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BRIEF COMMUNICATION

Identification and Characterization of the *FT*/*TFL1* Gene Family in the Biofuel Plant *Jatropha curcas*

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Abstract The transition from vegetative to reproductive growth is one of the most important developmental steps made by flowering plants. At the molecular level, the genes in the *FLOWERING LOCUS T* (*FT*)/*TERMINAL FLOWER 1* (*TFL1*) family, which encode proteins with high similarity to phosphatidyl ethanolamine-binding proteins, function as flowering promoters or repressors. Here, we isolated six members of the *FT*/*TFL1* family from *Jatropha curcas*, a plant with considerable potential for various uses including biofuels. All members of this gene family display a common exon-intron organization. Sequence comparisons and phylogenetic analysis with homologous genes from other plant species group *Jatropha FT*/*TFL1* genes into three major subfamilies: one into the *FT*-like, three into the *TFL1*-like, and two into the *MOTHER OF FT AND TFL1* (*MFT*)-like subfamilies. Expression analysis indicates differences in the expression patterns of these six genes at the temporal and spatial levels. *JcFT*, the *Jatropha FT* homolog, is primarily expressed in the reproductive organs. *JcTFL1a* and *JcTFL1c*, two genes in the *TFL1*-like subfamily, are mainly expressed in the roots of juvenile plants, whereas *JcTFL1b* transcripts are abundantly accumulated in the fruits. In addition, two *JcMFT* genes are primarily expressed in the fruits. The differential expression of the *FT*/*TFL1* gene family in *Jatropha* suggests that this gene family plays multifaceted roles in plant growth and development.

Keywords Physic nut · *FLOWERING LOCUS T* · *TERMINAL FLOWER 1* · *MOTHER OF FT AND TFL1* · Phosphatidyl ethanolamine-binding protein

Abbreviations

AP1	*APETALA 1*
ATC	*ARABIDOPSIS THALIANA CENTRORADIALIS HOMOLOGUE*
BFT	*BROTHER OF FT AND TFL1*
FUL	*FRUITFULL*
FT	*FLOWERING LOCUS T*
MFT	*MOTHER OF FT AND TFL1*
PEBP	Phosphatidyl ethanolamine-binding protein
qRT-PCR	Quantitative reverse transcriptase-polymerase chain reaction
SOC1	*SUPRESSOR OF OVEREXPRESSION OF CONSTANS 1*
TF	Transcription factor
TFL1	*TERMINAL FLOWER 1*
TSF	*TWIN SISTER OF FT*

Introduction

One of the key developmental processes in flowering plants is the floral initiation, the transition from vegetative to

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reproductive growth (Pin and Nilsson 2012). This transition is regulated through the integration of multiple environmental and endogenous signals (Imamura et al. 2011). Recent studies on the facultative long-day annual plant *Arabidopsis thaliana* demonstrated that floral initiation is controlled by five major flowering time regulatory pathways: photoperiod, temperature and vernalization, gibberellin, autonomous, and aging pathways (Srikanth and Schmid 2011). These pathways regulate the expression of a few flowering signal integrators such as the mobile florigen *FLOWERING LOCUS T* (*FT*) and *SUPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*), which further promote the expression of floral meristem identity genes (Posé et al. 2012). *FT* belongs to the *FT*/*TFL1* gene family, similar to the phosphatidyl ethanolamine-binding protein (PEBP) gene family, which are found in all taxa from bacteria to animals and plants (Bradley et al. 1997; Chautard et al. 2004). Many members of the *FT*/*TFL1* gene family act as key regulators of flowering transition and other developmental processes in plants (Karlgren et al. 2011).

In *Arabidopsis*, the *FT*/*TFL1* gene family includes six members that belong to three major subfamilies: the *FT*-like, the *TERMINAL FLOWER1* (*TFL1*)-like, and the *MOTHER OF FT AND TFL1* (*MFT*)-like subfamilies. *FT* and *TWIN SISTER OF FT* (*TSF*) belong to *FT*-like; *TFL1*, *ARABIDOPSIS THALIANA CENTRORADIALIS HOMOLOGUE* (*ATC*) and *BROTHER OF FT AND TFL1* (*BFT*) belong to *TFL1*-like; and *MFT* belongs to *MFT*-like (Kobayashi et al. 1999; Chardon and Damerval 2005). Despite their sequence similarities, these genes have different roles in diverse plant developmental processes, such as flowering control (Matsoukas et al. 2012; Xu et al. 2012; Jaeger et al. 2013), stomatal control (Kinoshita et al. 2011), plant architecture (Bradley et al. 1997; Yoo et al. 2010), and seed germination (Xi et al. 2010). FT protein acts as a mobile florigen that interacts with FD, a bZIP transcription factor (TF), to promote flowering in *Arabidopsis* through activation of several downstream TF genes, such as *APETALA 1* (*AP1*), *FRUITFULL* (*FUL*), and *SOC1* (Abe et al. 2005; Wigge et al. 2005). Besides flowering, FT proteins also mediate stomatal control in *Arabidopsis* (Kinoshita et al. 2011). In addition, the potato FT homolog controls tuber formation (Navarro et al. 2011) and onion FT homologs control bulb formation (Lee et al. 2013). *TSF*, the paralog of *FT*, also seems to act as a floral pathway integrator and promotes flowering redundantly with *FT* but makes a distinct contribution under short-day conditions (Yamaguchi et al. 2005). *TSF* mediates the *Arabidopsis* response to cytokinin treatment to promote flowering under noninductive short-day conditions (D'Aloia et al. 2011). *MFT* may have a redundant role in flowering promotion because its overexpression causes a slight reduction of flowering time (Yoo et al. 2004). *MFT* also regulates seed germination via the abscisic acid and gibberellin signaling pathways in *Arabidopsis* (Xi et al. 2010). Contrary to *FT*, *TSF*, and *MFT* function in flowering promotion, *TFL1* contributes to the maintenance of indeterminate shoot identity and the delay of flowering transition in *Arabidopsis* (Bradley et al. 1997; Shannon and Meeks-Wagner 1991; Ratcliffe et al. 1998). *ATC* is functionally redundant with *TFL1* and acts as a short-day-induced floral inhibitor (Mimida et al. 2001; Huang et al. 2012). Finally, *BFT* is suggested to have *TFL1*-like activity and functions redundantly with *TFL1* in inflorescence meristem development and acts as a floral repressor under high salinity conditions (Yoo et al. 2010; Ryu et al. 2011, 2013). Therefore, all three subfamilies of the *FT*/*TFL1* genes can function as floral activators or inhibitors.

Jatropha curcas (physic nut) is a monoecious woody plant that belongs to Euphorbiaceae family, with male and female flowers on the same inflorescence. *Jatropha* has been recognized as a biofuel plant because of its high oil content seeds, easy propagation, drought tolerance, and adaptability to marginal lands (Akashi 2012). In addition, *Jatropha* oil contains high levels of polyunsaturated fatty acids, and it is therefore suitable for biofuel production (Ong et al. 2011; Khalil et al. 2013). However, *Jatropha* exhibits low seed yield as a result of unreliable and poor flowering (Ghosh et al. 2010; Pan and Xu 2011). Molecular breeding would be a good genetic improvement method to obtain high-yielding *Jatropha* cultivars. Little research has been done on the genetic mechanism regulating floral transition in this perennial plant. It is expected that the *FT*/*TFL1* gene family plays an important role in this development process. In this study, we isolated six *Jatropha* genes that are highly similar to *Arabidopsis FT*/*TFL1* genes and investigated their expression patterns throughout the vegetative and reproductive developmental stages. The information provided by this study may help to elucidate the biological functions of the *FT*/*TFL1* gene family in *Jatropha*.

Materials and Methods

Plant Materials and Growth Conditions

Mature *Jatropha* seeds were collected from Xishuangbanna Tropical Botanical Garden of the Chinese Academy of Sciences, Mengla County, Yunnan Province, China. Seeds were planted in pots with peat soil and incubated at 28±2 °C under 14/10 h (light/dark) photoperiod with lighting provided by cool white fluorescent lights for germination. Ten-day-old *Jatropha* seedlings were sampled. Four-month-old *Jatropha* trees were sampled as post-seedling juvenile plants. *Jatropha* roots, stems, leaves, flower buds, flowers, and fruits (10 days after pollination) were collected during summer from Xishuangbanna and the mature seeds were collected in autumn. All tissues prepared for quantitative reverse

transcriptase-polymerase chain reaction (qRT-PCR) were immediately frozen in liquid nitrogen (N_2) and stored at −80 °C until needed.

Cloning of the *Jatropha FT*/*TFL1* Homologs

RNA samples extracted from various organs were used to isolate as many *FT*/*TFL1* gene family members as possible. RNA was extracted using the protocol described by Ding et al. (2008). First-strand complementary DNA (cDNA) was synthesized using M-MLV-reverse transcriptase (Takara, Dalian, China) according to the manufacturer's instructions. Partial cDNA sequences of an *FT* and three *TFL1*-like genes were amplified using degenerate primers. One *MFT*-like cDNA (*JcMFT1*, GenBank FM894171.1) sequence was obtained from a cDNA library of immature *Jatropha* embryos (Chen et al. 2011). Based on the amplified fragments, we designed gene specific primers of the five genes and conducted rapid amplification of cDNA ends (RACE) with a SMART^{TM}RACE cDNA Amplification Kit (PT3269-1) (Clontech, USA) according the manufacturer's instructions to amplify the cDNA 5′ and 3′ ends. Another *MFT*-like cDNA sequence (*JcMFT2*) was obtained from a *Jatropha* genome database created by Sato et al. (2011). The open reading frame sequences of the *Jatropha FT*/*TFL1* homologs were obtained by PCR amplification using the primers listed in Table S1.

To analyze the genomic structure of these genes, we obtained the genomic DNA (gDNA) sequences from amplified *Jatropha* DNA using the same PCR primers as listed in Table S1. The DNA was isolated from *Jatropha* leaves using the improved CTAB method (Doyle et al. 1990). The amplified PCR products were cloned into a pMD19-T simple vector and sequenced. Three clones of each amplified fragment were completely sequenced and compared.

Sequence Comparison and Phylogenetic Analysis

Sequence chromatograms were examined and edited using Chromas Version 2.23 (http://technelysium.com.au/). Related sequences were identified with BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). To determine the amino acid identities, sequences from the alignment were pairwise compared using DNAMAN 6.0 (http://www.lynnon.com/). A phylogenetic tree based on the protein sequences was constructed using MEGA 5.0 (http://www.megasoftware.net/). The amino acid sequences of the *FT*/*TFL1* family were assembled with ClustalX (http://www.clustal.org/). A neighbor-joining phylogenetic tree was generated with MEGA 5.0, using the Poisson model with gamma-distributed rates and 10, 000 bootstrap replicates.

Expression Pattern Analyses by qRT-PCR

To investigate the spatial and temporal expression patterns of each homolog, qRT-PCR experiments were performed on various organs. Total RNA was extracted from each tissue and first-strand cDNA was synthesized with a PrimeScript® RT Reagent Kit with gDNA Eraser (Takara, Dalian, China) according to the manufacturer's instructions. qRT-PCR was performed with SYBR® Premix Ex Taq™ II (Takara) on the Roche 480 Real-Time PCR Detection System (Roche Diagnostics).

Primers used for the qRT-PCR are listed in Table S2. qRT-PCR was performed with two independent biological replicates and three technical replicates for each sample. Data was analyzed using the $2^{-\Delta\Delta CT}$ method as described by Livak and Schmittgen (2001). Expression levels of specific genes were normalized to *Jatropha Actin* (*JcActin*) (Zhang et al. 2013).

Results

Isolation and Identification of *Jatropha FT*/*TFL1* Homologs

Based on the conserved sequence of the known members of the *FT/TFL1* family, four full-length cDNA clones encoding FT/TFL1 proteins in *Jatropha*, designated *JcFT*, *JcTFL1a*, *JcTFL1b*, and *JcTFL1c* were isolated by a combination of RT-PCR and 3′ and 5′-RACE techniques. *JcMFT1* cDNA was cloned using RACE based on an EST sequence (GenBank FM894171.1) from *Jatropha* embryos (Chen et al. 2011). *JcMFT2* cDNA was obtained using RT-PCR according to a sequence (Jcr4S00105.190) from a *Jatropha* genome database (Sato et al. 2011). The sequences of the six *Jatropha FT*/*TFL1* homologs were deposited with the following GenBank accession numbers: *JcFT* (KF113881), *JcTFL1a* (KF944349), *JcTFL1b* (KF944350), *JcTFL1c* (KF944351), *JcMFT1* (KF944348), and *JcMFT2* (KF944352). To study the structure of the *Jatropha FT/TFL1* genes, we cloned the genomic sequences of the six members from *Jatropha* gDNA with the same primers as were used for their cDNA cloning. The sequences of the six *Jatropha FT*/*TFL1* family genes were deposited with the following GenBank accession numbers: *JcFT* (KJ130139), *JcTFL1a* (KJ130140), *JcTFL1b* (KJ130141), *JcTFL1c* (KJ130142), *JcMFT1* (KJ130143), and *JcMFT2* (KJ130144).

Comparison of the gDNA and cDNA sequences revealed that all six genes comprised four exons with three introns at conserved positions identical to *FT*/*TFL1* genes from other species; however, the introns differed in length (Bradley et al. 1997; Carmona et al. 2007; Sato et al. 2009; Igasaki et al. 2008; Imamura et al. 2011; Harig et al. 2012) (Figs. 1 and 2). In *Jatropha*, exons I and IV varied from 195 to 207 bp and

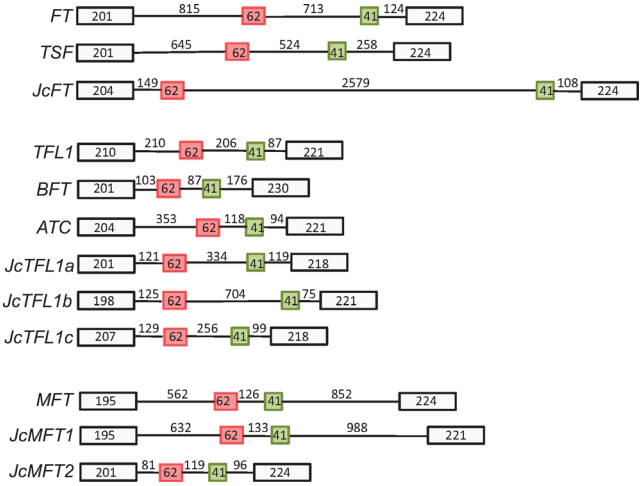

Fig. 1 Genomic organizations of members of the *FT*/*TFL1* family in *Jatropha* and *Arabidopsis*. Boxes represent exons and lines represent introns. *Numbers* indicate the lengths of exons and introns in base pairs

from 218 to 224 bp, respectively. Whereas exons II and III were conserved in length with 62 and 41 bp, respectively, in all genes examined. The exon-intron structures of the six *Jatropha* genes were compared with the *Arabidopsis FT*/*TFL1* genes shown in Fig. 1.

Comparisons of deduced *Jatropha* FT/TFL1 protein sequences with those from *Arabidopsis* (Fig. 2) revealed that the identity percentage of JcFT to FT is 78 %. JcTFL1a, JcTFL1b, and JcTFL1c to TFL1 are 74, 72, and 64 %, respectively, and JcMFT1 and JcMFT2 to MFT are 77 and 58 %, respectively.

Phylogenetic Analysis of *Jatropha FT*/*TFL1* Homologs

To analyze the phylogenetic relationships between members of the *FT*/*TFL1* homologous genes, we performed phylogenetic analysis of genes from *Jatropha* and other angiosperms. A neighbor-joining phylogenetic tree was generated with three major subfamilies: *JcFT* is in the *FT*-like subfamily; *JcTFL1a*, *JcTFL1b*, and *JcTFL1c* are in the *TFL1*-like subfamily; and *JcMFT1* and *JcMFT2* are in the *MFT*-like subfamily (Fig. 3). The analysis revealed that *Jatropha* FT/TFL1 proteins (indicated by a red box) were more closely related to those from perennial woody plants, such as *Populus nigra* and *Vitis vinifera*.

JcFT, the putative homolog of *Arabidopsis* FT, displayed all of the characteristic features of the FT-like protein subfamily (Ahn et al. 2006). This includes the conservation of Tyr85 and Gln140 (Tyr86 and Gln141 in JcFT, respectively) and the highly conserved amino acid sequences LGRQTVYAPGWRQN and LYN, corresponding to the binding regions of FT with FD present in exon IV (Abe et al. 2005; Wigge et al. 2005) (Fig. 2). A second subfamily, JcTFL1a, JcTFL1b, and JcTFL1c, related to *Arabidopsis* TFL1, ATC, BFT, and their putative homologs identified in other plant species (Fig. 3). All of them bear conserved residues His88 and Asp144 in similar positions to TFL1 (Hanzawa et al. 2005) (His85 and Asp140 in JcTFL1a, His84 and Asp140 in JcTFL1b, and His87 and Asp142 in JcTFL1c) (Fig. 2). The two additional *Jatropha* genes were classified in the third subfamily with MFT (Fig. 3). They bear a critical amino acid residue (Trp) that differs from Tyr and His in FT or TFL1 (Fig. 2). Conserved Pro is in the C-terminal of JcMFT1 and JcMFT2 (Fig. 2), which was not found in either the FT-like subfamily or TFL-like subfamily (Hedman et al. 2009).

Expression Patterns of *Jatropha FT*/*TFL1* Homologs

To understand the functions of *FT*/*TFL1* genes in *Jatropha* development, we studied their temporal and spatial expression

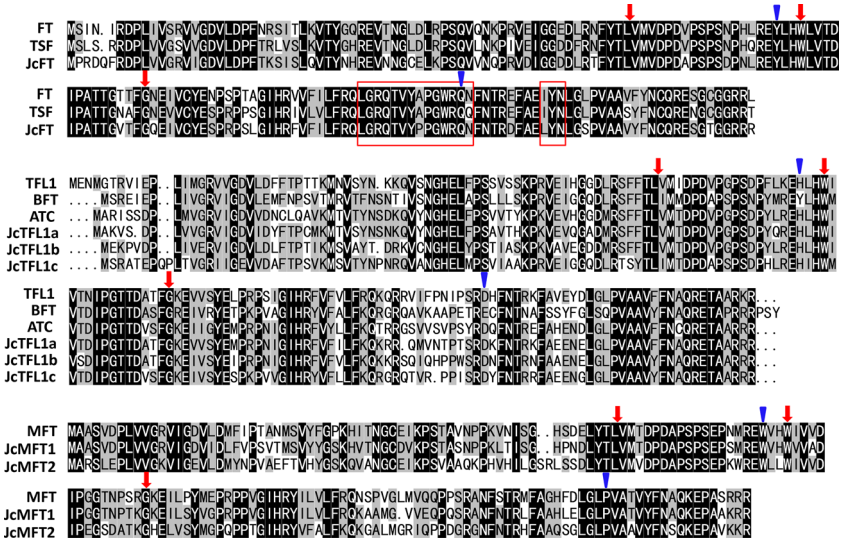

Fig. 2 Alignments of the deduced amino acid sequences of the *FT*/*TFL1* family products in *Jatropha* and *Arabidopsis*. A *black background* indicates a homology level of 100 % and a *gray background* indicates a homology level between 50 and 100 %. *Dots* indicate gaps. Intron positions are indicated by *red arrows* above sequences. *Blue arrowheads* indicate amino acids that are critical to define FT, TFL1, or MFT-like proteins. The two *red boxes* indicate the important amino acid sequences in exon IV of FT-like proteins

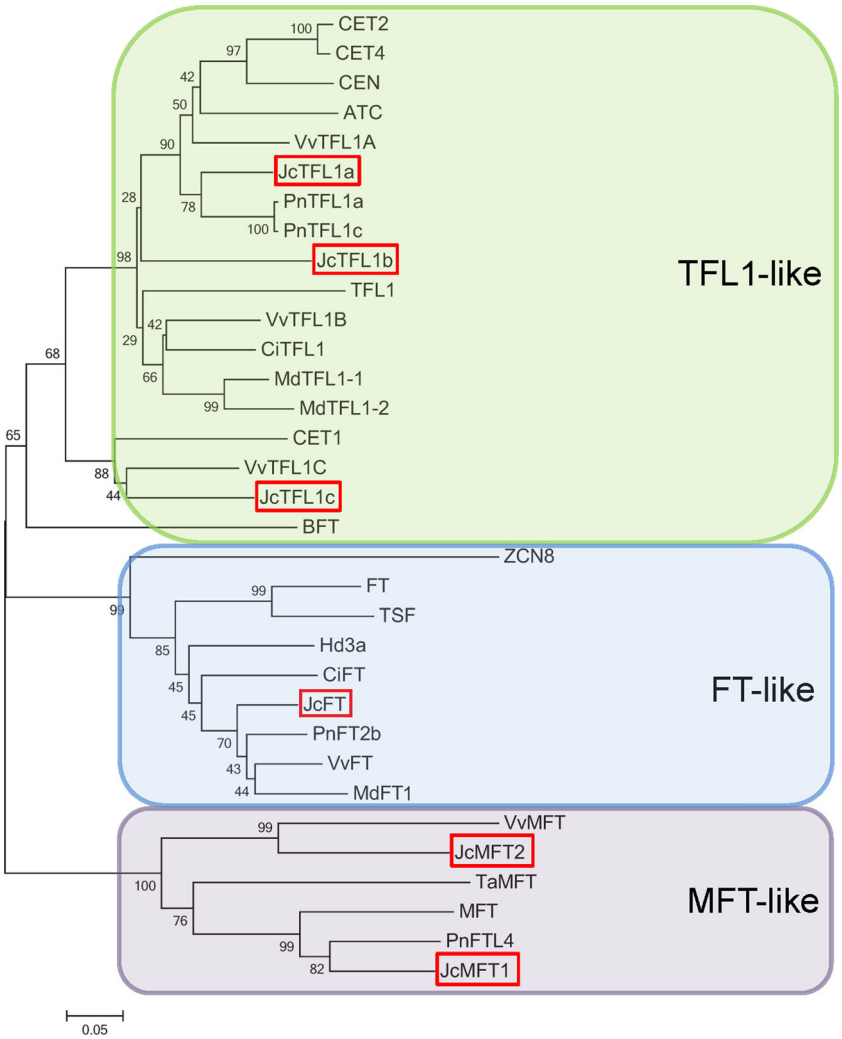

Fig. 3 Phylogenetic analysis of the *FT*/*TFL1* family members in *Jatropha* and other angiosperms. The tree was constructed by a neighbor-joining (N-J) method. The three subfamilies are indicated on the *right*. GenBank accession numbers are as follows: *Antirrhinum majus CEN* (S81193); *A. thaliana FT* (AF152096), *TSF* (AF152907), *TFL1* (U77674), *MFT* (AF147721), *ATC* (AB024714), and *BFT* (NM_125597); *Citrus unshiu CiFT* (AB027456) and *CiTFL1* (AY344245); *Malus* × *domestica MdFT1* (AB161112), *MdTFL1-1* (AB052994), and *MdTFL1-2* (AB162046); *Nicotiana tabacum CET1* (AF145259), *CET2* (AF145260), *CET4* (AF145261); *Oryza sativa Hd3a* (AB052944); *P. nigra PnFT1b* (AB161109), *PnFT2b* (AB109804), *PnTFL1a* (AB181183), *PnTFL1c* (AB104629), *PnTFL3b* (AB181240), and *PnFTL4* (AB181241); *Triticum aestivum TaMFT* (AB571513); *V. vinifera VvFT* (ABI99465), *VvMFT* (ABI99469), *VvTFL1A* (ABI99467), *VvTFL1B* (ABI99467), and *VvTFL1C* (ABI99468); *Zea mays ZCN8* (EU241988)

patterns during vegetative and reproductive development using qRT-PCR. *JcFT* was expressed mainly in the reproductive organs, while *JcFT* expression levels in several vegetative organs, such as the roots, stems, and leaves, were very low (Fig. 4a). In addition, *JcFT* was practically undetectable in the organs of post-seedling juvenile plants (Fig. 4a).

In the *TFL1*-like subfamily, the three *Jatropha TFL1* homologs exhibited different expression patterns. *JcTFL1a* was strongly expressed in the seedling roots (Fig. 4b), and *JcTFL1c* was highly expressed in juvenile plant roots (Fig. 4d). *JcTFL1b* was highly expressed in the fruits (Fig. 4c). *JcTFL1b* expression levels were also high in the stems of plants in the reproductive phase and post-seedling juvenile plants (Fig. 4c).

JcMFT1 and *JcMFT2*, two members of the *Jatropha MFT*-like subfamily, exhibit different expression patterns (Fig. 4e, f). Although *JcMFT1* and *JcMFT2* both reached their highest expression in the fruits, *JcMFT1* was also highly expressed in the seeds (Fig. 4e), whereas *JcMFT2* in seedling roots (Fig. 4f).

Discussion

In the present study, we identified six members of the *Jatropha FT*/*TFL1* gene family, as revealed by comparisons of sequences and genomic organizations (Figs. 1 and 2). The entire *Jatropha* genome has been sequenced (Sato et al. 2011; Hirakawa et al. 2012), and we found five *FT*/*TFL1* members in this genome database, *JcTFL1c* was not in the database, which may be accounted for by the fact that the genome database has only 95 % gene coverage. Thus, it is likely that all members of the *Jatropha FT*/*TFL1* gene family were identified in this study. And our study provides a comprehensive description about the genomic structures and expression patterns of the *Jatropha FT*/*TFL1* gene family.

Our phylogenetic analysis showed that the six genes belong to three subfamilies: one to the *FT*-like subfamily, three to the *TFL1*-like subfamily, and two to the *MFT*-like subfamily (Fig. 3). As in grape vines (Carmona et al. 2007), only one *FT*-related sequence has been found in *Jatropha*, whereas duplication and divergence of this sequence has been

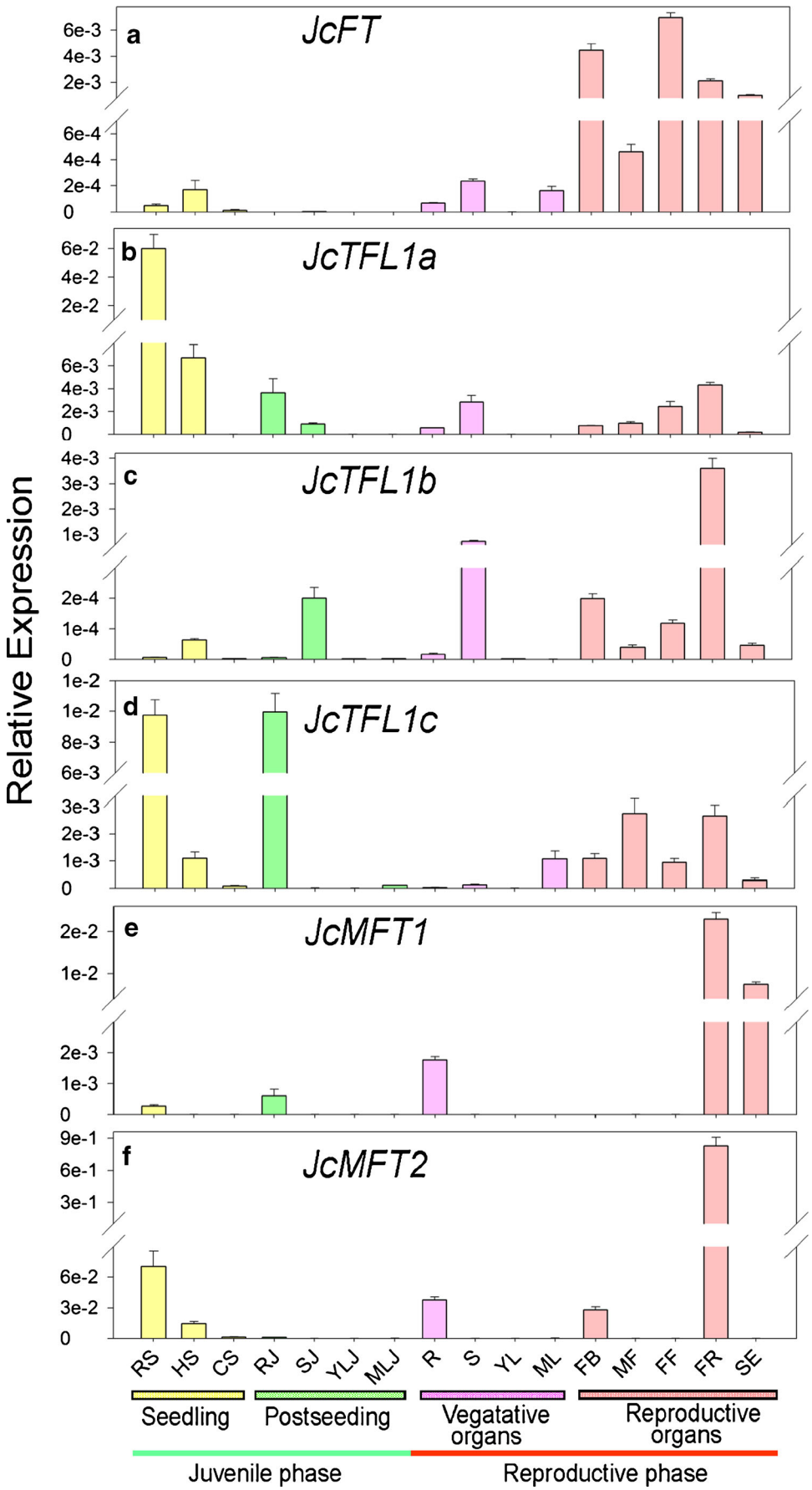

Fig. 4 Expression of genes in the *FT*/*TFL1* family in various *Jatropha* organs. (**a**) to (**f**) are expression patterns of *JcFT*, *JcTFL1a*, *JcTFL1b*, *JcTFL1c*, *JcMFT1* and *JcMFT2*, respectively. The qRT-PCR results were obtained from two biological replicates and three technical replicates for each sample. The levels of detected amplification were normalized using the amplified products of the *JcActin* gene as a reference. *RS*, *HS*, and *CS* represent seedling roots, hypocotyls, and cotyledons, respectively. *RJ*, *SJ*, *YLJ*, and *MLJ* represent roots, stems, young leaves, and mature leaves of post-seedling juvenile plants, respectively. *R*, *S*, *YL*, *ML*, *FB*, *MF*, *FF*, *FR*, and *SE* represent roots, stems, young leaves, mature leaves, flower buds, male flowers, female flowers, fruits, and seeds of reproductive phase plants, respectively

frequently observed in other botanical families, such as the apple (Kotoda et al. 2010), poplar (Igasaki et al. 2008), and saffron crocus (Tsaftaris et al. 2013). However, there are two members of the *MFT*-like subfamily in *Jatropha*, whereas only one member has been found in other dicot plants, such as *Arabidopsis* (Yoo et al. 2004), grape vines (Carmona et al. 2007), and poplar (Igasaki et al. 2008). More than one has been identified in some fully sequenced monocot genomes, two in rice (Chardon and Damerval 2005) and three in maize (Danilevskaya et al. 2008).

JcFT expression levels were highest in female flowers, but low in leaves. A florigen-encoding gene is supposed to be highly expressed in the leaves (Fig. 4a), suggesting that *JcFT* might be involved in the development of reproductive organs like *VvFT* in grapes (Carmona et al. 2007) and *ProFT* in *Protea* (Smart and Roden 2013). Three members of the *Jatropha TFL1*-like subfamily exhibited different expression patterns. *JcTFL1a* and *JcTFL1c* were highly expressed in juvenile plant roots, whereas *JcTFL1b* was highly expressed in the stems and fruits of plants in the reproductive phase (Fig. 4b–d). The *TFL1* homologs in other plants also exhibit divergent expression patterns. In the apple, *MdTFL1*and *MdTFL1a* transcripts were observed in the tissues of juvenile apple seedlings (Mimida et al. 2009). Unlike *MdTFL1* and *MdTFL1a*, *MdCENa*, another member of the *MdTFL1*-like subfamily, was expressed in both reproductive organs (fruit receptacles) and vegetative tissues (roots) (Mimida et al. 2009). *CsTFL*, correlated with juvenility in *Citrus*, was detected in all floral organs of adult plants (Pillitteri et al. 2004). These results suggest that *TFL1* homologs may play multifaceted roles in plant development. In the *Jatropha MFT*-like subfamily, *JcMFT*s expression levels were highest in the fruits, and *JcMFT1* was highly expressed in the mature seeds (Fig. 4e, f). Similar expression patterns to *MFT* were detected in *Arabidopsis* (Xi et al. 2010), suggesting that *JcMFT*s may play an important role in seed germination. Recently in a patent application by Chua et al. (2013), five genes of the *Jatropha FT*/*TFL1* family corresponding to *JcFT*, *JcTFL1a*, *JcTFL1b*, *JcMFT1*, and *JcMFT2* in this study, were cloned and analyzed for potential roles in flowering time control by using transgenic *Arabidopsis* and *Jatropha* plants. Chua et al. (2013) found that both *JcFT* and *JcTFL1* functioned as flowering promoters in *Jatropha*, which is inconsistent with previous studies showing that *TFL1* was a flowering repressor gene in various plants (Karlgren et al. 2011).

In summary, we isolated six members of the *FT*/*TFL1* family from *Jatropha* and analyzed their temporal and spatial expression patterns. Further studies to investigate the functions of these genes by overexpressing them in transgenic *Jatropha* plants might provide information about their involvement in floral transition and seed germination. Elucidation of the flowering mechanism in *Jatropha* would be helpful for the molecular breeding of high-yielding *Jatropha* cultivars.

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Conflicts of Interest The authors declare they have no conflicts of interest.

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