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A Drought-Inducible Transcription Factor Delays Reproductive Timing in Rice^{1[OPEN]}

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The molecular mechanisms underlying photoperiod or temperature control of flowering time have been recently elucidated, but how plants regulate flowering time in response to other external factors, such as water availability, remains poorly understood. Using a large-scale Hybrid Transcription Factor approach, we identified a bZIP transcriptional factor, *O. sativa* ABA responsive element binding factor 1 (*OsABF1*), which acts as a suppressor of floral transition in a photoperiod-independent manner. Simultaneous knockdown of both *OsABF1* and its closest homologous gene, *OsbZIP40*, in rice (*Oryza sativa*) by RNA interference results in a significantly earlier flowering phenotype. Molecular and genetic analyses demonstrate that a drought regime enhances expression of the *OsABF1* gene, which indirectly suppresses expression of the *Early heading date 1* (*Ehd1*) gene that encodes a key activator of rice flowering. Furthermore, we identified a drought-inducible gene named *OsWRKY104* that is under the direct regulation of *OsABF1*. Overexpression of *OsWRKY104* can suppress *Ehd1* expression and confers a later flowering phenotype in rice. Together, these findings reveal a novel pathway by which rice modulates heading date in response to the change of ambient water availability.

Flowering time (or heading date) and drought resistance are two major yield traits in crops, especially rice (*Oryza sativa*). As global climatic change looms, drought has become the biggest abiotic stress to limit crop yields. Breeders have capitalized on naturally occurring genetic variations to improve or maintain crop yield in times or areas of drought by different strategies (Eisenstein, 2013). Manipulation of floral transition has been a promising way to maximize crop yield during dry periods. This strategy has been successful due to extensive identification of genetic loci and elucidation

of molecular mechanisms that control flowering time under diverse or unpredictable environments.

Heading date in rice is influenced by many environmental cues such as day length (photoperiod), temperature, nutrition, and water availability. Molecular mechanisms that underlie photoperiod regulation of flowering time have already been characterized, probably because day length is more predictable than other environmental factors during seasonal changes. Rice is a facultative short-day plant that flowers earlier in short days (SDs) than in long days (LDs). *Heading date 3a* (*Hd3a*) and *RICE FLOWERING LOCUS T1* (*RFT1*) are two paralogous genes in rice encoding “florigen” molecules expressed in the phloem of leaves and transported to the shoot apical meristem to promote flowering (Tamaki et al., 2007; Komiya et al., 2008, 2009; Tsuji et al., 2013). *Heading date 1* (*Hd1*, counterpart of *Arabidopsis* [*Arabidopsis thaliana*] *CONSTANS*, *CO*), *Days to heading 2* (*DTH2*, encoding another CO-like protein), and *Early heading date 1* (*Ehd1*) regulate expression of these two “florigen” genes. *Hd1* acts as a transcription activator for *Hd3a* in SDs but as a repressor in the presence of functional *O. sativa* Phytochrome B in LDs (Hayama et al., 2003; Ishikawa et al., 2011). *DTH2* acts in LDs to suppress flowering (Wu et al., 2013). *Ehd1* encodes a rice-specific B-type response factor that acts in both LDs and SDs to promote *Hd3a* and *RFT1* mRNA expression, thus inducing floral initiation (Doi et al., 2004; Komiya et al., 2009; Sun et al., 2014). Many repressors or activators, including *Ehd2*,

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B.L. designed and supervised the experiments, analyzed the data, and wrote the article with contributions of all the authors; C.L. conceived the original screening and research plans; C.Z., J.L., and T.Z. performed most of the experiments; C.L., C.Y., H.L., J.L., and Y.Y. provided material and technical assistance to C.Z.; A.G. complemented the writing.

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Ehd3, *Ehd4*, *Ghd7*, *OsMADS51*, *OsLFL1*, and *OsPPR37/DTH7*, regulate the expression and activity of *Ehd1* itself in day length-dependent or -independent manners (Doi et al., 2004; Kim et al., 2007; Peng et al., 2007; Xue et al., 2008; Wei et al., 2010; Matsubara et al., 2011; Koo et al., 2013; Tsuji et al., 2013; Yan et al., 2013; Gao et al., 2014; Kwon et al., 2015). It appears that *Ehd1* is under the convergent regulation of SD activators, LD repressors, and photoperiod-independent regulators to control the heading date of rice in response to the internal development “clock” and environmental cues.

Water availability is another critical environmental factor affecting flowering time. Plants from xeric or mesic areas may utilize self-appropriate flowering strategies to cope with water deficits (Chaves et al., 2003). Many plants (*Arabidopsis*, *Phyllostachys heterocycla*, *Mimulus guttatus*, etc.) accelerate flowering to complete life cycle under drought conditions (Peng et al., 2013; Wolfe and Tonsor, 2014; Kooyers et al., 2015), a phenomenon referred to as drought escape (DE). Because drought triggers phytohormone abscisic acid (ABA) biosynthesis and signal transduction, it has been proposed that ABA mediates DE response. Consistent with this hypothesis, an ABA biosynthesis knockout mutant, *aba1-6*, was shown to flower later under LDs (Riboni et al., 2013). External application of ABA also delays flowering (Cheng et al., 2002; Achard et al., 2006; Domagalska et al., 2010; Wang et al., 2013), suggesting a dual effect of ABA on floral transition. Interestingly, drought accelerates flowering of *Arabidopsis* only under LDs but not SDs. Genetic studies have revealed that *GIGANTEA* (*GI*) knockout abolishes the drought-induced early flowering behavior of *Arabidopsis* under LDs (Han et al., 2013; Riboni et al., 2013), but the mechanism of *GI*-mediated photoperiod-dependent DE response has not been elucidated. The biological networks connecting drought perception, ABA signaling, and flowering gene regulation also remain unclear. In contrast to *Arabidopsis* species, rice cultivars tend to delay flowering in response to drought treatment (Wopereis et al., 1996; Ndjioudjop et al., 2010), but the underlying mechanism is less characterized.

Genome-wide investigations of transcription profiles have revealed that drought influences plant development and physiological responses by inducing hierarchical regulation of gene expression (Ding et al., 2012; Su et al., 2013; Huang et al., 2014; Maruyama et al., 2014). Several classes of transcription factors (TFs) play critical roles in orchestrating ABA-dependent or -independent drought responses (Jin et al., 2014; Todaka et al., 2015). One such class of TFs consists of a subgroup of bZIP proteins, which contain a basic region for DNA binding and a Leu zipper motif for dimerization. The DNA binding region preferentially interacts with ABA-responsive elements (ABREs), which are predominantly located in the promoter regions of ABA-inducible genes (Jakoby et al., 2002; Nijhawan et al., 2008). A great deal of genetic evidence shows that these ABRE-associated bZIP TFs are involved in drought tolerance response (Kang et al., 2002; Oh et al., 2005; Xiang et al., 2008; Lu et al., 2009; Hossain et al., 2010a,

2010b; Tang et al., 2012). Until now, none had been shown to regulate rice floral transition in response to drought stress. Here, we revealed that the bZIP TF, *O. sativa* ABA responsive element binding factor 1 (OsABF1), functions redundantly with OsbZIP40 to delay floral transition upon water deficit, which established a direct link between external water availability and plant developmental program.

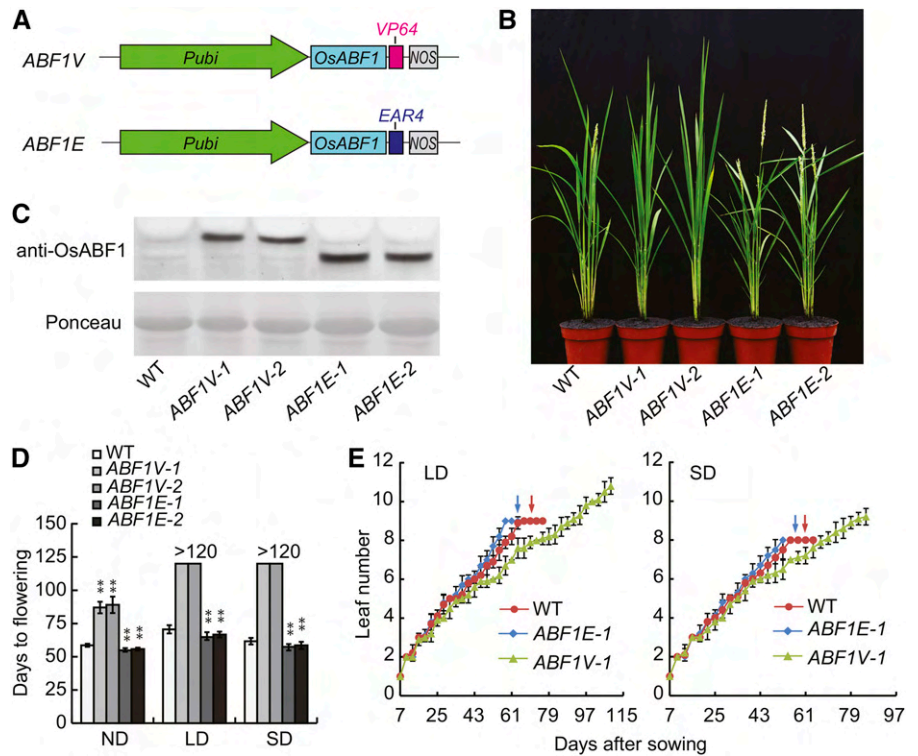
RESULTS

OsABF1 Is a Negative Regulator of Floral Initiation

Previously, we used a large-scale Hybrid Transcription Factor (HTF) strategy to examine roles of different TFs in plant growth and development (Zhao et al., 2015). In the HTF approach, TF coding sequences are fused with a universal transcriptional activation module VP64 (tetrameric repeats of VP16) or a repression module EAR4 (tetrameric repeats of EAR). The HTF strategy aims to probe for quantifiably distinct transgenic phenotypes by activating transcription in the TF-VP64 group or by repressing transcription in the TF-EAR4 group. Through surveying the phenotypes of about 50,000 transgenic lines, we identified a pair of HTFs, named OsABF1-VP64 (ABF1V) and OsABF1-EAR4 (ABF1E; Fig. 1A). Respective overexpression of ABF1V and ABF1E in rice under the control of the maize ubiquitin promoter (*Pubi*) results in flowering time phenotypes that are effectively opposite to one another (Fig. 1B). Hereafter, the *Pubi:OsABF1-VP64* and *Pubi:OsABF1-EAR4* transgenic lines are designated as *ABF1V* and *ABF1E*, respectively. In total, 52 *ABF1Vs* and 9 *ABF1Es* were obtained, with 36 *ABF1Vs* and 8 *ABF1Es* recapitulated for at least two generations demonstrating late or early flowering phenotypes, respectively. The overexpression of ABF1V or ABF1E in these transgenic lines was verified by immunoblotting probed with anti-OsABF1 antibody (Fig. 1C; two representative lines of each construct were shown). The statistical results showed that *ABF1Vs* were flowering later while *ABF1Es* were flowering earlier than wild type under the nature day (ND, summer in Beijing), LD, or SD conditions (Fig. 1D). To be noted, *ABF1Vs* were still not flowering for 120 d in the controlled growth chamber under LD/SD treatment. The different severity of flowering time phenotype under LDs/SDs or NDs is probably because the light intensity or temperature in the controlled chamber is lower than that in nature field. Moreover, the number of main shoot leaves of *ABF1V-1* was significantly more abundant than that of *ABF1E-1* or wild type at maturation stage under both LDs and SDs (Fig. 1E).

To further test the role of OsABF1 in flowering regulation, we made a *Pubi:OsABF1-3Flag* construct (Supplemental Fig. S1A) and obtained 32 independent OsABF1 overexpression lines (*ABF1Fs*) that displayed a late flowering phenotype under ND conditions (Supplemental Fig. S1B). The overexpression of ABF1F in transgenic plants was validated by immunoblotting probed with anti-Flag antibody (Supplemental Fig.

Figure 1. OsABF1 HTFs lead to opposite flowering time phenotypes. **A**, Diagrams of the OsABF1 HTF constructions. *ABF1V*, *Pubi:OsABF1-VP64*; *ABF1E*, *Pubi:OsABF1-EAR4*. **B**, Representative flowering image of indicated genotypes under NDs in summer at Beijing. **C**, Protein expression analysis of *ABF1V* or *ABF1E* in indicated lines. Kita-ake wild-type plants (WT) were used as control. The immunoblot was probed with anti-OsABF1 antibody. The ponceau staining bands of Rubisco large subunit was used as loading control. **D**, Flowering time of each genotype under different day length conditions. Mean values \pm SD were shown. The value of each genotype was compared to that of wild type (Student's *t* tests, ***P* < 0.01, *n* = 20). LDs (14 h light/10 h dark); SDs (10 h light/14 h dark). >120 indicates *ABF1V* transgenic plants cultured in growth chambers were not flowering after sowing for 120 d. **E**, Leaf number of each genotype grown under LDs or SDs in growth chambers. Red and blue arrows indicate the days of flowering of wild type and *ABF1E-1*, respectively.



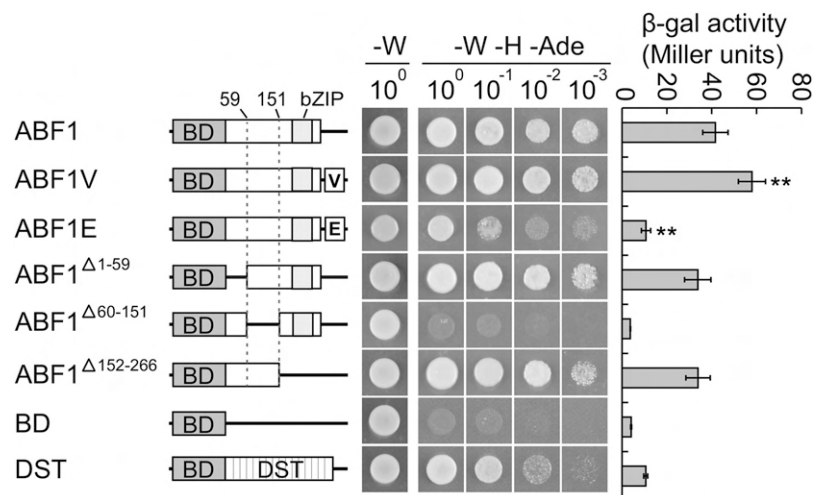
S1C, two representative lines were shown). Statistical analysis revealed that both *ABF1V-1* and *ABF1V-2* flower significantly later than wild type under ND, LD, or SD conditions (Supplemental Fig. S1D), demonstrating that OsABF1 acts as a negative regulator of floral initiation.

OsABF1 Acts as a Transcription Activator

To gain insight into how *ABF1V* and *ABF1E* result in opposite flowering time phenotypes, we performed a Yeast One-Hybrid assay to test the transcriptional activity of OsABF1, *ABF1V*, or *ABF1E*. The result showed

that OsABF1 is a transcriptional activator with an activation domain located between amino acids 60 and 151 of the protein (Fig. 2). Significant alteration in activity was observed when OsABF1 was fused with different effector modules (Fig. 2, left and middle). Transcriptional activation activity was increased by approximately 50% when OsABF1 was fused with VP64, while fusion with EAR4 resulted in repression of transcriptional activation activity by about 70% (Fig. 2, right). This result suggested that the opposite phenotypes of *ABF1Vs* and *ABF1Es* were due to the bilateral alteration of OsABF1 transcriptional activity when fused with VP64 or EAR4, respectively.

Figure 2. Yeast one-hybrid assay of transcriptional activation activity. Left, Diagrams of yeast one-hybrid bait constructs comprising OsABF1 (*ABF1*) fragments fused with the GAL4 DNA binding domain (BD), VP64 (V), or EAR4 (E) as indicated. BD and BD fused with DST were used as negative and positive controls, respectively. Middle, Plate auxotroph assays showing transcriptional activation activity of each protein. W, Trp; H, His; Ade, adenine. Right, Quantitative yeast one-hybrid assays define transcriptional activation activity of each protein. Values are means of β -galactosidase activity \pm SD, and the value of *ABF1V* or *ABF1E* was compared to that of *ABF1* (Student's *t* tests, ***P* < 0.01, *n* = 3).



OsABF1 Delays Floral Initiation through Inhibition of *Ehd1*

Next, we sought to identify genes that confer opposite flowering phenotypes in *ABF1V* versus *ABF1E* genotypes by investigating the mRNA expression profiles of 16 well-documented flowering-associated genes in rice in a time-course manner under both SDs and LDs (Supplemental Figs. S2 and S3). The result indicated that transcription levels of *Ehd1*, *Hd3a*, and *RFT1* clearly vary between the genotypes and that variation is not apparent in the other flowering-associated genes. Expression levels of these three genes increased in *ABF1E* compared with wild type, but sharply decreased to an undetectable level in *ABF1V*. We then used incrementally aged plant groups to show that *Ehd1*, *Hd3a*, and *RFT1* expression continually increased over time from week 3, but then decreased around weeks 7 and 8 in wild type and *ABF1E*. Again, we saw greater expression levels in *ABF1E* compared to wild type under both LDs and SDs, but wild-type expression levels also increased over time (Fig. 3). Expression levels in *ABF1V* were undetectable throughout the entire growth period (Fig. 3). Such expression patterns are consistent with the opposite flowering phenotype of *ABF1V*s and *ABF1E*s. Interestingly, expression of the *OsABF1* gene incrementally decreased with age under both LDs and SDs. This negative correlation between *OsABF1* and *Ehd1* mRNA levels suggests that *OsABF1* may delay flowering by suppressing *Ehd1* transcription.

To test if *ABF1V* suppresses flowering through down-regulation of *Ehd1* expression, we generated 12 and 18 *Pubi:Ehd1-Flag* transgenic lines in wild-type background (*Ehd1-OX*) and *ABF1V-1* background (*Ehd1-OX/ABF1V*), respectively. Among them, 8 *Ehd1-OX* and 11 *Ehd1-OX/ABF1V* lines at T1 generation were flowering earlier than wild type under NDs (two representative lines of each genotype were shown in Fig. 4, A and B), demonstrating that overexpression of *Ehd1* can efficiently suppress the later flowering phenotype of *ABF1V*. To measure and compare the flowering times between *Ehd1-OX* and *Ehd1-OX/ABF1V*, two independent lines of each genotype expressing equal levels of *Ehd1-Flag* protein were selected for the investigation (Fig. 4C). The results showed that *Ehd1-OX/ABF1V* lines were flowering about 4 d later than *Ehd1-OX* lines grown in NDs. This slight flowering difference might be due to the dosage effect of *Ehd1* mRNA, because the transcription of endogenous *Ehd1* was suppressed in *Ehd1-OX/ABF1V* but not in *Ehd1-OX*. Alternatively, *ABF1V* may not repress flowering in a manner entirely dependent on the *Ehd1* pathway. Furthermore, we tested if *ABF1E* accelerates flowering through enhancing *Ehd1* expression. In total, 18 and 15 *Ehd1-RNAi* transgenic lines in wild-type background (*Ehd1R*) and *ABF1E-1* background (*Ehd1-RNAi/ABF1E*) were generated, respectively. Among them, 15 *Ehd1R* and 12 *Ehd1R/ABF1E* lines at T1 generation were similarly flowering later than wild type under NDs (two representative lines of each genotype were shown in Supplemental Fig. S4), demonstrating that knockdown of *Ehd1* can efficiently suppress the early

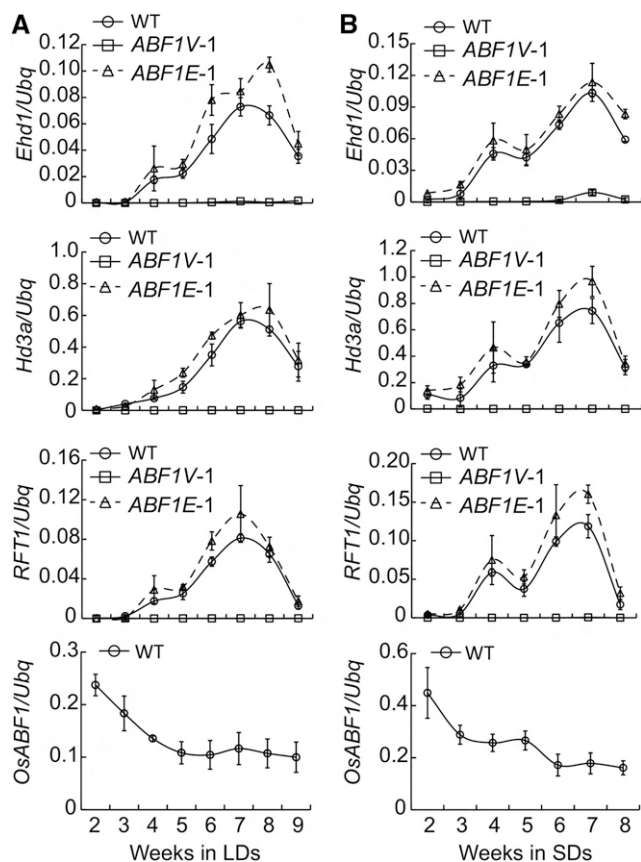


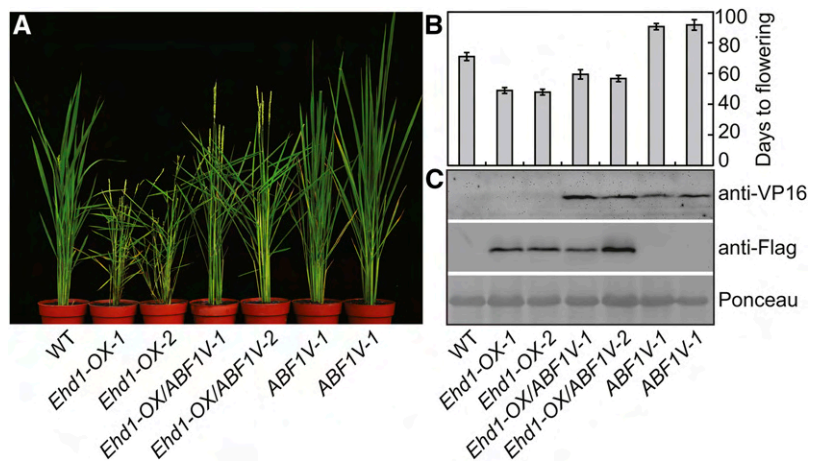
Figure 3. Transcriptional levels of indicated flowering-associated genes in each genotype at different developmental stages. A, Plants were grown in LDs. Samples were the latest fully expanded leaves that were collected just at the end of dark period. Three biological replicates of qRT-PCR experiment were performed using *Ubq* gene as the internal control and the representative results were shown. Values were shown as mean \pm SD ($n = 3$). B, Plants were grown in SDs and qRT-PCR experiments were performed as in A.

flowering phenotype of *ABF1E*. Taken together, our molecular and genetic results support the hypothesis that *OsABF1* regulates flowering time through the *Ehd1*-mediated flowering pathway.

OsABF1 Acts Redundantly with *OsbZIP40* to Delay Rice Flowering upon Drought Stress

To test if *OsABF1* loss of function affects rice flowering time, we obtained a new T-DNA insertion mutant *Osabf1-3* (Supplemental Fig. S5A). Gene expression analysis showed that the transcription of *OsABF1* is undetectable in the *Osabf1-3* mutant (Supplemental Fig. S5B). Nevertheless, *Osabf1-3* mutant plants displayed no obvious flowering time phenotype under all experimental conditions (NDs, LDs, and SDs), which is probably due to functional redundancy. To test this possibility, an RNA interference (RNAi) approach was applied to knockdown *OsABF1* and its closest homologous gene *OsbZIP40* simultaneously (Fig. 5; Supplemental Fig. S6; Nijhawan

Figure 4. Overexpression of *Ehd1* suppresses the late flowering phenotype of *ABF1V*. A, The flowering phenotypes of indicated genotypes grown in NDs. All plants are in Kita-ake background. *Ehd1-OX* indicates the *Pubi:Ehd1-Flag* transgenic line in T1 generation. *Ehd1-OX/ABF1V-1* and *Ehd1-OX/ABF1V-2* were two independent T1 lines generated by stacking transformation of *Pubi:Ehd1-Flag* construct into *ABF1V-1* homozygotes line. B, Statistical analysis of flowering time of indicated genotypes as in A. C, Protein expression analysis of OsABF1V or Ehd1-Flag in each genotype. OsABF1V or Ehd1-Flag was detected using anti-VP16 antibody or anti-Flag antibody, respectively. The ponceau staining band of Rubisco large subunit was used as loading control.

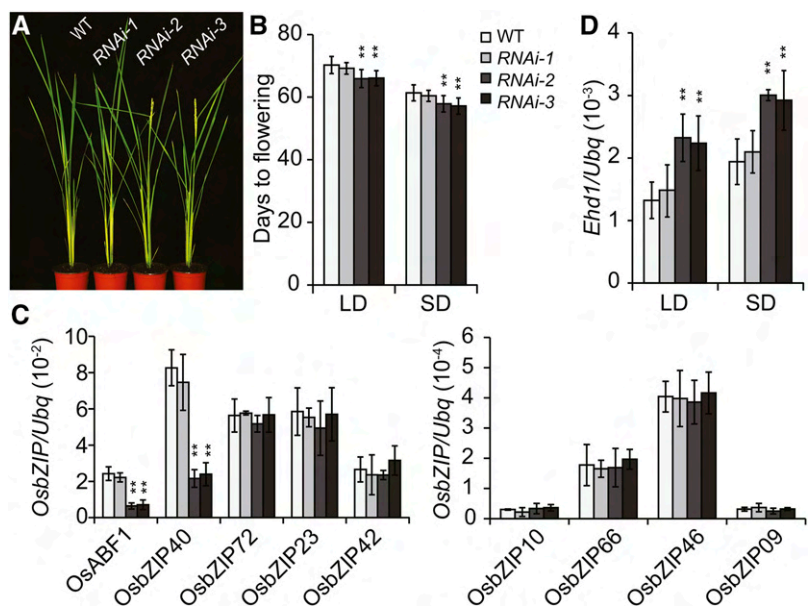


et al., 2008). A total of 15 RNAi lines were obtained, and 11 of them showed early flowering phenotype at T1 generation under both SDs and LDs (Fig. 5, A and B). Gene expression analysis revealed that the mRNA levels of both *OsABF1* and *OsZIP40* significantly decreased in the RNAi effective lines (*RNAi-2* or *RNAi-3*) showing early flowering phenotype, but not in wild type or in the RNAi ineffective line (*RNAi-1*). Expression levels of other *OsABF1* homologous genes were not altered, demonstrating that just *OsABF1* and *OsZIP40* were specifically silenced in the RNAi effective lines (Fig. 5C). Consistent with this result, the level of *Ehd1* mRNA was significantly up-regulated in the RNAi effective lines (Fig. 5D).

Given that polyethylene glycol (PEG) (or ABA) treatment can trigger and enhance the expression of *OsABF1* (Fig. 6A; Amir Hossain et al., 2010), we tested the expression of *Ehd1* in the similar conditions. The results demonstrated that the mRNA level of *Ehd1* rapidly decreased upon dehydration treatment (Supplemental Fig.

S5C), which is in sharp contrast to the enhanced expression pattern of *OsABF1* mRNA (Fig. 6A). Moreover, the expression level of *Ehd1* was significantly higher in *Osabf1-3* than in wild type (Factorial ANOVA, $P < 0.001$, $n = 3$; Supplemental Fig. S5C), suggesting that *OsABF1* may act as a drought-inducible suppressor of floral transition. To test this hypothesis, we compared the flowering time phenotype of indicated genotypes under normal and drought conditions (Fig. 6B). The results demonstrated that RNAi effective lines (*RNAi-2*, *RNAi-3*) were flowering earlier than wild type and RNAi ineffective line (*RNAi-1*) under both conditions (Fig. 6C). Moreover, drought treatment delayed flowering in wild type and RNAi ineffective line more efficiently than the RNAi effective lines (Fig. 6D), demonstrating that *OsABF1*-RNAi attenuates drought delay of flowering. Together, these results suggested that *OsABF1* and *OsZIP40* function redundantly in mediating drought inhibition of flowering through the *Ehd1* pathway.

Figure 5. Simultaneous knockdown of *OsABF1* and *OsZIP40* by RNAi approach results in early flowering phenotype. A, Representative flowering image of indicated genotypes under SDs. B, Flowering days of each genotype grown under LDs or SDs. Mean values \pm SD are shown. The value of each genotype was compared to that of wild type (Student's *t* tests, $**P < 0.01$, $n = 20$). C, qRT-PCR analysis of *OsABF1* or the homologous genes in indicated lines. Values were shown as mean \pm SD (Student's *t* tests, $**P < 0.01$, $n = 3$). D, Expression analysis of *Ehd1* in indicated genotypes grown under LDs or SDs for 4 weeks. Samples were the latest fully expanded leaves that were collected just at the end of dark period. Values were shown as mean \pm SD (Student's *t* tests, $**P < 0.01$, $n = 3$).



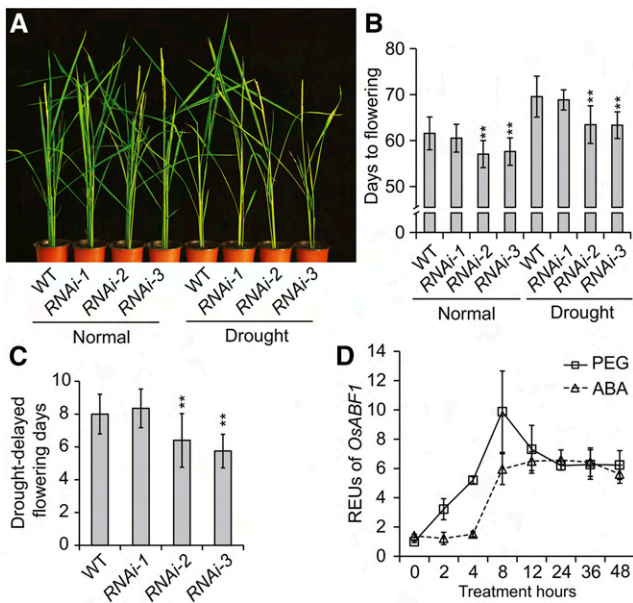


Figure 6. Flowering phenotype of *OsABF1*-RNAi lines in response to drought treatment. A, Dynamic transcription of *OsABF1* in wild type under PEG or ABA treatment for the indicated periods. The seedlings were grown in continuous light for 3 weeks and then subjected to PEG or ABA treatment. The Relative Expression Units (REUs) were calculated by the formula: $[(OsABF1/ubq) \text{ of each time point}] / [(OsABF1/ubq) \text{ of time point } 0]$. B, Representative flowering image of indicated genotypes under normal or drought conditions. *RNAi-1* is an RNAi infective transgenic line used for negative control. C, Flowering days of each genotype as in A. Mean values \pm SD were shown. The value of each genotype was compared to that of wild type under similar growth condition (Student's *t* tests, $**P < 0.01$, $n = 20$). D, The value of drought-delayed flowering days of each genotype was calculated with the flowering days under drought regime minus that under normal conditions. The significant difference between each genotype and wild type was calculated (Student's *t* tests, $**P < 0.01$, $n = 20$).

OsABF1 May Delay Flowering by Directly Activating *OsWRKY104* Gene

Given that *OsABF1* is a transcriptional activator and that *Ehd1* transcription is up-regulated in the *Osabf1-3* mutant and *OsABF1*-RNAi lines, *Ehd1* is unlikely under the direct regulation of *OsABF1*. We speculate that *OsABF1* may activate the expression of an unknown TF that acts as a repressor of *Ehd1* in response to drought treatment. Therefore, we interrogated the transcriptional profiles of rice TF genes in response to drought treatment (NCBI database, GEO accession no. GSE65022) and found 271 drought inducible TF genes (DTFs). By overlapping analysis with the heading dates of the large HTF transgenic population (Zhao et al., 2015), we found 29 DTFs that could confer abnormal flowering time phenotype. We further tested these DTF expression levels in different genotypes and found that a gene named *OsWRKY104* was up-regulated in *ABF1V*, but down-regulated in *ABF1E* and *OsABF1*-RNAi lines (Fig. 7A; Supplemental Fig. S7, D and E). Moreover, in contrast to the early flowering phenotype

of *EAR4*-*OsWRKY104* (*EWRKY104*) HTF transgenic lines (Supplemental Fig. S7), overexpression of *OsWRKY104* (*W104-OX*) resulted in a later flowering phenotype (Fig. 7B; Supplemental Fig. S8). Next, we tested if *OsWRKY104* is a direct target gene of *OsABF1*. Chromatin immunoprecipitation (ChIP) using *ABF1V* transgenic plants and anti-VP16 antibody showed that *ABF1V* robustly binds to a, b, and d sites that contain the ACGT core sequence of the ABRE motif (Ono et al., 1996; Hobo et al., 1999; Nijhawan et al., 2008; Fig. 7D). Furthermore, we performed ChIP experiments using wild-type plants and the anti-*OsABF1* antibody. The results also showed similar robust binding signals at a, b, and d sites (Fig. 7E), validating that *OsWRKY104* is a direct target gene under the regulation of *OsABF1*. All the above results together with the fact that *Ehd1* expression is down-regulated in *W104-OX* lines (Fig. 7F) but up-regulated in *EWRKY104* lines (Supplemental Fig. S7F) suggest that *OsABF1* suppresses *Ehd1* expression at least partially through activating *OsWRKY104* transcription in response to drought stress (Fig. 7, G and H).

DISCUSSION

Drought Inhibition of Flowering, a Rice Unique Drought Avoidance Strategy

Terrestrial plants, being sessile, grow adaptively in response to limited water availability by flexible strategies, including DE (via rapid flowering and reproduction before severe water shortage leads to lethality), drought avoidance (via developmental adaptations to reduce water consumption), or drought tolerance (via adjusting osmosis, enhancing oxidative capacity, or strengthening cell walls to withstand dehydration; Chaves et al., 2003; Yue et al., 2006). For example, *Arabidopsis* has a short life cycle and naturally grows in xeric environments, so it relies on DE response when encountering drought stress (Supplemental Fig. S9) (Riboni et al., 2013). In contrast, lowland rice is historically grown in mesic conditions and tends to delay flowering in response to temporary drought stress (Wopereis et al., 1996; Ndjiondjop et al., 2010). Therefore, the two model plants (*Arabidopsis* and rice) seem to display opposite flowering time responses to drought stress. Moreover, other environmental cues also differentially affect their flowering behavior upon water scarcity. The study of *Arabidopsis* showed that drought-induced acceleration of flowering is dependent on LD condition (Han et al., 2013; Riboni et al., 2013). Our study in rice showed that drought delayed flowering is photoperiod independent, while other factors such as light intensity or temperature may affect the severity of drought-delayed flowering time mediated by *OsABF1* (Fig. 1D). Therefore, how plants decide time of flowering seems to be ecologically diversified, and further investigations are warranted for a better understanding and discrimination of the mechanistic basis underlying the integration of environmental signals for reproductive success of different plant species.

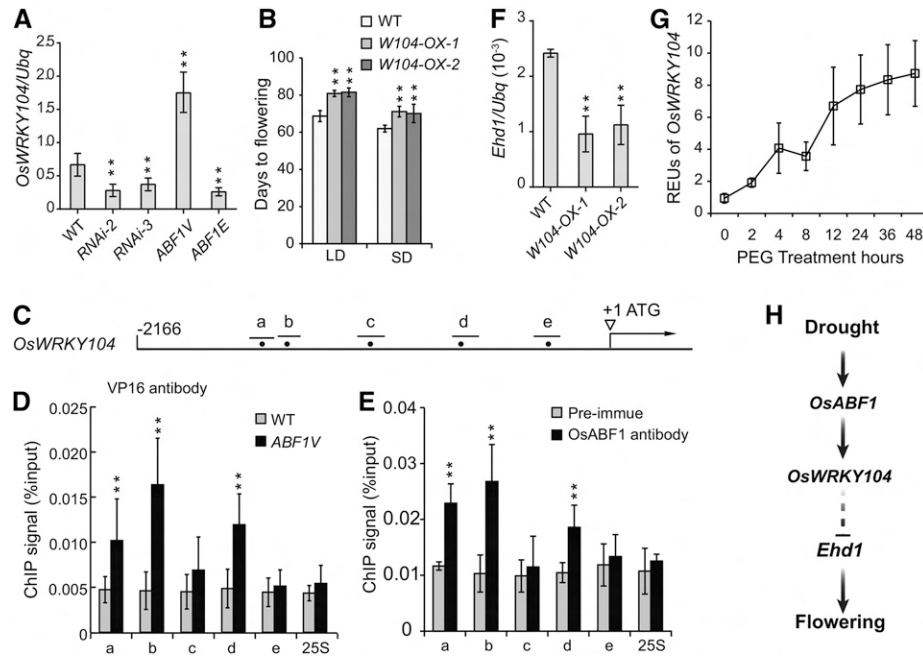


Figure 7. *OsWRKY104* is a direct target of *OsABF1* that delays flowering. **A**, qRT-PCR analysis of *OsWRKY104* expression in indicated lines. The means \pm SD (Student's *t* tests, $**P < 0.01$, $n = 3$) are shown. **B**, Flowering days of *OsWRKY104* over-expression lines. The means \pm SD ($n = 15$) are shown. **C**, A diagram representing the promoter region of *OsWRKY104* gene. The bars represent the distribution of DNA fragments containing ACGT core as indicated by dots. **D**, Verification of *ABF1V* direct binding sites in the *OsWRKY104* promoter by ChIP-qPCR analysis. ChIP samples were prepared using *ABF1V* and wild-type plants, precipitated with anti-VP16 antibody, and subjected to qPCR analysis. Results of ChIP-qPCR were quantified by normalization of the immunoprecipitation signal with the corresponding input signal. The binding to 25S rDNA was used as negative control. The means \pm SD ($n = 3$) are shown. **E**, Verification of *OsABF1* binding sites in the *OsWRKY104* promoter by ChIP-qPCR analysis. ChIP samples were prepared using wild-type rice and precipitated with anti-*OsABF1* antibody or with the preimmune serum as negative control. The means \pm SD ($n = 3$) are shown. **F**, qRT-PCR analysis of *Ehd1* expression in *OsWRKY104* over-expression lines. Values were shown as mean \pm SD ($n = 3$). **G**, Dynamic transcription of *OsWRKY104* in wild type under PEG treatment for the indicated periods. The seedlings were grown in continuous light for 3 weeks and then subjected to PEG treatment. The REUs were calculated by the formula: $[(OsWRKY104/ubq) \text{ of each time point}] / [(OsWRKY104/ubq) \text{ of time point } 0]$. **H**, A working model depicts how *OsABF1* modulates flowering time in response to drought stress. The transcription of *OsABF1* is up-regulated under water deficit. Consequently, *OsABF1* instigates the expression of *OsWRKY104*, which further delays flowering through inhibiting *Ehd1* expression.

In this report, we tested the effect of drought on rice floral transition using both *indica* and *japonica* cultivars grown in well-controlled growth chambers to avoid the effects of other environmental parameters. The result indicated that the flowering time of all tested cultivars was significantly delayed by drought regime in both LDs and SDs (Supplemental Figure S10), suggesting that drought-delayed flowering response may have already existed prior to genetic divergence of *indica* and *japonica* cultivars. Rice is more susceptible to drought sensitivity during reproductive stages (Lanceras et al., 2004), so we hypothesize that rain-fed ancestors of contemporary rice species may have postponed the transition from vegetative phase to reproductive phase as they sensed temporary water deficits and reduced water consumption. Such a drought avoidance strategy may enable rice to enhance survival by postponing blossoming until the upcoming rainy season. Although most rice cultivars tend to delay flowering under drought stress conditions, acceleration of flowering

under drought conditions has also been documented in some modern rice varieties (Xu et al., 2005; Vikram et al., 2015). These examples demonstrate the complexity and diversity of rice genotype-environment interactions as a result of domestication.

OsABF1 Mediates Drought-Derived Signal to Regulate Rice Flowering Time

In this study, we showed that *OsABF1* acts as a transcriptional activator to delay rice floral transition. We checked the expressions of previously known flowering associated genes and demonstrated that only *Ehd1*, *Hd3a*, and *RFT1* genes, which close to the final step of the rice flowering pathway, were down-regulated by *OsABF1*. Several repressors of *Ehd1* (including *Ghd7*, *OsPPR37/DTH7*, *DTH8*, *OsLFL1*, and *OsCOL4*) have been identified previously. In the Kita-ake cultivar, *Ghd7* and *OsPPR37/DTH7* are functionally deficient (Itoh et al.,

2010; Gao et al., 2014), while the mRNA expression levels of *DTH8*, *OsLFL1*, and *OsCOL4* showed no significant difference in *ABF1V*, *ABF1E*, and wild-type plants (Supplemental Figs. S2 and S3). Therefore, OsABF1 may not inhibit *Ehd1* expression through up-regulation of any these repressors. By conjoint analyses of the drought-induced transcription profile and our large-scaled HTF transgenic population, we identified a direct target gene of OsABF1, named *OsWRKY104*, which belongs to a large WRKY TF gene family consisting of more than 100 members in rice (Berri et al., 2009). WRKY TFs have been documented to regulate a range of biological processes, including biotic or abiotic response, senescence, and development (Rushton et al., 1996; Hara et al., 2000; Johnson et al., 2002; Maré et al., 2004; Miao et al., 2004; Cai et al., 2014). Here, we showed that drought stress does enhance the expression of *OsWRKY104*, which may further repress floral transition. Provided that *OsWRKY104* overexpression lines and *EAR4-OsWRKY104* HTF transgenic lines show opposite flowering time phenotype, *OsWRKY104* probably acts as a transcriptional activator in rice. Therefore, we proposed that drought stress enhances the transcription of *OsABF1* and *OsWRKY104*, then further activates the expression of an unknown repressor of *Ehd1* to delay rice heading date (Fig. 7H). The increased understanding of how rice regulates flowering time in response to water availability will benefit the development of high performance rice lines in drought-prone regions.

MATERIALS AND METHODS

Plant Materials

To generate *ABF1V* and *ABF1E* overexpression lines, *OsABF1* cDNA was inserted into pBCV and pBCE expression vectors respectively (Zhao et al., 2015), using the Gateway cloning system (Invitrogen). To generate *OsABF1*, *Ehd1* and *OsWRKY104* overexpression lines, respective cDNA was inserted into the pHCF vector (Supplemental Figure S6) at *Pst*I site using the Infusion system (Clontech). To generate *OsABF1-RNAi* or *Ehd1-RNAi* plants, a 267-bp fragment of the *OsABF1* gene (from 506 to 772 bp) or a 270-bp fragment of the *Ehd1* gene (from 56 to 325 bp) was inserted into the pANDA vector using the Gateway cloning system (Miki and Shimamoto, 2004). The above constructs were introduced into *Agrobacterium tumefaciens* strain EHA105 and transformed into wild type rice (*Oryza sativa japonica* cv Kita-ake) or indicated background. The T-DNA insertion mutant *Osabf1-3* (rice cv Hwayoung background) was identified from the Salk Institute Genomic Analysis Laboratory (<http://signal.salk.edu/cgi-bin/RiceGE>) (Jeong et al., 2006). Homozygous line of *Osabf1-3* was characterized by genotyping PCR with *OsABF1* gene-specific and T-DNA specific primers.

Flowering Time Analyses

To investigate the flowering phenotypes, plants were grown under NDs in Beijing (39°54'N, 116°23'E, nursery on May 6), China, or under LDs (14 h light, 28°C; 10 h dark, 24°C) or SDs (10 h light, 28°C; 14 h dark, 24°C) in plant growth chambers or green house. To investigate the effect of drought stress on rice flowering time, the indicated rice genotypes were sown and cultured with 1/10 Murashige and Skoog culture solution in transparent boxes for 2 weeks and then transferred to boxes containing wet soil for resurrounding. For drought treatment, the plants were not irrigated until the soil moisture content achieved approximately 30%, and then water was added to the boxes until the soil was saturated (Supplemental Figure S12). The drought regime was imposed until flowering. The control plants were grown in boxes maintaining a water level above the soil surface.

Sample Collection and Gene Expression Analyses

Seeds of wild-type rice and transgenic lines were germinated for 2 d on wet filter paper in petri dishes at 37°C. The uniformly germinated seeds were picked up and sown in bottomless 96-well plates and hydroponically grown (distiller water with 1/10 Murashige and Skoog). To test the mRNA expression of flowering-associated genes in a time course manner under LDs or SDs, plants were grown for 4 weeks and samples were collected every 4 h from the beginning of the light period. To test the mRNA level with increment of age, the latest fully expanded leaves were collected just at the end of dark period from 2 to 9 weeks. To test *OsABF1* or *Ehd1* mRNA expression in response to PEG treatment, the roots of seedlings grown under continuous light conditions were submerged into 20% PEG4000 solution for the hours indicated. To test *OsABF1* mRNA expression in response to ABA treatment, the roots were submerged in a 100- μ M ABA solution and the leaves were sprayed with same solution. RNA extraction and quantitative reverse transcription PCR (qRT-PCR) were performed as previously described with three biological replicates (Meng et al., 2013).

Transactivation Activity Assay in Yeast

To test the transactivation activity, the indicated coding DNA sequence (CDS) was fused with GAL4 DNA-binding domain in the pGBKT7 vector and transformed into the yeast strain AH109. The empty vector (BD) and BD-DST vector were used as negative and positive controls, respectively. Measurement of the β -galactosidase activity was performed according to the Yeast Protocols Handbook (Clontech) using chlorophenol red- β -D-galactopyranoside (Roche Biochemical) as the substrate.

Immunoblot Analyses

The anti-OsABF1 polyclonal antibody was generated by inoculating rabbits with TF-His-OsABF1 recombinant protein (Bio-med). The anti-VP16 polyclonal antibody was generated by inoculation of rabbits with a synthesized antigenic peptide consisting of hexameric repeats of VP16 (DALDDFDLML DALDDFDLML DALDDFDLML DALDDFDLML DDFDL DDFDL; Abmart). To extract the total protein for immunoblot, the young leaves were ground in liquid nitrogen and mixed with 5X SDS-PAGE loading buffer [250 mM Tris (pH 6.8), 10% (w/v) SDS, 0.5% (w/v) bromophenol blue, 50% (v/v) glycerol, 5% (v/v) 2-mercaptoethanol], boiled for 5 min, and spun at 12,000 rpm for 5 min at room temperature. The supernatants were fractionated by 10% SDS-PAGE, and the membrane was probed with the indicated antibody.

ChIP Assay

Seedlings of 4-week-old *ABF1V-1* and wild-type plants grown under continuous light were used for ChIP assays. Three g of leaves was cross-linked in 35 mL formaldehyde buffer [0.4 M Suc, 10 mM Tris-HCl (pH 8.0), 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM EDTA, and 1% (v/v) formaldehyde] and vacuumed twice for 15 min. To stop the reaction, 1.6 mL of 2 M Gly was added and vacuumed for 5 min. Then the samples were washed in water and ground to powder in liquid nitrogen prior to being suspended in 15 mL of Honda buffer [0.44 M Suc, 1.25% Ficoll, 2.5% Dextran T40, 20 mM HEPES KOH (pH 7.4), 10 mM MgCl₂, 0.5% Triton X-100, 5 mM dithiothreitol, 1 mM PMSF, and 1 tablet/50 mL of protease inhibitor cocktail]. The nuclei were filtered through two layers of Miracloth, precipitated by centrifugation, and suspended in nuclei lysis buffer [50 mM Tris-HCl (pH 8.8), 10 mM EDTA, 1% SDS, 1 mM PMSF, and 1 tablet/50 mL of protease inhibitor cocktail]. The chromatin complexes were sonicated and incubated with anti-VP16 antibody or anti-OsABF1 antibody as described. The precipitated DNA was recovered in water for further experiments. For ChIP-qPCR assays, the DNA samples were analyzed by qPCR with SYBR Premix Ex Taq (Takara) for at least three replicates and the value was normalized to that of input DNA (% of input).

Accession Numbers

Sequence data from this article can be found in the MSU Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/analyses_search_locus.shtml) databases (Kawahara et al., 2013) under the following accession numbers: *OsABF1* (LOC_Os01g64730), *Ehd1* (LOC_Os10g32600), *Hd1* (LOC_Os06g16370), *Hd3a* (LOC_Os06g06320), *RFT* (LOC_Os06g06300), *OsPHYB* (LOC_Os03g19590), *OsCOL4* (LOC_Os02g39710), *DTH8* (LOC_Os08g07740), *SE5* (LOC_Os06g40080), *LFL1*

(LOC_Os01g51610), OsMADS56 (LOC_Os10g39130), OsGI (LOC_Os01g08700), Ehd2 (LOC_Os10g28330), Ehd3 (LOC_Os08g01420), Ehd4 (LOC_Os03g02160), OsMADS50 (LOC_Os03g03070), OsMADS51 (LOC_Os01g69850), and OsWRKY104 (LOC_Os11g02520).

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. Overexpression of *OsABF1* confers later flowering phenotype.

Supplemental Figure S2. Transcriptional levels of flowering-associated genes under LDs.

Supplemental Figure S3. Transcriptional levels of flowering-associated genes under SDs.

Supplemental Figure S4. Knockdown of *Ehd1* suppresses the early flowering phenotype of *ABF1E*.

Supplemental Figure S5. Analysis of *Ehd1* mRNA expression in *Osabf1-3* mutant.

Supplemental Figure S6. Phylogenetic tree of OsABF1 and homologous proteins.

Supplemental Figure S7. EAR-OsWRKY104 HFT leads to early flowering phenotype.

Supplemental Figure S8. Overexpression of *OsWRKY104* confers later flowering phenotype.

Supplemental Figure S9. Drought accelerates flowering in Arabidopsis.

Supplemental Figure S10. Drought delays flowering in rice.

Supplemental Figure S11. Vector map and sequence of pHCOMPARE WITH.

Supplemental Figure S12. The soil moisture content during drought regime experiment.

Supplemental Table S1. Oligonucleotide primers used in this study.

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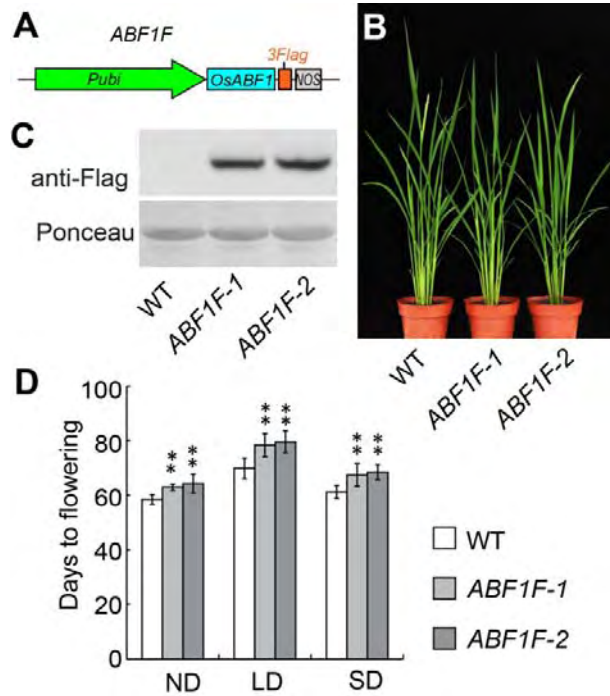
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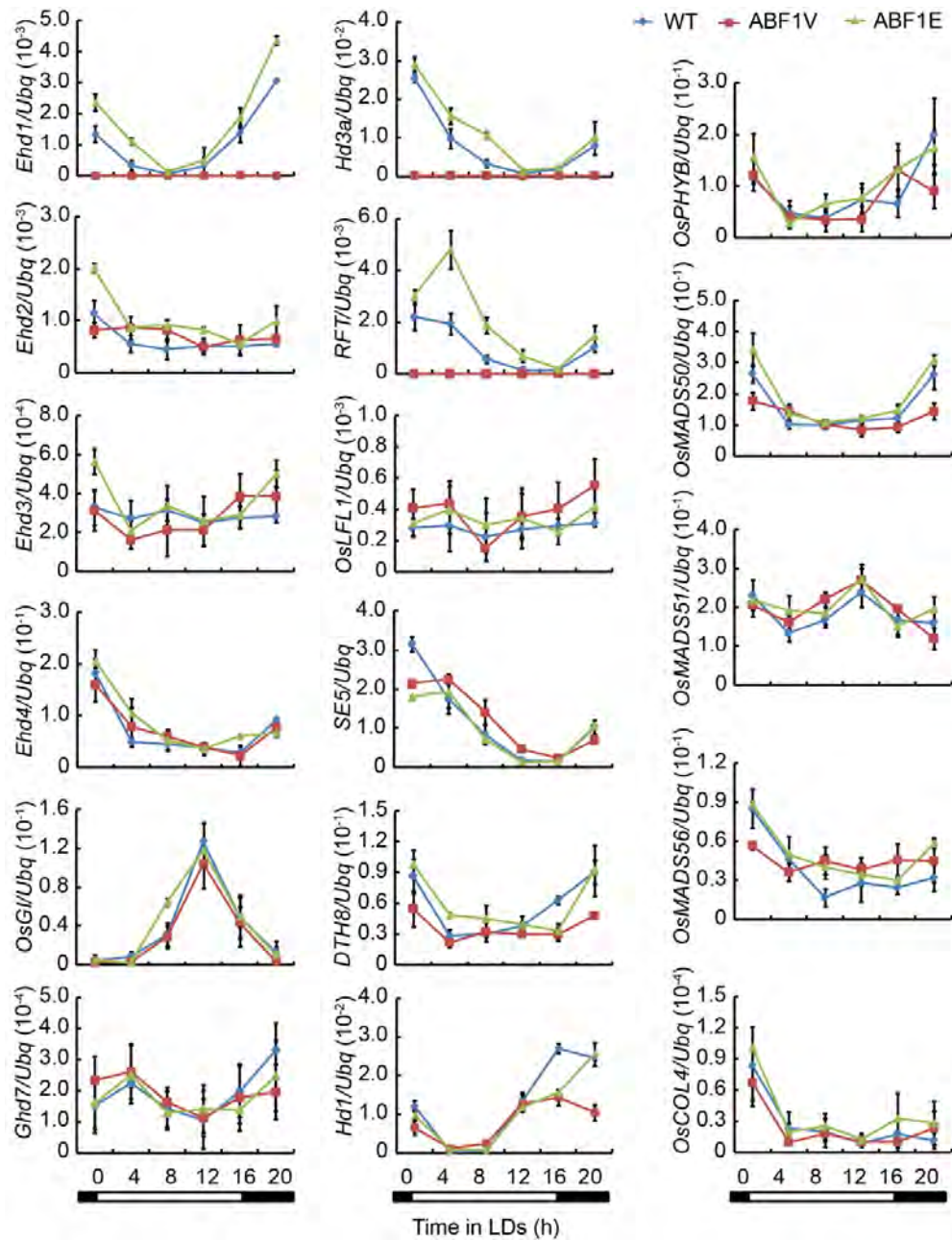
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Supplemental Data

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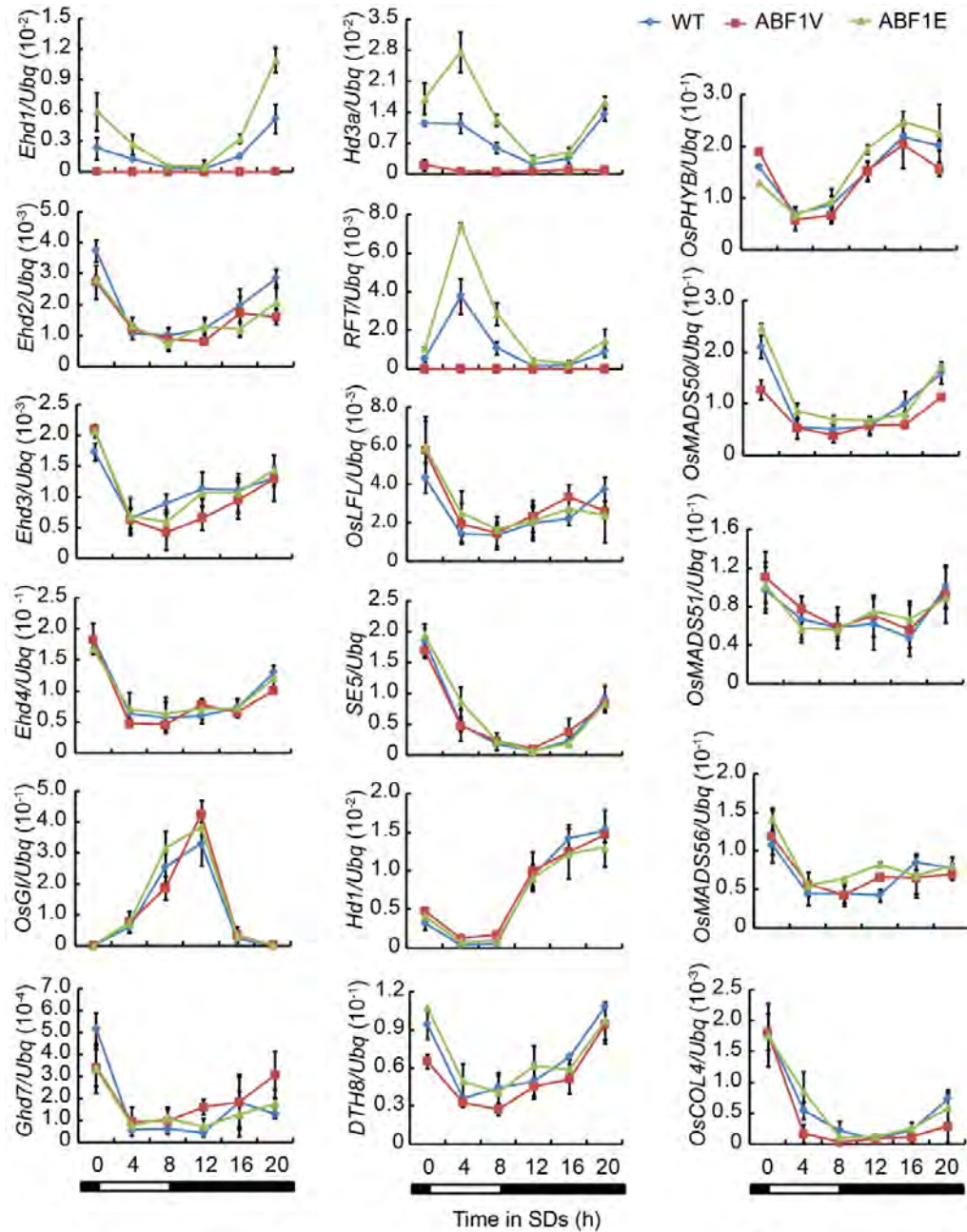


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7 **Supplemental Figure S1.** Overexpression of *OsABF1* confers later flowering
8 phenotype. A, Diagrams of the *OsABF1* overexpression construct. *ABF1F*,
9 *Pubi:OsABF1-3Flag*. B, Representative flowering image of indicated genotypes
10 under NDs. C, Protein expression analysis of *ABF1F* in indicated lines. The
11 immunoblot was probed with anti-Flag antibody. D, Flowering days of each genotype
12 grown under NDs, LDs or SDs. Mean values \pm s.d. were shown. The value of each
13 genotype was compared to that of WT (Student's *t* tests, ***P* < 0.01, n=20).
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Supplemental Figure S2. Transcriptional levels of flowering-associated genes under LDs. Plants were grown for 4 weeks. Samples were collected every 4 hours from the beginning of light period. Three biological replicates of qRT-PCR experiment were performed using *Ubq* gene as the internal control and the representative results were shown. Values were mean \pm s.d. (n = 3).

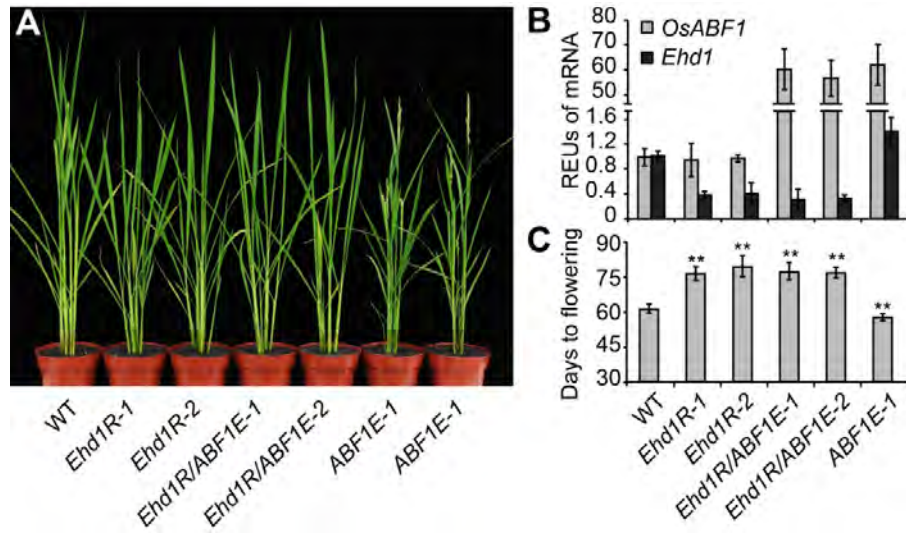


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25 **Supplemental Figure S3.** Transcriptional levels of flowering-associated genes under
 26 SDs. Plants were grown for 4 weeks. Samples were collected every 4 hours from the
 27 beginning of light period. Three biological replicates of qRT-PCR experiment were
 28 performed using *Ubq* gene as the internal control and the presentative results were
 29 shown. Values were mean \pm s.d. (n = 3).

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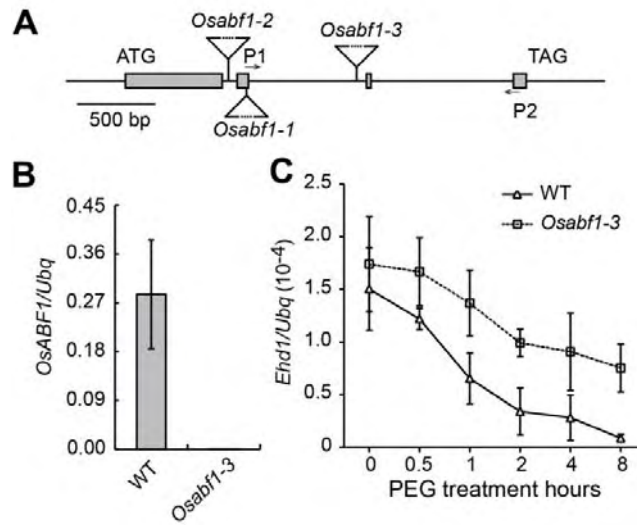


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33 **Supplemental Figure S4.** Knockdown of *Ehd1* suppresses the early flowering
 34 phenotype of *ABF1E*. A, The flowering phenotypes of indicated genotypes grown
 35 under NDs. All plants are in Kita-ake background. *Ehd1R* indicates the *Ehd1-RNAi*
 36 transgenic line in T1 generation. *Ehd1R/ABF1E-1* and *Ehd1R/ABF1E-2* were two
 37 independent T1 lines generated by stacking transformation of *Ehd1-RNAi* construct
 38 into *ABF1E-1* homozygous line. B, qRT-PCR analysis of *OsABF1* or *Ehd1* mRNA
 39 expression in each genotype. The latest fully expanded leaves of 4-week-old seedlings
 40 grown under LDs were sampled at the end of dark period. The relative expression
 41 units (REUs) were calculated by the formula: [(*gene/ubq*) of each genotype]/
 42 [(*gene/ubq*) of WT]. C, Statistic analysis of flowering time of indicated genotypes as
 43 in A.

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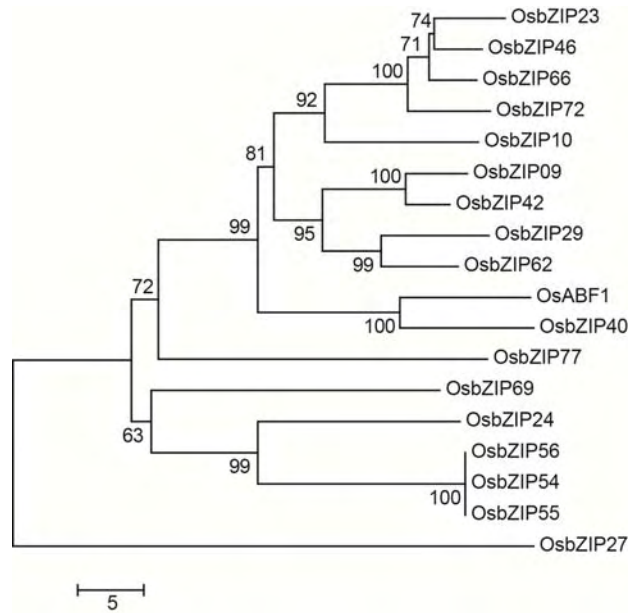


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47 **Supplemental Figure S5.** Analysis of *Ehd1* mRNA expression in *Osabf1-3* mutant. A,
 48 Schematic diagram showing the T-DNA insertion positions in *Osabf1-1*, *Osabf1-2* and
 49 *Osabf1-3* mutant alleles. The boxes and solid lines present exons and introns
 50 respectively. B, Analysis of *OsABF1* mRNA level in WT and *Osabf1-3* by qRT-PCR
 51 using a pair of primers P1 and P2 as indicated in A. C, Dynamic transcription of *Ehd1*
 52 in WT under PEG treatment for the indicated periods. The seedlings were grown
 53 under continue light for 4 weeks and then subjected to PEG treatment.

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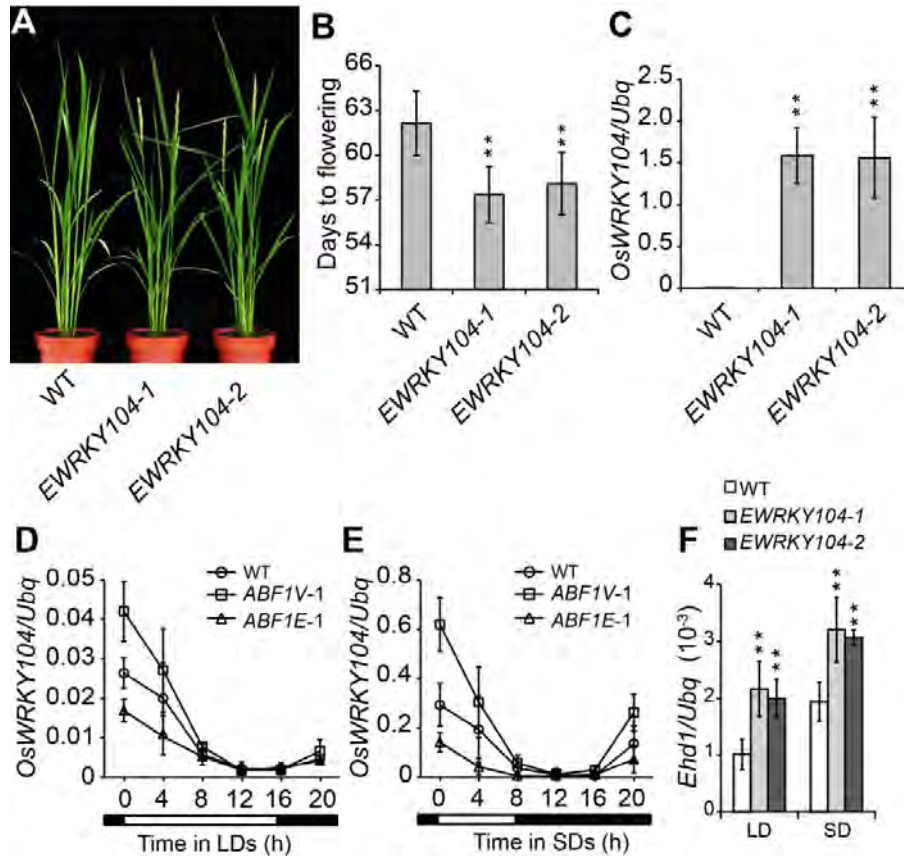


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57 **Supplemental Figure S6.** Phylogenetic tree of OsABF1 and homologous proteins.
 58 Protein sequences were downloaded from the MSU Rice Genome Annotation Project
 59 (http://rice.plantbiology.msu.edu/analyses_search_locus.shtml) databases and then
 60 used for Neighbor-joining phylogenetic analysis (MEGA5.2). The scale bar indicates
 61 substitutions per site.

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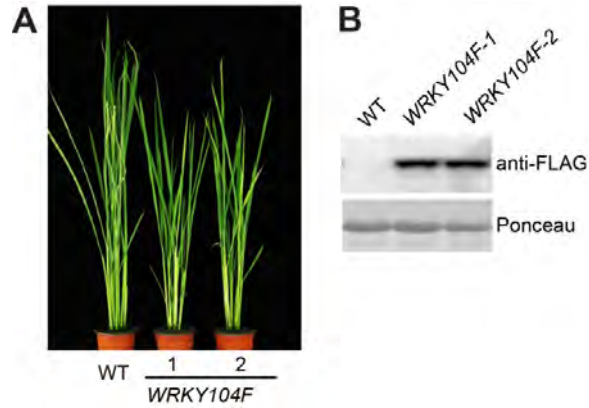


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65 **Supplemental Figure S7.** EAR-OsWRKY104 hybrid transcription factor leads to
 66 early flowering phenotype. A, Representative flowering image of indicated genotypes
 67 cultured under NDs. *EWRKY104*, *Pubi:EAR4-OsWRKY104*. B, Flowering time of
 68 each genotype as in A. Mean values \pm s.d. (standard deviations) were shown. The
 69 value of each genotype was compared to that of WT (Student's *t* tests, ***P* < 0.01,
 70 *n*=20). C, qRT-PCR analysis of *OsWRKY104* mRNA level in each genotype. The
 71 leaves of 4-week-old seedlings were sampled just at the end of dark period under SDs.
 72 D and E, qRT-PCR analysis of the dynamic expression of *OsWRKY104* mRNA under
 73 LDs or SDs. F, qRT-PCR analysis of *Ehd1* mRNA level in each genotype cultured
 74 under LDs or SDs. The samples were collected just at the end of dark period.

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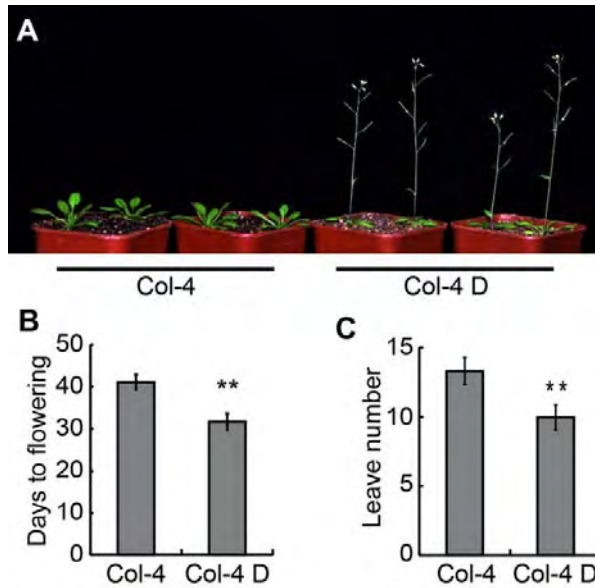
78 **Supplemental Figure S8.** Overexpression of *OsWRKY104* confers later flowering

79 phenotype. A, Representative flowering image of indicated genotypes under LDs.

80 *WRKY104F*, *Pubi:OsWRKY104-3Flag*. B, Protein expression analysis of *WRKY104F*

81 in indicated lines. The immunoblot was probed with anti-Flag antibody.

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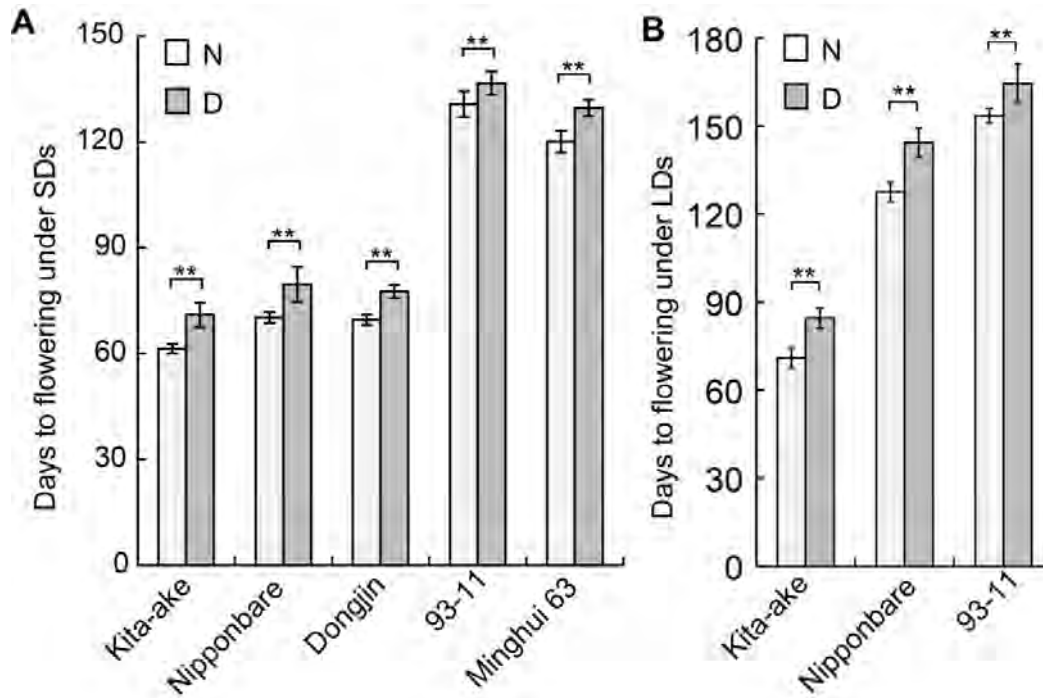


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85 **Supplemental Figure S9.** Drought accelerates flowering in Arabidopsis. A, Image of
86 5-week-old Columbia ecotype (Col-4) plants grown in normal (Left) or drought (right,
87 Col-4 D) condition under LDs. B, Flowering time of the plants as in A (Student's *t*
88 tests, ** $P < 0.01$, $n = 20$). C, Leaf number of the plants as in A (Student's *t* tests, ** P
89 < 0.01 , $n = 20$).

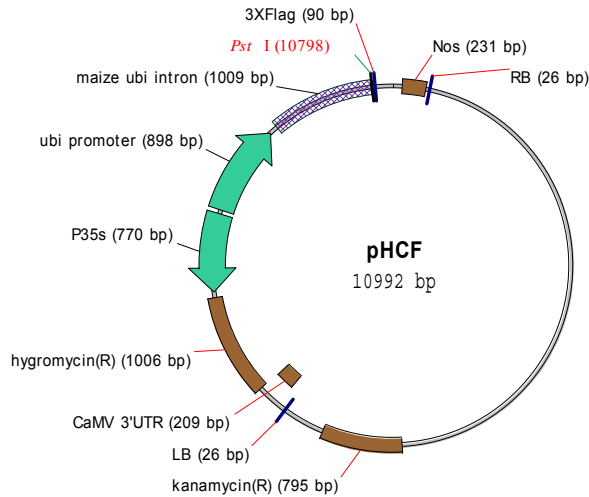
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93 **Supplemental Figure S10.** Drought delays flowering in rice. A, Flowering time of
 94 *japonica* cultivars (Kita-ake, Nipponbare, Dongjin) and *indica* cultivars (93-11,
 95 Minghui 63) grown in normal (N) or drought condition (D) under SDs (Student's *t*
 96 tests, ** $P < 0.01$, $n = 20$). B, Flowering time of indicated cultivar grown in normal (N)
 97 or drought condition (D) under LDs (Student's *t* tests, ** $P < 0.01$, $n = 20$).
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101 > pHCF sequence

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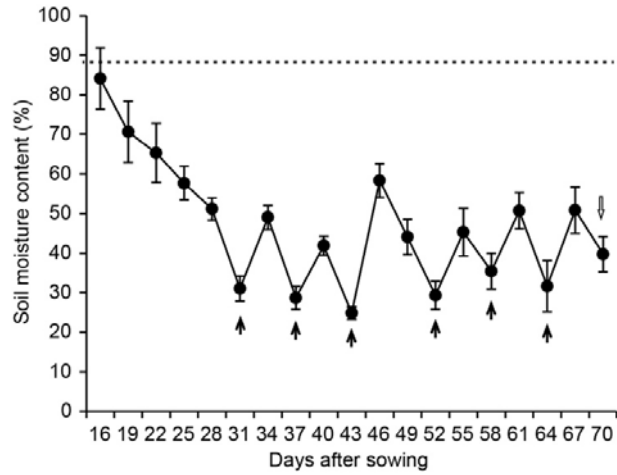
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216 ataattttgtatcttgataactggatgatggcatatgcagcagctatatgtggatttttttagcctcctcacaacgctattttgttgg
217 actgttctttgtgatgctcaccctgttgttgtgttacttctgcactaggtacctgcaggtcagcggatccatcgataaccgtcagatggact

218 acaagacgatgacgataaagtcgagatggactacaagacgatgacgataaagtcgagatggactacaagacgatgacgataaagtc
219 gaggggggcccctgaggatcccgggaattctaagaggagtccacatggtagatctgactagtgttaacg

220

221 **Supplemental Figure S11.** Vector map and completed sequence of pHCF.

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224

225 **Supplemental Figure S12.** The soil moisture contents during drought regime
 226 experiment. The arrows and the hollow arrow indicate irrigation time and flowering
 227 time of WT under drought treatment respectively. The dash line indicates saturated
 228 soil moisture content. The soil moisture content was calculated by the formula: $[(\text{total soil weight}) - (\text{dry soil weight})]/(\text{dry soil weight})$. Values were mean \pm s.d. (n = 5).

229

230

Supplemental Table S1. Oligonucleotide primers used in this study.

Experiment	Primer name	Sequence
qRT-PCR	Ubq-QF	5' AACCAGCTGAGGCCCAAGA 3'
	Ubq-QR	5' ACGATTGATTTAACCAGTCCATGA 3'
	Ehd1-QF	5' CCTACAGTGATTATGGCTTCA 3'
	Ehd1-QR	5' GTGCTGCCAAATGTTGCTC 3'
	Hd1-QF	5' TCAGCAACAGCATATCTTTCTCATCA 3'
	Hd1-QR	5' TCTGGAATTTGGCATATCTATCACC 3'
	Ehd2-QF	5' CGACGACAATAGCTCGATCGC 3'
	Ehd2-QR	5' GTGCATGGTCACGGAGCCTT 3'
	Ehd3-QF	5' GGACCACCTCGTCACCTACAA 3'
	Ehd3-QR	5' CGCCGTTGGCCATGAG 3'
	Ehd4-QF	5' CAGCCAGCGGAATCATCAC 3'
	Ehd4-QR	5' CCAAATCCATCAGACCTACTCCT 3'
	OsMADS50-QF	5' CAGGCCAGGAATAAGCTGGAT 3'
	OsMADS50-QR	5' TTAGGATGGTTTGGTGTGATTGC 3'
	OsMADS51-QF	5' GTTTGCTCTGCTCCTACTC 3'
	OsMADS51-QR	5' ACTCCTCCTCCAGCATTGAA 3'
	OsMADS56-QF	5' GACCGCTATAAAGCATAACACA 3'
	OsMADS56-QR	5' TCATGTGGTTAGCCACCAGC 3'
	OsGI-QF	5' GATAGACGGCACTTCAGCAGAT 3'
	OsGI-QR	5' TGGAGAAAGGTTGTGGATGC 3'
	COL4-QF	5' GGAATCAAGGCCATCGGG 3'
	COL4-QR	5' CGAGCCC GCCGACAG 3'
	SE5-QF	5' AGGACTCCCAAGCTTTTATC 3'
	SE5-QR	5' CTCCAGAATACGAGAACGAC 3'
	OsphyB-QF	5' ATGGAACAGACACAATGCTT 3'
	OsphyB-QR	5' AGCATAACCATATCAGCTT 3'
	DTH8-QF	5' CAGGAGTGC GTGTCGGAGTT 3'
	DTH8-QR	5' GGTCGTCGCCGTTGATGGT 3'
	LFL-QF	5' TTGTCATCGGAGCAAAGAAGG 3'
	LFL-QR	5' TGCCATGGGAGAGAGATAGTCA 3'
	Hd3a-QF	5' GCTCACTATCATCATCCAGCATG 3'
	Hd3a-QR	5' CCTTGCTCAGCTATTTAATTGCATAA 3'
	RFT1-QF	5' TGACCTAGATTCAAAGTCTAATCCTT 3'
	RFT1-QR	5' TGCCGGCCATGTCAAATTAATAAC 3'
	Ghd7-QF	5' GTCGCCAAATTATCAGGTGAAAA 3'
	Ghd7-QR	5' ACAGGCCACATCCTTCTCCG 3'
	OsABF1-QF	5' GGAGGAGAACGCCAAGATGT 3'
	OsABF1-QR	5' GATCTCGTGCTGACGTTTTCC 3'
	OsABF1-RNAi-QF	5' GGAGATGACGCTGGAGGACTT 3'
	OsABF1-RNAi-QR	5' CCATCACCACCTGCCCT 3'
	OsZIP40-QF	5' AGGAGAAAAATCAAAGAGGCTTAA 3'

	OsZIP40-QR	5' TTCCATTGAGTTGGTTCTTCGTA 3'
	OsZIP72-QF	5' AGATATCATGGAAATGCAGAAAAAT 3'
	OsZIP72-QR	5' GGACCAGTCAGTGTCTTCGC 3'
	OsZIP23-QF	5' AAATGTTGGAGCAGCAAAAGAA 3'
	OsZIP23-QR	5' CCCGTCAGAGTCCTCCGAA 3'
	OsZIP10-QF	5' CGCTCGTCTCAAAGAGGCAG 3'
	OsZIP10-QR	5' ATTCATCTTCTCCTTGGACTGCT 3'
	OsZIP66-QF	5' CATCTTCTTCGGAACTACGG 3'
	OsZIP66-QR	5' GACATCAGCCTATTCCCATG 3'
	OsZIP46-QF	5' GCAGAAAAAGCAGGTGGAAAT 3'
	OsZIP46-QR	5' GTCAGTGTTCGTCCGAGGC 3'
	OsZIP09-QF	5' TTCAAGAGCCAGGAAGCAGG 3'
	OsZIP09-QR	5' GAAATCTGCGGAGCTTGTCTC 3'
	OsZIP42-QF	5' CAGGGGAGATTGCAGATAAGCT 3'
	OsZIP42-QR	5' CGAGCTTGTTCGCCTGAGTT 3'
	WRKY104-QF	5' GCTTGCTCTTCAACTCTTGGGA 3'
	WRKY104-QR	5' AAGATTGGTCAGCTTTTTAGTGTCTC 3'
	WRKY104-ChIP-QFa	5' TCACAACACAAACATGGTGAGCAA 3'
	WRKY104-ChIP-QRa	5' TTCGTTTTCCCTTTAGCTTCTG 3'
	WRKY104-ChIP-QFb	5' CATGCCGAACCTTAGGTGGA 3'
	WRKY104-ChIP-QRb	5' GTCGTTTTTAGTTCGCTGTTGTGT 3'
	WRKY104-ChIP-QFc	5' GAGAAGATCTACCATTGCTCTATAGCT 3'
	WRKY104-ChIP-QRc	5' CGAGTGGATTATTACTAGCTTAGTGAAT 3'
	WRKY104-ChIP-QFd	5' CCACAAGTGACCCCATCCAT 3'
	WRKY104-ChIP-QRd	5' CCTGAGCATATCATTAAAAAATATCT 3'
	WRKY104-ChIP-QFe	5' TTAGGATGAAGTTCTGAGCAAAAAC 3'
	WRKY104-ChIP-QRe	5' GGGTTCTATTCTAGGATGTCTCTCC 3'
	25s-ChIP-QF	5' GGGACTACCCGCTGAGTTTA 3'
	25s-ChIP-QR	5' GGACGCCTCTCCAGACTACA 3'
Gene cloning	pCold-OsABF1-F	5' ATCCGAATTCAAGCTT ATGATGGCGTCGAGGGTG 3'
	pCold-OsABF1-R	5' GCAGGTCGACAAGCTT CTACCACTCCATCGAGTTTGTCT 3'
	pDONR-OsABF1-F	5' CAAAAAAGCAGGCTTC ATGATGGCGTCGAGGGTG 3'
	pDONR-OsABF1-R	5' CAAGAAAGCTGGGTC CCACTCCATCGAGTTTGTCT 3'
	attB adaptor-F	5' GTGGGGACAAGTTTGTACAAAAAAGCAGGCTT C 3'
	attB adaptor-R	5' GTGGGGACCACTTTGTACAAGAAAGCTGGGTC 3'

OsABF1-OX-F	5' TCTGCACTAGGTACCTGCAG ATGATGGCGTCGAGGGTG 3'
OsABF1-OX-R	5' ATGGATCCGTCGACCTGCAG CCACTCCATCGAGTTTGTCT 3'
OsABF1-RNAi-F	5' CAAAAAAGCAGGCTTC GCAGGAGCAGGAAGAGGCA 3'
OsABF1-RNAi-R	5' CAAGAAAGCTGGGTC GATCTCGTGCTGACGTTTCC 3'
Ehd1-OX-F	5' TCTGCACTAGGTACCTGCAG ATGGATCACCGAGAGCTGTGG 3'
Ehd1-OX-R	5' ATGGATCCGTCGACCTGCAG GAAATTCCAAAAACATGGTCCAT 3'
Ehd1-RNAi-F	5' CAAAAAAGCAGGCTTC TTGATTTCTGCTAAAACCTGTG 3'
Ehd1-RNAi-R	5' CAAGAAAGCTGGGTC CGAGGTCTCGTATGTCCGTGA 3'
WRKY104-OX-F	5' TCTGCACTAGGTACCTGCAG ATGAAAATTCTCGAATCTTTTGGTC 3'
WRKY104-OX-R	5' ATGGATCCGTCGACCTGCAG TCCCATATGCATATTGGACTG 3'
pDONR-WRKY104-F	5' CAAAAAAGCAGGCTTC ATGAAAATTCTCGAATCTTTTGGTC 3'
pDONR-WRKY104-R	5' CAAGAAAGCTGGGTC TCCCATATGCATATTGGACTG 3'
OsABF1-BD-F	5' CATGGAGGCCGAATTC ATGATGGCGTCGAGGGTGATGG 3'
OsABF1-BD-R	5' GGATCCCCGGGAATTC CTACCACTCCATCGAGTTTGT 3'
OsABF1-HA-BD-R	5' GGATCCCCGGGAATTC TCTGGCACGTCGTAGGGGTA 3'
OsABF1-Δ1-BD-F	5' CATGGAGGCCGAATTC GGTGACAGGCCGATGTGCG 3'
OsABF1-Δ3-BD-R	5' GGATCCCCGGGAATTC CTGCCCTTGGCCGC 3'
OsABF1-Δ2-BD-R	5' CCATCACCAC GCCGACGAGGGGCAGC 3'
OsABF1-Δ2-BD-F	5' CCTCGTCGGC GTGGTGATGGGGTTCCTGAA 3'
DST-BD-F	5' CATGGAGGCCGAATTC ATGGACTCCCCGTCGCCT 3'
DST-BD-R	5' GGATCCCCGGGAATTC CTAGAGGCTCAAGTTGAGGTCA 3'