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BROTHER OF FT AND TFL1 (BFT), a member of the *FT/TFL1* family, shows distinct pattern of expression during the vegetative growth of Arabidopsis

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Transition to the flowering stage is precisely controlled by a few classes of regulatory molecules. *BROTHER OF FT AND TFL1* (*BFT*) is a member of *FLOWERING LOCUS T* (*FT*)/*TERMINAL FLOWER 1* (*TFL1*) family, an important class of flower development regulators with unidentified biochemical function. *BFT* has a *TFL1*-like activity and plays a role in axillary inflorescence development. To elucidate the expression pattern of *BFT,* we analyzed the subcellular localization and conditional expression of *BFT* in this study. We generated *35S::BFT:GFP* plants to investigate the subcellular localization of BFT protein. *35S::BFT:GFP* plants showed late flowering, similarly as did *35S::BFT* plants. BFT:GFP fusion protein was localized in the nucleus and the plasma membrane, which was different from the localization pattern of FT and TFL1. *BFT* expression was induced by abiotic stress conditions. ABA, drought, and osmotic stress treatments induced *BFT* expression, whereas cold, salt, and heat stress conditions did not, suggesting that *BFT* plays a role in regulating flowering time and inflorescence structure under drought conditions. The induction pattern of *BFT* was different from those of other *FT/TFL1* family genes. Our studies indicated that BFT showed a distinct expression pattern from its homologous genes during the vegetative growth in Arabidopsis.

The *FLOWERING LOCUS T* (*FT*)/*TERMINAL FLOWER 1* (*TFL1*) family is a small gene family whose members play a pivotal role in flower development in Arabidopsis. The family includes *FT*, *TFL1*, *TWIN SISTER OF FT* (*TSF*), *Arabidopsis thaliana CENTRORADIALIS* homologue (*ATC*), *MOTHER OF FT AND TFL1* (*MFT*) and *BROTHER OF FT AND TFL1* (*BFT*).[3,5,6,9,15,17] *FT* is a floral promoter that integrates signal inputs from various pathways that regulate flowering time in Arabidopsis.[5,6] *TFL1* plays an antagonistic role to that of *FT*, functioning as a floral inhibitor. Unlike *FT*, *TFL1* also plays an important role in controlling plant architecture by regulating the expression of *LEAFY* (*LFY*) and *APETALA1* (*AP1*), two important floral meristem identity genes in the shoot apical meristem (SAM).[3,7] *TSF* regulates flowering by a mechanism similar to that of *FT*, although a lesion in *TSF* does not have an apparent effect on the determination of flowering time. *MFT* has a weak *FT*-like activity.[17] *ATC* acts as a floral repressor, and its role is similar to that of *TFL1*.[9] Finally, *BFT* has a *TFL1*-like activity, in spite of its amino acid homology to *FT*,[2,4,16] and functions redundantly with *TFL1* in inflorescence meristem development in Arabidopsis.[16] Although genetic studies elucidated an intricate role of the *FT*/*TFL1* family genes, not much is known about the expression pattern of the remaining members except *FT* and *TFL1*. Here, we report that *BFT* expression showed a distinct pattern from its homologous genes during the vegetative phase. BFT:GFP fusion protein was detected in the nucleus and the plasma membrane. *BFT* expression was induced by abiotic

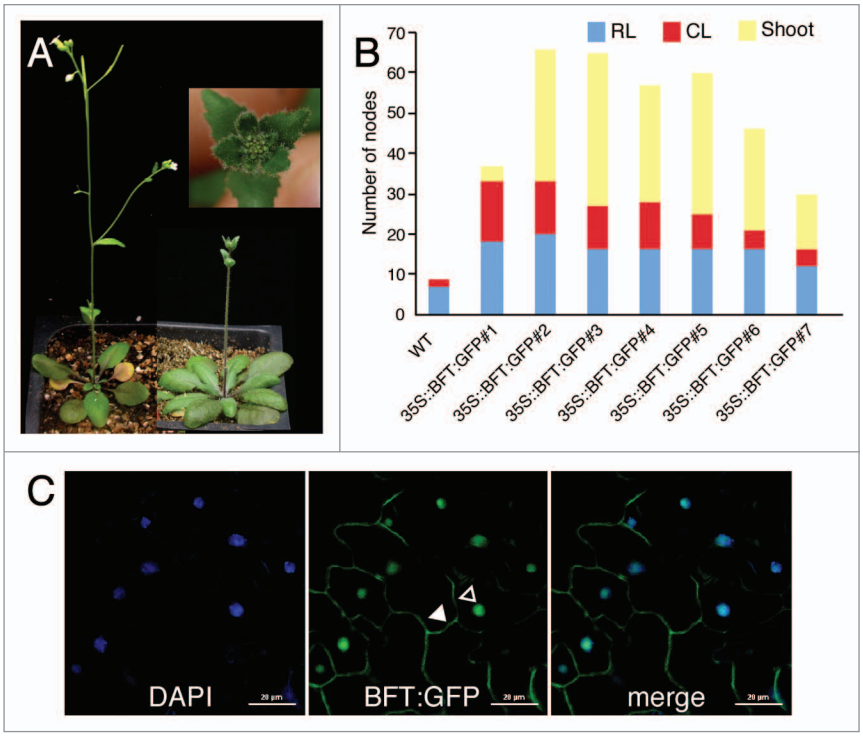

Figure 1. Phenotype of *35S::BFT:GFP* plants and the subcellular localization of BFT:GFP fusion protein. (A) Morphology of a *35S::BFT:GFP* transgenic plant (right) and a wild-type Columbia plant (left). Inset showed a close-up view of the primary inflorescence of *35S::BFT:GFP* plants. Note that overall phenotype of *35S::BFT:GFP* plants is very similar to that of a strong line of *35S::BFT* plants.[16] (B) Number of nodes formed in the primary shoot of *35S::BFT:GFP* transgenic plants. Results from different lines in the T1 generation are shown. CL, cauline leaf; RL, rosette leaf; WT, wild-type Columbia plants. (C) The subcellular localization of BFT in the leaf of 12-day-old *35S::BFT:GFP* plants. Note that BFT:GFP fusion protein is detected in the nucleus (open arrowhead) and the plasma membrane (closed arrowhead). Scale bar: 20 μm.

stress conditions, distinct from other *FT/TFL1* family genes, raising the possibility that *BFT* plays a role in regulating flowering time and inflorescence structure under drought conditions.

BFT is Localized in the Nucleus and the Plasma Membrane

To investigate the subcellular localization of BFT, we expressed a *BFT:GFP* chimeric gene under the control of the 35S promoter in wild-type Columbia plants. *35S::BFT:GFP* transgenic plants showed late flowering (24.4 ± 7.1 leaves) under long-day (LD) conditions (cf. wild-type plants = 10.0 ± 0.8 leaves) (**Fig. 1A**), similarly as did *35::BFT* plants.[16] In addition, *35S::BFT:GFP* plants produced compact inflorescences in the primary inflorescence filled with leaf primordia surrounded by serrated leaves with trichomes, which is very similar to that produced in *TFL1*-overexpressing plants.[13] *35S::BFT:GFP* plants also showed a delayed conversion from shoot to flower, thus producing more nodes (**Fig. 1B**). This suggested that the phase transition in both the vegetative phase and the adult phase was delayed. These observation suggested that functional BFT protein was produced in *35S::BFT:GFP* plants. When we analyzed GFP signal under confocal laser microscopy, we identified BFT:GFP signal localized in the nucleus and the plasma membrane (**Fig. 1C**). This expression pattern was different from those of FT:GFP and HA:TFL1 reported previously. FT:GFP fusion protein was localized in the nucleus and the cytoplasm,[1] whereas HA:TFL1 fusion protein was localized in the plasma membrane, vacuole, and dense vesicles approximately 100 nm in diameter.[11] Subcellular localization of the remaining *FT/TFL1* family members is unknown. The nuclear and plasma membrane localization of BFT:GFP indicated that BFT expression pattern was distinct but had some of the features of both FT and TFL1. Considering that FT directly binds to FD, a bZIP transcription factor,[1,14] to activate the floral identity gene *AP1*, it is tempting to speculate that BFT may compete with FT to bind to FD within the nucleus.

Induction of *BFT* Expression Under Abiotic Stress Conditions

Since the *BFT* expression level was low in wild-type plants,[16] we tested the possibility that *BFT* expression was conditional. Among the abiotic stress conditions that we tested, *BFT* expression was upregulated by the ABA stress, drought, and osmotic stress treatments (**Fig. 2**), whereas the cold, salt stress, and heat stress conditions did not induce *BFT* expression. This result demonstrated that *BFT* expression was significantly enhanced by water-related stress. We also monitored the expression levels of other *FT/TFL1* family genes. The *ATC* and *TFL1* expression levels were not significantly altered under the above-mentioned stress conditions. *FT* expression was not induced by the conditions under which *BFT* expression was enhanced. On the contrary, *FT* expression was weakly downregulated under salt, osmotic, and heat stress conditions. A notable result was that *TSF* expression was markedly induced under cold and drought conditions. Collectively, it was apparent that none of the *FT/TFL1* family genes showed induction patterns similar to that of *BFT*, suggesting that *BFT* also plays a role in regulating flowering time and inflorescence structure under drought conditions.

It has been suggested that there is a close positive correlation between flowering time and water-use physiology.[8] The degree of plasticity of flowering time is suggested to be an important component of fitness.[12] Thus, *BFT* may be suggested to be one of the genes that pleiotropically affect drought physiology and are responsible for differences in flowering time. We are currently conducting experiments to test whether drought tolerance is closely associated with alteration in *BFT* activity and whether *BFT* is involved in abiotic stress signaling.

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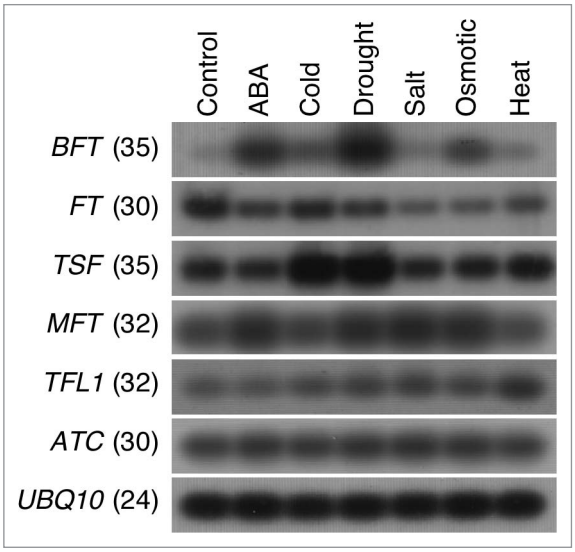

Figure 2. Induction of BFT expression revealed by semi-quantitative RT-PCR. For this experiment, wild-type Columbia plants were planted on half-strength Murashige and Skoog (MS) media and grown for 8 days under LD conditions. For abscisic acid (ABA) stress treatment, wild-type seedlings were placed in 100 μM ABA solution for 3 h. For cold treatment, the plants were placed under white light for 40 h in a cold room maintained at 4°C. Drought treatment was provided in a drying station as described previously.[10] The seedlings were placed in 100 mM NaCl solution and 100 mM mannitol for 3 h for salt stress and osmotic stress treatments, respectively. For heat stress treatment, the seedlings were placed under white light for 1 h in a growth chamber maintained at 37°C. Expression patterns of the other FT/TFL1 family genes were also monitored. Numbers in parenthesis indicate the number of RT-PCR cycles. UBQ10 was used as an internal control.

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