|---|
| PC | phosphatidylcholine |
| PCA | principle component analysis |
| PCNA2 | PROLIFERATING CELL NUCLEAR ANTIGEN 2 |
| PEBPs | phosphatidylethanolamine-binding proteins |
| PFK | phosphofructokinases |
| PGY2/3 | PIGGYBACK 2/3 |
| PHYC | PHYTOCHROME C |
| PI | PISTILLATA |
| PIP3 | PLASMA MEMBERANE INTRINSIC PROTEIN 3 |
| PPD-H1 | Barley PHOTOPERIOD1 |
| ra1,2,3 | ramosa1, 2, 3 |
| RCN1/2 | Rice CEN-LIKE 1/2 |
| RKIP | Raf kinase inhibitor protein |
| RPS 6/13 | RIBOSOMAL PROTEIN S 6/13 |
| SAM | shoot apical meristem |
| SAMS3 | S-ADENOSYLMETHIONINE SYNTHETASE 3 |
| sdw1 | semidwarf1 |
| SEP1/2/3/4 | SEPALLATA1/2/3/4 |
| SFT | SINGLE FLOWER TRUSS |
| SHI | SHORT INTERNODES |
| SIS | SALT INDUCED SERINE RICH PROTEIN |
| SL | strigolactone |
| SNB | SUPERNUMERARY BRACT |
| SOC1 | SUPPRESSOR OF OVEREXPRESSION OF CO1 |
| SP | SELF-PRUNING |
| SP5G | SELF PRUNING 5G |
| STM | SHOOT MERISTEMLESS |
| SVP | SHORT VEGETATIVE PHASE |
| TAD1 | TILLERING AND DWARF 1 |
| TaPPD-1 | Wheat PHOTOPERIOD-1 |
| TAW1 | TAWAWA1 |
| TB1 | TEOSINTE BRANCHED 1 |
| TE | TILLER ENHANCER |
| TFL1 | TERMINAL FLOWER 1 |
| TPS1 | trehalose-6-phosphatase |
| TSF | TWIN SISTER OF FT |
| UFO | UNUSUAL FLORAL ORGANS |
| VRN3 | VERNALIZATION 3 |
| VRS1/2/2/4/5 | SIX-ROWED SPIKE1/2/3/4/5 |
| WFP | WEALTHY FARMER'S PANICLE |
| WRKY | WRKY DNA-binding proteins |
| ZCN2,4,5,8 | Zea CENTRORADIALIS 2,4,5,8 |
| ZTL | ZEITLUPE |

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# VIII. Erklärung

Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit – einschließlich Tabellen, Karten und Abbildungen -, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegt worden ist, sowie, dass ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde. Die Bestimmungen dieser Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. Maria von Korff Schmising und Professor Dr. Rüdiger Simon betreut worden.

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