

# The Floral Repressor BROTHER OF FT AND TFL1 (BFT) Modulates Flowering Initiation under High Salinity in *Arabidopsis*

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Floral transition is coordinately regulated by both endogenous and exogenous cues to ensure reproductive success under fluctuating environmental conditions. Abiotic stress conditions, including drought and high salinity, also have considerable influence on this developmental process. However, the signaling components and molecular mechanisms underlying the regulation of floral transition by environmental factors have not yet been defined. In this work, we show that the *Arabidopsis* BROTHER OF FT AND TFL1 (*BFT*) gene, which encodes a member of the FLOWERING LOCUS T (FT)/TERMINAL FLOWER 1 (TFL1) family, regulates floral transition under conditions of high salinity. The *BFT* gene was transcriptionally induced by high salinity in an abscisic acid (ABA)-dependent manner. Transgenic plants overexpressing the *BFT* gene (35S:*BFT*) and *BFT*-deficient mutant (*bft-2*) plants were phenotypically indistinguishable from Col-0 plants in seed germination and seedling growth under high salinity. In contrast, although the floral transition was delayed significantly in Col-0 plants under high salinity, that of the *bft-2* mutant was not affected by high salinity. We also observed that expression of the *APETALA1* (*AP1*) gene was suppressed to a lesser degree in the *bft-2* mutant than in Col-0 plants. Taken together, our observations suggest that BFT mediates salt stress-responsive flowering, providing an adaptive strategy that ensures reproductive success under unfavorable stress conditions.

## INTRODUCTION

Higher plants undergo a series of consecutive developmental phase transitions during their life cycles. Among these, the transition from the vegetative phase to the reproductive phase is the most prominent process in plant development and is essential for reproductive success (Bäurle and Dean, 2006; Jung et al., 2011; Poethig, 2003). Therefore, this developmental transition step is elaborately regulated in response to both internal and external cues (Amasino, 2010; Levy and Dean, 1998; Simpson and Dean, 2000).

External factors that affect the reproductive phase transition include changes in day length and ambient temperature, as well as vernalization (that is, exposure to a long period of low but nonfreezing temperatures) (Blázquez et al., 2003; Corbesier and Coupland, 2006; Kumar and Wigge, 2010). It has recently been reported that salt stress also influences floral transition. High salinity causes a delay in floral transition, which allows the plant time to enhance its resistance responses (Achard et al., 2006). The effects of high salinity on flowering initiation are largely dependent on the DELLA proteins involved in gibberellic acid (GA) signaling. The stability of the DELLA proteins is elevated under high salinity (Achard et al., 2006). Accordingly, although salt stress extends vegetative growth and thus delays flowering in control plants, this effect has not been observed in multiple mutants lacking *DELLA* genes, such as *GA-INSENSITIVE* (*GAI*), *REPRESSOR OF GA1-3* (*RGA*), *RGA-LIKE 1* (*RGL1*), and *RGL2* (Achard et al., 2006). Moreover, exogenous application of GA restores the salt responsiveness of floral transition (Li et al., 2007), further supporting the hypothesis that salt dependence of flowering is dependent on GA signaling.

Additional examples of salt regulation of flowering initiation include the role played by the transcription factor NTM1-LIKE 8 (*NTL8*), a member of the NAM, ATAF1/2, CUC2 (*NAC*) transcription factor family. The *NTL8* gene is induced by high salinity (Kim and Park, 2007; Kim et al., 2007). Transgenic plants that overproduce a transcriptionally active *NTL8* protein (35S:*8.1C*) exhibit delayed flowering with associated suppression of *FT* gene expression. The *FT* gene is consistently down-regulated under high salinity, and the salt repression of the *FT* gene is diminished in the *ntl8-1* mutant (Kim et al., 2007), indicating that FT plays a role in salt-responsive flowering.

Notably, the CONSTANS (CO)-FT regulatory module also plays a role in salt regulation of floral transition (Li et al., 2007). The *CO* and *FT* genes are considerably suppressed under high salinity. In addition, the salt responsiveness of flowering is impaired in the *co-2* mutant and in 35S:*CO* transgenic plants (Li et al., 2007), showing that CO is involved in the salt regulation of floral transition. It is now widely thought that high salinity delays floral transition via multiple signaling routes. However, the mechanisms by which high salinity influences flowering

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initiation are only poorly understood at the molecular level. In particular, the underlying intermolecular signaling schemes are largely unknown.

The FT/TFL1 protein family includes FT, TFL1, BFT, MFT (MOTHER OF FT AND TFL1), ATC (ARABIDOPSIS THALIANA CENTRORADIALIS HOMOLOGUE), and TSF (TWIN SISTER OF FT), all of which play an important role in the temporal control of flowering (Kardailsky et al., 1999; Kobayashi et al., 1999; Mimida et al., 2001). Among the FT/TFL1 members, FT is the best characterized.

FT serves as a floral integrator that incorporates both developmental and environmental signals into genetic flowering pathways (Michaels et al., 2005; Moon et al., 2005; Turck et al., 2008). It has recently been demonstrated that the FT protein also acts as a long-distance signaling florigen (Corbesier et al., 2007; Jaeger and Wigge, 2007). However, the TFL1 protein has an antagonistic activity in flowering time control (Kobayashi et al., 1999) despite its high amino acid sequence similarity to FT (Ahn et al., 2006). It also regulates establishment of shoot meristem identity, contributing to plant architecture (Conti and Bradley, 2007; Liljegren et al., 1999). The TSF protein acts redundantly with FT as a floral activator through association with the CO and FD proteins (Jang et al., 2009; Michaels et al., 2005; Yamaguchi et al., 2005). MFT also accelerates flowering initiation (Yoo et al., 2004). However, ATC acts as a floral repressor (Mimida et al., 2001). Overall, it is evident that although the FT/TFL1 family members are closely associated with floral transition, they each work through somewhat different molecular mechanisms.

In addition to their roles in flowering time control, the FT/TFL1 proteins are also interconnected with diverse growth hormonal signaling events. The MFT protein plays a critical role in seed germination via ABA and GA signaling by forming a negative feedback loop with the ABA-INSENSITIVE3 (ABI3) and ABI5 proteins (Xi et al., 2010). The TSF protein is activated by cytokinins in promoting flowering when days are short (D'Aloia et al., 2011). It has recently been shown that the BFT protein functions redundantly with the TFL1 protein in inflorescence meristem development (Yoo et al., 2010). Remarkably, the *BFT* gene is regulated by both developmental signals and abiotic stress conditions (Chung et al., 2010), suggesting that it may play a role in floral transition under stressful conditions.

In this study, we observed that the *BFT* gene was induced significantly under high salinity. The salt induction of *BFT* expression was reduced in ABA biosynthetic and signaling mutants, indicating that the *BFT* gene is regulated by ABA-mediated salt stress signaling at least at the transcriptional level. Transgenic plants overexpressing the *BFT* gene exhibited delayed flowering. In agreement with the effects of high salinity on the *BFT* gene expression, flowering of the *BFT*-deficient *bft-2* mutant was largely insensitive to high salinity. We therefore propose that BFT contributes to the modulation of floral transition under high salinity.

## MATERIALS AND METHODS

### Plant materials and growth conditions

All *Arabidopsis thaliana* lines used were in a Columbia background (Col-0). Plants were grown in a controlled culture room set at 22°C with a relative humidity of 55% under long day conditions (LDs, 16-h light/8-h dark) with white light illumination (120  $\mu$ mol photons/m<sup>2</sup>s) provided by fluorescent FLR40D/A tubes (Osram, Korea). The *BFT*-deficient *bft-2* mutant (GABI-331D12) was obtained from a T-DNA insertional mutant pool deposited in the Nottingham *Arabidopsis* Stock Centre at the

University of Nottingham. The absence of gene expression in the mutant was verified by RT-PCR before use.

To produce transgenic plants overexpressing the *BFT* gene, full-size *BFT* cDNA was subcloned into the binary pB2GW7 vector under the control of the Cauliflower Mosaic Virus (CaMV) 35S promoter. *Agrobacterium*-mediated *Arabidopsis* transformation was performed according to a modified floral dip method (Clough and Bent, 1998).

### Analysis of gene transcript levels

Quantitative real-time RT-PCR (qRT-PCR) was employed for measuring relative levels of gene transcripts. Total RNA sample preparation, reverse transcription, and quantitative polymerase chain reaction were conducted based on the rules that have been proposed to ensure reproducible and accurate measurements of gene transcripts (Udvardi et al., 2008). Methods for extraction of total RNA samples from the appropriate plant materials and RT-PCR conditions have been described previously (Kim et al., 2006). Total RNA samples were pretreated extensively with an RNase-free DNase in order to eliminate contaminating genomic DNA and thus to achieve accurate measurements of gene transcripts.

qRT-PCR was carried out in 96-well blocks with an Applied Biosystems 7500 Real-Time PCR System using SYBR Green I master mix in a volume of 25  $\mu$ l. The PCR primers were designed using the Primer Express Software installed on the system. The two-step thermal cycling profile used consisted of 15 s at 94°C followed by 1 min at 68°C. The gene *elf4A* (At3g-13920) was included in the assays as an internal control for normalizing the variations in cDNA amounts used (Gutierrez et al., 2008). All of the qRT-PCR reactions were carried out in biological triplicates using RNA samples extracted from three independent plants that were grown under identical growth conditions. The comparative  $\Delta\Delta C_T$  method was used to evaluate the relative quantities of each amplified product in the samples. The threshold cycle ( $C_T$ ) for each reaction was automatically determined by the system set with default parameters. The specificity of each PCR reaction was determined by melt curve analysis of the amplified products using the standard method installed in the system.

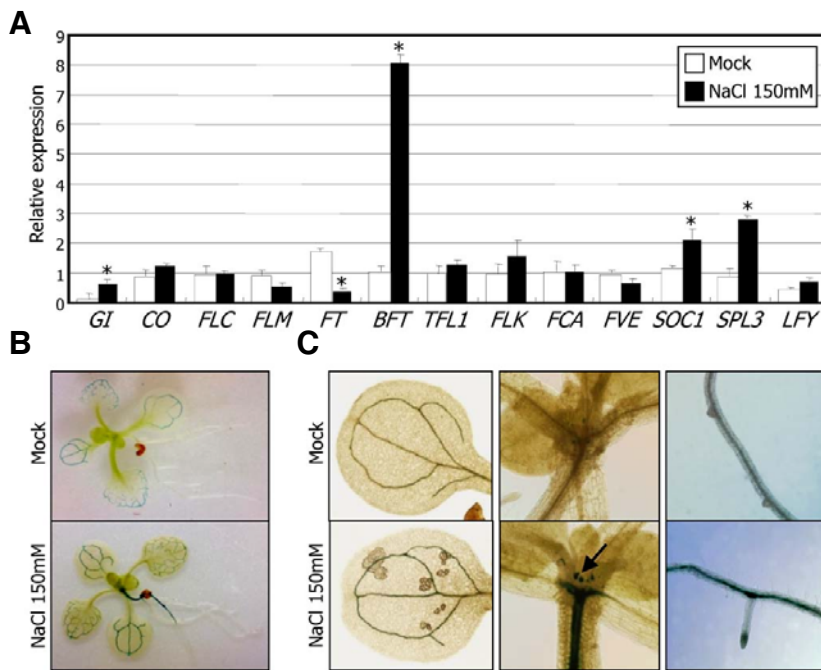
### Histochemical staining

The primers used to subclone the *BFT* gene promoter region were P<sub>BFT</sub>:GUS-F (5'-AAAAAGCAGGCTCGATCAAGCGTTT AGGTATGAT) and P<sub>BFT</sub>:GUS-R (5'-AGAAAGCTGGGTTTTT TCTTTGCTCCAATGTGT). The PCR product was subcloned into the pHGWFS7 vector (Invitrogen, USA), and the fusion construct was transformed into *Arabidopsis* plants.

For histochemical analysis of  $\beta$ -glucuronidase (GUS) activity, plant materials were incubated in 90% acetone for 20 min on ice, washed twice with rinsing solution (50 mM sodium phosphate, pH 7.2, 0.5 mM K<sub>3</sub>Fe(CN)<sub>6</sub>, 0.5 mM K<sub>4</sub>Fe(CN)<sub>6</sub>), and subsequently incubated at 37°C for 18–24 h in rinsing solution containing 2 mM 5-bromo-4-chloro-3-indolyl- $\beta$ -D-glucuronide (X-Gluc) (Duchefa, The Netherlands). The plant materials were then incubated in a series of ethanol solutions ranging from 15% to 80% in order to remove chlorophylls from the plant tissues. The samples were then mounted on microscope slides and visualized using a Nikon SMZ 800 microscope (Nikon, Japan).

### Treatments with growth hormones and abiotic stresses

Two-week-old plants grown on 1/2 X Murashige and Skoog (MS)-agar plates (hereafter referred to as MS-agar plates) were transferred to MS-agar plates supplemented with appropriate



MS-liquid cultures supplemented with 150 mM NaCl for 6 h. Whole seedlings (B) or individual plant organs (C) were subject to GUS staining.

**Fig. 1.** Effects of high salinity on expression of flowering time genes. (A) Expression of flowering time genes under high salinity. Col-0 plants grown on MS-agar plates for 2 weeks under long days (LDs) were incubated in MS-liquid cultures supplemented with 150 mM NaCl for 6 h. Salt treatments were initiated at zeitgeber time (ZT) 10. Whole plants were harvested for the extraction of total RNA ( $n = 25$ ). Transcript levels were determined by quantitative real-time RT-PCR (qRT-PCR). Biological triplicates using total RNA extracted from different plant samples grown under identical conditions were averaged and statistically treated using Student's *t*-test (\* $P < 0.01$ ). Bars indicate standard error of the mean. (B, C) Effects of high salinity on localized expression of the *BFT* gene in different plant organs. Two-week-old transgenic plants expressing a *BFT-GUS* gene fusion, in which the promoter region of the *BFT* gene consisting of approximately 3-kbp sequence region upstream of the transcriptional start site was fused transcriptionally to the  $\beta$ -glucuronidase (GUS)-coding sequence, were incubated in

growth hormones. ABA, indole-3-acetic acid (IAA), and methyl jasmonic acid (mJA) were each used at a final concentration of 20  $\mu$ M.

For the assays of the effects of drought on gene expression, two-week-old plants grown on MS-agar plates were put on a dry 3MM paper at room temperature for appropriate time periods. To examine the effects of high salinity on gene expression, two-week-old plants grown on MS-agar plates were soaked in MS liquid cultures containing 150 mM NaCl and incubated under constant light for appropriate time periods. For cold treatments, two-week-old plants grown on MS-agar plates were transferred to a cold chamber set at 4°C and incubated for appropriate time periods before plant materials were harvested. Whole plants were used for total RNA extraction unless otherwise specified.

## RESULTS

### The *BFT* gene is induced by high salinity

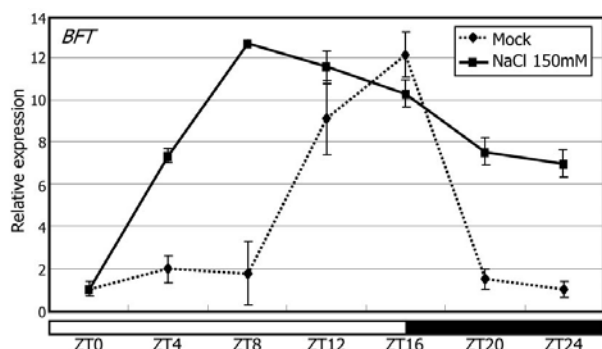
It has been reported that high salinity delays flowering time, although some variations have been noticed in individual experiments depending on plant genotypes and growth conditions (Achard et al., 2006; Li et al., 2007). To obtain molecular clues to how high salt stress regulates floral transition at the molecular level, we examined the effects of high salinity on the expression of key flowering time genes by using qRT-PCR. Col-0 plants grown on MS-agar plates for 2 weeks under LDs were incubated in MS-liquid cultures supplemented with 150 mM NaCl for 6 h, and total RNA was extracted from whole plants. The genes examined included those encoding floral promoters, such as *GIGANTEA* (*GI*), *CO*, *FT*, *SUPPRESSOR OF OVER-EXPRESSION OF CO 1* (*SOC1*), *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 3* (*SPL3*), and *LEAFY* (*LFY*), and floral repressors, including *FLC*, *FLM*, *BFT*, and *TFL1*.

The results showed that many of the genes examined were not noticeably affected by salt treatments. In contrast, the

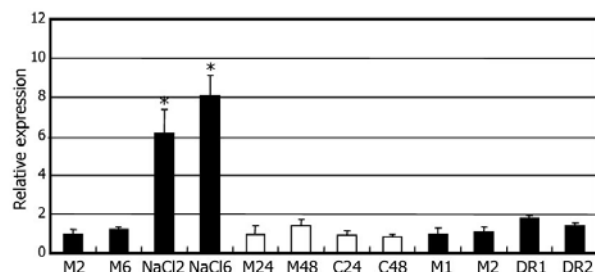
genes *FT*, *SOC1*, *SPL3*, and *BFT* were influenced to varying degrees by high salinity. The *FT* gene was suppressed significantly under high salinity, as has been reported previously (Kim et al., 2007; Li et al., 2007). Notably, expression of the *BFT* gene was elevated 8-fold under identical conditions (Fig. 1A), suggesting that it might be related with salt-mediated flowering. Expression of the *SOC1* and *SPL3* genes was also induced detectably by high salinity. However, the salt induction of the *SOC1* and *SPL3* genes, which act as floral promoters, was not correlated with the salt repression of flowering, suggesting that these genes are not involved directly in salt-responsive flowering. We therefore chose the *BFT* gene, which represses the initiation of flowering for further study (Yoo et al., 2010).

We next examined the effects of high salinity on *BFT* gene expression in different plant tissues using transgenic plants expressing the *BFT* promoter-*GUS* gene fusion, in which a *GUS*-coding sequence was transcriptionally fused to the *BFT* gene promoter sequence covering an approximately 3-kb region upstream of the transcription start site. The transgenic plants were grown on MS-agar plates for 2 weeks under LDs, and then incubated in MS-liquid cultures supplemented with 150 mM NaCl for 6 h, after which whole seedlings and dissected plant parts were subject to GUS staining. Under normal growth conditions, GUS activity was detected mostly in the veins of leaves (Fig. 1B). In plants grown under high salinity, GUS activity was also present in other plant organs, such as the vascular tissues of hypocotyls and the primary roots (Figs. 1B and 1C). Notably, GUS activity was elevated to a high level in the shoot apical region (indicated by arrow in Fig. 1C), as has been reported previously (Yoo et al., 2010).

To examine diurnal expression of *BFT* gene under high salinity, Col-0 plants grown on MS-agar plates for 2 weeks under LDs were incubated in MS-liquid cultures supplemented with 150 mM NaCl for 6 h, and whole plants were harvested at zeitgeber time (ZT) points for up to 24 h. qRT-PCR assays showed that the levels of *BFT* transcripts exhibited a rhythmic pattern



**Fig. 2.** Diurnal expression patterns of the *BFT* gene under high salinity. Col-0 plants grown on MS-agar plates for 2 weeks under LDs were incubated for 24 h in MS-liquid cultures supplemented with 150 mM NaCl. Salt treatments were initiated at ZT0. Whole plants were harvested at various ZT points for the extraction of total RNAs ( $n = 20$ ). Transcript levels were examined and statistically analyzed as described in Fig. 1A. Bars indicate standard error of the mean.



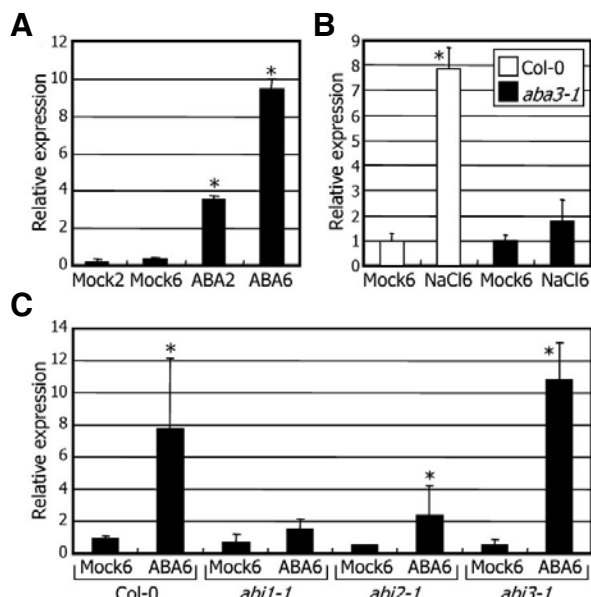
**Fig. 3.** Effects of abiotic stresses on expression of the *BFT* gene. Col-0 plants grown on MS-agar plates for 2 weeks under LDs were subject to treatments with abiotic stresses for the indicated time periods in units of hours. Whole plants were harvested for the extraction of total RNAs. Transcript levels were examined and statistically analyzed as described in Fig. 1A. Bars indicate standard error of the mean (\* $P < 0.01$ ). M, mock; C, cold; DR, drought.

and peaked at ZT12-ZT16 under normal growth conditions (Fig. 2), as has been reported previously (Yoo et al., 2010). In contrast, *BFT* expression was maintained at a high level without rhythmic fluctuations under high salinity (Fig. 2).

We also examined the effects of environmental factors other than high salinity on *BFT* gene expression. The results revealed that the *BFT* gene is influenced specifically by high salinity but is also slightly affected by other environmental factors, such as cold and drought (Fig. 3). These results indicate that the *BFT* gene is involved in the response to salt stress.

#### ABA is required for salt induction of the *BFT* gene

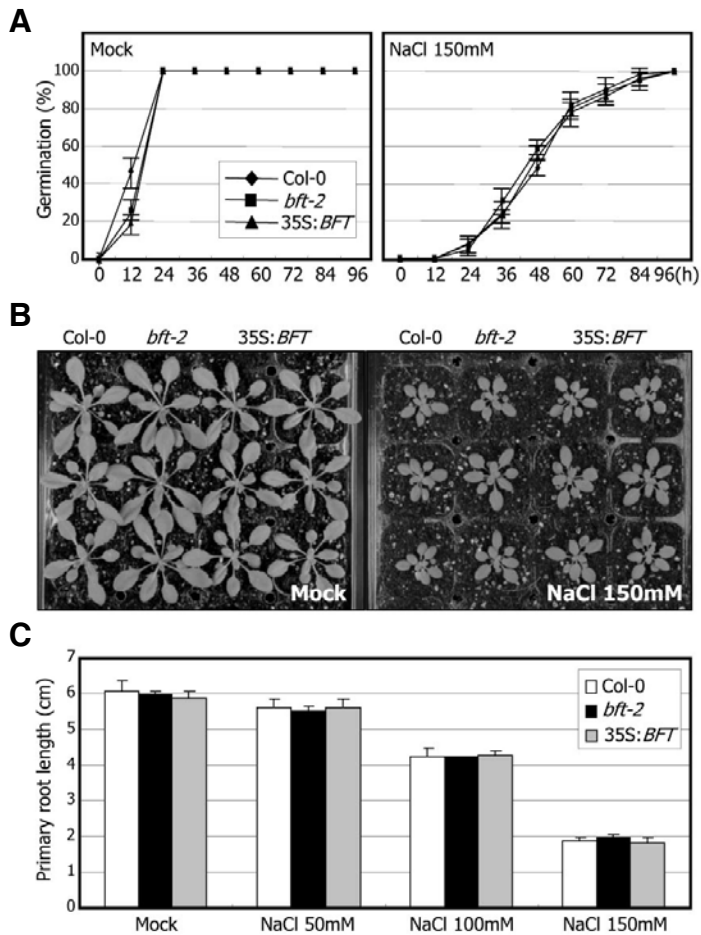
Growth hormones play a critical role in plant responses to environmental stresses. In particular, ABA is intimately involved in salt stress response and signaling (Shinozaki et al., 2003; Yamaguchi-Shinozaki and Shinozaki, 2006). It exerts its role either independently or synergistically through signaling interactions with other growth hormones, such as auxin, salicylic acid (SA), and GA (Jiang et al., 2010; Park et al., 2011; Subbiah and Reddy, 2010; Yaish et al., 2010). Therefore, we investigated whether the *BFT* gene is linked with growth hormonal signals or not.



**Fig. 4.** Effects of ABA and high salinity on expression of the *BFT* gene in ABA-deficient (*aba3-1*) or ABA signaling (*abi1-1* to *abi3-1*) mutants. Plants grown on MS-agar plates for 2 weeks were transferred to liquid MS cultures containing either 20  $\mu$ M ABA or 150 mM NaCl and gently shaken for the indicated numbers of hours. Whole plant materials were harvested. Transcript levels were examined and statistically analyzed as described in Fig. 1A. Bars indicate standard error of the mean (\* $P < 0.01$ ). (A) Effects of ABA on the *BFT* gene. (B) Expression of the *BFT* gene in *aba3-1* mutant under high salinity. (C) Expression of the *BFT* gene in *abi1-1*, *abi2-1*, and *abi3-1* mutants treated with 20  $\mu$ M ABA.

To examine whether the *BFT* gene is affected by growth hormones, Col-0 plants were incubated in the presence of various growth hormones, and gene transcript levels were examined by qRT-PCR. We found that among the growth hormones examined, such as ABA, GA, IAA, SA, cytokinins, and JA, only ABA induced transcription of the *BFT* gene (Fig. 4A), similar to *BFT* induction by high salinity.

We next asked whether ABA is necessary for the salt-induced transcription of the *BFT* gene. We employed the ABA-deficient *Arabidopsis* mutant *aba3-1* (Bittner et al., 2001). Both Col-0 and *aba3-1* mutant plants were exposed to high salinity (150 mM NaCl), and the expression of the *BFT* gene was examined. It was found that under high salinity, while the *BFT* gene was induced significantly in Col-0 plants, the degree of gene induction was drastically reduced in the *aba3-1* mutant (Fig. 4B). This result indicates that salt induction of the *BFT* gene is largely mediated by ABA. We also assayed the effects of high salinity on the *BFT* gene expression in ABA signaling mutants, such as *abi1-1*, *abi2-1*, and *abi3-1* (Kurup et al., 2000; Leung et al., 1997). Whereas salt-induced *BFT* expression was diminished in the *abi1-1* and *abi2-1* mutants, as observed in the ABA-deficient *aba3-1* mutant, it was not changed in the *abi3-1* mutant (Fig. 4C). It has been shown that the *ABI1*- and *ABI2*-encoded protein phosphatases constitute the ABA receptor complex and function in earlier steps of ABA signaling, but the *ABI3*-encoded transcription factor functions in later steps of ABA signaling cascades (Fujii et al., 2009; Kurup et al., 2000; Ma et al., 2009). Together with the previous work, our data demonstrate that the *BFT* gene is interrelated with ABA, possi-



**Fig. 5.** Effects of high salinity on seed germination, seedling growth, and root development in 35S:*BFT* transgenic and *bft* loss-of-function mutant plants. (A) Effects of high salinity on seed germination. Seeds were cold-imbibed and allowed to germinate on MS-agar plates containing 150 mM NaCl. The percentages of germinated seeds were counted at the indicated time points. Radicle emergence was used as a morphological marker for germination. Approximately 80 seeds were counted at each time point. Biological triplicates were averaged. Bars indicate standard error of the mean. h, hour. (B) Effects of high salinity on seedling growth. Plants were grown in soil containing 150 mM NaCl for 3 weeks before taking photographs. Two independent transgenic lines were examined. (C) Primary root growth. Plants grown on MS-agar plates for 5 days were transferred to MS-agar plates supplemented with various concentrations of NaCl and incubated for 2 weeks. The lengths of the primary roots of approximately 50 seedlings were averaged. Bars indicate standard error of the mean.

bly in earlier signaling steps.

#### **BFT is not involved in seed germination and vegetative growth**

We found that the *BFT* gene is induced by high salinity, particularly in the shoot apical region. To investigate the physiological role of the *BFT* gene, we produced transgenic plants overexpressing the *BFT* gene under the control of the CaMV 35S promoter (35S:*BFT*). We also obtained the T-DNA insertional knockout mutant *bft-2* from the Nottingham *Arabidopsis* Stock Centre (University of Nottingham). Overexpression of the *BFT* gene in the 35S:*BFT* transgenic plants and lack of gene expression in the *bft-2* mutant were verified by RT-PCR before use (data not shown).

We first examined the germination phenotypes of the transgenic and mutant seeds in the presence of 150 mM NaCl. The results showed that the germination of both the transgenic and mutant seeds was delayed to a similar degree as observed with Col-0 seeds under high salinity (Fig. 5A). The growth of both the transgenic and mutant seedlings was also indistinguishable from that of control plants in the presence of high salt (Fig. 5B). These observations indicate that the *BFT* gene is not associated with seed germination and plant growth under high salinity.

Root growth is a developmental trait that is profoundly affected by soil salinity. Both primary root growth and lateral root development are reduced under high salinity (Achard et al., 2006). We therefore examined the root growth of the transgenic and mutant plants on MS-agar plates supplemented with 150

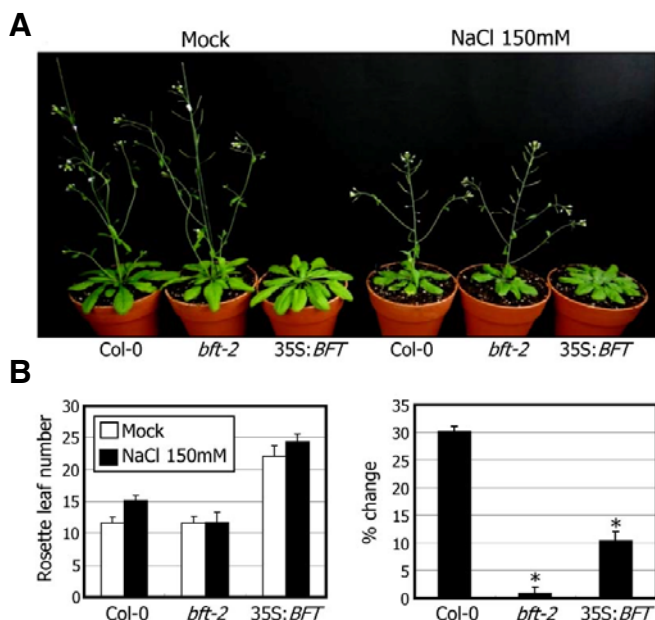
mM NaCl. The primary roots of both the transgenic and mutant plants were reduced, as was observed in Col-0 plants (Fig. 5C). Lateral root growth was also influenced to a similar degree to that observed in Col-0 plants (data not shown). Together, these observations indicate that the *BFT* gene is not related with salt regulation of root growth.

#### **Flowering initiation of the *bft-2* mutant is insensitive to high salinity**

It has been reported that the *BFT* gene plays a role in floral transition and inflorescence meristem development (Yoo et al., 2010). We found that the *BFT* gene is induced by high salinity. We therefore hypothesized that the *BFT* gene might be involved in salt regulation of flowering initiation.

To examine the hypothesis, plants were grown in soil under LDs until flowering. The 35S:*BFT* transgenic plants exhibited significantly delayed flowering (Fig. 6A), as has been previously described (Yoo et al., 2010). In contrast, the flowering phenotype of the *bft-2* mutant was not significantly different from that of Col-0 plants under normal growth conditions. To compare the flowering times, rosette leaf numbers were counted. The measurements revealed that flowering of both Col-0 plants and the 35S:*BFT* transgenic plants was delayed under high salinity (Fig. 6B). Interestingly, flowering of the *bft-2* mutant was not discernibly affected by high salinity.

To more accurately examine the effects of high salinity on flowering initiation, percent changes in the rosette leaf numbers were calculated. The numbers of rosette leaves increased by



**Fig. 6.** Flowering phenotypes of 35S:*BFT* transgenic and *bft* loss-of-function mutant plants under high salinity. (A) Phenotypic comparison of flowered plants. Plants were grown in soil in either the presence or the absence of 150 mM NaCl for 5 weeks under LDs before taking photographs. (B) Measurements of flowering times. Flowering times of the plants shown in (A) were measured by counting rosette leaf numbers at bolting. Thirty plants were counted and averaged (left panel). Bars indicate standard error of the mean. To better compare the flowering times, the rates of increase in the leaf numbers in plants grown under high salinity were calculated and statistically analyzed using Student's *t*-test (\**P* < 0.01) (right panel).

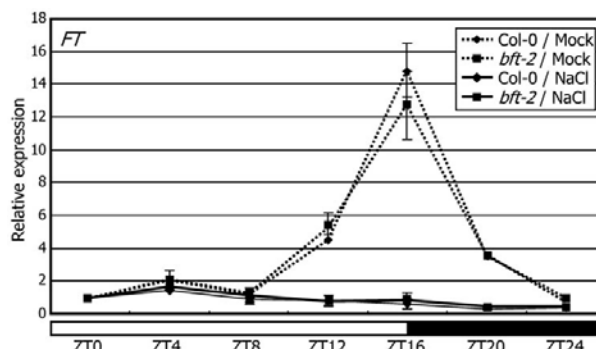
30% in Col-0 plants and by 10% in the 35S:*BFT* transgenic plants when they were grown under high salinity (Fig. 6C). In contrast, the number of rosette leaves was unchanged in the *bft-2* mutant. Collectively, these data demonstrate that the *BFT* gene plays a role in modulating the effects of high salinity during floral transition.

#### AP1 may be involved in BFT-mediated salt-responsive flowering

The floral integrator *FT* is linked with salt-responsive flowering (Kim et al., 2007; Li et al., 2007), and the *FT* gene is suppressed by high salinity (Fig. 7; Kim et al., 2007; Li et al., 2007). However, it has been shown that the *FT* gene is not associated with *BFT* function (Fig. 7; Yoo et al., 2010). Instead, *BFT* action has been implicated in the regulation of the genes *APETALA1* (*AP1*) and *LFY* during induction of floral meristem identity (Yoo et al., 2010).

To further investigate the role of the genes *AP1* and *LFY* in *BFT*-mediated salt signaling, we examined the expression patterns of the *AP1* and *LFY* genes in the 35S:*BFT* transgenic plants and the *bft-2* mutant plants grown in the presence of high salt. Compared to the level of *AP1* expression in wild-type (Col-0) plants, the *AP1* gene was down-regulated slightly in the *bft-2* mutant and significantly in the 35S:*BFT* transgenic plants under normal growth conditions (Fig. 8A), which is consistent with the delayed flowering of the transgenic plants. Under high salinity, the *AP1* gene was suppressed significantly in Col-0 plants but was not influenced in the *bft-2* mutant. This result is consistent with the salt insensitivity of flowering in the mutant (Fig. 6B), supporting that the *AP1* gene is interrelated with *BFT* regulation of flowering under high salinity.

It has been reported that the *LFY* gene is suppressed by salt stress (Achad et al., 2006; Li et al., 2007). Under normal growth conditions, the levels of the *LFY* gene transcripts were significantly lowered in the 35S:*BFT* transgenic plants, but the levels in the *bft-2* mutant plants were comparable to those in Col-0 plants (Fig. 8B). In addition, we found that the *LFY* gene in Col-0 plants was suppressed slightly only after long-term salt treatments (Fig. 8B). Furthermore, the effects of high salt on *LFY* gene expression were maintained in the *bft-2* mutant. These



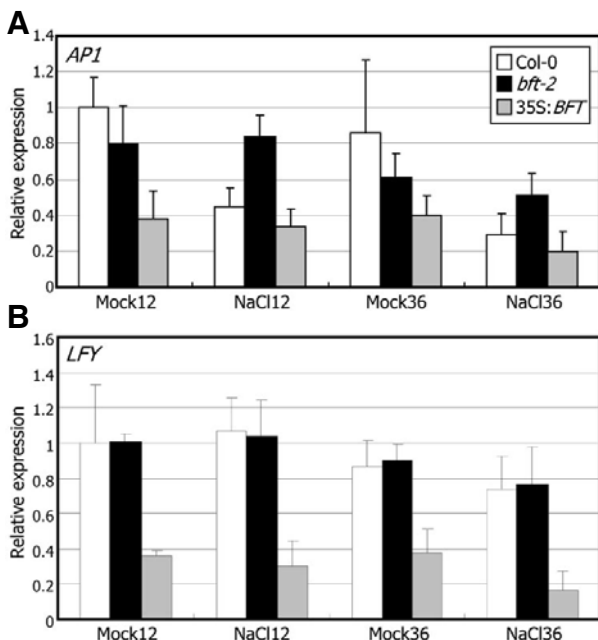
**Fig. 7.** Diurnal expression patterns of the *FT* gene under high salinity. Plants grown on MS-agar plates for 2 weeks under LDs were incubated for 24 h in MS-liquid cultures supplemented with 150 mM NaCl. Salt treatments were initiated at ZT0. Whole plants were harvested at various ZT points for the extraction of total RNA. Transcript levels were examined and statistically analyzed as described in Fig. 1A. Bars indicate standard error of the mean.

observations indicate that the *LFY* gene is not directly related with the *BFT*-mediated salt regulation of flowering.

In conclusion, our data show that the floral repressor *BFT* mediates the salt regulation of flowering initiation, possibly via the *AP1* gene. Although this signaling scheme may not be essential under normal growth conditions, it would help plants to adjust their flowering time in a way that optimizes reproductive success by synchronizing floral transition with plant growth under high salinity.

## DISCUSSION

Adverse environmental conditions affect almost all aspects of plant growth and development. Of particular interest are the effects of stressful growth conditions on the timing of flowering, which is essential for successful reproduction. Floral transition is influenced significantly by various abiotic stresses, including



**Fig. 8.** The expression of *AP1* and *LFY* genes in 35S:*BFT* transgenic and *bft* loss-of-function mutant plants. Plants grown on MS-agar plates for 2 weeks were transferred to liquid MS cultures containing 150 mM NaCl and incubated for the indicated time periods before harvesting whole plant materials. The transcript levels were examined as described in Fig. 1A. (A) *AP1* gene expression. (B) *LFY* gene expression.

soil salinity, drought, and low temperatures. The genes and associated molecular mechanisms underlying ambient temperature regulation of flowering time have been elucidated fairly well in recent years by analyzing *Arabidopsis* mutants with defects in flowering time control under low temperature conditions (Lee et al., 2007; Yoo et al., 2007). Although soil salinity has been shown to have an effect on floral transition in several plant species, direct evidence for the salt regulation of floral transition at the molecular level is still elusive.

It has been recently suggested that the members of the FT/TFL1 family, which play an important role in reproductive development, are also involved in environmental stress regulation of flowering initiation. Expression of the *FT* gene is suppressed by high salt, osmotic, and heat stresses (Chung et al., 2010; Li et al., 2007). In addition, the *TSF* gene is induced by cold and drought stresses (Chung et al., 2010), supporting the hypothesis that there is extensive crosstalk between environmental stress signals and floral transition via the FT/TFL1 members.

Here, we demonstrate that the *BFT* gene is also involved in salt stress responses. While it has been previously reported that the *BFT* gene is induced by drought and osmotic stresses (Chung et al., 2010), our careful examination revealed that high salinity also regulates the *BFT* gene. Hyperosmotic stress caused by high salinity may influence *BFT* transcription. The discrepancy between the previous and our own observations could be due to different concentrations of NaCl used and/or to different procedures for sample preparations. The *BFT* gene is also closely associated with ABA signaling. Our observations provide direct evidence supporting the hypothesis that ABA signaling is interconnected with the regulation of floral transition under high salinity. In addition, it is evident that BFT-mediated

salt signaling is related specifically to flowering time control. In contrast, it is not linked with seed germination or seedling growth under high salinity.

The *BFT* gene is expressed in leaves, shoot apical regions, and inflorescence meristems. It regulates the meristem identity genes, such as *LFY* and *AP1*, and plays a critical role in inflorescence meristem development. It has been shown that transgenic plants overexpressing the *BFT* gene exhibit delayed auxiliary inflorescence formation and impaired floral architecture, which is also observed in *TFL1*-overexpressing transgenic plants (Yoo et al., 2010). In contrast, although the *bft-2* single mutant does not show any visible phenotypes, the *bft-2 tfl1-20* double mutants show precociously terminated primary inflorescence and accelerated formation of auxiliary inflorescences (Yoo et al., 2010). These observations indicate that BFT acts redundantly with TFL1 in regulating inflorescence meristem development. Our observations suggest that the BFT regulation of *AP1* gene expression also functions in salt-mediated floral repression. It will be interesting to investigate whether salt regulation of inflorescence meristem development is also mediated by the *BFT* gene. Furthermore, our findings will shed light on how plants optimize their reproductive development under stressful conditions, such as high salinity.

The FT/TFL1 family includes the phosphatidylethanolamine-binding proteins (PEBP) (Schoentgen et al., 1987). The PEBP proteins are widely conserved from prokaryotes to many eukaryotes (Yang et al., 2004). It is known that PEBP proteins bind to phosphatidylethanolamine and nucleotides (Serre et al., 1998), but their physiological roles are largely unknown. Their interactions with as yet unidentified regulatory proteins seem to be important for their function. The bZIP transcription factor FD is critical for FT function. The FT protein activates expression of regulatory targets, such as *AP1* and *LFY*, by interacting with FD (Abe et al., 2005; Wigge et al., 2005). In addition, TFL1, which has an antagonistic activity in floral transition, also interacts with FD in yeast cells (Abe et al., 2005), indicating that dynamic interactions, as well as competition, may constitute the functional mechanisms of the FT/TFL1 members. Our data suggest that the *AP1* genes are putative regulatory targets of the BFT protein. However, the biochemical activity of the BFT protein and the underlying molecular mechanisms are still unclear. Searching for interacting partners of BFT and investigating the molecular and biochemical functions of BFT will be helpful for further understanding of BFT-mediated salt signaling in flowering time control.

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