Molecular and Functional Characterization of PEBP Genes in Barley Reveal the Diversification of Their Roles in Flowering[1][W][OA]

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Five barley (*Hordeum vulgare*) PEBP (for phosphatidylethanolamine-binding protein) genes were analyzed to clarify their functional roles in flowering using transgenic, expression, and quantitative trait locus analyses. Introduction of *HvTFL1* and *HvMFT1* into rice (*Oryza sativa*) plants did not result in any changes in flowering, suggesting that these two genes have functions distinct from flowering. Overexpression of *HvFT1*, *HvFT2*, and *HvFT3* in rice resulted in early heading, indicating that these *FT*-like genes can act as promoters of the floral transition. *HvFT1* transgenic plants showed the most robust flowering initiation. In barley, *HvFT1* was expressed at the time of shoot meristem phase transition. These results suggest that *HvFT1* is the key gene responsible for flowering in the barley *FT*-like gene family. *HvFT2* transgenic plants also showed robust flowering initiation, but *HvFT2* was expressed only under short-day (SD) conditions during the phase transition, suggesting that its role is limited to specific photoperiodic conditions in barley. Flowering activity in *HvFT3* transgenic rice was not as strong and was modulated by the photoperiod. These results suggest that *HvFT3* functions in flowering promotion but that its effect is indirect. *HvFT3* expression was observed in Morex, a barley cultivar carrying a dominant allele of *Ppd-H2*, a major quantitative trait locus for flowering under SD conditions, although no expression was detected in Steptoe, a cultivar carrying *ppd-H2*. *HvFT3* was expressed in Morex under both long-day and SD conditions, although its expression was increased under SD conditions. *HvFT3* was mapped to chromosome 1HL, the same chromosome that carries *Ppd-H2*. Genomic sequence analyses revealed that Morex possesses an intact *HvFT3* gene, whereas most of this gene has been lost in Steptoe. These data strongly suggest that *HvFT3* may be identical to *Ppd-H2*.

Floral transition (i.e. the change from a vegetative meristem to the reproductive stage) is a critical event in the life cycle of seed-propagated plants. Several pathways promote flowering, including vernalization, photoperiod, and autonomous and gibberellin pathways (Boss et al., 2004). In Arabidopsis (*Arabidopsis thaliana*), *FLOWERING LOCUS T* (*FT*), which encodes a protein with a unique phosphatidylethanolamine-binding protein (PEBP) domain (domain accession pfam01161), promotes flowering. *FT* plays a central role in the integration of flowering signals from the vernalization and photoperiod pathways (Kardailsky et al., 1999; Kobayashi et al., 1999). Arabidopsis is a long-day (LD) plant, and its flowering is induced under LD conditions. Under LD conditions, *CONSTANS* (*CO*), which is regulated by circadian clock factors such as GIGANTEA (GI) and which encodes a transcription factor with two B-box zinc fingers, induces *FT* expression, causing early flowering (Yanovsky and Kay, 2003). Recent studies indicate that the FT protein functions as a systemic signaling molecule from leaf to apex, a so-called "florigen" (Corbesier et al., 2007; Jaeger and Wigge, 2007; Mathieu et al., 2007). The FT protein interacts with the bZIP transcription factor FD at the apex and activates the floral meristem identity gene *APETALA1* (Abe et al., 2005; Wigge et al., 2005).

Like the Arabidopsis *FT* gene, the rice (*Oryza sativa*) *FT*-like gene *Hd3a* was identified as a flowering-time quantitative trait locus (QTL), which promotes flowering under short-day (SD) conditions (Yano et al., 2001; Monna et al., 2002). *Hd3a* expression is regulated by *Hd1*, the rice ortholog of *CO*. However, the expression and regulation of *Hd3a* are completely opposite from those of *FT* in Arabidopsis. *Hd3a* is involved in the promotion of heading (the developmental stages responsible for the initial emergence of the inflorescence

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from the boot in grass plants) under SD conditions, and its expression is completely inhibited under LD conditions (Kojima et al., 2002; Hayama et al., 2003; Hayama and Coupland, 2004). This is consistent with the fact that rice is a SD plant that flowers earlier under SD conditions than LD conditions. These results obtained for Arabidopsis and rice show that *FT* expression is common to flowering induction in higher plants and that variation in the *CO-FT* interaction is a key difference between LD and SD flowering induction. Recently, in wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*), a vernalization gene, *VRN3*, was shown to encode a homolog of Arabidopsis *FT* (Yan et al., 2006). Analyses of transgenic plants showed that *VRN3* functions as a flowering promoter, like Arabidopsis *FT* and rice *Hd3a*. This finding indicates that the same type of genetic system controls the flowering process in wheat and barley.

Recent advances in plant biology have provided access to the complete genome sequences of flowering plant species, including those of two model organisms, Arabidopsis and rice (Arabidopsis Genome Initiative, 2000; International Rice Genome Sequencing Project, 2005). Using these sequence data, genome-wide searches have been carried out to discover all PEBP gene families present in plant genomes. In Arabidopsis, *FT* forms a small gene family with five other genes: *TERMINAL FLOWER1* (*TFL1*), *TWIN SISTER OF FT* (*TSF*), *BROTHER OF FT AND TFL1* (*BFT*), *ARABIDOPSIS THALIANA CENTRORADIALIS HOMOLOGUE* (*ATC*), and *MOTHER OF FT AND TFL1* (*MFT*; Kardailsky et al., 1999; Kobayashi et al., 1999). Some of these genes are postulated to have important functions in the flowering pathway of Arabidopsis. On the other hand, Chardon and Damerval (2005) identified 19 PEBP genes, including the *FT* homolog *Hd3a* in the rice genome. Recently, Danilevskaya et al. (2008) identified 25 PEBP genes in maize (*Zea mays*). The number of PEBP family genes in cereal is three to four times greater than that in Arabidopsis, indicating that this family is much more complex in cereal plants than in Arabidopsis. This PEBP gene redundancy in cereals raises questions about the functional diversification that remain to be elucidated.

In barley, a study using EST database searches and bacterial artificial chromosome library screening identified at least five *FT*-like genes, *HvFT1* to *HvFT5* (Faure et al., 2007). This raised questions about the roles of these *FT*-like genes in the flowering process. Moreover, it is interesting to compare the functional roles of these *FT*-like genes between rice and barley, because barley is more closely related to rice than to Arabidopsis but is a LD plant like Arabidopsis. Although barley is classified as a LD plant, its photoperiod response is facultative (quantitative response). Therefore, flowering in barley is not only promoted by a suitable LD condition but can also occur even under an inappropriate SD condition, although it is delayed. In barley, two major photoperiod response genes, *Ppd-H1* and *Ppd-H2*, are located on chromosomes 2HS and 1HL, respectively (Laurie, 1997). *Ppd-H1* promotes flowering in response to increasing daylength under LD conditions, whereas *Ppd-H2* affects flowering under SD conditions but has little effect under LD conditions. Recently, the *Ppd-H1* gene was cloned and identified as a member of the pseudoresponse regulator family. This gene may modulate daylength induction of *FT*-like genes by controlling *CO*-like activity in barley (Turner et al., 2005).

In this study, we performed expression and transgenic studies to clarify the functional roles of three *FT*-like genes and two other PEBP genes with regard to the flowering time of barley using two cultivars with different photoperiod response behaviors and different genotype combinations for the major photoperiod-sensitive *Ppd* genes, Steptoe and Morex. Three *FT*-like genes had already been identified by Faure et al. (2007), and the other two were novel genes. Here, we demonstrate the functional differentiation between these genes in controlling flowering using rice plants overexpressing barley PEBP genes. Some of these genes carry out the same function for flowering as their orthologs in Arabidopsis and rice, whereas others do not function in flowering in the rice genetic background. We also show the expression profiles of barley *FT*-like genes under LD and SD conditions in relation to the photoperiod response genes, *Ppd-H1* and *Ppd-H2*. Furthermore, we reveal the close relationship between one barley *FT*-like gene and *Ppd-H2*, a major photoperiod response QTL that promotes flowering under SD conditions (Laurie et al., 1995; Laurie, 1997).

RESULTS

Search for Barley PEBP Genes and Phylogenetic Analyses

To identify PEBP genes in barley, we performed an in silico search of an in-house sequence database of full-length barley cDNA libraries, which were constructed from the mixed cDNAs of various tissues of a Japanese two-row variety, Haruna-Nijo (T. Matsumoto, H. Kanamori, K. Kurita, T. Bito, A. Kikuta, K. Kamiya, M. Yamamoto, Y. Mukai, H. Ikawa, N. Fujii, H. Sakai, T. Itoh, K. Sato, and S. Nakamura, unpublished data). The protein sequence of *HvFT1* was used as the query (accession no. DQ100327). Five entries were identified as PEBP genes by this TBLASTN search: NIASHv3142C18, NIASHv3064E22, NIASHv1003I22, NIASHv2071G09, and NIASHv3007O09. During the course of this study, Faure et al. (2007) published their results based on the same kind of survey for *FT*-like genes in barley. We compared our cDNA clones with their barley *FT*-like genes. Our three full-length cDNAs, NIASHv3142C18, NIASHv3064E22, and NIASHv1003I22, corresponded to their *HvFT1*, *HvFT2*, and *HvFT3* genes, respectively. Our other two full-length cDNAs, NIASHv2071G09 and NIASHv3007O09, did not corre-

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spond to any *FT*-like genes found by Faure et al. (2007). However, these cDNAs showed extensive similarity to Arabidopsis *TFL1* and *MFT*, respectively. Therefore, we named these two cDNA clones (NIASHv2071G09 and NIASHv3007O09) *HvTFL1* (accession no. AB447465) and *HvMFT1* (accession no. AB447466), respectively.

Phylogenetic analysis of an amino acid sequence alignment of the PEBP domains from PEBP genes in barley, rice, and Arabidopsis (Chardon and Damerval, 2005; Faure et al., 2007; this study) revealed that the PEBP gene family contains three major clades. *HvFT1*, *HvFT2*, and *HvFT3* belonged to the *FT*-like clade, whereas *HvTFL1* and *HvMFT1* were members, respectively, of the *TFL1*-like and *MFT*-like clades, as expected (Fig. 1; Supplemental Fig. S1). Protein sequence alignment also revealed an amino acid residue change from Tyr in barley FT-like proteins (HvFT1, HvFT2, and HvFT3) to His in HvTFL1 (Supplemental Fig. S1), which was a key residue for the functional difference between FT and TFL1 in Arabidopsis (Hanzawa et al., 2005).

Genetic Mapping of Barley PEBP Genes and Analysis of QTLs for Heading

All five barley PEBP genes in a doubled haploid (DH) population from the cross between two barley cultivars, Steptoe and Morex, were mapped (Fig. 2). *HvFT1* and *HvFT2* were mapped to a position 4.7 cM distal from ABC158 on the short arm of chromosome 7H and to a position 4.4 cM proximal to ABG399 on chromosome arm 3HS, respectively. These data correspond well to previous reports (Yan et al., 2006; Faure et al., 2007). *HvTFL1* and *HvMFT1* were also mapped to a position 5.4 cM distal from ksuA3A on chromosome arm 5HS and to the end of the short arm of chromosome 3H, 8.5 cM from MWG571C, respectively. Barley chromosome 3H is syntenic to rice chromosome 1, the short arm of which contains *OsMFT2* at its end (Chardon and Damerval, 2005). Phylogenetic analysis showed that *OsMFT2* and *HvMFT1* were located in the same branch (Fig. 1). These results suggest that these two genes are orthologs in rice and barley, respectively.

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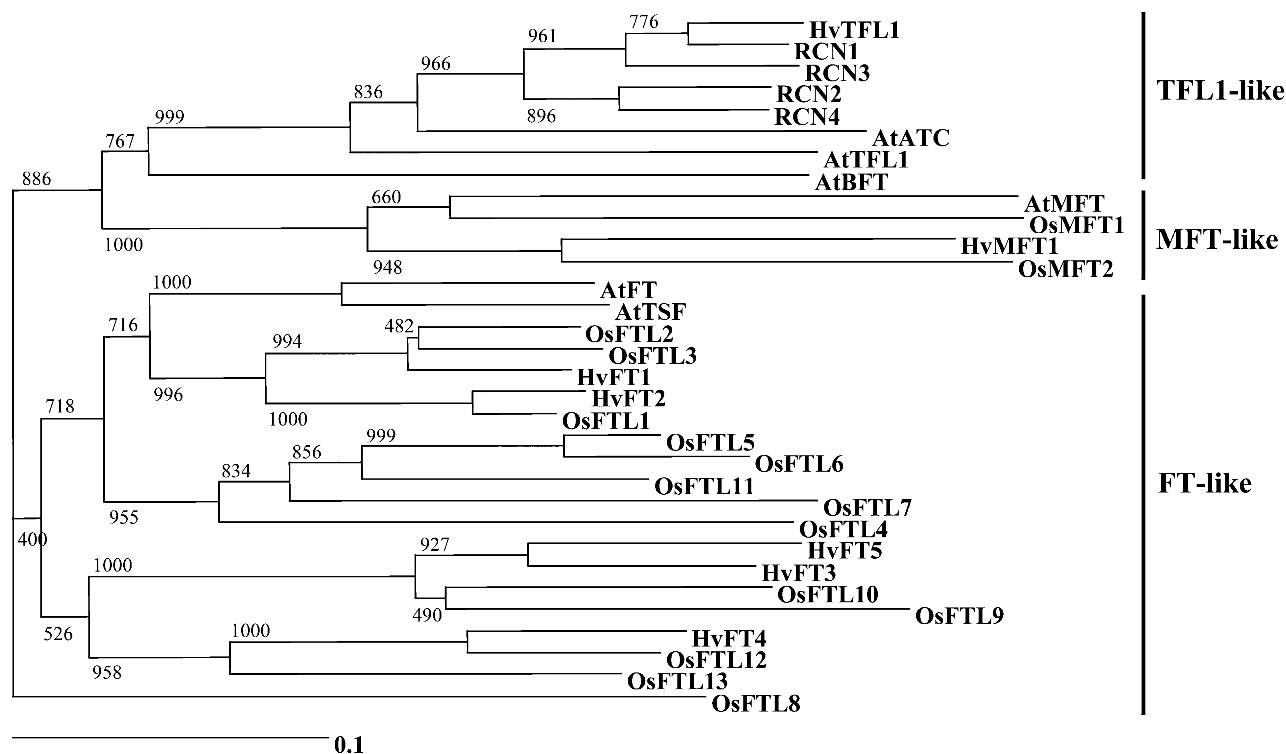

Figure 1. Phylogenetic tree of the PEBP proteins from barley, rice, and Arabidopsis. Sequences were aligned with ClustalX, and the results are displayed graphically using TreeView. Bootstrap values out of 1,000 bootstrap resamplings are shown at the nodes to assess the robustness of the tree. Accession numbers of the genes used for the phylogenetic analysis are as follows: barley HvFT1 (DQ100327), HvFT2 (DQ297407), HvFT3 (DQ411319), HvFT4 (DQ411320), HvFT5 (EF012202), HvTFL1 (AB447465), and HvMFT1 (AB447466); and Arabidopsis FT (AB027505), TSF (AB027506), TFL1 (AAM27944), MFT (AF147721), BFT (BAB10165), and ATC (NM_128315). TIGR loci in rice are as follows: OsFTL1 (Os01g11940), OsFTL2 (Hd3a; Os06g06320), OsFTL3 (RFT1; Os06g06300), OsFTL4 (Os09g33850), OsFTL5 (Os02g39064), OsFTL6 (Os04g41130), OsFTL7 (Os12g13030), OsFTL8 (Os01g10590), OsFTL9 (Os01g54490), OsFTL10 (Os05g44180), OsFTL11 (Os11g18870), OsFTL12 (Os06g35940), OsFTL13 (Os02g13830), OsMFT1 (Os06g30370), OsMFT2 (Os01g02120), RCN1 (Os11g05470), RCN2 (Os02g32950), RCN3 (Os12g05590), and RCN4 (Os04g33570).

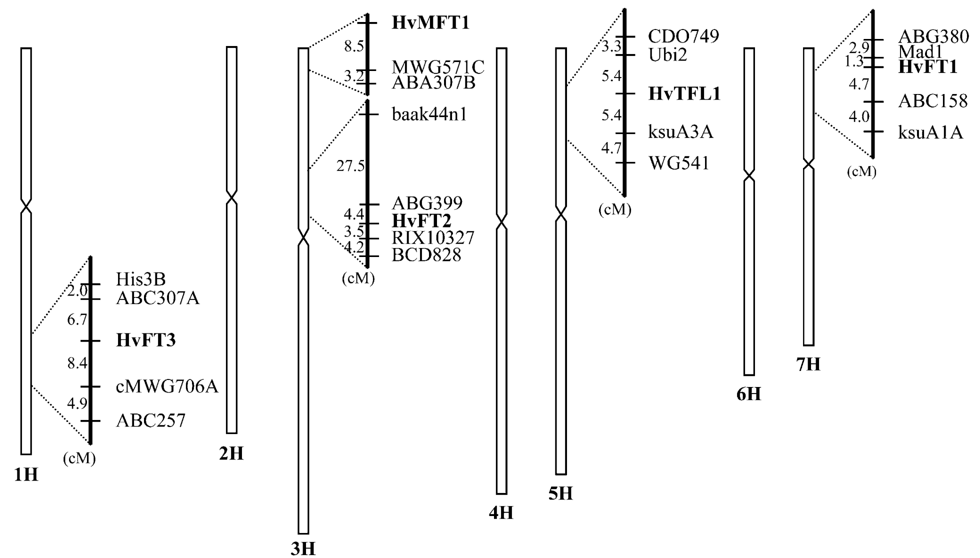

Figure 2. Chromosomal localization of the PEBP genes in barley. Solid lines indicate the partial maps that are drawn to larger scale and marked with the approximate positions of barley chromosomes. All chromosomes were mapped using the DH population derived from the cross between Steptoe and Morex.

The chromosomal position of *HvFT3* is 6.7 cM distal from ABC307A and 8.4 cM proximal to cMWG706A on the long arm of chromosome 1H (Figs. 2 and 3), consistent with the results of Faure et al. (2007). As pointed out by Faure et al. (2007), it is interesting that *HvFT3* maps to the same region as *Ppd-H2*, which is a major QTL for heading time under SD conditions in barley (Laurie et al., 1995; Laurie, 1997). Therefore, we conducted a QTL analysis for flowering time under SD conditions (12 h of light/12 h of dark) in a growth chamber at 20°C ± 2°C using the Steptoe/Morex DH population. Figure 3A shows the log of the odds (LOD) curve obtained for chromosome 1H using interval mapping for flowering time under SD conditions. One major peak for flowering time was detected in the region containing *HvFT3*. The LOD score for this peak was 11.3, which explained 30.4% of the phenotypic variation. We also identified a second QTL peak between markers ABC482 and ABG391 on the long arm of chromosome 5H. This peak demonstrated a LOD score of 4.13, explaining 13.1% of the phenotypic variation (Fig. 3B). Although *HvFTL1* was located on the short arm of chromosome 5H (Fig. 2), there was no association between *HvFTL1* and this minor QTL on 5H (Fig. 3B).

Overexpression of Barley PEBP Genes in Rice Plants

To investigate their possible functional roles, especially pertaining to flowering, five barley PEBP genes, *HvFT1*, *HvFT2*, *HvFT3*, *HvTFL1*, and *HvMFT1*, were introduced into rice plants under the control of the cauliflower mosaic virus (CaMV) 35S promoter.

Overexpression of *HvTFL1* or *HvMFT1* had no effects on flowering or other phenotypes in rice plants (Fig. 4B for flowering time; data not shown for other phenotypes). On the other hand, *HvFT1* and *HvFT2* transgenic rice plants displayed much earlier heading than control plants under both LD and SD conditions (Fig. 4). There were no differences in the heading times of *HvFT1* and *HvFT2* transgenic plants between LD and SD conditions. These results indicate that *HvFT1* and *HvFT2* function similarly as strong inducers of flowering in rice plants, whereas *HvTFL1* and *HvMFT1* demonstrated no obvious function for flowering.

HvFT3-overexpressing rice plants also showed earlier heading compared with control plants, as did *HvFT1*- and *HvFT2*-overexpressing plants (Fig. 4), suggesting that *HvFT3* also plays a role in flowering. However, the effect of *HvFT3* on heading time was weaker than those of *HvFT1* and *HvFT2*. Interestingly, the heading time for *HvFT3* transgenic rice plants differed between LD and SD conditions. Heading of *HvFT3* transgenic rice plants under LD conditions was delayed by 14.7 d compared with heading under SD conditions (Fig. 4B). This difference was identical to that of the control plants (15.4 d). These results revealed that *HvFT3* transgenic rice plants maintain the same flowering photoperiod response as control plants, suggesting that the role of *HvFT3* in flowering is indirect and that flowering activity can be modulated by the photoperiod signals, in contrast to the cases of *HvFT1* and *HvFT2*.

Expression Pattern of Barley *FT*-Like Genes during Different Developmental Stages

To determine whether *FT*-like gene expression is associated with the photoperiodic response in barley, quantitative reverse transcription (RT)-PCR was applied to two barley cultivars, Steptoe and Morex. Steptoe is a late-heading cultivar under field conditions (autumn sowing), whereas Morex exhibits early heading in the field. However, Morex heading is delayed under controlled LD conditions compared with Steptoe. Turner et al. (2005) revealed that Steptoe

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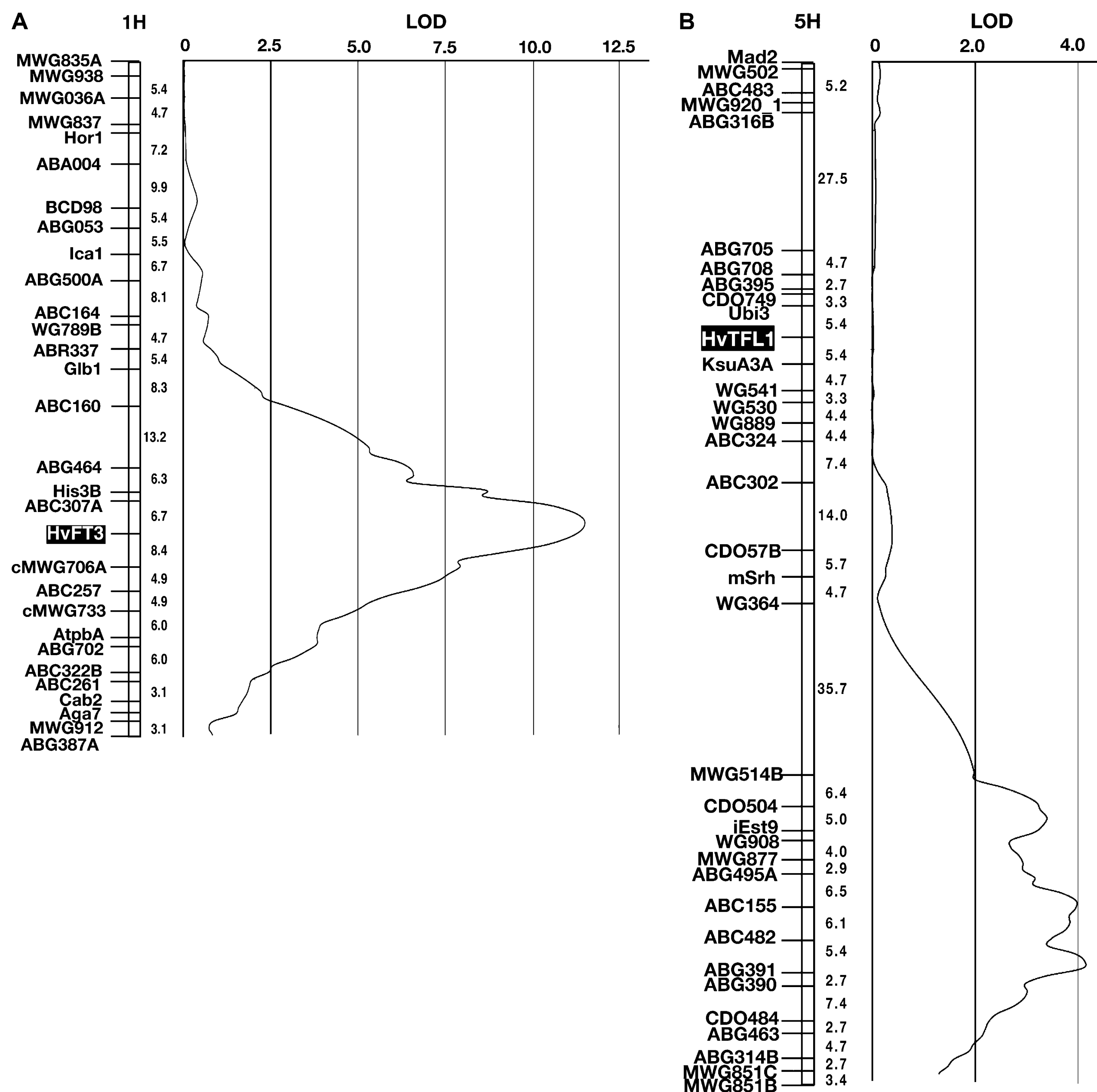

Figure 3. Linkage maps of barley chromosomes 1H (A) and 5H (B) constructed from the DH population derived from Steptoe × Morex and QTL graphs for flowering time under SD conditions (12 h of light) with a threshold of LOD 2.5 and 2.0 using interval mapping, respectively. Genetic distances are shown in centimorgans.

possesses *Ppd-H1*, which promotes flowering under LD conditions, but Morex carries *ppd-H1*. Therefore, the heading delay of Morex under LD conditions can be explained by the difference in the *Ppd-H1* genotype. In contrast, in the field and under controlled SD conditions, Morex undergoes heading earlier than Steptoe. As shown before, we identified a major QTL for SD heading on chromosome 1HS that may correspond to *Ppd-H2*; the positive and dominant allele for this QTL was derived from Morex. Therefore, we postulate that Steptoe and Morex carry *ppd-H2* and *Ppd-H2*, respectively, which could explain the early heading of Morex under SD conditions.

To obtain an overall picture of barley *FT*-like gene expression, we compared expression patterns between Steptoe and Morex through different developmental stages under both photoperiodic conditions (LD and SD). Under LD conditions, the expression levels of *HvFT1* and *HvFT2* were higher in Steptoe than in Morex (Fig. 5, A and C). This result supports the hypothesis that *Ppd-H1* expression in response to LD photoperiods in Steptoe induces *HvFT1* and *HvFT2*

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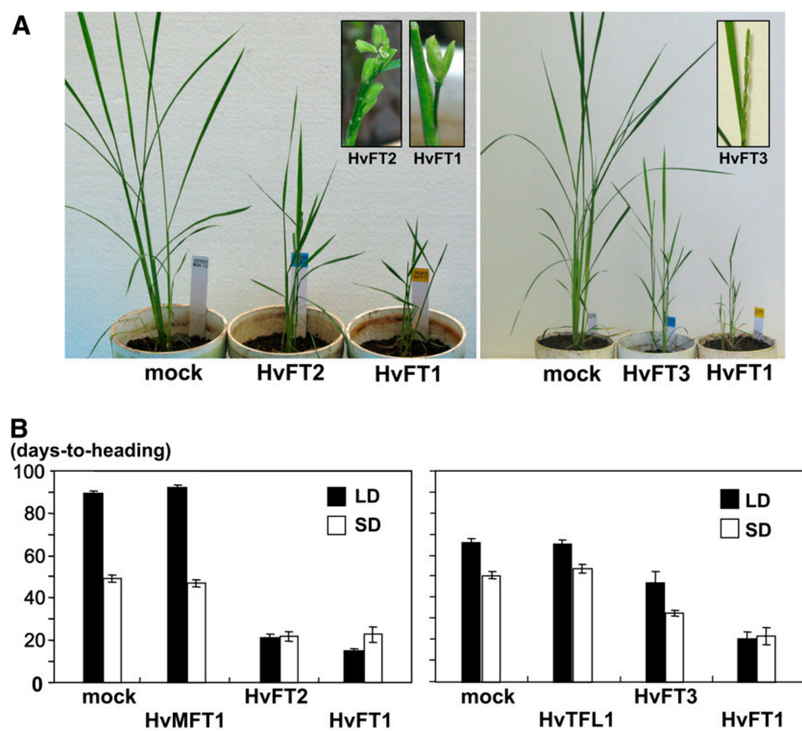

Figure 4. Phenotype (A) and flowering time (B) of transgenic rice plants overexpressing barley PEBP genes. A, Photographs show the transgenic plants at the heading stage under LD conditions as an example. The insets show enlarged photographs of panicles on the heads of transgenic rice plants. *HvTFL1* and *HvMFT1* transgenic rice are not shown because they exhibited no differences compared with mock controls. B, The average number of days to heading ± SE of 10 to 20 transgenic rice plants under LD conditions (black bars) and SD conditions (white bars). Two independent experiments were performed for the transgenic rice plants (see "Materials and Methods" for details). Mock indicates the transgenic plant containing only the vector construct (negative control).

expression and then promotes flowering under LD conditions. *HvFT1* was expressed at an earlier stage (one-leaf stage, 1 week after sowing), whereas the expression of *HvFT2* was detected later (after the four-leaf stage). Morex, which carries *ppd-H1*, showed extremely low *HvFT1* and *HvFT2* expression under LD conditions (Fig. 5, A and C), consistent with the late heading of Morex compared with Steptoe under LD conditions. These results suggest that *HvFT1* plays a primary role in flowering induction in barley cultivars carrying *Ppd-H1* under LD conditions.

Under SD conditions, Morex showed higher *HvFT1* and *HvFT2* expression than Steptoe, although their expression levels in Morex under SD conditions were lower than in Steptoe under LD conditions (Fig. 5, B and D). These results explain why Morex heads earlier than Steptoe under SD conditions. Under SD conditions, *HvFT1* was expressed in Morex at an earlier stage (one-leaf stage), similar to Steptoe under LD conditions, indicating that *HvFT1* plays an important role in flowering induction under SD conditions. These findings suggest that under SD conditions, *Ppd-H2* induces the expression of *HvFT1*, equivalent to *Ppd-H1* under LD conditions. This results in earlier heading, even under SD conditions. Moreover, we detected the expression of *HvFT2* at the one-leaf stage, and its expression level was nearly the same as that of *HvFT1* (Fig. 5, B and D), indicating that *HvFT2* can cooperate with *HvFT1* as a flowering inducer under SD conditions. However, unlike *HvFT1*, the expression of *HvFT2* under SD conditions was observed not only in Morex but also in Steptoe. This suggests that *Ppd-H2* has no effect on the expression of *HvFT2* and that *HvFT2* expression is regulated by other photoperiodic pathway(s).

The expression of *HvFT3* was observed only in Morex under both LD and SD conditions (Fig. 5, E and F). We could not detect any *HvFT3* expression in Steptoe under either LD or SD conditions. The *HvFT3* expression level in Morex was several times higher under SD conditions than under LD conditions, although its expression was detected under both LD and SD conditions in Morex. Relatively high *HvFT3* expression preceded the expression of *HvFT1* and *HvFT2*; the levels decreased when *HvFT1* and *HvFT2* expression increased (Fig. 5, E and F).

On the other hand, *HvTFL1* and *HvMFT1* transcripts were not detected in leaves in either cultivar under either condition (data not shown). These results suggest that the two genes are not expressed in leaves and that they may not be associated with flowering initiation.

Diurnal Rhythmic Expression Patterns of *HvFT1*, *HvFT2*, and *HvFT3*

To investigate the relationship among the three barley *FT*-like genes, *HvFT1*, *HvFT2*, and *HvFT3*, their diurnal expression patterns were examined at the two-leaf and three-leaf stages, during which the shoot apical meristem (SAM) begins its transition from the vegetative to the reproductive phase, under LD and SD conditions, respectively. The experiment was performed using two barley cultivars, Steptoe and Morex, by quantitative RT-PCR.

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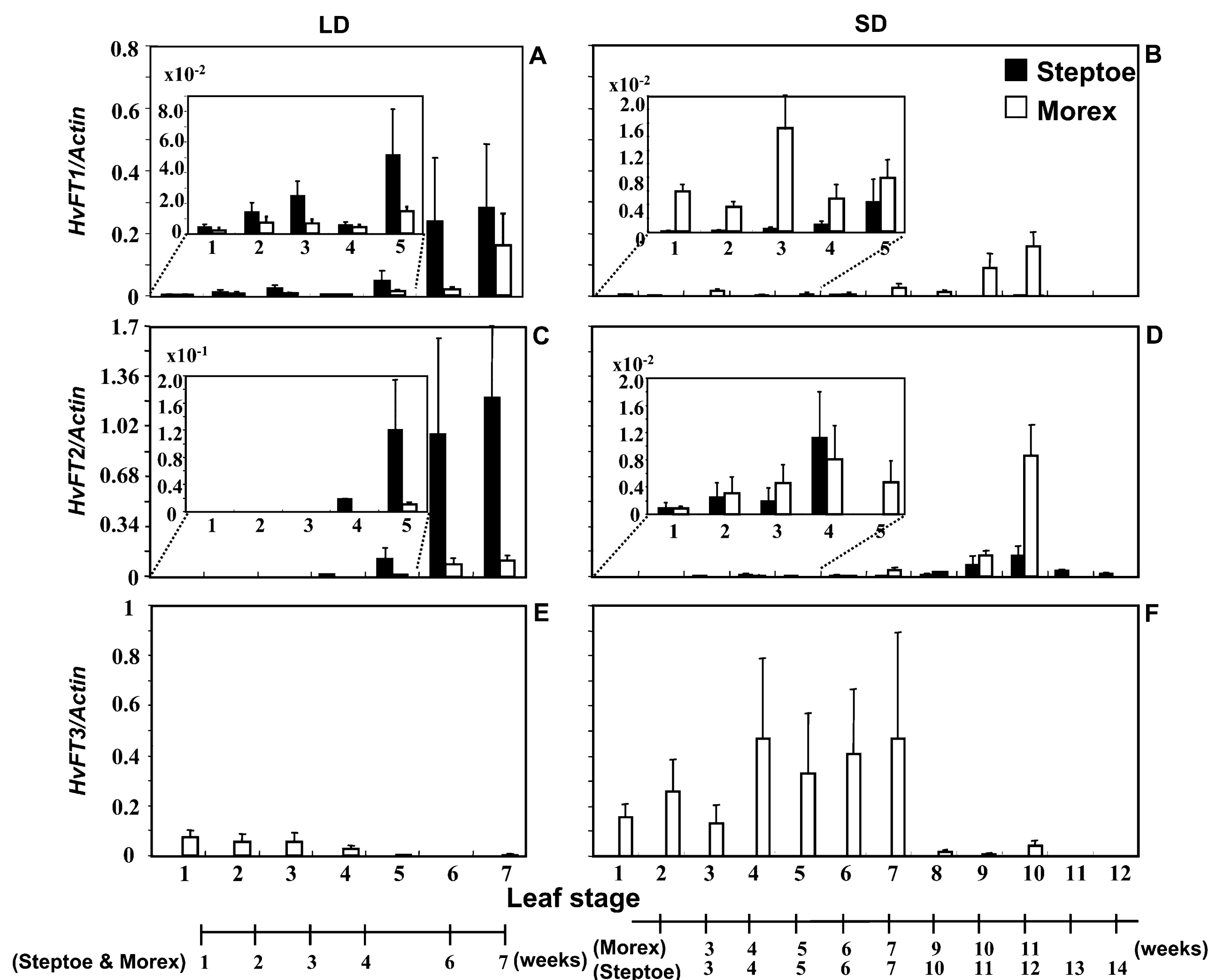

Figure 5. Expression of barley *FT*-like genes, *HvFT1* (A and B), *HvFT2* (C and D), and *HvFT3* (E and F), in Steptoe (*Ppd-H1/ppd-H2*) and Morex (*ppd-H1/Ppd-H2*) at each leaf stage under LD conditions (16 h of light/8 h of dark) and SD conditions (12 h of light/12 h of dark), respectively. The insets show enlarged graphs for earlier stages. Black bars indicate Steptoe and white bars indicate Morex. Each mRNA was quantified relative to *HvActin* mRNA. Data were standardized over four separate experiments (means ± SE). The numbers at the bottom indicate each developmental stage as a leaf stage, with the time scales showing approximate weeks after sowing.

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In Morex, *HvFT1* was transcribed beginning at dawn, and its expression peaked in the middle of the light phase under LD and SD conditions (Fig. 6, A and B). This diurnal oscillation of barley *HvFT1* is nearly identical to that of the rice ortholog *Hd3a* under SD conditions (Kojima et al., 2002; Hayama et al., 2003). The expression of *HvFT3* also demonstrated a diurnal oscillation in Morex under both photoperiod conditions (Fig. 6, E and F). However, the expression pattern of *HvFT3* in Morex was different from that of *HvFT1*, which began to increase in the middle of the light phase, with expression peaking at dusk, early in the dark phase (Fig. 6, E and F). On the other hand, *HvFT2* showed nearly the same pattern of diurnal oscillation under SD conditions as *HvFT3*, and it peaked at the beginning of the dark phase (Fig. 6D).

As discussed above (Fig. 5, B and F), transcripts of *HvFT1* and *HvFT3* in Steptoe were barely detectable under SD conditions during either the daytime or the nighttime (Fig. 6, B and F). However, the expression of *HvFT2* in Steptoe exhibited a distinct diurnal oscillation, which was nearly the same as that observed in Morex (Fig. 6D).

Genomic Structure of *HvFT3* in Steptoe, Morex, and Their DH Lines

To investigate the cause of the differences in *HvFT3* expression between Steptoe and Morex shown in Figures 5 and 6, we determined the genomic structures of *HvFT3* in both barley cultivars (Fig. 7A). We cloned a 1,966-bp region containing *HvFT3* from Morex and

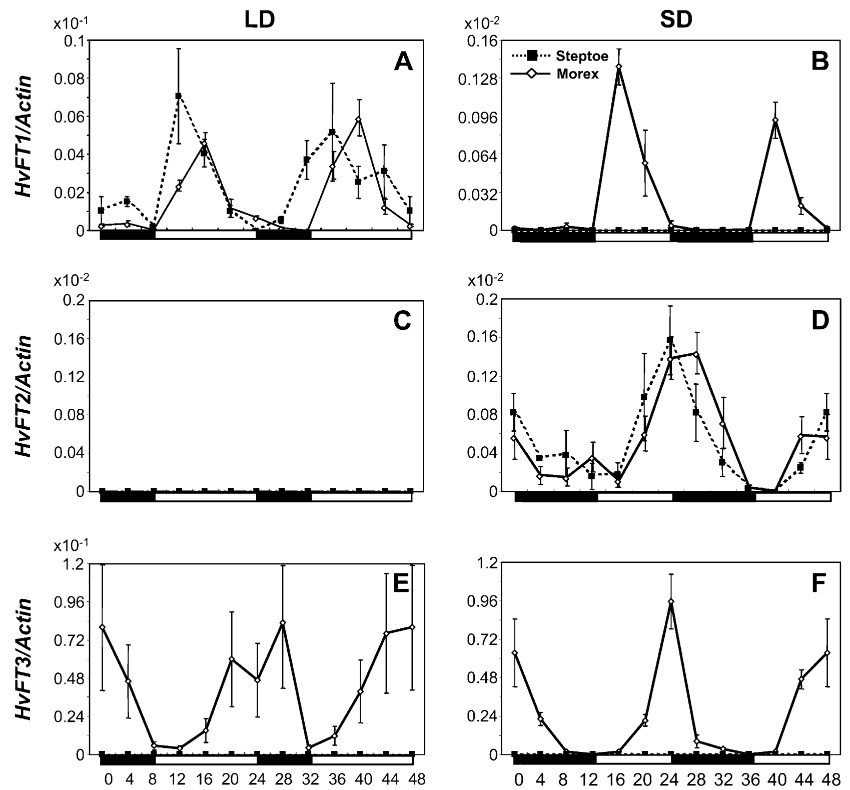

Figure 6. Diurnal expression of barley *FT*-like genes, *HvFT1* (A and B), *HvFT2* (C and D), and *HvFT3* (E and F), in Steptoe (*Ppd-H1/ppd-H2*) and Morex (*ppd-H1/Ppd-H2*) under LD and SD conditions, respectively. Dotted lines with black squares represent Steptoe, and solid lines with white diamonds represent Morex. Leaves were harvested from plants at 4-h intervals during the two-leaf and three-leaf stages for 2 d. The vertical axis shows relative mRNA levels of *FT*-like genes normalized to *HvActin*. The mean quantified values ± SE for two independent experiments are shown. White and black bars at the bottom indicate light and dark periods, respectively.

identified the complete *HvFT3* gene within this fragment (accession no. AB476614). The *HvFT3* gene in Morex has four exons and three introns, a structure that is conserved across most of the *FT*-like genes in higher plants. However, we could not amplify the *HvFT3* region from Steptoe using primer pairs HvFT3/F1 and HvFT3/R1 or HvFT3/F2 and HvFT3/R1, whereas these pairs worked when Morex was used as the template (Fig. 7B). Only the primer pair HvFT3/F3 and HvFT3/R1 produced a fragment from Steptoe (Fig. 7B). Therefore, we tried to isolate the *HvFT3* region in Steptoe and finally obtained a 1,532-bp sequence from the *HvFT3* region in Steptoe (Fig. 7A; accession no. AB476615). The Steptoe *HvFT3* region contains only the 3′ portion of exon 4 (189 bp; Morex exon 4 was 245 bp), and it has lost an upstream region, including three exons (exons 1–3). Instead of the *HvFT3* genomic region, an unknown sequence was

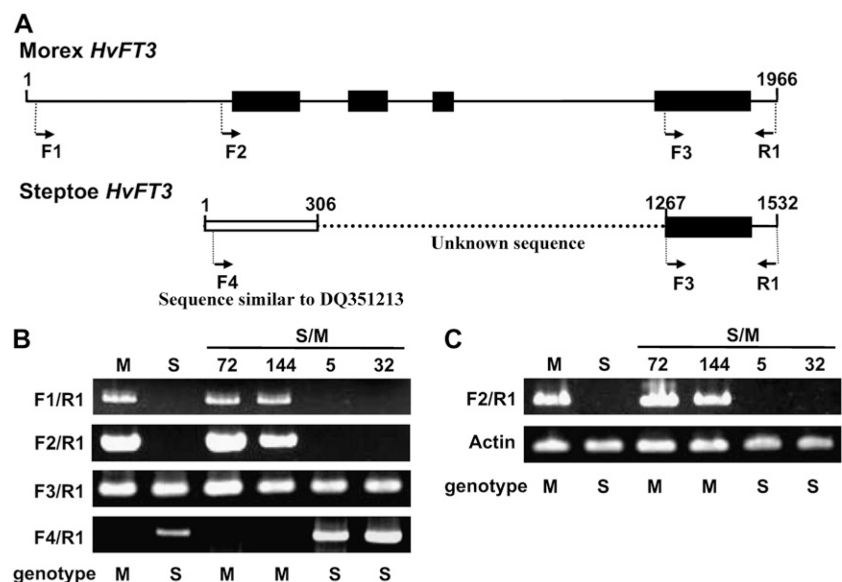

Figure 7. Genomic structure of *HvFT3* in Morex and Steptoe. A, Schematic diagram of the *HvFT3* locus. Black boxes indicate exons, and arrows show the positions of primers. The white box indicates a sequence similar to DQ351213, and the unknown sequence is shown as a dotted line. B, Genomic PCR of Morex, Steptoe, and DH lines (S/M-72, -144, -5, and -32). C, RT-PCR of Morex, Steptoe, and DH lines (S/M-72, -144, -5, and -32) at the four-leaf stage under SD conditions.

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found upstream of the truncated exon 4 sequence (Fig. 7A). These sequence data indicate that Steptoe has no functional *HvFT3* and that this locus is a null allele in Steptoe.

HvFT3 is postulated to be closely associated with *Ppd-H2* (Fig. 3). Therefore, we analyzed the *HvFT3* sequences from four DH lines (S/M-5, -32, -72, and -144) derived from the cross between Steptoe and Morex in addition to those from the parental cultivars (Table I). All four DH lines carried the Steptoe genotype for markers ABC482 and ABG391, which are linked to the 5HL QTL for flowering time under SD conditions. However, the DH lines were segregated between Steptoe type (S/M-5 and -32) and Morex type (S/M-72 and -144) for markers ABC307A and cMWG706A, which lie adjacent to *Ppd-H2*. They were also segregated with regard to heading time, because S/M-72 and -144 displayed early heading under SD conditions (like Morex) and S/M-5 and -32 exhibited a late-heading phenotype (like Steptoe). The presence of an intact copy of the *HvFT3* gene and its normal expression correlated with the segregation of the heading phenotype and the marker genotype (Fig. 7, B and C). Early-heading DH lines (S/M-72 and -144) possessed an intact *HvFT3* gene and demonstrated similar expression to that of Morex, whereas late-heading and Steptoe-type genotype DH lines (S/M-5 and -32) carried no intact copies of *HvFT3* and showed no expression of the corresponding transcript.

DISCUSSION

Compared with dicot plants, which have smaller gene families of approximately six to eight members, monocots possess large families of PEBP genes (e.g. 19 members in rice [Chardon and Damerval, 2005] and 25 members in maize [Danilevskaya et al., 2008]). The quantity of family members raises questions about the functional diversification and conservation of genes within the PEBP family in cereals.

In this study, we identified five PEBP genes in barley, three of which belong to the *FT*-like clade: *HvFT1*, *HvFT2*, and *HvFT3*. Two other genes, *HvTFL1* and *HvMFT1*, were classified in the *TFL1*-like and *MFT*-like clades, respectively (Fig. 1). Each PEBP gene was subjected to further expression and transgenic analyses to reveal its functional role in flowering.

tfl1 mutants show early flowering and the promotion of terminal floral meristem formation in Arabidopsis (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992). *TFL1* encodes a protein with a PEBP domain (Bradley et al., 1997; Ohshima et al., 1997), and *FT* was later identified as a *TFL1* homolog (Kardailsky et al., 1999; Kobayashi et al., 1999). In transgenic Arabidopsis plants that ectopically overexpress *TFL1*, both vegetative and reproductive phases are greatly extended (Ratcliffe et al., 1998). Amaya et al. (1999) overexpressed *TFL1* and *Antirrhinum CENTRORADIALIS* (*CEN*) in tobacco (*Nicotiana tabacum*), which is a homolog of *TFL1* (Bradley et al., 1996). Tobacco plants overexpressing *CEN* display an extended vegetative phase, whereas overexpression of *TFL1* in tobacco plants does not significantly delay flowering time. This may reflect a divergence of the CEN and TFL1 proteins, or plants may possess a pathway for altering the phase change mechanism that involves TFL1-like proteins, and the pathway may differ between species. Two *TFL1*-like genes, *RCN1* and *RCN2*, were detected in rice (Nakagawa et al., 2002). Overexpression of *RCN1* and *RCN2* in rice yielded similar phenotypes to overexpression of *TFL1* in Arabidopsis (i.e. delayed flowering and altered panicle morphology). These results suggest that rice possesses the same type of molecular mechanism responsible for controlling the meristem phase transition as Arabidopsis.

In this study, we identified a barley *TFL1*-like gene, *HvTFL1*, which demonstrates the greatest similarity to rice *RCN1* (Fig. 1). However, rice plants overexpressing *HvTFL1* exhibited neither a flowering delay nor alteration of panicle morphology (data not shown). In

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Table I. *Heading date under SD conditions and genotypes of Ppd-H1 and DNA markers near SD-flowering QTLs*

n.d., Not determined.

Line	Heading Date[a]	Genotype				
		Ppd-H1[b]	1HL QTL		5HS QTL	
			ABC307A	cMWG706A	ABC482	ABG391
Steptoe	104.8	*Ppd-H1*	S	S	S	S
Morex	69.4	*ppd-H1*	M	M	M	M
DH line						
S/M-5	104.0	n.d.	S	S	S	S
S/M-32	105.7	n.d.	S	S	S	S
S/M-72	62.5	n.d.	M	M	S	S
S/M-144	76.3	n.d.	M	M	S	S

[a]Days to heading after sowing under SD conditions (12 h of light/12 h of dark). [b]According to the data of Turner et al. (2005).

addition, the *HvTFL1* transcript was not detected in the leaves of any of the barley cultivars under any growth conditions, whereas *RCN1* is expressed in leaves (Nakagawa et al., 2002). These results for *HvTFL1* and *RCN1* in transgenic rice are interesting and indicate the divergence of orthologous proteins and the differentiation of the flowering pathway between rice and barley, similar to the heterologous expression of *CEN* and *TFL1* in tobacco (Amaya et al., 1999).

Similar observations were obtained from the study of *HvMFT1*, a homolog of Arabidopsis *MFT*. We overexpressed *HvMFT1* in rice under the control of the CaMV 35S promoter. The *HvMFT1*-overexpressing plants showed no notable differences in flowering time or plant architecture when compared with the wild type (data not shown). Little is known about the role of *MFT* genes in other plants or even in Arabidopsis. Yoo et al. (2004) suggested that Arabidopsis *MFT* functions as a floral inducer but that it may act redundantly in determining flowering time based on observations using gain-of-function and loss-of-function alleles of *MFT*. However, we could not detect any induction of flowering when *HvMFT1* was overexpressed in rice, suggesting that *HvMFT1* does not function in flowering. Recently, Danilevskaya et al. (2008) revealed the seed-specific expression of two *MFT*-like genes in maize, *ZCN9* and *ZCN10*. These two *MFT*-like genes are closely related to one of the *MFT*-like genes, *OsMFT2*, in rice. *HvMFT1* may be orthologous to *OsMFT2*, as evidenced by sequence similarity and chromosome mapping (Figs. 1 and 2; Supplemental Fig. S1). *HvMFT1* was mapped to the end of the short arm of barley chromosome 3H (Fig. 2), whereas *OsMFT2* lies in the syntenous region of the short arm of rice chromosome 1. Expression analysis showed that *HvMFT1* is not expressed, at least not in leaves. Together with the transgenic data and expression analysis obtained in this study and other reports, the data suggest that these cereal *MFT*-like genes may function not in the flowering pathway but rather in the grain maturation pathways, as hypothesized by Chardon and Damerval (2005) and Danilevskaya et al. (2008).

We characterized three barley *FT*-like genes: *HvFT1*, *HvFT2*, and *HvFT3*. Transgenic rice plants possessing *HvFT1* and *HvFT2* demonstrated much earlier heading than control plants, regardless of the daylength (Fig. 4). The early-heading phenotypes of *HvFT1* and *HvFT2* transgenic rice plants were very similar to those of rice plants overexpressing *Hd3a*, the rice ortholog of *FT* (Kojima et al., 2002). These results indicate that *HvFT1* and *HvFT2* function similarly to rice *Hd3a*, a strong inducer of flowering in rice and probably also in barley. *HvFT1* was mapped to the short arm of chromosome 7H (Fig. 2), which is syntenous with rice chromosome 6, which contains *Hd3a*. The results from gene-mapping and phylogenetic studies (Fig. 1) suggest that *HvFT1* is an ortholog of rice *Hd3a*. The expression of *HvFT1* in barley has been observed before SAM transition from the vegetative to the reproductive phase (i.e. the two-leaf and three-leaf stages under LD and SD conditions, respectively), although the expression level under LD conditions was higher than that under SD conditions (Fig. 5, A and B). On the other hand, *HvFT1* expression seems to be regulated by the major photoperiod response genes, *Ppd-H1* under LD conditions and *Ppd-H2* under SD conditions, because very low expression was observed in cultivars possessing the recessive alleles of these *Ppd* genes, *ppd-H1* (Morex) and *ppd-H2* (Steptoe), under LD and SD conditions, respectively (Fig. 5, A and B). Taken together, the evidence indicates that *HvFT1* plays a major role in flowering initiation in barley and that this role is regulated by the major response pathways during both photoperiod conditions.

HvFT2 is postulated to be orthologous to one of the rice *FT*-like genes, *OsFTL1*, based on phylogenetic data (Fig. 1) and gene-mapping data indicating that barley 3H, which contain *HvFT2*, is syntenous to rice chromosome 1, which contains *OsFTL1* (Fig. 2). *OsFTL1* is involved in floral promotion in rice, similar to *Hd3a* (Izawa et al., 2002). In wild-type rice, *Hd3a* mRNA expression was up-regulated under inductive SD conditions but not under LD conditions. Under SD conditions, *Hd3a* mRNA was diurnally expressed, exhibiting a peak before dawn. In contrast, *OsFTL1* mRNA expression was detected under both LD and SD conditions, although mRNA levels were higher under SD conditions. These results indicate that these rice *FT*-like genes are expressed differentially in response to different photoperiods (Izawa et al., 2002). Transgenic analysis revealed that *HvFT2* has a functional role during flowering initiation. However, the expression pattern of *HvFT2* in barley differed from that of *HvFT1*. Under LD conditions, the expression of *HvFT2* was delayed compared with *HvFT1* and it was not detected at the two-leaf stage, during which SAM transitions from the vegetative to the reproductive phase (Fig. 5C). On the other hand, under SD conditions, *HvFT2* expression was observed at the three-leaf stage in both Steptoe and Morex (Fig. 5D). However, *HvFT2* expression alone does not appear to be sufficient to induce flowering under SD conditions. Steptoe, as well as Morex, shows *HvFT2* expression under SD conditions, but Steptoe heading is greatly delayed compared with that of Morex. This may be due to the difference in *HvFT1* expression between these two cultivars. These results suggest that *HvFT2* functions as a floral activator specifically under SD conditions but that its function is supplementary to *HvFT1* function. The results also suggest that *HvFT2* participates in a separate pathway from the *Ppd-H2*-dependent photoperiod pathway, unlike *HvFT1*. Barley has an adaptive mechanism to adjust flowering according to photoperiod changes using a combination of different *FT*-like genes.

Faure et al. (2007) postulated that *HvFT3* is a good candidate gene for *Ppd-H2*, which affected flowering

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time in a SD greenhouse experiment (10 h of light) and in an autumn-sown field experiment but was not detectable under LD conditions (Laurie et al., 1995). We identified this major QTL under SD conditions (12 h of light) using a DH population derived from a cross between Steptoe and Morex (Fig. 3).

By examining its overexpression in rice plants, *HvFT3* was found to function as a flowering inducer, but its effect was weaker than that of *HvFT1* or *HvFT2* (Fig. 4). Unlike *HvFT1* and *HvFT2*, heading of *HvFT3* transgenic rice plants was sensitive to the photoperiod. The difference in heading time in *HvFT3* transgenic rice plants under LD compared with SD conditions (14.7 d) was nearly identical to that of control plants (15.4 d; Fig. 4). These results clearly show that *HvFT3* transgenic rice maintained the same photoperiod response for flowering as control plants. Due to the weak flowering induction effect and the same level of photoperiod response as the control plant, we hypothesize that *HvFT3* functions indirectly to promote flowering and that its activity can be modulated by photoperiod signals. This hypothesis is supported by the finding that *HvFT1* and *HvFT2* expression mirrors that of *HvFT3* under SD conditions (Fig. 5).

HvFT3 transcripts in Morex, which carries *Ppd-H2*, were detected under both LD and SD conditions, but its expression levels were higher under SD than under LD conditions (Fig. 5, E and F). Especially high *HvFT3* expression was observed in Morex at the early stage of development before the SAM transition under SD conditions, similar to *HvFT1* (Fig. 5E). In contrast, Steptoe, a cultivar that contains *ppd-H2*, demonstrated no expression of *HvFT3* under any photoperiod conditions (Fig. 5, E and F) and very low expression of *HvFT1* under SD conditions (Fig. 5B). The difference between Steptoe and Morex in flowering time under SD conditions is thought to be due to this difference in *HvFT1* expression, which also suggests that *HvFT3* expression facilitates the induction of *HvFT1* expression. The expression patterns of *HvFT3* and *HvFT1* under SD conditions in Morex showed a clear diurnal rhythm but displayed their own oscillation phase (Fig. 6, B and F). Expression of *HvFT3* begins during the middle of the light phase and peaks at dusk, whereas *HvFT1* is expressed in the morning and peaks in the middle of the light phase. This phase difference is not inconsistent with the idea that *HvFT3* expression participates in the induction of *HvFT1* expression. Sequence analysis revealed that Morex had an intact and functional copy of *HvFT3* but Steptoe had lost most of the gene, rendering it functionless (Fig. 7).

The heading behavior of *HvFT3* transgenic rice, the chromosomal position of *HvFT3*, and the expression profile and structural analysis of *HvFT3* using *Ppd-H2* and *ppd-H2* cultivars strongly support the hypothesis that *HvFT3* is a good candidate gene for *Ppd-H2*, which was reported by Faure et al. (2007). Further studies, including fine mapping and complementation tests with a functional *HvFT3* gene, should be performed to verify this hypothesis.

In conclusion, this study of the barley PEBP genes reveals their presence and structure, which are well conserved among cereal plants, whereas their expression and function have diverged between rice and barley. To understand the molecular mechanism of flowering in a specific plant like barley, it is important to integrate the knowledge obtained from general studies that focus on model plants like Arabidopsis or rice with insights from specific studies of particular plants of interest.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Three varieties of barley (*Hordeum vulgare* 'Steptoe', 'Morex', and 'Haruna-Nijo') were used in this study. DH lines developed from the F1 cross between Steptoe and Morex were also used for gene mapping and QTL analysis (North American Barley Genome Mapping Project; Kleinhofs et al., 1993). The heading dates and genotypes of *Ppd-H1* and DNA markers proximal to SD-flowering QTLs in Steptoe, Morex, and the DH lines used in this study are shown in Table I. Plants were grown in a growth chamber at 20°C ± 2°C (175 μmol m^{-2} s^{-1}) under LD (16 h of light/8 h of dark) or SD (12 h of light/12 h of dark) conditions. For the expression study, leaves from first to last were harvested at the middle of the light period. For the diurnal expression study, the leaves of plants at the two- and three-leaf stages were harvested every 4 h for 2 d under LD and SD conditions, respectively.

Phylogenetic Analysis of PEBP Genes

Phylogenetic analysis using amino acid sequence alignment of the PEBP domain from PEBP genes in barley, rice (*Oryza sativa*), and Arabidopsis (*Arabidopsis thaliana*; Chardon and Damerval, 2005; Faure et al., 2007; this study) was conducted using ClustalX (http://www-igbmc.u-strasbg.fr/BioInfo/ClustalX/Top.html; Thompson et al., 1997) and the neighbor-joining method (Saitou and Nei, 1987). Bootstrap analysis for 1,000 replicates was performed to provide confidence estimates for the tree topologies using the neighbor-joining option in ClustalX. Results were displayed graphically using TreeView (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html).

Genetic Mapping of PEBP Genes

HvFT1, *HvFT2*, *HvFT3*, *HvTFL1*, and *HvMFT1* were mapped in the DH population from the F1 cross between Steptoe and Morex (North American Barley Genome Mapping Project; Kleinhofs et al., 1993). All genes except *HvFT3* were mapped using the cleaved-amplified polymorphic sequence method. *HvFT3* was mapped as a presence/absence polymorphism. The primers and experimental details are shown in Supplemental Table S1. Linkage map construction and QTL analyses were performed using MAPMAKER/EXP.

Plasmid Construction and Rice Transformation

Genomic sequences of *HvFT1*, *HvFT3*, and *HvTFL1* and cDNA sequences of *HvFT2* and *HvMFT1* were amplified using Morex genomic DNA or Haruna-Nijo cDNA, respectively, as templates and specific gene primer pairs (Supplemental Table S2) and then cloned into the entry vector pKS221MCS (Wakasa et al., 2006). The LR reaction (Gateway System; Invitrogen) was carried out in order to transfer the inserts from the entry vector into the expression vector (pSTARH302GateA), which included the CaMV 35S promoter (H. Ichikawa, H. Nakamura, M. Hakata, Y. Nishizawa, and M. Kazikawa, unpublished data). The MCS from pKS221MCS was inserted into pSTARH302GateA to generate a control plasmid. The constructed plasmids were then introduced into *Agrobacterium tumefaciens* strain EHA101. Rice cv Nipponbare was used for *Agrobacterium*-mediated transformation (Toki, 1997). Plants regenerated from the transformed callus (R0) were selected on MS solid medium (Murashige and Skoog, 1962) containing 50 mg L^{-1} hygromycin at 27°C under LD conditions at 40 μmol m^{-2} s^{-1}. Regenerated plants were grown in a growth chamber or greenhouse for further experiments.

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Growth Conditions for Transgenic Rice Plants

Twenty rice plants transformed with expression vectors containing *HvFT1*, *HvFT2*, *HvMFT1*, or a control vector (mock) were transplanted to soil in a growth chamber under SD conditions with a 28°C day and a 25°C night (9 h of light/15 h of dark; 270 μmol m^{-2} s^{-1}) or under LD conditions in a greenhouse with a 28°C day and a 24°C night under natural light from the end of May until the end of August (approximately 14 h of light/10 h of dark). Twenty rice plants transformed with the expression vectors for *HvFT1*, *HvFT3*, *HvTFL1*, or the control vector (mock) were transplanted to soil in a growth chamber under LD conditions with a 28°C day and a 25°C night (13.5 h of light/10.5 h of dark; 270 μmol m^{-2} s^{-1}) or in a greenhouse under SD conditions at 28°C under natural light from the beginning of August to the beginning of October (approximately 12.5 h of light/11.5 h of dark). The number of days from transplanting to the appearance of the first panicle was recorded.

Expression Analysis

Total RNA was extracted from leaves with the Get Pure RNA Kit (Dojindo). First-strand cDNA was synthesized from 1 μg of each RNA sample in a 20-μL reaction solution using the TaKaRa RNA PCR kit (AMV) version 3.0 (Takara Bio). Real-time PCR was carried out using Mx3000P (Stratagene Products Division, Agilent Technologies) with Brilliant II SYBR Green QPCR Master Mix (Stratagene) according to the manufacturer's recommendations. A diluted series of pCR2.1-TOPO vectors (Invitrogen) containing the partial fragments of barley *FT*-like genes or the barley actin (*HvActin*) gene was used to generate the standard curve. The barley *FT*-like gene transcripts were amplified with each specific primer pair (Supplemental Table S2). The value for the *FT*-like genes was normalized using *HvActin* as an internal standard. Real-time PCR results reflect the results of three independent experiments.

Genomic Sequencing of the *HvFT3* Region

Genomic DNA was extracted from the leaves of Morex, Steptoe, and DH lines and used as template for genomic PCR with each *HvFT3* primer pair (Fig. 7; Supplemental Table S1). PCR was performed using 35 cycles under the following conditions: denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 2 min. To isolate the *HvFT3* upstream region in Steptoe, we used the Genome Walker System provided by Clontech Laboratories with gene-specific primers (Supplemental Table S1).

Sequence data from this article can be found in the DNA Data Bank of Japan sequence database under accession numbers AB447465 (*HvTFL1*), AB447466 (*HvMFT1*), AB476614 (*HvFT3* in Morex), and AB476615 (*HvFT3* in Steptoe).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Multiple alignment of the PEBP domains of Arabidopsis, barley, and rice FT-, TFL-, and MFT-like proteins.

Supplemental Table S1. Mapping of barley *FT*-like genes.

Supplemental Table S2. Sequences of primers used in this study for plasmid construction, quantitative RT-PCR, and structural analysis of barley *FT*-like genes.

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LITERATURE CITED

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

Alvarez J, Guli CL, Yu X, Smyth DR (1992) *Terminal flower*: a gene affecting inflorescence development in *Arabidopsis thaliana*. Plant J **2:** 103–116

Amaya I, Ratcliffe OJ, Bradley DJ (1999) Expression of *CENTRORADIALIS* (*CEN*) and *CEN*-like genes in tobacco reveals a conserved mechanism controlling phase change in diverse species. Plant Cell **11:** 1405–1417

Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature **408:** 796–815

Boss PK, Bastow RM, Mylne JS, Dean C (2004) Multiple pathways in the decision to flower: enabling, promoting, and resetting. Plant Cell (Suppl) **16:** S18–S31

Bradley D, Carpenter R, Copsey L, Vincent C, Rothstein S, Coen ES (1996) Control of inflorescence architecture in *Antirrhinum*. Nature **376:** 791–797

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

Chardon F, Damerval C (2005) Phylogenomic analysis of the PEBP gene family in cereals. J Mol Evol **61:** 579–590

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

Danilevskaya ON, Meng X, Hou Z, Ananiev EV, Simmons CR (2008) A genomic and expression compendium of the expanded PEBP gene family from maize. Plant Physiol **146:** 250–264

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

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

Hayama R, Coupland G (2004) The molecular basis of diversity in the photoperiodic flowering responses of Arabidopsis and rice. Plant Physiol **135:** 677–684

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

International Rice Genome Sequencing Project (2005) The map-based sequence of the rice genome. Nature **436:** 793–800

Izawa T, Oikawa T, Sugiyama N, Tanisaka T, Yano M, Shimamoto K (2002) Phytochrome mediates the external light signal to repress *FT* orthologs in photoperiodic flowering of rice. Genes Dev **16:** 2006–2020

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

Kardailsky I, Shukla VK, Ahn JH, Dagenais N, Christensen SK, Nguyen JT, Chory J, Harrison MJ, Weigel D (1999) Activation tagging of the floral inducer *FT*. Science **286:** 1962–1965

Kleinhofs A, Kilian A, Saghai Maroof MA, Biyashev RM, Hayes P, Chen FQ, Lapitan N, Fenwick A, Blake TK, Kanazin V, et al (1993) A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. Theor Appl Genet **86:** 705–712

Kobayashi Y, Kaya H, Goto K, Iwabuchi M, Araki T (1999) A pair of related genes with antagonistic roles in mediating flowering signals. Science **286:** 1960–1962

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

Laurie DA (1997) Comparative genetics of flowering time. Plant Mol Biol **35:** 167–177

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

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

Monna L, Lin HX, Kojima S, Sasaki T, Yano M (2002) Genetic dissection of a genomic region for a quantitative trait locus, *Hd3*, into two loci, *Hd3a*

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and *Hd3b*, controlling heading date in rice. Theor Appl Genet **104:** 772–778

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant **15:** 473–497

Nakagawa M, Shimamoto K, 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

Ohshima S, Murata M, Sakamoto W, Ogura Y, Motoyoshi F (1997) Cloning and molecular analysis of the *Arabidopsis* gene *Terminal flower 1*. Mol Gen Genet **254:** 186–194

Ratcliffe OJ, Amaya I, Vincent CA, Rothstein S, Carpenter R, Coen ES, Bradley DJ (1998) A common mechanism controls the life cycle and architecture of plants. Development **125:** 1609–1615

Saitou N, Nei N (1987) A neighbor-joining method: a new method for constructing phylogenetic tree. Mol Biol Evol **44:** 406–425

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

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res **25:** 4876–4882

Toki S (1997) Rapid and reliable *Agrobacterium*-mediated transformation in rice. Plant Mol Biol Rep **15:** 16–21

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

Wakasa Y, Yasuda H, Takaiwa F (2006) High accumulation of bioactive peptide in transgenic rice seeds by expression of introduced multiple genes. Plant Biotechnol J **4:** 499–510

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

Yan L, Fu D, Li C, Blechl A, Tranquilli G, Bonafede M, Sanchez A, Valarik M, Yasuda S, Dubcovsky J (2006) The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*. Proc Natl Acad Sci USA **99:** 16336–16341

Yano M, Kojima S, Takahashi Y, Lin H, Sasaki T (2001) Genetic control of flowering time in rice, a short-day plant. Plant Physiol **127:** 1425–1429

Yanovsky MJ, Kay SA (2003) Living by the calendar: how plants know when to flower. Nat Rev Mol Cell Biol **4:** 265–275

Yoo SY, Kardailsky I, Lee JS, Weigel D, Ahn JH (2004) Acceleration of flowering by overexpression of *MFT* (*MOTHER OF FT AND TFL1*). Mol Cells **17:** 95–101

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