Three *FLOWERING LOCUS T*-like genes function as potential florigens and mediate photoperiod response in sorghum

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Summary

- Sorghum is a typical short-day (SD) plant and its use in grain or biomass production in temperate regions depends on its flowering time control, but the underlying molecular mechanism of floral transition in sorghum is poorly understood.
- Here we characterized sorghum *FLOWERING LOCUS T* (*SbFT*) genes to establish a molecular road map for mechanistic understanding. Out of 19 PEBP genes, *SbFT1*, *SbFT8* and *SbFT10* were identified as potential candidates for encoding florigens using multiple approaches.
- Phylogenetic analysis revealed that *SbFT1* clusters with the rice *Hd3a* subclade, while *SbFT8* and *SbFT10* cluster with the maize *ZCN8* subclade. These three genes are expressed in the leaf at the floral transition initiation stage, expressed early in grain sorghum genotypes but late in sweet and forage sorghum genotypes, induced by SD treatment in photoperiod-sensitive genotypes, cooperatively repressed by the classical sorghum maturity loci, interact with sorghum 14-3-3 proteins and activate flowering in transgenic Arabidopsis plants, suggesting florigenic potential in sorghum.
- SD induction of these three genes in sensitive genotypes is fully reversed by 1 wk of long-day treatment, and yet, some aspects of the SD treatment may still make a small contribution to flowering in long days, indicating a complex photoperiod response mediated by *SbFT* genes.

Introduction

Floral transition is a major phase change in flowering plants where developmental programs switch from vegetative growth to reproductive growth in which gametes are formed to ensure continuity to the next generation. Thus, plants coordinate the timing of their flowering with environmental changes to achieve reproductive success. Several environmental factors and endogenous developmental signals converge to determine reproductive competence and flowering. At the heart of this competence is a flowering hormone called florigen, originally proposed by Chailakhyan *c*. 80 yr ago (Chailakhyan, 1936). Florigen is a leaf-derived, graft-transmissible signal that under inductive conditions moves from the leaf to the shoot apex through the phloem to induce transition to the reproductive phase (Chailakhyan, 1936, 1937; Zeevaart, 1976). The long-sought-after florigen has now been widely accepted to be the protein encoded by the *FLOWERING LOCUS T* (*FT*) gene of Arabidopsis and its orthologs (Lifschitz *et al*., 2006; Corbesier *et al*., 2007; Jaeger & Wigge, 2007; Tamaki *et al*., 2007; Giakountis & Coupland, 2008; Zeevaart, 2008). *FT* encodes a protein with similarity to the mammalian phosphatidylethanolamine binding protein (PEBP) (Kardailsky *et al*., 1999; Kobayashi *et al*., 1999). Under long-day (LD) conditions, Arabidopsis *FT* is up-regulated in the leaf by *CONSTANS* (*CO*) (Samach *et al*., 2000), which encodes a B-box zinc finger transcription factor with a CCT domain (Putterill *et al*., 1995). *CO* is diurnally regulated by photoreceptors and the circadian clock, and its protein accumulates towards the end of day in LDs but is degraded in short days (SDs) (Suárez-López *et al*., 2001; Valverde *et al*., 2004). In response to CO accumulation at dusk, *FT* transcript abundance peaks at the end of day in LDs but not in SDs (Kardailsky *et al*., 1999; Suárez-López *et al*., 2001). The FT protein is then transported from the leaf through the vasculature to the shoot apex (Corbesier *et al*., 2007; Jaeger & Wigge, 2007; Lin *et al*., 2007; Mathieu *et al*., 2007), where it forms a complex with FLOWERING LOCUS D (FD) to activate floral transition (Abe *et al*., 2005; Wigge *et al*., 2005). FD is a bZIP transcription factor that in complex with FT activates the transcription of floral meristem identity genes such as *APETALA1* (*AP1*) and *LEAFY* (*LFY*). *FT* homologs have been reported from several species of both dicots and monocots with LD, SD or day-neutral requirements for floral induction.

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In the SD plant rice, *Heading date 3a* (*Hd3a*), the ortholog of *FT*, is activated by *Heading date1* (*Hd1*), the ortholog of *CO*, in SD photoperiods to induce flowering (Kojima *et al.*, 2002) analogous to the *CO-FT* activity in Arabidopsis. A second florigen, *Rice flowering locus T1* (*RFT1*), has also been identified to promote flowering under LD conditions (Komiya *et al.*, 2008). *Rice indeterminate1* (*RID1*), also called *OsID1* or *Ehd2*, a C2H2 zinc finger transcription factor homologous to the maize *ID1* (Colasanti *et al.*, 1998) gene, promotes flowering by activating *Early heading date1* (*Ehd1*) independent of day length (Matsubara *et al.*, 2008; Park *et al.*, 2008; Wu *et al.*, 2008). *Ehd1* is a B-type response regulator induced by SD photoperiods and promotes flowering by activating *Hd3a/FT*-like genes independent of *Hd1* (Doi *et al.*, 2004). On the other hand, *Grain number, plant height* and *heading date7* (*Ghd7*) (Xue *et al.*, 2008), encoding a CCT domain protein homologous to wheat VRN2 (Yan *et al.*, 2004), represses flowering in LDs by down-regulating *Ehd1* and *Hd3a* (Itoh *et al.*, 2010). Thus, the promoter and repressor activities of Ehd1 and Ghd7, respectively, enable the control of *Hd3a* transcription with a critical day-length threshold in rice to a resolution of 30 min (Itoh *et al.*, 2010). The proteins of both Hd3a and RFT1 are shown to move to the shoot apex (Tamaki *et al.*, 2007; Komiya *et al.*, 2009). In the shoot apex, 14-3-3 proteins bind to Hd3a as intracellular receptors, and the resulting complex is translocated to the nucleus to bind to OsFD1, homolog of FD, forming a ternary florigen activation complex (FAC), which induces transcription of *OsMADS15*, a homolog of *AP1*, leading to flowering (Taoka *et al.*, 2011).

In maize, *INDETERMINATE1* (*ID1*), a C2H2 zinc finger transcription factor, and *DELAYED FLOWERING1* (*DLF1*), a homolog of FD, activate flowering in the leaf and shoot apex, respectively (Colasanti *et al.*, 1998; Muszynski *et al.*, 2006). *ZMM4*, a homolog of *AP1*, promotes flowering downstream of *DLF1* in the shoot apex (Danilevskaya *et al.*, 2008a). Out of 25 *FT*-like genes, *ZCN8* (*Zea mays CENTRORADIALIS 8*) has been identified as the best maize florigen candidate activating flowering (Danilevskaya *et al.*, 2008b; Lazakis *et al.*, 2011; Meng *et al.*, 2011). *ZCN8* is diurnally regulated and its transcript is induced after 7 d exposure to SD conditions in tropical maize but photoperiod sensitivity is attenuated in day-neutral temperate maize, activating flowering independent of day length (Danilevskaya *et al.*, 2011). *ZCN8* functions downstream of *ID1* and upstream of *DLF1* and *ZMM4*, analogous to the rice flowering pathway.

In sorghum (*Sorghum bicolor*), flowering time is a key agronomic trait that determines whether it can be used as a grain or biomass crop. Sorghum is a multipurpose crop grown in many parts of the world, especially in arid and semiarid regions for food, feed, fuel and fiber. Sorghum is a typical SD plant with substantial photoperiod sensitivity. However, like maize, photoperiod-insensitive genotypes have been selected by breeders for grain production in temperate regions. As a result, temperate sorghum can be classified as grain sorghum with attenuated photoperiod response, biomass sorghum (includes forage and energy sorghum) and sweet sorghum. Biomass and sweet sorghums require a SD photoperiod for early flowering and flower very late under LD conditions, and were selected for increased biomass yield through longer duration of vegetative growth in temperate regions (Rooney *et al.*, 2007; Olson *et al.*, 2012). Grain sorghums, on the other hand, were selected for early flowering irrespective of day length to optimize grain yield production.

Despite this critical importance of flowering time for sorghum agronomy and the existence of > 40 flowering time quantitative trait loci (Mace *et al.*, 2013), very little is known about the molecular mechanism of flowering time control in sorghum. Six maturity loci (named *Ma1–Ma6*) that modify photoperiod sensitivity have been identified by genetic analysis (Quinby & Karper, 1945; Quinby, 1966; Rooney & Aydin, 1999; Morgan & Finlayson, 2000) in which dominance at each locus delays flowering under LD conditions. *Ma1* was identified as *SbPRR37*, a pseudoresponse regulator ortholog of rice *OsPRR37* (Koo *et al.*, 2013) and barley *Ppd-H1* (Turner *et al.*, 2005) which represses flowering under LD conditions (Murphy *et al.*, 2011). *Ma3* encodes phytochrome B (Childs *et al.*, 1997), while *Ma6* corresponds to *SbGhd7* (Murphy *et al.*, 2014), ortholog of the rice floral repressor *Ghd7*. Thus *SbPRR37*, *SbPhyB* and *SbGhd7* are floral repressors and confer photoperiod sensitivity upstream of the floral activators, ortholog of *Ehd1* and *FT*-like genes *SbCN8* and *SbCN12* (Murphy *et al.*, 2011; Yang *et al.*, 2014b).

Here we cloned and characterized 13 *FT*-like genes from sorghum to establish a molecular road map for understanding the mechanism of flowering time control in sorghum. With comprehensive analysis of spatial and temporal expression pattern, genotype-specific expression patterns including commonly used cultivars and natural mutants, photoperiod response, protein–protein interaction patterns and transgenic analysis, we identified that out of 19 PEBP genes in the sorghum genome, three genes, designated here as *SbFT1*, *SbFT8* and *SbFT10*, behave as functional *Hd3a/RFT1/ZCN8* orthologs, suggesting that sorghum probably has three florigens. We show that the three *SbFT* genes are induced by 1 wk SD treatment in photoperiod-sensitive genotypes and mediate photoperiod response, but the SD induction can be reversed by transition to LDs.

Materials and Methods

Plant materials and growth conditions

Sorghum bicolor (L.) Moench genotypes; grain sorghum (BTx623 and Tx430), sweet sorghum (Theis and Rio) and forage sorghum (FS000504 and FS000991) with wide differences in photoperiod response and flowering time were grown in 1 gallon pots in the glasshouse under LD conditions with a 16 : 8 h, light : dark cycle and a temperature of 27–30°C, and in the growth chamber under SD conditions with an 8 : 16 h, light : dark cycle and a temperature of 24–27°C with 70–80% relative humidity and 150 μmol m^2 light intensity. In addition, early-flowering mutants of sorghum, 38M (*ma*, *ma2*, *ma3^R*), 44M (*ma2*, *ma3^R*), 90M (*ma3*) and the control 100M (*Ma1*, *Ma2*, *Ma3*, *Ma4*) were grown under LD conditions in the glasshouse. Arabidopsis plants, Landsberg *erecta* (*Ler*) ecotype and *ft-1* mutant were grown in the growth room under LD conditions with a 16 : 8 h, light : dark cycle at 23–25°C.

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Plant tissue sample collection

The developmental stages of sorghum in relation to timing of morphological changes during the course of plant development have been well described (Vanderlip & Reeves, 1972). Six growth stages (S0–S6) were selected to collect samples for transcript analyses. S0 represents seedling emergence, where coleoptile is just visible; S1 is 10 d after emergence when the collar of the third leaf is visible; S2 is when the collar of the fifth leaf is visible 20 d after emergence; S3 is the growing point differentiation with seven to 10 leaf collars *c.* 30 d after emergence; S5 is booting stage at 50 d after emergence where the head is extended into the flag leaf sheath; and S6 is the half blooming (anthesis) stage at 60 d after emergence (Vanderlip & Reeves, 1972). Five samples were collected and pooled from fully expanded top leaves or other tissues as specified.

For diurnal expression analysis, young, fully expanded leaf samples were harvested from three randomly selected BTx623 plants every 4 h during 52 h in the 24 h diurnal cycle. Samples were analyzed by real time quantitative reverse transcription polymerase chain reaction (qRT-PCR) using actin as control. Leaf samples from grain, sweet and forage sorghum genotype were also collected separately for varietal-based analysis of *SbFT* transcript abundance. Samples were collected after midnight when the highest transcript accumulation of *SbFT* genes was found. Collected samples were immediately snap-frozen in liquid nitrogen and stored at −80°C until processing.

RNA extraction, gene cloning and transformation

Total RNA was isolated using TRIzol Reagent (Invitrogen) for cDNA synthesis. Reverse transcription was performed using RNA treated with DNase I (Invitrogen), an oligo(dT) primer and SuperScript III reverse transcriptase (Invitrogen) according to the manufacturer's instructions. Expression pattern analyses were performed using semiquantitative RT-PCR. Full-length *SbFT1*, *SbFT2*, *SbFT6*, *SbFT8*, *SbFT9* and *SbFT10* coding sequences were amplified by RT-PCR using total RNA extracted from leaf and apex tissues. Cloning was performed in the pMDC32 gateway destination vector with 2x35S promoter for *SbFT1* and *SbFT8*, while leaf-specific STF promoter (Tadege *et al.*, 2011) was used for the *SbFT10* construct and the resulting plasmids were transformed into *Agrobacterium tumefaciens* strain GV3101 by the freeze–heat shock method. Arabidopsis was transformed using the floral dipping method (Clough & Bent, 1998).

Multiple sequence alignment and phylogenetic analysis

Multiple sequence alignment of 19 PEBP proteins of sorghum and homologs from other related species was performed using BioEdit software and the ClustalW program (http://www.mbio.ncsu.edu/bioedit/bioedit.html). A neighbor-joining phylogenic tree was constructed using MEGA6.0 default settings with 1000 bootstrap replications (http://www. megasoftware.net/). All gene sequences used in this study were listed as Supporting Information Notes S1.

Yeast two-hybrid (Y2H) assays

Yeast two-hybrid analysis was performed using the ProQuest Two-Hybrid system (Invitrogen) following the manufacturer's instructions. The full-length proteins of AtFD and SbFD1 were cloned in pGBKT7-GW as bait, while the full-length proteins of SbFT1, SbFT8 and SbFT10 were cloned in pGADT7-GW as prey, and sets of constructs were cotransformed into Y2H Gold yeast strain (Clontech, Mountain View, CA, USA). Yeast transformants were selected on synthetic minimal double dropout medium deficient in *SD/-Leu/-Trp*, and protein interactions were assessed on quadruple dropout medium deficient in *SD/-His/-Trp/-Leu/-Ade*.

Bimolecular florescence (BiFC) analysis and confocal microscopy

Bimolecular florescence assays were conducted according to Lu *et al.* (2010). Briefly, SbFT1, SbFT8 and SbF10 were cloned into pEARLEYGATE201-YN, while AtFD and Sb14-3-3 were cloned into pEARLEYGATE202-YC, by LR reaction. For the SbFD1 BiFC assay, SbFT1, SbFT8, SbF10 and Sb14-3-3 were cloned into pEARLEYGATE202-YC, while SbFD1 was cloned into pEARLEYGATE201-YN. Each construct was introduced into *Agrobacterium* by the freeze–heat shock method. Pairs of combinations were coinfiltrated into 4-wk-old *Nicotiana benthamiana* leaves. P19 was used to inhibit transgenic silencing. The yellow fluorescent protein (YFP) signal was observed after 48–60 h of infiltration using a TCS SP2 AOBS confocal laser scanning microscope (Leica Microsystems, Buffalo Grove, IL, USA).

Results

Multiple sequence alignment and phylogenetic analysis of sorghum FT/TFL1/MFT-Like proteins with homologs from other related species

BLAST search of the *S. bicolor* genome v2.1 using *Arabidopsis FT* and rice *Hd3a* sequences identified 19 phosphatidylethanolamine binding protein (PEBP) family genes showing significant homology to both *FT* and *Hd3a* at the DNA and amino acid sequence level. Of these 19 PEBP sequences, 13 are *FLOWERING LOCUS T* (*FT*)-like, four are *TERMINAL FLOWER LIKE1* (*TFL1*)-like and two are *MOTHER OF FT* and *TFL1* (*MFT*)-like. On the basis of sequence homology to *Hd3a*, we designated the 13 *FT*-like genes *SbFT1* to *SbFT13*, the four *TFL1*-like genes *SbTFL1-1* to *SbTFL1-4* and the two *MFT*-like genes *SbMFT1* and *SbMFT2* (Table 1). SbFT1, SbFT8 and SbFT10 correspond to SbCN15, SbCN12 and SbCN8, respectively, reported in previous studies (Yang *et al.*, 2014a,b). The 19 sorghum PEBP genes show limited homology to each other (Table S1), but have close homologs in both the maize and rice genomes (Table S2). The conserved PEBP domain displayed very high homology among sorghum PEBPs and *FT*-like genes from other species (Fig. S1) in which the functionally important tyrosine (Y) at position 87 in SbFT1 was conserved in all FT-like proteins but substituted by a

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Table 1 Summary of 19 sorghum PEBP genes compared with *FT* genes from Arabidopsis, maize, rice and wheat

Designation		Alias name	Protein size	Full-length amino acid identity (%)					
				AtFT	*ZCN8*	*ZCN12*	*ZCN15*	*Hd3a*	*VRN3*
1	*SbFT1*	(Sb10g003940)	179	72	62	65	97	93	92
2	*SbFT2*	(Sb03g001700)	173	72	58	60	82	79	82
3	*SbFT3*	(Sb06g020850)	174	73	56	57	70	70	71
4	*SbFT4*	(Sb04g025210)	174	70	57	56	69	68	70
5	*SbFT5*	(Sb0010s003120)	177	70	57	57	70	70	71
6	*SbFT6*	(Sb02g029725)	178	67	52	54	62	63	61
7	*SbFT7*	(Sb04g008320)	182	63	58	62	64	64	65
8	*SbFT8*	(Sb03g034580)	177	59	79	94	65	64	64
9	*SbFT9*	(Sb10g021790)	173	62	59	60	63	62	62
10	*SbFT10*	(Sb09g025760)	175	53	97	83	66	69	66
11	*SbFT11*	(Sb08g008180)	177	62	51	51	62	60	62
12	*SbFT12*	(Sb06g012260)	185	58	57	59	59	59	61
13	*SbFT13*	(Sb03g002500)	187	61	55	56	63	62	62
14	*SbTFL1-1*	(Sb04g021650)	173	56	49	53	58	60	58
15	*SbTFL1-2*	(Sb08g003210)	173	43	39	57	50	62	61
16	*SbTFL1-3*	(Sb05g003200)	173	54	47	55	63	60	59
17	*SbTFL1-4*	(Sb06g015490)	173	54	51	53	60	60	60
18	*SbMFT1*	(Sb03g008270)	171	57	46	47	55	57	56
19	*SbMFT2*	(Sb10g013070)	181	43	37	39	37	50	51

conserved histidine (H) residue (Hanzawa *et al.*, 2005) in TFL1-like proteins (Figs 1a, S1). Among the 13 SbFT proteins, only SbFT5 had an asparagine (N) substitution at the equivalent position (Fig. 1a). This position also appeared not to be conserved in MFT-like proteins (Fig. 1a).

Phylogenetic analysis revealed that sorghum PEBPs are grouped into three major clades: the FT clade containing SbFT1to SbFT13, the TFL1 clade containing SbTFL1-1 to SbTFL1-4, and the MFT clade containing SbMFT1 and SbMFT2 (Fig. 1b). The FT clade could be further subdivided into three groups. The functionally characterized monocot florigens, except maize ZCN8, clustered together in the Hd3a subclade. SbFT1 and SbFT2 belong to this group. The second subgroup, represented by AtFT, included SbFT3-6 and SbFT11. The third subgroup represented by ZCN8, included SbFT7-10, 12 and 13 (Fig. 1b). As *AtFT*, *Hd3*a and *ZCN8* are well characterized major components of the FAC and the *SbFT* genes are distributed in all of these three subclades, it is not a trivial task to determine which of these *SbFT* genes function as activators of floral transition in sorghum.

Three sorghum *FT* genes are highly expressed in grain sorghum leaves at the floral transition stage

To understand which sorghum *FT-like* genes play functional roles in regulating flowering time, we assessed the temporal and spatial expression patterns of the 19 PEBP genes in different tissues at different developmental stages in grain sorghum BTx623 genotype grown under LD conditions. The developmental stages of grain sorghum in relation to timing of morphological changes have been well described (Vanderlip & Reeves, 1972). We selected six growth stages (S0–S6) to collect samples for transcript analyses (see the Materials and Methods section). Expression analysis in different tissues by semiquantitative RT-PCR using gene-specific primers (Table S3) revealed that most *SbFT/TFL1*-like genes were expressed amongst many of the different tissues tested (Fig. 2a). *SbFT3*, *SbFT4* and all the four *SbTFL1*-like gene transcripts were detected in roots at variable expression levels. *SbFT1*, *SbFT3*, *SbFT6*, *SbFT8*, *SbFT9* and *SbFT10* genes were variably expressed in leaf from seedling stage (S0) to transition stage (S3). Of these six genes, five (*SbFT1*, *SbFT6*, *SbFT8*, *SbFT9* and *SbFT10*) showed strong transcript accumulation during the critical floral transition period (S3). However, *SbFT6* and *SbFT9* also showed strong expression in the leaf at earlier developmental stages (S0–S2), leaving *SbFT1*, *SbFT8* and *SbFT10* as the only candidates that were specifically and strongly induced near the floral transition stage (Fig. 2a). Expression of *SbFT1* and *SbFT3* in the shoot apex at stage S3 was very weak but *SbFT2*, *4*, *5*, *9*, *11* and all of *SbTFL1s*, except *SbTFL1-4*, were strongly expressed in the shoot apex (Fig. 2a). On the other hand, *SbFT2*, *SbTFL1-1* and *SbTFL1-3* were detected in the stem at variable levels. *SbTFL1-4* was only very weakly expressed in root and shoot apex. *SbFT1*, *SbFT2*, *SbFT6*, *SbFT8*, *SbFT10*, *SbFT11*, *SbTFL1-1* and *SbTFL1-2* transcripts were also detected in the floral head at booting (S5) and in florets at blooming (S6) stages at various levels (Fig. 2a). The remaining five genes, *SbFT7*, *SbFT12*, *SbFT13*, *SbMFT1* and *SbMFT2*, were not detectable in the tissues analyzed. The strong and specific transcript accumulation of *SbFT1*, *SbFT8* and *SbFT10* in the leaf near the time of the critical floral transition period suggests that these three genes could be the sources of sorghum florigen, although this does not exclude the possibility that others may also have a contribution. For this reason, we focused on the three genes, *SbFT1*, *SbFT8* and *SbFT10*, to further characterize their involvement in flowering.

To determine if sorghum *FT* gene expression follows diurnal cycling, we assessed the diurnal expression patterns of *SbFT1*, *SbFT8* and *SbFT10* in grain sorghum BTx623 under LD conditions using real-time qRT-PCR. Our results indicated that

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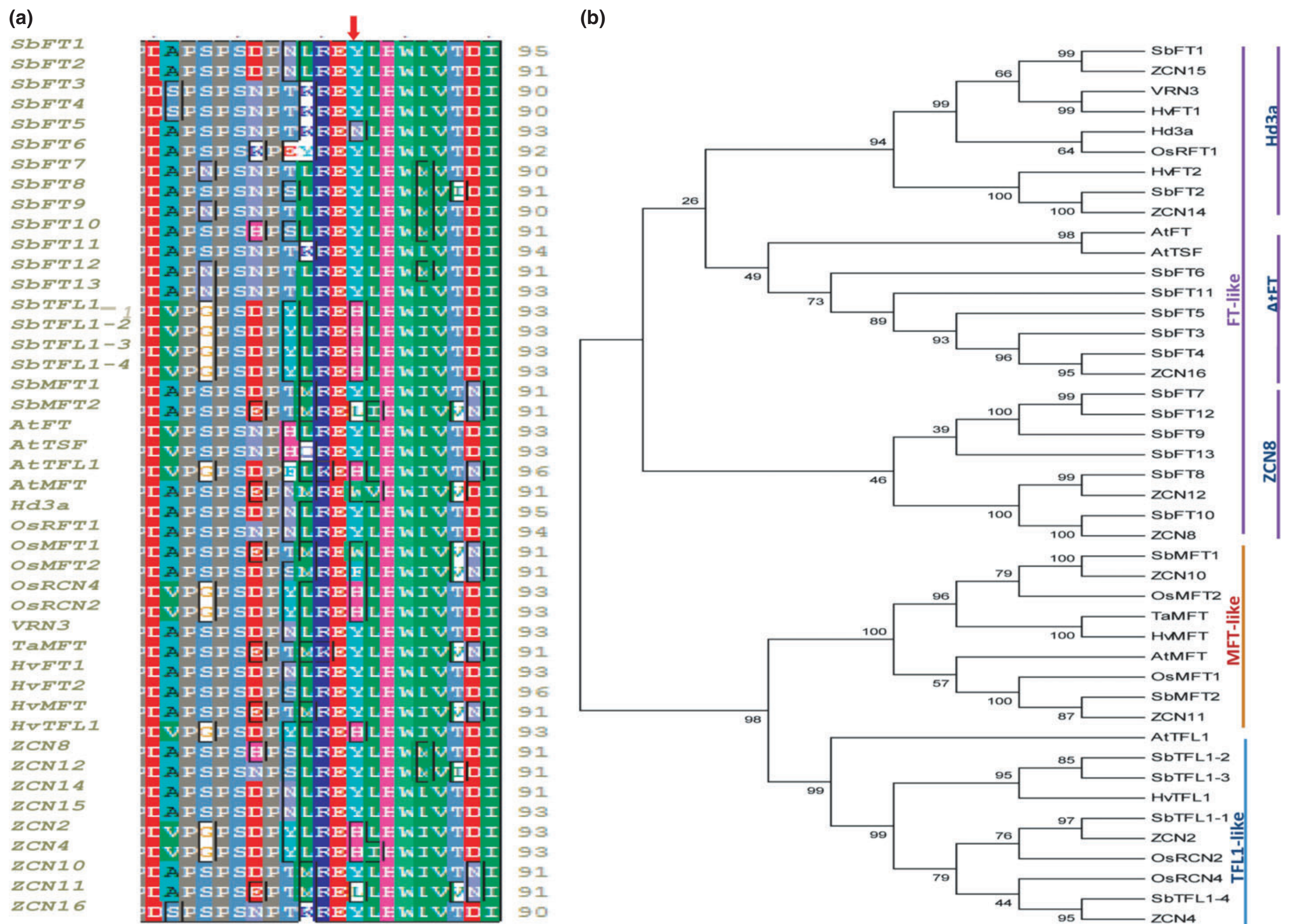

Fig. 1 Amino acid sequence alignment and phylogenetic analysis of 19 sorghum phosphatidylethanolamine binding proteins (PEBPs) and related PEBP proteins. (a) Multiple sequence alignment showing a portion of the PEBP domain where key differences were observed between FT and TFL1-like sequences. The red arrow points to conserved *Tyrosine* (*Y*) residue in FT-like or *Histidine* (*H*) in TFL1-like sequences. (b) Phylogenetic analysis of *SbFT*, *SbTFL1* and *SbMFT* genes based on the amino acid sequence of the full-length protein. Thirteen sorghum FT-like, four TFL1-like and two MFT-like proteins group into three clades with the corresponding proteins from maize, wheat, barley, rice and Arabidopsis, each node being indicated by solid lines of different color. The FT-like clade is further subdivided into ZCN8, AtFT and Hd3a subclades. Numbers on branches indicate bootstrap values for 1000 replicates.

accumulation of transcripts of *SbFT1*, *SbFT8* and *SbFT10* started to increase at *c.* 4 h after the light was turned off, peaked within 2 h and started to decline gradually, reaching basal levels at *c.* 2 h after the light was turned on (Fig. 2b,c). All three genes showed a similar pattern but *SbFT10* showed the greatest, while *SbFT1* showed the least, induction in the dark. The *SbFT1* induction peak was slightly broader, extending more into dawn (Fig. 2b). These results suggest that all the three *SbFT* genes are regulated with a similar diurnal pattern but with different induction strength.

SbFT1, *SbFT8* and *SbFT10* are expressed in grain sorghum and early-flowering mutants but not in sweet sorghum and forage sorghum genotypes under LD conditions.

Grain sorghum genotypes Tx430 and BTx623 are photoperiod-insensitive and flower early irrespective of day length, whereas sweet sorghum genotypes Theis and Rio as well as commercial forage sorghum hybrids FS000504 and FS000991 are photoperiod-sensitive and flower early under SD conditions but late under LD conditions. We tested the expression patterns of *SbFT1*, *SbFT8* and *SbFT10* in these six different genotypes grown under LD conditions to evaluate if sorghum *FT* genes account for variation in flowering time and photoperiod sensitivity. Fully expanded top leaf samples were collected from each genotype at dawn at a growth stage corresponding to the transition period in grain sorghum. We found that the transcripts of *SbFT1*, *SbFT8* and *SbFT10* were highly abundant in grain sorghum genotypes, but consistently very weak or absent in sweet and forage sorghum genotypes (Fig. 3a). On the other hand, expression of a nonflorigen candidate, *SbFT9*, showed no difference between photoperiod-sensitive and -insensitive genotypes (Fig. 3a).

We further determined the expression patterns of these three *SbFTs* in the available sorghum classical flowering mutants.

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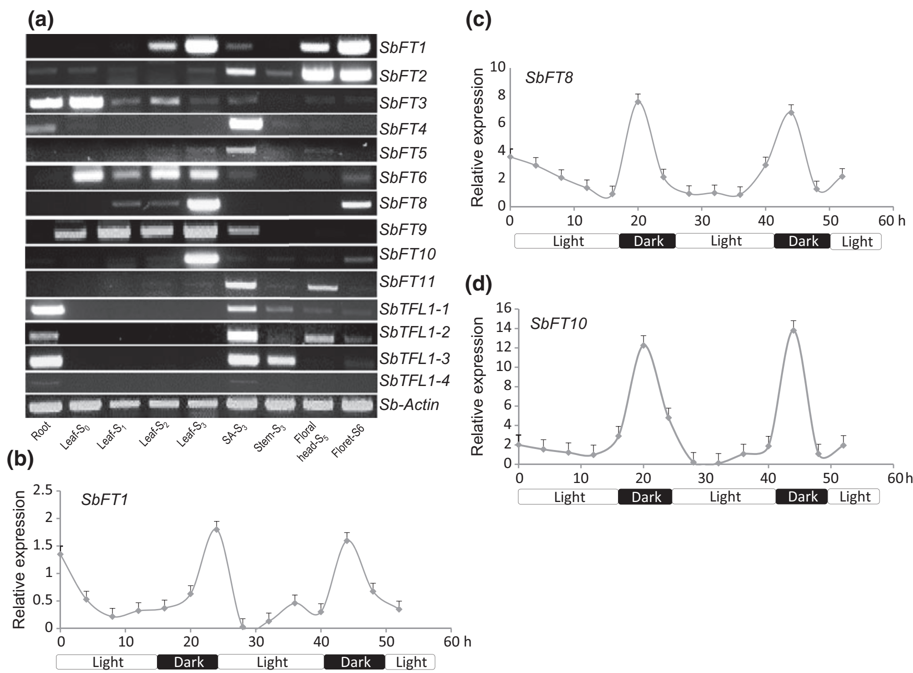

Fig. 2 Spatial and diurnal expression pattern analyses of sorghum PEBP genes. (a) Expression pattern analysis of sorghum *SbFT/SbTFL1*-like genes in different tissues of grain sorghum BTx623 at different growth stages analyzed by semi-quantitative reverse transcription polymerase chain reaction (RT-PCR). S0–S6 refer to the sorghum developmental stages. SA, shoot apex. (b–d) Diurnal expression pattern of *SbFT1* (b), *SbFT8* (c) and *SbFT10* (d) in fully expanded leaf blades of grain sorghum of BTx623 under long-day (LD) conditions near the floral initiation period. Transcripts were analyzed by real-time quantitative RT-PCR during 52 h in the 24 h diurnal cycle relative to the ACTIN control. Error bars, ± SE. The light periods are shown in white boxes, and the dark periods are shown in black boxes.

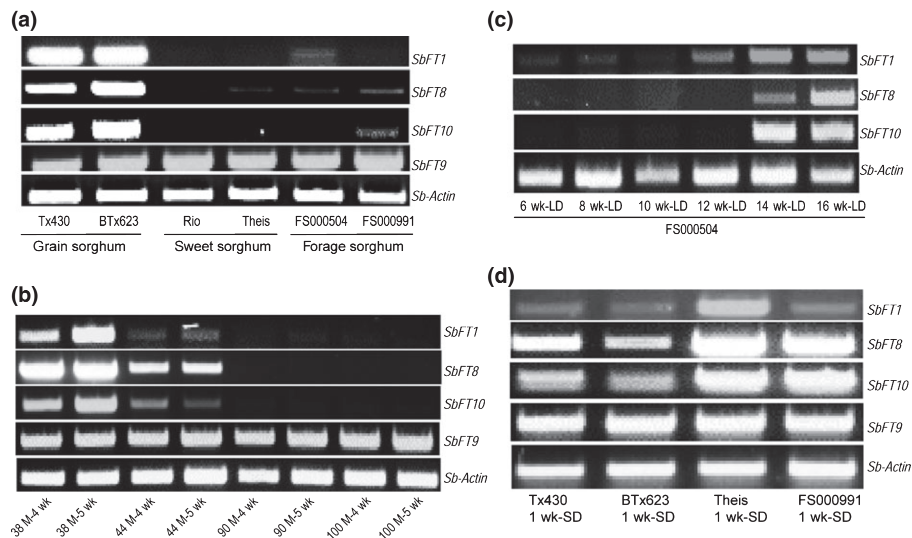

Fig. 3 Expression level of four *SbFT-Like* genes in leaves of different sorghum genotypes under short-day (SD) and long-day (LD) conditions. (a) Expression levels of *SbFT1*, *SbFT8*, *SbFT9* and *SbFT10* in the leaves of grain, sweet and forage sorghum genotypes 4 wk after germination under LD conditions. (b) Expression levels of *SbFT1*, *SbFT8*, *SbFT9* and *SbFT10* in the leaves of different sorghum natural mutant lines and a parental control at 4 and 5 wk after germination under LD conditions. (c) Expression patterns of *SbFT1*, *SbFT8* and *SbFT10* in the leaves of late-flowering forage sorghum (FS000504) at different stages of development under LD conditions.(d) Expression levels of *SbFT1*, *SbFT8*, *SbFT9* and *SbFT10* in the leaves of grain, sweet and forage sorghum at the seedling stage in response to 1 wk SD treatment. Transcript expression levels were determined by semiquantitative RT-PCR using sorghum ACTIN as a loading control.

Sorghum flowering mutants have long been identified by genetic selection based on the presence (*Ma*) or absence (*ma*) of one or more maturity loci that modify photoperiod sensitivity (Quinby, 1973). The early-flowering mutants of sorghum, 38M (*ma1*, *ma2*, *ma3*^{R}), 44M (*ma2*, *ma3*^{R}), near control 90M (*ma3*) and the control 100M (*Ma1*, *Ma2*, *Ma3*, *Ma4*) were grown under LD glasshouse conditions. *ma3*^{R} is a strong allele of *ma3*. Leaf samples were collected from each line at 4 and 5 wk after emergence for transcript analysis. We found that the transcripts of *SbFT1*, *SbFT8* and *SbFT10* were highly abundant in the earliest flowering line 38M, followed by 44M, but consistently below detection in 90M and 100M at this developmental stage (Fig. 3b). 38M is slightly earlier heading than 44M and even this difference was reflected by the expression levels of the three *SbFT*

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genes. By contrast, *SbFT9* expression showed no difference in all the tested lines (Fig. 3b). Together these results demonstrate that expression of the three sorghum *FT* genes, *SbFT1*, *SbFT8* and *SbFT10*, is additively repressed by maturity loci, suggesting that these *SbFT* genes are involved in promoting flowering in sorghum and may account for differences in flowering time and photoperiod sensitivity between sorghum genotypes.

Expression of three sorghum *FT* genes is induced by plant age and SD photoperiod in late-flowering genotypes

The forage hybrid FS000504 is the latest flowering line we have and takes 22 wk to heading under our LD (16 : 8 h, light : dark cycle) conditions. We determined the time at which the *SbFT* genes are expressed in this genotype under LD conditions by collecting leaf samples at dawn with 2 wk intervals. We found that *SbFT1* was first detected at 12 wk after emergence and *SbFT8* and *SbFT10* were also clearly induced by 14 wk (Fig. 3c). As this genotype heads in 22 wk, 14 wk probably represents the initiation of the floral transition period and suggests that the three sorghum *FT* genes are induced at the time of floral transition in FS000504, although this occurred 10 wk later than that of grain sorghum.

To determine if SD photoperiod can induce the expression of sorghum *FT* genes in both photoperiod-insensitive and -sensitive genotypes, we examined the expression patterns of *SbFT1*, *SbFT8* and *SbFT10* in grain, sweet and forage sorghum genotypes grown under SD (8 : 16 h, light : dark cycle) conditions. Leaf samples were collected from each genotype at dawn at 1 wk after emergence. Interestingly, the transcripts of *SbFT1*, *SbFT8* and *SbFT10* were detected in most genotypes with the highest induction observed in the sweet sorghum Theis (Fig. 3d). Induction of *SbFT1* appeared to be the weakest and *SbFT8* the strongest in all the genotypes tested, but generally the sweet and forage sorghums appeared to induce all the three *FT* genes more strongly than the grain sorghums (Fig. 3d), suggesting that *SbFT1*, *SbFT8* and *SbFT10* promote flowering in photoperiod-sensitive genotypes in response to short days, and SD induction of these genes, especially that of *SbFT1*, is attenuated in grain sorghum genotypes. Rio and FS000504 require 3 wk to induce at least *SbFT8* and *SbFT10* in response to SDs (Fig. S2), suggesting a minimum SD saturation requirement for *SbFT* gene induction depending on genotype.

We next asked whether the SD treatment can be remembered and the 1 wk high induction of *FT* genes in Theis is sufficient for floral promotion after transferring to LDs. To address this, we grew Theis under SD conditions for one to several weeks and at the end of each treatment, plants were transferred to LD conditions. Leaf samples were collected at the end of each treatment while plants were still on SDs and after transfer to LDs at the specified period, and *FT* transcript abundances were compared with controls grown continuously under LD conditions. Expression of *SbFT1*, *SbFT8* and *SbFT10* genes was highly induced in Theis within 1 wk of SD conditions as described earlier and remained high at least in the second week of SDs (Fig. 4a). However, when SD-treated plants were transferred to LD conditions for 1 wk, the expression level of all three genes reduced dramatically to undetectable levels (Fig. 4a), indicating that 1 wk growth under LD conditions was sufficient to completely reverse induction even after 6 wk of SD photoperiods. This suggests that there is no long-term memory for *SbFT* induction by SDs and the three *SbFT* transcripts were probably quickly destabilized by LD photoperiods in Theis.

However, the growth response appeared to be more complex. Theis grown constantly under LD conditions took *c.* 140 d to heading, while this was achieved in *c.* 70 d when grown constantly under SD conditions (Fig. 4d). When plants were transferred from 1 to 4 wk growth under SDs to LDs, vegetative growth continued with the same shoot (Fig. 4c), but when plants were transferred at the fifth week and after, growth of the original shoot was arrested and new shoot growth was initiated from a lower node (Fig. 4d–h). This happened even when plants were transferred after heading (Fig. 4h). Interestingly, plants transferred after 7 wk under SD conditions started heading at the ninth week under LD conditions (Fig. 4f) while the control was still vegetative (Fig. 4b). These results suggest that the LD photoperiod is dominant in reversing *SbFT* induction and growth stages established under SDs, but some aspects of the floral transition may still be activated by the SD treatment provided that the SD treatment lasts 7 wk or more in the case of Theis.

Functional analyses of sorghum *FT* genes in transgenic Arabidopsis

To confirm that the biological function of *SbFT1*, *SbFT8* and *SbFT10* is indeed activation of flowering, we transformed each of these genes into L*er* driven by the 2x35S promoter. Under LD conditions, all *SbFT1* transgenic lines had at most two rosette leaves (1.1 ± 0.9) at flowering compared with eight to nine (8.4 ± 1.0) in L*er* (Fig. 5a–c,i), where 36% flowered without forming any rosette leaves, indicating strong activation of flowering. In addition, 10 of these transgenic lines were evaluated in the T2 generation for early flowering under SD conditions. All of the 10 T2 lines flowered with two to three rosette leaves compared with 10–12 in L*er* (Fig. 5d), indicating activation of flowering in transgenic Arabidopsis both under LD and SD conditions. We further tested the ability of *35S:SbFT1* to complement the late-flowering Arabidopsis *ft-1* mutant. Complemented transgenic plants flowered early with zero to two rosette leaves at bolting under LD conditions compared with the *ft-1* mutant, which produced 12–15 rosette leaves at bolting (Figs 5i, S3a,c). This indicates that *SbFT1* ectopic expression not only complements the *ft-1* late-flowering phenotype but also induces early flowering in the mutant background.

On the other hand, all *35S::SbFT8* transgenic lines in L*er* flowered before bolting with two tiny up-curled cauline leaves (Fig. 5e,i) and died without setting seeds, suggesting that *SbFT8* is probably more active and its high expression is lethal for the plants. Alternatively, *SbFT8* may regulate vegetative growth and plant architecture in addition to activation of flowering in sorghum. Furthermore, *35S::SbFT8* expression in the *ft-1* mutant

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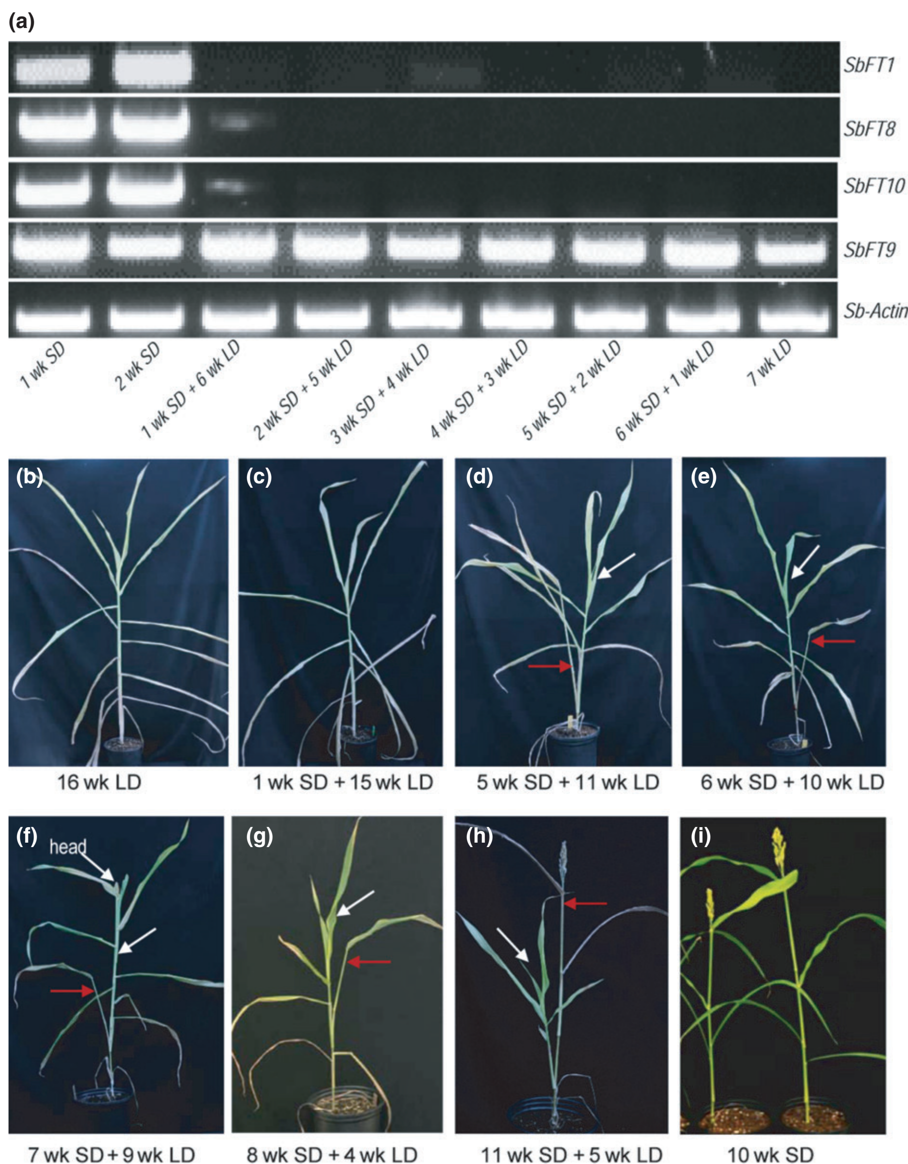

Fig. 4 *SbFT* gene expression and growth response of Theis to short-day (SD) and long-day (LD) treatments. (a) Transcript accumulation of *SbFT1*, *SbFT8*, *SbFT9* and *SbFT10* in the leaves of Theis after exposure to SD, LD or SD + LD conditions for specified periods. For SD + LD treatment, plants were grown under SD conditions for 1–6 wk and transferred to LD conditions for 6–1 wk before sampling. (b, i) Phenotype of Theis plants grown continuously under LD and SD conditions for 16 and 10 wk, respectively. (c–h) Phenotype of Theis plants first grown under SD conditions for the specified 1–11 wk and shifted to LD conditions for the specified 4–15 wk. Note that in (d–h), the first shoot grown under SD conditions is arrested (red arrow) and a new shoot then developed from the lower node (white arrow) after transfer to the LD conditions.

resulted in very early flowering with zero to two rosette leaves at bolting under LD conditions (Figs 5f,i, S3d,e), suggesting that *SbFT8* is, indeed, a strong activator of flowering. The fact that *SbFT8*-expressing *ft-1* mutant plants survived better than *SbFT8*-expressing L*er* plants suggests that the very high expression of *SbFT8* is most probably the cause for the observed defects.

As we used a vector (pMDC32) that drives very high expression with 2x35S promoter in all our transformations, high expression of *SbFT10* led to an even worse phenotype and resulted in lethality, and therefore we were unable to obtain enough *SbFT10* transgenic lines for further analysis. Consequently, we resorted to a weaker promoter, *STENOFOLIA* (*STF*) from *Medicago truncatula*, to obtain transgenic lines for *SbFT10*. The *STF* promoter drives expression in the leaf margin and middle mesophyll at the adaxial–abaxial junction including the vascular region but not in the shoot apical meristem (Tadege *et al.*, 2011). *STF*::*SbFT10* expression resulted in early flowering with four phenotypic classes: flowering without bolting with two up-curled leaves (17%) which died without setting seeds (Fig. 5g); bolting with two to three up-curled leaves and terminal flower (50%) (Fig. S3f); bolting with four rosette leaves (23.5%) (Fig. S3g); and later-flowering, bushy plants bolting with nine or more rosette leaves (9.5%). The *STF::SbFT10* construct also complemented the *ft-1* mutant in LD conditions with an average of 1.8 rosette leaves at bolting (Fig. 5h,i). These results suggest that *SbFT10* activates flowering in Arabidopsis perhaps more strongly than *SbFT8* because a weaker promoter (*STF*) was required to obtain *SbFT10*-expressing transgenic Arabidopsis plants. Alternatively, *SbFT10* may have other functions in addition to activation of flowering which became detrimental in Arabidopsis with a very strong constitutive promoter. These transgenic analyses are consistent with the spatial, temporal and diurnal expression patterns, as well as the day length response and genotype-dependent expression of *SbFT* genes, and together

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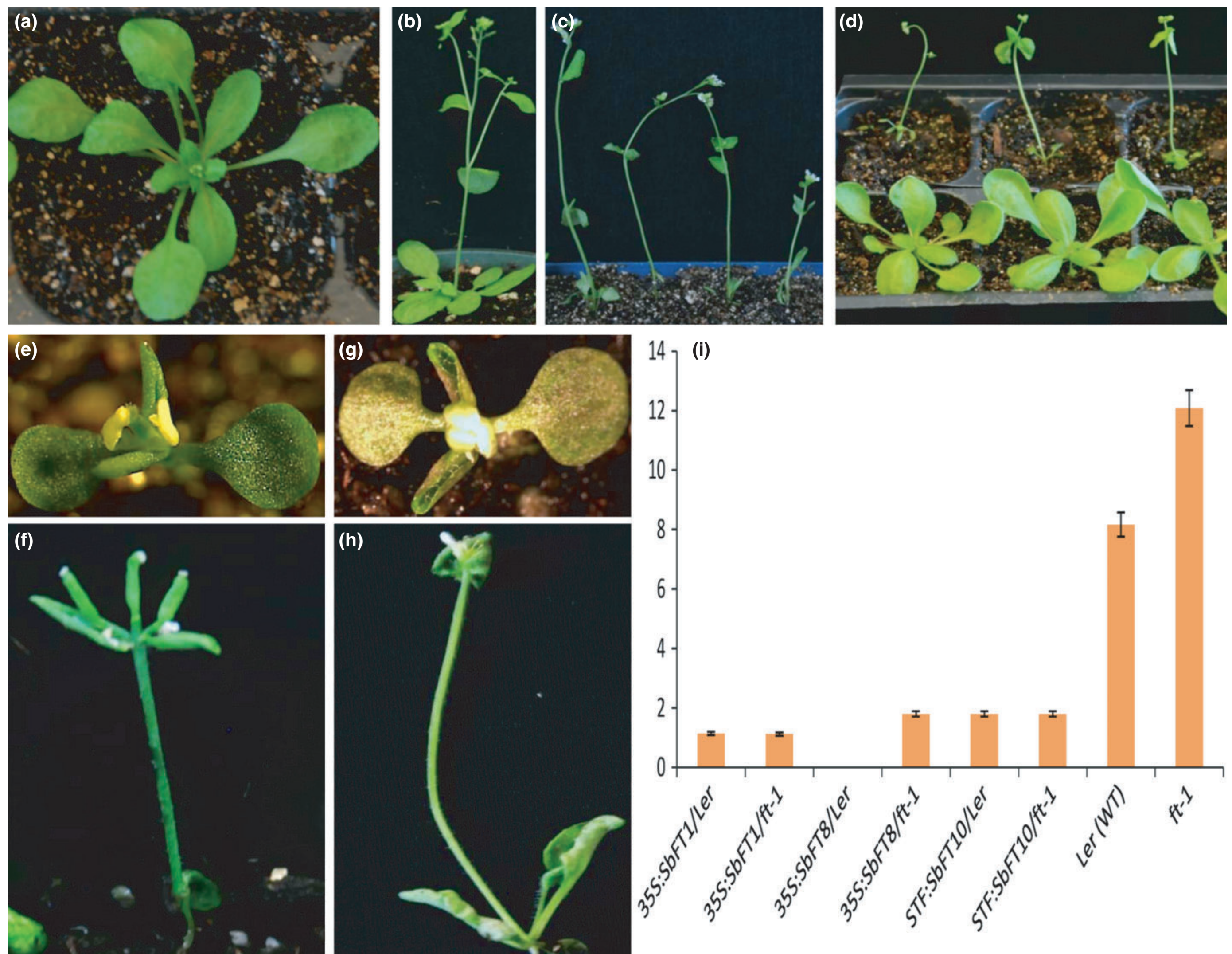

Fig. 5 Phenotype of transgenic Arabidopsis plants transformed with sorghum *FT* genes. (a, b) Untransformed Landsberg *erecta* (L*er*) control. (c) Phenotype of *35S::SbFT1* transgenic plants under long-day (LD) conditions. (d) Phenotype of *35S::SbFT1* transgenic plants (upper panel) vs L*er* (lower panel) under short-day (SD) conditions. (e) Phenotype of *35S::SbFT8* transgenic plant flowering at the cotyledon stage without bolting under LD conditions. (f) Phenotype of *ft-1* mutant transformed with *35S::SbFT8* showing early flowering with a terminal flower phenotype under LD conditions. (g) Strong early-flowering phenotype of transgenic L*er* plant transformed with *STF::SbFT10* under LD conditions. (h) Phenotype of *ft-1* mutant transformed with *STF::SbFT10* showing early flowering under LD conditions. (i) Flowering time of *SbFT1*, *SbFT8* and *SbFT10* transgenic Arabidopsis plants compared with L*er* and *ft-1* mutant under LD conditions measured by rosette leaf number at bolting. Error bars, ± SE.

suggest that sorghum has at least three functional *FT* genes that can promote floral activation.

SbFT2, *SbFT6* and *SbFT9* genes do not induce flowering in transgenic Arabidopsis

As *SbFT2* is expressed in the shoot apex at the floral transition stage and *SbFT6* and *SbFT9* are expressed in leaves at all stages of development (Fig. 2a), these three genes were further tested for their potential candidacy. The three genes were introduced into L*er* and the *ft-1* mutant individually driven by the 35S promoter. Our results revealed that none of these genes activated flowering in L*er* or rescued the late-flowering *ft-1* mutant (Fig. S4), suggesting that *SbFT2*, *SbFT6* and *SbFT9* are probably not involved in the activation of flowering in sorghum.

SbFT1, SbFT8 and SbFT10 proteins may interact with SbFD1 and/or Sb14-3-3

We performed Y2H and BiFC assays to determine whether the *SbFT* encoded proteins are capable of interacting with AtFD, SbFD1 and Sb14-3-3. Our results showed that SbFT1 but not SbFT8 and SbFT10 proteins interact with AtFD and SbFD1 in the Y2H assay under stringent conditions (Fig. S5a). We also tested whether SbFT1, SbFT8 and SbFT10 interact with each other. SbFT1 interacted with both SbFT8 and SbFT10 and also showed self-interaction, while SbFT8 and SbFT10 neither interacted with each other nor showed self-interaction in the Y2H assay (Fig. S5b). However, the BiFC assay using split YFP complementation in *N. benthamiana* leaf cells identified that the three sorghum FTs can interact with AtFD and SbFT1 interacted with

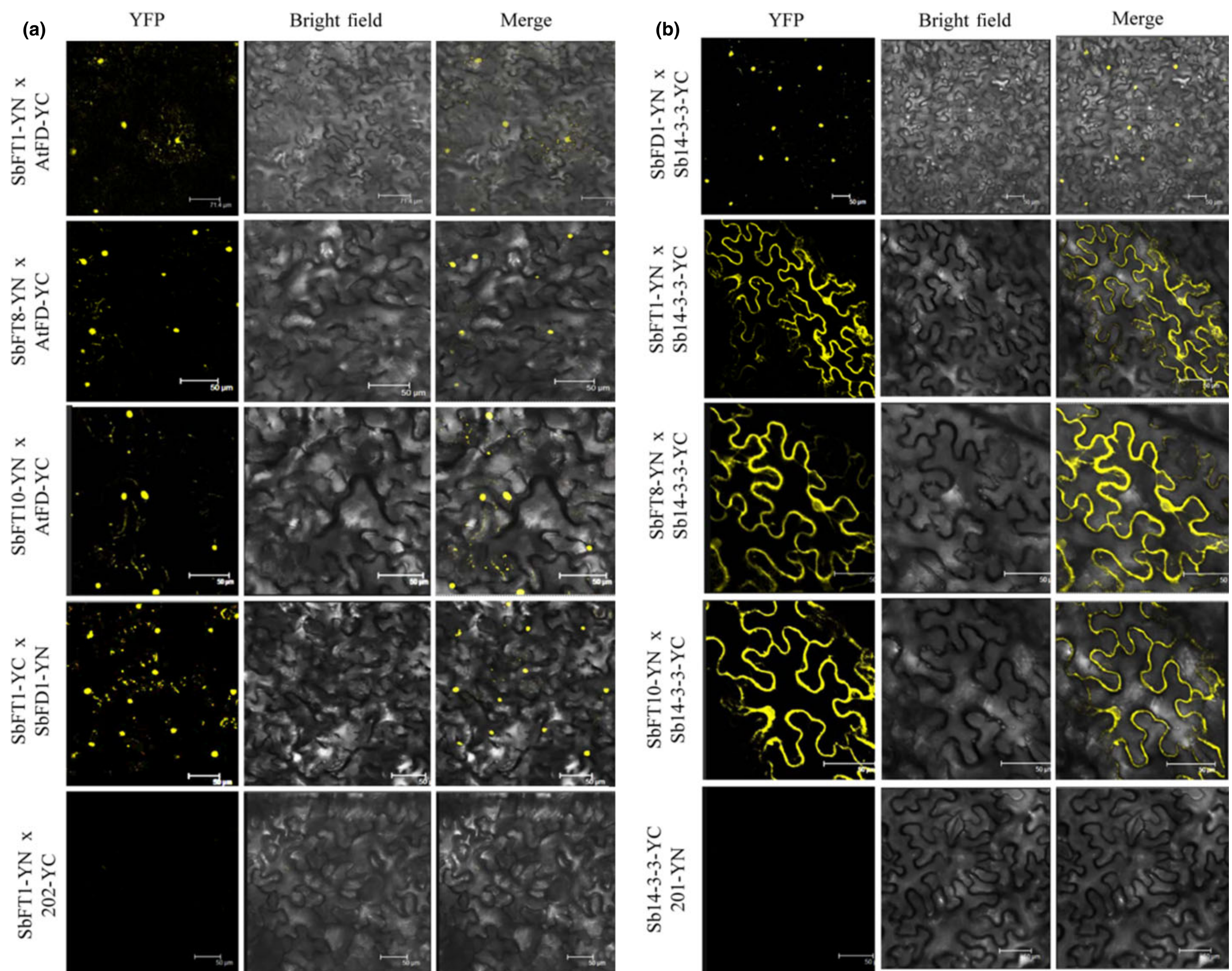

Fig. 6 Bimolecular fluorescence complementation (BiFC) assay in tobacco leaf cells testing protein–protein interaction between SbFT and AtFD, SbFD1 and Sb14-3-3 proteins. (a) Split yellow fluorescent protein (YFP) BiFC assay between Arabidopsis FD and sorghum FT proteins, as well as between SbFT1 and SbFD1: SbFT1 with AtFD (top panel), SbFT8 with AtFD (second panel, from top), SbFT10 with AtFD (third panel) and SbFT1 with SbFD1 (fourth panel) where the FTs were fused to the N-terminal half while FD was fused to the C-terminal half of YFP. Reconstitution of yellow fluorescence shows positive interaction in the nucleus, and absence of fluorescence in the negative control (bottom panel) shows no interaction between SbFT1 fused to YN and YC alone of YFP. (b) BiFC interaction assay of Sb14-3-3 with SbFD1 and SbFT proteins: SbFD1-YN and Sb14-3-3-YC (top panel), SbFT1-YN and Sb14-3-3-YC (second panel), SbFT8-YN and Sb14-3-3-YC (third panel), and SbFT10-YN and Sb14-3-3-YC (fourth panel). Reconstitution of yellow fluorescence shows positive signal in the nucleus for the interaction of SbFD1 with Sb14-3-3 and in the cytoplasm for the interaction of Sb14-3-3 with SbFTs. The control (bottom panel) shows an absence of interaction between Sb14-3-3-YC and YN alone.

SbFD1 (Fig. 6a). However, SbFT8 and SbFT10 did not show interaction with SbFD1 in the BiFC assay (Fig. S6a). We also cloned the sorghum homolog of rice GF14C, Sb14-3-3, and tested interactions with SbFTs in the Y2H and BiFC assays. In the Y2H assay, only SbFT1 but not SbFT8 and SbFT10 interacted with Sb14-3-3 (Fig. S5a), but in the BiFC assay SbFT1, SbFT8 and SbFT10 all interacted with Sb14-3-3 in the cytoplasm, and SbFD1 interacted with Sb14-3-3 in the nucleus (Fig. 6b). SbFT1, SbFT8 and SbFT10 were localized in both the nucleus and the cytoplasm, while Sb14-3-3 was exclusively localized in the cytoplasm (Fig. S6b), but interaction of Sb14-3-3 with SbFD1 occurred in the nucleus, suggesting that all three SbFT proteins are potentially capable of forming a floral activation complex with Sb14-3-3 and SbFD1 in the nucleus of living plant cells. Consistent with this, ectopic expression of *SbFT1*, *SbFT8* and *SbFT10* but not *SbFT9* induced expression of floral meristem identity genes *AP1* and *LFY* in transgenic Arabidopsis (Fig. S7).

Discussion

Control of flowering time is a major agronomic trait in sorghum that dictates its usage in grain or biomass production in temperate regions. Owing to its original domestication in tropical East

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Africa, sorghum exhibits strong photoperiod response. However, sorghum has been introduced into temperate agriculture where photoperiod-insensitive varieties have been developed for optimum seed production in the warm summer months. We examined 19 PEBP sequences from the sorghum genome (version 2.1) and identified 13 FT-like genes, *SbFT1–SbFT13*, in an effort to understand the mechanistic control of flowering time in sorghum. Of the 13 *FT*-like genes, *SbFT1*, *SbFT8* and *SbFT10* were identified as potential candidates for encoding florigens that activate floral transition and mediate photoperiodic responses. *SbFT1* is most closely related and syntenic to rice *Hd3a*, and was previously reported as sorghum *FT* (Murphy *et al.*, 2011), whereas *SbFT8* and *SbFT10* were reported as *SbCN12* and *SbCN8*, respectively, analogous to the maize *ZCN12* and *ZCN8* genes (Yang *et al.*, 2014a,b). As ZCN8 appeared to be the only florigen candidate in maize (Lazakis *et al.*, 2011; Meng *et al.*, 2011) but there are three candidates in sorghum, we retained the original naming '*FT*' to avoid confusion. Our results indicated that although *SbFT1* exhibits the lowest transcript accumulation under SD conditions, all three genes, *SbFT1*, *SbFT8* and *SbFT10*, are induced by SDs especially in photoperiod-sensitive genotypes, expressed in leaves at the time of floral transition irrespective of genotype and day length, expressed early in early-flowering lines and late in late-flowering lines under LD conditions, interact with sorghum 14-3-3 in BiFC assays, and strongly activate flowering in transgenic Arabidopsis. These observations are consistent with all three genes being floral transition activators, and may function redundantly to control flowering in sorghum.

Having two florigens is not uncommon, as exemplified by *FT* and *TSF* in Arabidopsis (Yamaguchi *et al.*, 2005) and *Hd3a* and *RFT1* in rice (Komiya *et al.*, 2008). However, it is intriguing that sorghum may have at least three functional *FT*s, while maize, with a much larger genome, appears to have one functional *FT*, *ZCN8* (Lazakis *et al.*, 2011; Meng *et al.*, 2011). Sorghum is a close relative of maize and the two are assumed to have shared a common ancestor as recently as 24 million yr ago (Thomasson *et al.*, 1986); therefore a one-to-one correspondence of sorghum to maize genes might be expected. Indeed, all of the sorghum PEBP genes, except *SbFT13*, have close homologs in maize (Table S2) (Danilevskaya *et al.*, 2008b). But, despite the existence of the *SbFT1* homolog with 97% amino acid identity, *ZCN15*, at the syntenic region on chromosome 6 of maize, this gene appears not to be involved in maize flowering (Meng *et al.*, 2011). However, *SbFT8* and *SbFT10* are more closely related to the maize *ZCN8* than to rice *Hd3a* or *RFT1* (Fig. 1b; Table 1), and are syntenic to *ZCN12* and *ZCN8*, respectively. It appears that sorghum has retained floral activation function for three genes, *SbFT1*, *SbFT8* and *SbFT10*, equivalent to maize *ZCN15*, *ZCN12* and *ZCN8* in which *SbFT1* is also collinear to rice *Hd3a* and maize *ZCN15* (Danilevskaya *et al.*, 2008a), although *ZCN15* appears not to play a role in floral activation in maize. However, not all *ZCN* genes are completely excluded from potential candidacy for florigen. For example, *ZCN12* is expressed in leaf and induced by SD treatment (Danilevskaya *et al.*, 2008a; Meng *et al.*, 2011), suggesting a potential for floral activation in maize. Thus, with the possibility of additional functional *ZCN* genes in maize, the regulation of floral transition in maize and sorghum may not be that different after all. Further experiments with mutants or reduced expression lines will be required to quantitatively determine the contribution of each of the three *SbFT* genes to sorghum flowering and photoperiod response.

Sorghum *FT* genes are additively repressed by maturity loci in LDs but repression is overcome by SD treatment

In the photoperiod-insensitive grain sorghum genotype, BTx623, the expression of *SbFT1*, *SbFT8* and *SbFT10* under LD conditions is regulated by plant age in which expression of all three genes is barely detectable at the seedling stage but sharply increases near the floral transition period (Fig. 2a), suggesting that endogenous factors regulate *FT* expression and flowering in this genotype. *SbFT1*, *8* and *10* are also highly expressed in leaves at the S3 stage in other early-flowering genotypes, such as 38M and Tx430, but not in 100M, forage and sweet sorghums (Fig. 3), suggesting repression by maturity loci. This repression appeared to be additive. In 38M (*ma1*, *ma2*, *ma3^R*), all three *SbFT* genes are highly expressed 5 wk after germination, but in 44M (*ma2*, *ma3^R*) expression of *SbFT1* and *SbFT10*, in particular, is highly reduced, and in 90M (*ma2*) or 100M (*Ma1*, *Ma2*, *Ma3*, *Ma4*), expression of the three genes is not detectable (Fig. 3b). This shows that the maturity loci, at least *Ma1*, *Ma2* and *Ma3*, may cooperatively repress the expression of *SbFT1*, *8*, *10* genes under LD conditions, consistent with the genetic data showing that maturity loci together have the strongest effect on flowering (Quinby & Karper, 1945; Quinby, 1966, 1973; Rooney & Aydin, 1999; Morgan & Finlayson, 2000). The two loci that have major effects on flowering, *Ma1* and *Ma3*, encode PRR37 and PHYB proteins, respectively (Foster *et al.*, 1994; Childs *et al.*, 1997; Murphy *et al.*, 2011). *PHYB* is an upstream activator of *PRR37* and represses *SbFT* expression by repressing the *SbFT* activator *SbEHD1* through *PRR37* (Murphy *et al.*, 2011; Yang *et al.*, 2014a,b). But there is a significant difference in *SbFT* expression between 38M and 44M (Fig. 3b), which suggests that *PHYB* affects *SbFT* expression in addition to its effect through *PRR37* accounting for cooperative repression. This may be through activating other repressors such as *SbGHD7* (Yang *et al.*, 2014a), other phytochromes, the circadian clock, or directly repressing *SbEHD1* or *SbFT*s. Further experiments will establish whether two or more of these possibilities are correct and whether the three *SbFT*s are differentially regulated by these upstream repressors.

Nevertheless, repression is fully overcome by growth under SD conditions. Our results with the SD to LD shift experiments suggest that there may not be intact long-term memory for SD treatment, as *SbFT* expression under SD conditions was completely repressed by just 1 wk at LD photoperiods (Fig. 4a). However, the SD memory is not all lost, as it makes some contribution to the early flowering of plants shifted to LDs (Fig. 4f) depending on the length of the SD treatment before shifting. A phenomenon related to this, called night break, is known in rice and other species, where a short light break during the long night

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interrupts the SD response in a dosage-dependent manner (Ishikawa *et al.*, 2004, 2005, 2009; Higuchi *et al.*, 2012). This situation in rice favors the possibility of the presence of a separate maintenance factor of Hd3a induction by SDs, which would be sensitive to dosage-dependent exposure to light. On the other hand, the strict day length control in rice mediated by repressor *GHD7* and activator *EHD1* functions on *Hd3a* expression (Itoh *et al.*, 2010) could tip the balance to the *GHD7* side with additional light exposure without necessarily having a separate factor for maintenance of *Hd3a* induction. *GHD7* and *EHD1* orthologs have been reported in sorghum (Murphy *et al.*, 2011; Yang *et al.*, 2014a,b) and, in fact, SbGHD7 is encoded by *Ma6*, one of the maturity loci that modifies the photoperiod response (Murphy *et al.*, 2014). Further molecular analyses are needed to understand the mechanism with which reversion of *FT* induction by LDs, memory of exposure to SDs, as well as de-repression of the axillary meristem during photoperiod switching are achieved in sorghum, which will provide novel mechanistic insights into the response of sorghum developmental programs to environmental signals.

All three SbFT proteins physically interact with Sb14-3-3 but not necessarily with SbFD1

In Arabidopsis, rice and maize, the FT–FD protein interaction in the shoot apical meristem is required to directly activate transcription of floral meristem identity target genes. In rice, Hd3a interacts with OsFD1 via a 14-3-3 protein (Taoka *et al.*, 2011; Hiroyuki *et al.*, 2013), which appears to be a cytoplasmic receptor for Hd3a (Taoka *et al.*, 2011, 2013). The 14-3-3-Hd3a complex translocates to the nucleus to bind with OsFD1 and thereby activate OsMADS15 transcription (Taoka *et al.*, 2011, 2013; Hiroyuki *et al.*, 2013). Our analysis suggests that this type of interaction may also be conserved in sorghum. SbFT1 but not SbFT8 and SbFT10 interact with SbFD1 in Y2H and BiFC assays (Figs 6, S5, S6). However, SbFT1 interacts with SbFT8 and SbFT10 in the Y2H assay, and all three SbFTs interact with Sb14-3-3 in the BiFC assays in the cytoplasm while SbFD1 interacts with Sb14-3-3 in the nucleus (Fig. 6b). It is thus likely that SbFT8 and 10 interact with SbFD1 in the nucleus through Sb14-3-3, analogous to the situation in rice. The significance of SbFT1 interaction with SbFT8 and 10 is unclear at this point, but it is possible that SbFT1 facilitates or stabilizes the SbFT8 and 10 interaction with SbFD1 in the nucleus. These observations together suggest that SbFT8 and SbFT10 may interact with SbFD1 through Sb14-3-3 and it is likely that a SbFT-Sb14-3-3-SbFD1 floral activation complex could form in the nucleus, although this remains to be demonstrated. Consistent with these, all three *SbFT*s induced expression of *AtLFY* and *AtAP1*, and activated early flowering in transgenic Arabidopsis.

Our results together demonstrate that sorghum has at least three *FT* genes that could potentially form an FAC and mediate genotype-dependent photoperiod response and flowering time variation in sorghum. As flowering is a key agronomic trait in sorghum, the three *SbFT* genes identified here can be used as valuable molecular markers in sorghum breeding programs.

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Author contributions

T.W.W., F.Z. and M.T. planned and designed the research. T.W.W., F.Z., L.N. and S.K. performed experiments. T.W.W., F.Z., P.B-M., M.G.M. and M.T. analyzed the data. T.W.W. and M.T. wrote the manuscript.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Sequence alignment of SbFT/TFL1/MFT-like and related PEBP proteins.

Fig. S2 Expression levels of *SbFT* genes in the leaves of late-flowering sweet sorghum Rio and forage sorghum FS000504 (FS) under SD conditions.

Fig. S3 Flowering phenotypes of *SbFT* transgenic Arabidopsis lines.

Fig. S4 Overexpression of *SbFT2*, *SbFT6* and *SbFT9* genes in transgenic Arabidopsis L*er* and *ft-1* mutant plants does not alter flowering time.

Fig. S5 Yeast two-hybrid (Y2H) protein interaction assay of sorghum FT proteins with AtFD, SbFD1, and Sb14-3-3, and with each other.

Fig. S6 BiFC assay and localization of SbFT and Sb14-3-3 proteins.

Fig. S7 Induction of floral meristem identity marker genes, *AtAP1* and *AtLFYi*, in *SbFT*-expressing transgenic Arabidopsis lines.

Table S1 Full-length amino acid sequence identity of sorghum PEBPs with maize and rice homologs

Table S2 Amino acid sequence identity within sorghum PEBP family proteins

Table S3 Lists of primers used in this study

Notes S1 Accession numbers or gene identifiers of sequences used in this study.

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