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The *Jatropha* FT ortholog is a systemic signal regulating growth and flowering time

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Abstract

Background: *Jatropha curcas* is being promoted as a new bioenergy crop in tropical and subtropical regions due to its high amount of seed oil and its potential capacity to grow on marginal land for biofuel production. However, the productivity of the plant is constrained by the unfavorable flowering time and inflorescence architecture, which render harvesting of seeds time-consuming and labor-intensive. These flowering-related traits have limited further widespread cultivation of *Jatropha*.

Results: We identified a *Jatropha curcas* homolog of *Flowering locus T* (*JcFT*) and demonstrated its function by genetic complementation of the *Arabidopsis ft* mutant. The *JcFT* expression level was found to be remarkably correlated with leaf age. Overexpression of *JcFT* in *Jatropha* reduced flowering time and altered plant architecture by producing more branches. Grafting experiments suggested that the earlyflowering and alteration of plant architecture traits were graft-transmissible. We also showed that the *FT*-overexpressing transgenic *Jatropha* can be used as a root stock for grafting of scions derived from other *Jatropha*.

Conclusion: We generated early flowering transgenic *Jatropha* plants that accumulate higher levels of the florigen FT. Not only early flowering but also plant growth was affected in *JcFT* overexpression lines. More seeds can be produced in a shorter time frame by shortening the flowering time in *Jatropha*, suggesting the possibility to increase seed yield by manipulating the flowering time.

Keywords: Biodiesel, Flowering locus T, *Jatropha*, Transgenic, Grafting

Background

Biofuels have been recognized as a national priority for many countries as an alternative source to meet their energy security needs. The demand for biofuel has placed increasing pressure on food production, which further raises the 'food vs fuel' debate. For instance, to satisfy the biofuel need for Germany in 2017 as mandated by the German government, all agricultural land for food production has to also be used for rape seed derived biodiesel production [1]. To ease the competition between food and fuel for arable land while satisfying the need for renewable fuels, it is well recognized that the first choice is to use marginal or degraded land for the production of biofuel crops [2].

Jatropha curcas, a small seed-propagated woody plant belonging to the family Euphorbiaceae, is a non-food plant used for biofuel production. This plant has the potential to solve in part the competition between food and fuel mainly because of its wide adaptability, drought tolerance and its ability to thrive on degraded soils [3,4]. At present, the key agronomic trait targeted by *Jatropha* breeders is the productivity of the plant [3]. Flowering is a prerequisite for crop production whenever fruits or seeds are harvested. The productivity of *Jatropha* is constrained by an unfavorable male-to-female flower ratio that has not been optimized by conventional breeding. *Jatropha* is monoecious and produces male and female flowers on the same inflorescence [5]. Not surprisingly, the productivity of the *Jatropha* plant is also highly affected by geographical and environmental conditions [6,7]. Physiological and genetic analysis of flowering has shown that multiple environmental and endogenous inputs influence the timing of the transition from vegetative to reproductive growth [7,8]. *Jatropha* mainly grows in tropical and subtropical regions between 30°N and 35°S [6,9]. It can produce limited amounts of seeds at

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high altitudes (>500 meters above sea level), where the majority of marginal land is located in the world. However, it is unable to flower and produce seeds in areas with low light intensity and poor light quality [9]. Lastly, unsynchronized flowering and fruit setting renders harvesting of *Jatropha* seeds highly labor-intensive; in fact, high labor cost is the primary fixed cost in *Jatropha* fruit production [3,4]. Therefore, it is essential to systematically study the *Jatropha* flowering biology so as to devise strategies to synchronize flowering and increase seed yield [9]. With a better understanding of molecular and genetic mechanisms of flowering, it will be possible to generate next-generation *Jatropha* plants by traditional breeding and/or genetic engineering.

At a certain point in their life cycle, annual plants undergo a major developmental transition in which they switch from vegetative to reproductive growth [9]. Although the draft genome sequence of *Jatropha* has been reported, little genetic and mechanistic research on regulation of flowering time or control of male/female flower ratio has been done [10]. The male-biased ratio within an inflorescence limits *Jatropha* seed production; hence, more female flowers in a plant will produce more fruits and seeds. Meanwhile, to precisely control the time of flowering, plants have evolved mechanisms to integrate seasonally predictable environmental cues (such as changes in photoperiod and prolonged periods of cold temperatures) and developmental cues (such as maturity).

In the model plant *Arabidopsis thaliana*, the FLOWERING LOCUS T (FT) protein serves as a flowering-inducing signal [11]. FT is a member of a protein family that contains a plant-specific phosphatidylethanolamine-binding protein (PEBP) domain. In *Arabidopsis*, the PEBP family is divided into three subfamilies, namely FT-like, TERMINAL FLOWER1 (TFL1)-like, and MOTHER of FT and TFL1 (MFT)-like. FT and TFL1 are thought to be molecular switches regulating vegetative and reproductive growth, whereas MFT is phylogenetically ancestral to the two proteins. In other plants, such as tomato, the balance between the activity of tomato FT homolog SINGLE FLOWER TRUSS and that of the TFL1 homolog SELF-FRUNING affects a variety of developmental processes, such as flowering response, reiterative growth, termination cycles, leaf maturation and stem growth [12-14]. In the sugar beet, flowering time is controlled by the interplay between two paralogs of the *Arabidopsis FT* gene that have evolved antagonistic functions. *BvFT2* is functionally conserved with *FT* and is essential for flowering. By contrast, *BvFT1* functions as a flowering terminator (TFL1) to repress flowering, and its down-regulation is crucial for vernalization in beets [15]. Therefore, all three subfamilies of PEBP genes can function as general developmental regulators rather than simple floral initiators or florigens [12].

In this study, we functionally identified an *FT* homolog *JcFT* gene from *Jatropha* by genetic complementation of the *Arabidopsis thaliana ft-10* mutant. We showed how manipulation of *Jatropha* flowering time can lead to yield increase. A rapid breeding technology for *Jatropha* using earlyflowering transgenic plants and marker-assisted selection is also discussed.

Results

Identification of *FT* genes from *Jatropha* and other Euphorbiacious plants

By analysing the database of a sequenced cDNA library prepared from *J. curcas* seeds [16], several *Flowering locus T*-related genes and one *Jatropha curcas Flowering locus T* homolog (*JcFT*) were identified. At the amino acid level *JcFT* is 86% identical to *Arabidopsis* FT. Using the amino acid sequence of *JcFT*, we further identified *FT*-like genes from other Euphorbiacious plants such as castor bean (*Ricinuscommunis*) and cassava (*Manihotesculenta*) by mining data in Phytozome [17]. Phylogenetic analysis of PEBP domain family proteins from various plants indicated that PEBP proteins fall into three groups: the FT group, which promotes flowering; the TFL1/CEN-like group, which prolongs vegetative identity; and the MFT group, which is ancestral to the FT and TFL1 groups. As the *JcFT* protein and FT-like proteins in Euphorbiacious plants are located in the FT-like group, we deduced that this *JcFT* is an FT-like protein (Figure 1A). Using the polymerase chain reaction (PCR) approach, we cloned an *FT* cDNA from a *Jatropha* leaf sample. Southern blot analysis, using the *JcFT* cDNA as a probe, revealed only one copy of the *FT* gene in the *Jatropha* genome (see Additional file 1). That there is only one *FT* homologous gene in the *Jatropha* genome was further confirmed through mining of both the deep sequencing data set of *Jatropha* performed at the Temasek Life Sciences Laboratory (Yan Hong, unpublished data) and the *Jatropha* genome database available online (Sato et al., [10]). Analysis of genomic structures of *FT* homologous genes from Euphorbiaceae showed that they are similar to that of the *Arabidopsis FT* gene (Figure 1B). The second intron of the *JcFT* and the *Ricinuscommunis FT* (*RcFT*) is very big in size (Figure 1B), and is similar to that of the sugar beet *FT* (*BvFT2*) [15].

We investigated the relationship between *JcFT* transcript levels in leaves of different ages and found that the *JcFT* transcript level increased from younger to older leaves (Figure 1C). These data suggest that *FT* expression is correlated with plant age and is important for *Jatropha* flowering regulation.

Transgenic Arabidopsis plants expressing *JcFT* with early flowering phenotype

To determine the roles of *JcFT in planta*, we tested the function of *JcFT* in the model plant *Arabidopsis thaliana*.

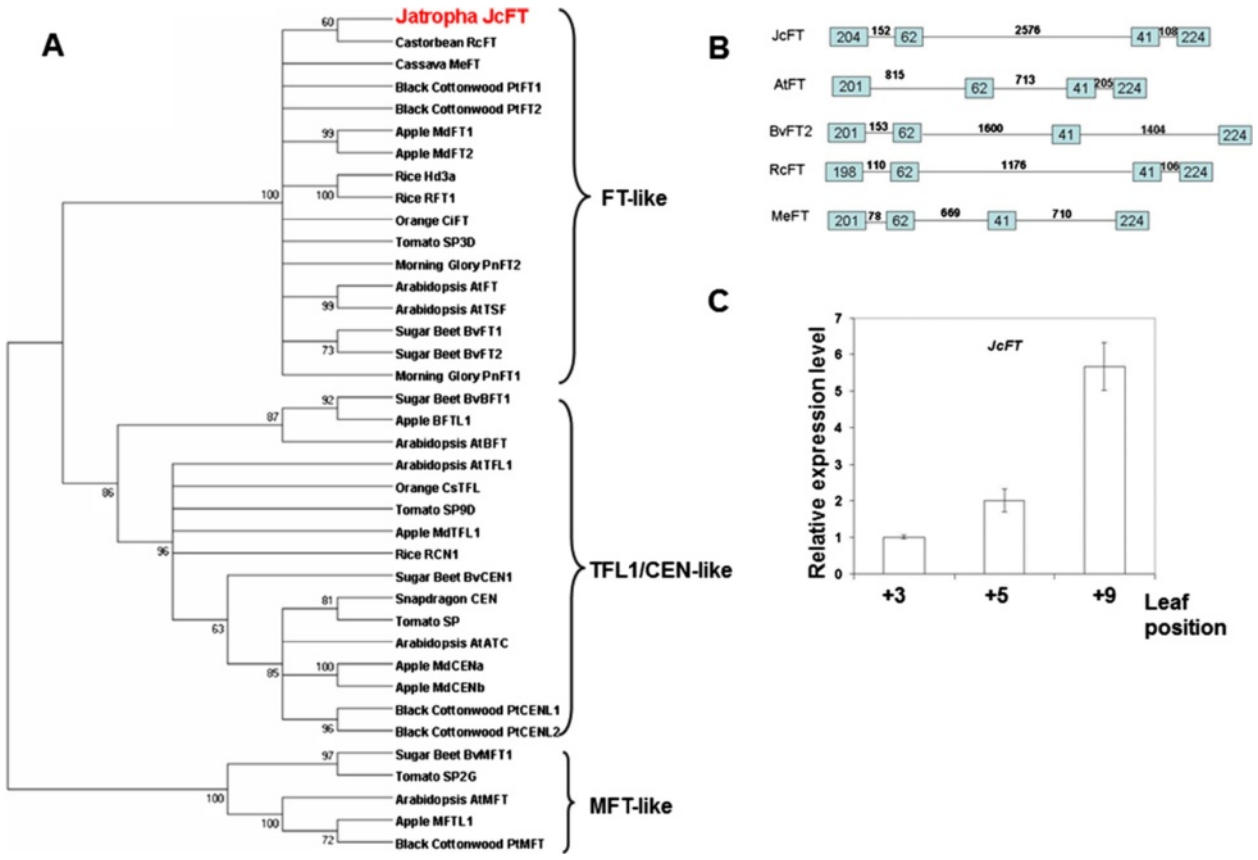

Figure 1 Identification of PEBP-related genes from several members of Euphorbiaceae. (A) Phylogenetic analysis of PEBP family proteins in plants. A Neighbor-Joining phylogenetic analysis of multiple members of the PEBP family from several plant species. Bootstrap values for 1,000 resamplings are shown on each branch. **(B)** Genomic organization of *FT* genes from *Jatropha curcas* (*Jc*), *Arabidopsis thaliana* (*At*), *Beta vulgaris* (*Bv*), *Ricinuscommunis* (*Rc*) and *Manihotesculenta* (*Me*). Boxes and lines represent exonic and intronic regions, respectively. Numbers refer to the length of DNA in base pairs. **(C)** Age-dependent expression of *JcFT* in *Jatropha* leaves. The newly formed leaf upon emergence of the first inflorescence was considered as +1. *JcFT* RNA levels from leaves at three different positions (+3, +5, +9) were measured and normalized with respect to *ubiquitin* mRNA. Values are mean ± SD (n = 3).

First, we constructed a binary vector pCAMBIA 2 × 35S: *JcFT*, containing the coding sequence of *JcFT* under the control of a double *CaMV35S* promoter. After transformation of *Arabidopsis* mutant *ft-10* with pCAMBIA 2 × 35S: *JcFT*, the late flowering phenotype was rescued as indicated by the number of plant leaves at bolting and the final plant size of the complemented mutant (Figure 2A, B and C); by contrast, the *ft-10* mutant remained in vegetative growth (Figure 2D). We further generated *JcFT*-overexpression lines in WT *Arabidopsis* Columbia (Col-0) background. All T1 plants showed expression of *JcFT* transcripts (see Additional file 2). Transgenic plants overexpressing *JcFT* displayed significantly earlyflowering phenotypes under either long day or short day conditions (Table 1). WT (Col-0) plants started bolting on average 16 days after sowing on soil, and their first flowers bloomed 21.5 days after sowing under long day conditions. By contrast, the *JcFT* overexpression #15 line required only 7.5 days for bolting and 13.8 days for blooming (Table 1, Figure 2E, F and G). Under long day conditions, *JcFT* overexpression significantly shortened the bolting time (only 47% of WT in #15) but had no effect on the time from bolting to blooming. The effect of *JcFT* overexpression on reducing the bolting time was even stronger under short day conditions (only 27% of WT in #15), whereas *JcFT* overexpression still did not have any effect on the time from bolting to blooming (Table 1). Overexpression of *JcFT* in *Arabidopsis* not only shortened plant flowering time, but also altered plant morphology such as upward curling of leaf and increased number of inflorescence branches (Figure 2G).

Early flowering by over expression of *JcFT* in *Jatropha*

We further tested the functional roles of *JcFT* in *Jatropha*. Transgenic shoots overexpressing *JcFT* from a *35S* promoter were found to flower as early as the shoot regeneration stage in tissue culture (Figure 3A and B). These shoots with early flowering trait failed to generate roots.

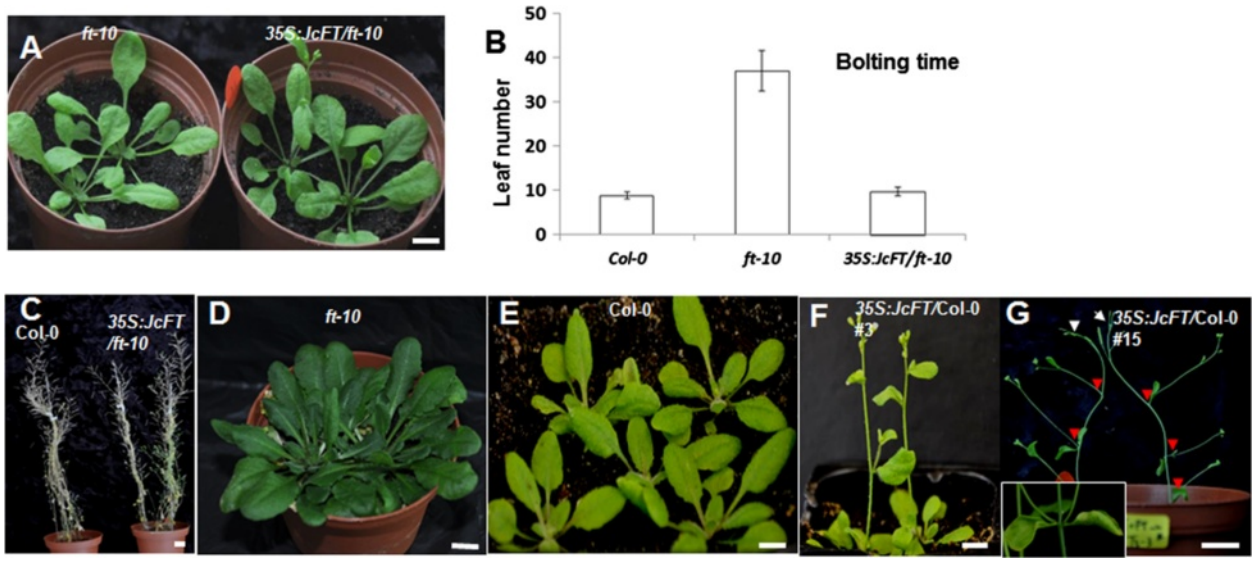

Figure 2 Functional analysis of *JcFT* in *Arabidopsis*. **(A)** Left pot: *Arabidopsis ft-10*; right pot: *35S:JcFT/ft-10*. **(B)** Flowering time for WT (Col-0) control, *ft-10* and *35S:JcFT/ft-10* plants. Values are mean ± SD (n = 8). **(C)** Comparison of plant size of WT and transgenic complementation line of *35S:JcFT/ft-10*. **(D)** *Arabidopsis ft-10* mutant remaining in vegetative growth. **(E)***Arabidopsis* WT (Col-0) control. **(F)** Transgenic line #3 expression *JcFT*. **(G)** Transgenic line #15 with *JcFT* strong expression of *35S:JcFT*/Col-0. The primary shoot is early-terminated by strong expression of *JcFT* (white arrow). Red arrows indicate axillary branches. Inset in **(G)** shows the typical upward curling cauline leaf phenotype in transgenic lines of *35S:JcFT*/Col-0. Bars: 1 cm.

Moreover, it was difficult to graft these shoots onto WT rootstock. These observations prompted us to replace the *35S* promoter with a weaker, synthetic *G10-90* promoter to express *JcFT* (see Additional file 3). We successfully generated 10 transgenic *Jatropha* lines carrying *G10-90::JcFT* transgene. Both Southernblot and reverse transcriptase (RT)-PCR analysis verified the presence of the transgene and *JcFT* overexpression in transgenic *Jatropha* plants (see Additional file 1 and Additional file 4). Figures 4A and 5A show that ectopic expression of *JcFT* strongly accelerated *Jatropha* flowering and fruit setting. Under normal growth conditions in a greenhouse, WT *Jatropha* required around 8 months to produce the first inflorescence. By contrast, only 3.5 months were needed

Table 1 Flowering time of transgenic *Arabidopsis* lines overexpressing *JcFT* under long day (LD) or short day (SD) light conditions

LINE	Bolting		Anthesis		
	Time (Days)	Leaf (Number)	Time (Days)	Rosette leaves (Number)	Cauline leaves (Numbers)
LD					
Col-0	16.00 ± 2.00	8.88 ± 0.83	21.50 ± 1.60	9.63 ± 0.74	1.63 ± 0.74
35S:JcFT/Col-0					
#3	10 ± 1.41	8.9 ± 0.74	16.8 ± 2.20	9.6 ± 1.07	2.9 ± 0.32
#9	11.1 ± 1.73	8 ± 1.41	17.3 ± 0.67	8.7 ± 1.77	2.9 ± 0.74
#15	7.5 ± 0.71	6.1 ± 0.99	13.8 ± 1.69	6.8 ± 0.79	1.7 ± 0.48
SD					
Col-0	42.5 ± 8.90	23.50 ± 8.60	52.50 ± 8.47	25.00 ± 7.33	5.63 ± 0.74
35S:JcFT/Col-0					
#3	11.8 ± 1.55	7.3 ± 0.95	23.3 ± 3.30	7.4 ± 0.84	6 ± 2.21
#9	14.5 ± 1.78	7.6 ± 1.35	25.4 ± 2.55	9.2 ± 1.40	5.1 ± 1.85
#15	11.3 ± 2.06	6.9 ± 70.74	22 ± 2.54	7.4 ± 0.70	2 ± 0.82

T4 homozygous seedlings were analyzed. The day of sowing was taken as day 0. Values are mean ± SD (n = 8).

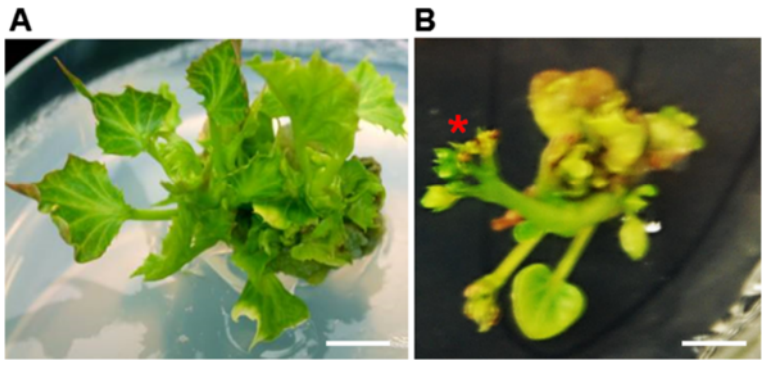

Figure 3 Early flowering phenotype of transgenic *Jatropha* expressing *35S:JcFT*. (A) Transgenic *J. curcas* shoots expressing *35S:GFP*. Bar = 1 cm. **(B)** Transgenic *J. curcas* shoots expressing *35S:JcFT*. *indicates inflorescences. Bar = 1 cm.

to obtain the first inflorescence for 10 primary independent *JcFT* overexpression lines (Figure 5A). Seeds of two early flowering T0 lines *JcFT* overexpression lines (#33 and #43) were germinated on plates and the seedlings transferred into soil at the two-true-leafstage together with the WT control. The flowering times of the T1 plants of #33 and #43 were dramatically shortened to 1 to 2 months, from 8 months (Figures 4E, 5B and Additional file 5). The great reduction of time to flowering considerably shortened the time for seed set, which is one of the key determinant factors for *Jatropha* yield. We further found that the *JcFT* expression level was correlated with flowering time in *Jatropha* (Figure 5B). However, there was no significant role of *JcFT* on the time from in florescene emergence to blooming, and the time from seed set to seed maturation (data not shown).

The time for flowering dormancy, a period between the initiation of inflorescence and the next, was also drastically reduced by *JcFT* overexpression as indicated by the number of leaves developed between the first to the second inflorescence (Compare Figure 4A with B, and Figure 5C). Besides early flowering, *JcFT*-overexpressing plants produced more leaves but with smaller leaf size (Figure 4A, E and Additional file 5).

Overexpression of *JcFT* was found to also change the *Jatropha* architecture. Figure 4D and F show a typical WT *Jatropha* sympodial unit structure including two inflorescences and two lateral shoots. The sympodial unit structure in *JcFT*-overexpressing plants included only one inflorescence and two shoots (Figure 4D, G and H). Due to the short dormancy time, more branches were also found in *JcFT*-overexpressing *Jatropha* plants (Figures 4A, I and 5D). Importantly, early flowering *JcFT*-overexpressing *Jatropha* consistently produced three times more seeds without compromising seed weight (Figures 4A, K, 5E, F and G), indicating the potential agronomic value of *JcFT* overexpression.

In addition to genetics, environmental conditions highly affect plant growth and flowering time, especially for *Jatropha*, a tropical plant. Our data showed that the early flowering time trait obtained by *JcFT* ectopic expression was not affected by lower temperature (22°C vs 28°C as optimal temperature, Figure 5H).

Graft-transmissible action of *JcFT*

In general, it takes 8 months for transgenic *Jatropha* to develop the first inflorescence under greenhouse conditions. Therefore, a method to shorten flowering time will be beneficial for the development of genetically modified *Jatropha*. As a mobile, long-distance signal, it is generally believed that FT is produced in the leaf and is transported to the shoot apex, where it triggers floral morphogenesis. We asked whether *Jatropha* FT protein can act as a mobile and graft-transmissible signal to promote flowering. To this end, we used *JcFT*-overexpressing plant (#43) as a root stock and a high oleic acid line of transgenic *Jatropha* (X8-FAD2 RNAi #34) as scion [18]. Figure 6 shows that FT overexpression in the root stock was capable of promoting flowering in the recipient scion. A parallel control experiment showed that a WT *Jatropha* root stock had no effect on flowering time of the grafted scion. The use of transgenic *JcFT*-overexpressing rootstock enabled us to save 4 months in seed production of the T0 generation of transgenic *Jatropha*. More interestingly, the role of *JcFT* on sympodial unit structure was also found to be graft-transmissible (Figure 6F).

Discussion

Regulation of flowering time of biodiesel plants has the potential to increase yield. Using *Miscanthussacchariflorus,* Jensen et al. (2013) found that delayed flowering results in a greater than 50% increase in biomass [19]. Here, we demonstrate that direct manipulation of the florigen FT could yield a larger number of seeds. The great reduction of time to flowering considerably shortened the time for seed set, which is one of the key determinant factors for *Jatropha* yield. Overexpression of *JcFT* was found to also change the *Jatropha* architecture to semidwarf stature

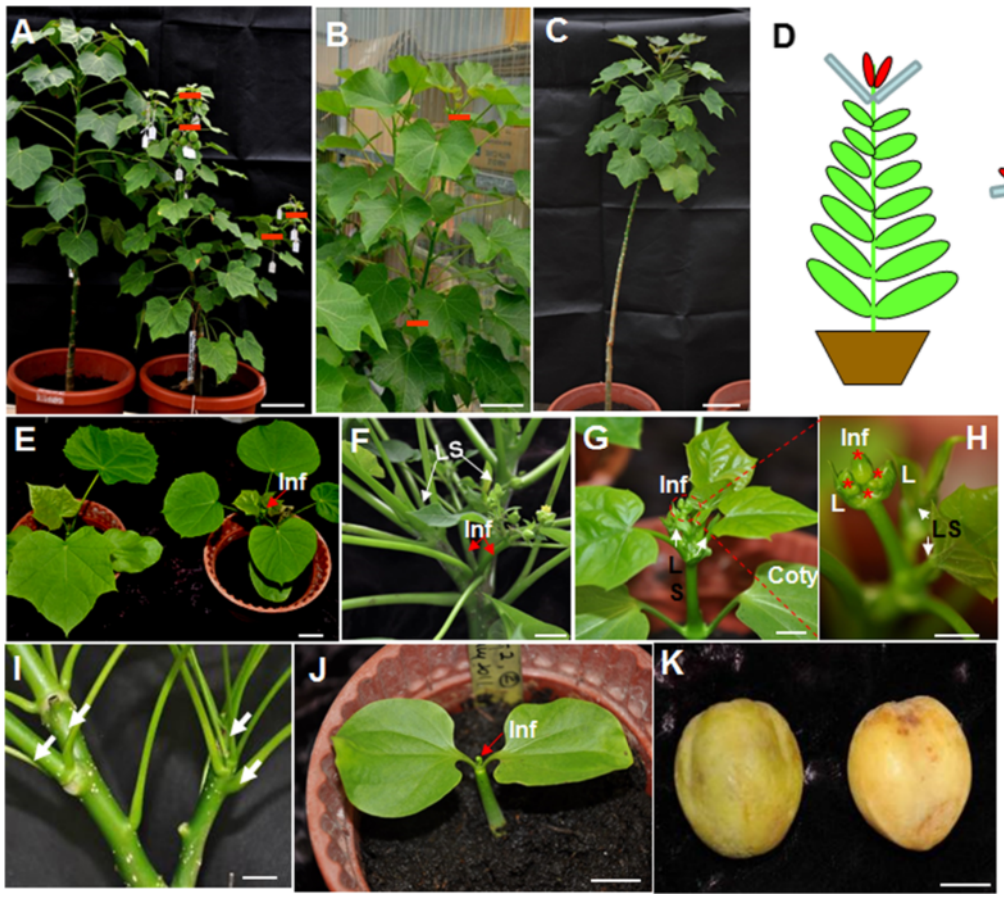

Figure 4 Functional analysis of *JcFT* in *Jatropha*. **(A)** Comparison of plant architecture of *JcFT* transgenic T1 plant (#43, right) with WT *Jatropha* plant. Red lines indicate the leaf position of the first and second inflorescences. Bar: 10 cm. **(B)**: A 12-month old WT *Jatropha* plant. Red lines indicate the leaf position of the first and second inflorescences. Bar: 10 cm. **(C)** Segregated *Jatropha* transgenic T1 plant (#43) showing similar flowering traits as WT *Jatropha* plant. Bar: 10 cm. **(D)** Schematic diagram of plant architectures in WT and *JcFT*-overexpressing *Jatropha* plants. **(E)** Comparison of WT *Jatropha* (left) and *JcFT*-overexpressing transgenic T1 plant (#33, right) with early inflorescence (Inf). Bar: 1 cm. Besides early flowering, *JcFT*-overexpressing plants produce smaller leaves but with higher leaf number. Bar: 1 cm. **(F)** Typical WT *Jatropha* sympodial unit structure: two inflorescences (Inf) and two lateral shoots (LS). **(G)** and **(H)** Enlarged view showing sympodial unit structure of *JcFT*-overexpressing transgenic T1 plant (#33): one inflorescence (Inf) and two lateral shoots (LS). Coty: cotyledon. White arrow: lateral shoots. Star: flower bud. L: leaf. Bar: 1 cm. **(I)** Increased branching in *JcFT*-overexpressing transgenic *Jatropha*. Bar: 1 cm. **(J)** A *JcFT* transgenic T1 plant (#33) with strong phenotype showing extreme early flowering. Note that the plant produces one single flower at the cotyledon stage. Bar: 1 cm. **(K)** Comparison of fruit size of WT *Jatropha* (left) and transgenic *Jatropha* plant overexpressing *JcFT* (right). Bar: 1 cm.

(Figure 4D), which is ideal for mechanical harvesting of seeds to reduce labor costs. Not only flowering time, but also the inflorescence structure was affected by overexpression of *JcFT* in plants with distinct growth habits, monopodial *Arabidopsis* and sympodial *Jatropha* (shown in Figures 2 and 4). Overexpression of *JcFT* resulted in one instead of two inflorescences in one sympodial unit in transgenic *Jatropha*. No branching inflorescence phenotype was found in the loss-of-function *Arabidopsis TFL1* mutant. TFL1 and FT have highly conserved amino acid sequences but opposing functions. Previous studies in *Arabidopsis* have suggested that an antagonistic interaction between the *TFL1* and floral meristem identity genes, such as *LEAFY* (*LFY*) and *APETALA1* (*AP1*), regulates the inflorescence branching pattern [9]. There are two TFL1-like proteins in the *Jatropha* genome [10,20]. The roles of *Jatropha TFL1* orthologs in the determination of flowering traits are being investigated by our group [20]. Nevertheless, the observed impact on plant architecture, that of increasing the number of branches in *Jatropha* by manipulation of the florigen FT, is likely an indirect consequence of the early and rapid initiation of flowering, rather than a direct effect on branch initiation, since each flowering event in *Jatropha* is accompanied by a subsequent branching event, each of which terminates in a second flowering event and subsequent branching.

Other than flowering, FT-like proteins in plants have also been recognized as major regulatory factors in a

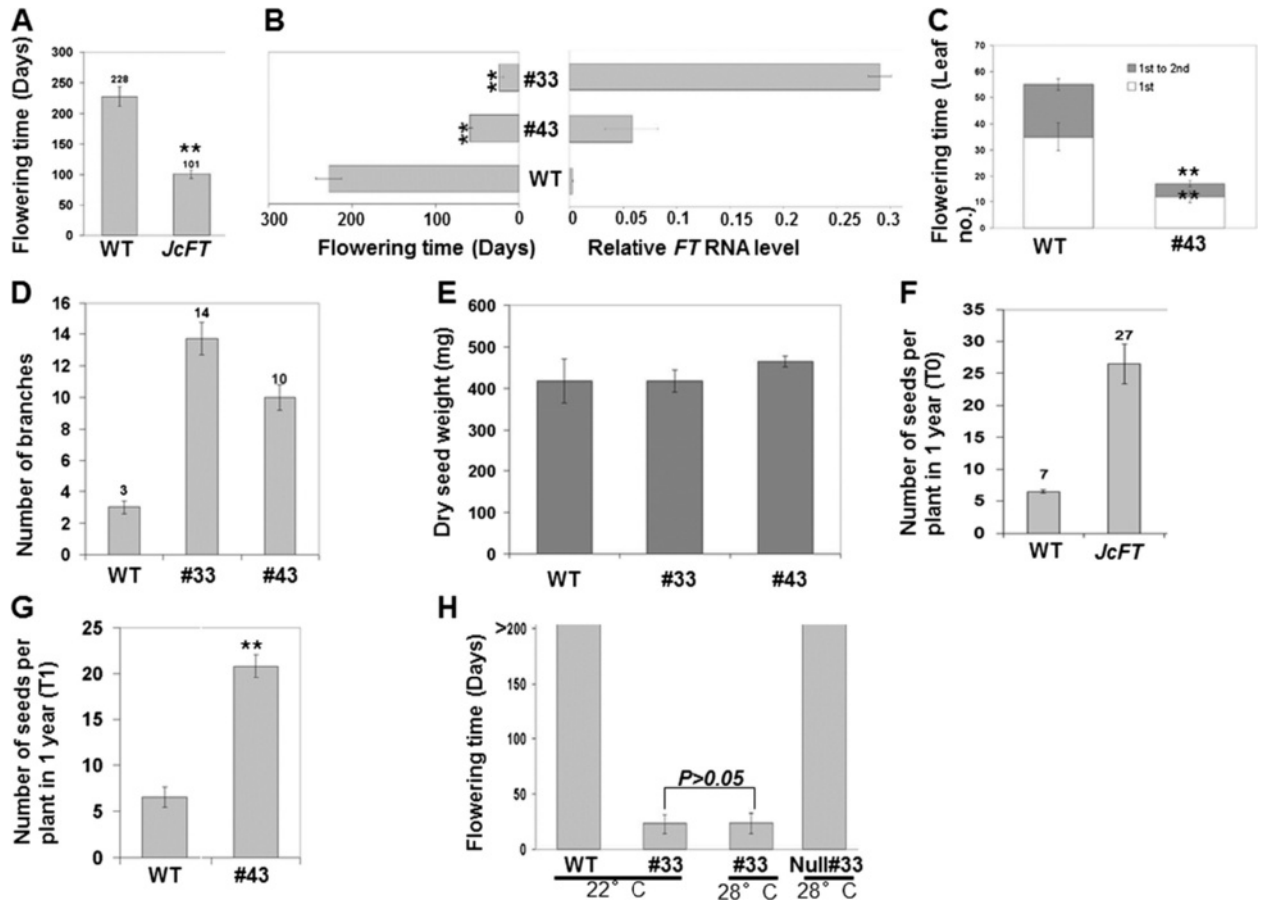

Figure 5 Agronomic traits of early flowering *Jatropha* by *JcFT* over expression. (A) Flowering time in T0 *Jatropha* plant overexpressing *JcFT*. Flowering time was scored by the number of days from transplantation to soil to the day of first inflorescence emergence. Values are mean ± SD (n = 10). **indicate $P < 0.01$. **(B)** Correlation of early flowering (left panel) with *JcFT* expression level (right panel) in T1 *Jatropha* plants (#33 and #43).Values are mean ± SD (n = 3).**indicate $P < 0.01$. **(C)** *JcFT* overexpression reduces the time for flowering dormancy. The dormancy time is indicated by the numbers of leaves formed between the first and the second inflorescence. Values are mean ± SD (n = 3). **indicate $P < 0.01$. **(D)** Comparison of branch number of WT plant and two T1 *JcFT* overexpression lines (#33 and #43). Values are mean ± SE (n = 5). **(E)** Comparison of dry seed weight of WT and two T1 *JcFT* overexpression lines (#33 and #43). Values are mean ± SE (n = 10). **(F)** Comparison of seed number of WT plant and T0 *JcFT* overexpression lines (n = 4). **(G)** Comparison of seed number of WT plant and T1 *JcFT* overexpression line #43. * and ** indicate $P < 0.05$ and $P < 0.01$, respectively. Values are mean ± SE (n = 5). **(H)** Early flowering trait induced by *JcFT* overexpression was insensitive to low temperature. Flowering time was scored by the number of days from seed germination to the day of first inflorescence emergence. T1 transgenic plants of line #33 were used. Values are mean ± SD (n = 3). Null segregant derived from #33 (Null #33) was used as a control.

number of developmental processes including stomatal control and tuberization. These multifunctional roles of FT-like proteins are derived from extensive gene duplication that occurred during evolution. As the gene evolutionary process occurred independently in nearly all modern angiosperm lineages, it is essential to determine the spatiotemporal pattern of ectopic FT expression to minimize its negative effect on normal vegetative growth in plants [21]. An alternative way is to induce expression of FT-like proteins by using, for example, an ethanol-inducible promoter [21,22]. Controllable flowering allows the synchronization of fruit set and collection, thus reducing labor cost, although more research is needed on the feasibility of chemical induction on an industrial scale.

Precise control of flowering time is a critical developmental process that determines the reproductive success of flowering plants. Our data demonstrated that JcFT is a mobile signal to control flowering time and a major flowering integrator in *Jatropha*. The earlyflowering lines reported here provide valuable germplasm, especially for marginal land and mountainous regions with conditions of poor light and high altitude (>500 meters above sea level), for example, the Sichuan, Yunnan and Guizhou provinces in China, to produce seeds in a cost-effective way. These lines can also be used to shorten the development time for other GM traits by grafting as shown in Figure 6. Transgenic seeds obtained from grafting can be used to integrate with

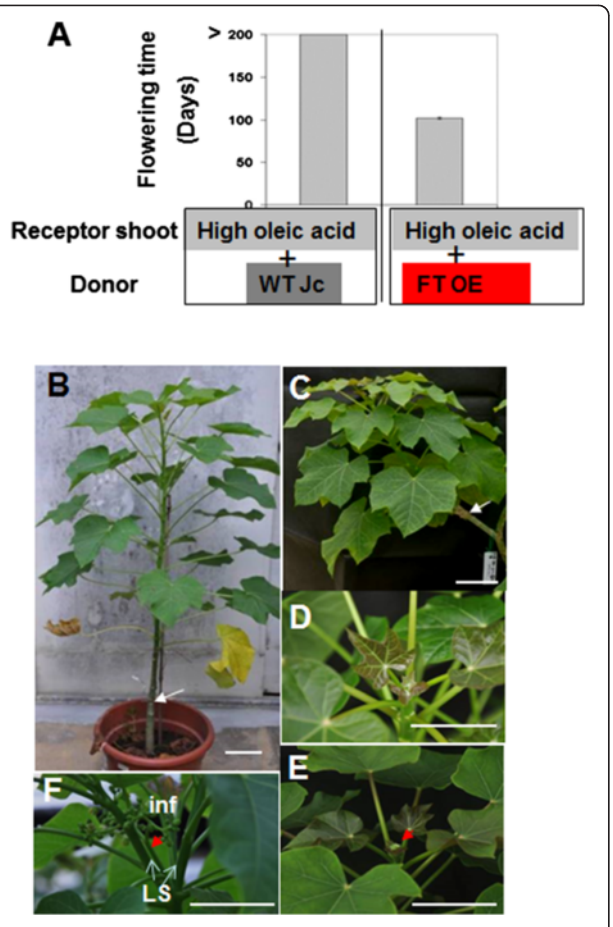

Figure 6 Graft-transmissible action of *JcFT* to reduce flowering time of other genetically modified *Jatropha* plants. (A) Comparison of flowering time of high oleic acid (*X8-34*) transgenic *Jatropha* shoot grafted to root stock of WT or *JcFT*-overexpressing plants. Flowering time refers to the number of days from the day of grafting to the day of emergence of the first inflorescence. **(B)** Flowering time of high oleic acid (*X8-34*) transgenic *Jatropha* not affected by grafting on WT *Jatropha* root stock. White arrow indicates the grafting site. **(C)** Flowering time of high oleic acid (*X8-34*) transgenic *Jatropha* was reduced by grafting onto *JcFT* transgenic T1 plant (#43) root stock. White arrow indicates the grafting site. **(D)** Comparison between plants grafted to WT control root stock and *JcFT* #43 root stock. **(D)**, WT control root stock. **(E)** and **(F)**, *JcFT* # 43 root stock. **(F)** Enlarged view of sympodial unit structure. LS: lateral shoots. Red arrow indicates the first inflorescence. Bar: 10 cm.

traditional breeding processes to accelerate advancement of transgenic traits.

In comparison to herbaceous plants, the breeding of trees is more time-consuming owing to their long generation time. Shortened juvenility and precocious flowering are therefore important breeding goals. Flower initiation has been intensively studied in *Arabidopsis*, and orthologs/homologs of genes for LFY, AP1, and TFL1 have been cloned from apple trees among others [23-25]. There are a few successful reports on a rapid breeding program in apple by genetic modification of the flowering pathway [26-28]. A similar strategy can be used in breeding programs of *Jatropha*. Viral-based systems such as virus- induced gene silencing (VIGS) or transient expression of flowering time genes can also be good alternatives to shorten flowering time to accelerate the breeding process [29-31].

Conclusions

We generated early flowering transgenic *Jatropha* plants that accumulate higher levels of the florigen FT. Not only early flowering but also plant growth was affected in *JcFT*-overexpressing lines. More seeds can be produced in a shorter time frame by reducing flowering time in *Jatropha*, suggesting the possibility to increase seed yield by flowering time manipulation.

Methods

Plant materials and growth condition

Seeds were obtained from *Jatropha curcas* (*Jc*-MD) elite plants preselected by Drs. Yan Hong and Chengxin Yi [32]. All control or transgenic plants were grown in a biosafety level 2 greenhouse [18]. Plant management, such fertilization, pesticide spraying, watering and artificial fertilization, was carried out according to normal practice.

JcFT cDNA isolation and plasmid construction

The *Jatropha curcas Flowering locus T* (*JcFT*) gene was first identified from the database of a sequenced cDNA library prepared from *Jatropha* seeds [16]. A full-length cDNA fragment was PCR-amplified with forward primer 5'-ATA AGTCGACATGAGGGATCAATTTAGAGA-3' and reverse primer 5'-TTATTTCTAGATCACCGTCTCCGTCCTCC GGT-3'. The PCR fragment was inserted in the sense orientation into the *Sal* I/*Xab*I sites of the pCABMIA1300-3HA vector.

To generate β-estradiol-inducible *JcFT* overexpression lines, we used a cDNA fragment encoding JcFT protein. This fragment was PCR-amplified with forward primer 5'-ATAACTCGAGATGAGGGATCAATTTAGAGA-3' and reverse primer 5'-TTATTACTAGTTCACCGTCTCCGT CCTCCGGT-3'. The PCR fragment was inserted in the sense orientation into the *Xho*I/*Spe*I sites of the pX7-GFP vector as described previously by Qu et al. [18]. The construct was named pX7-JcFT.

Jatropha transformation

We used the transformation protocol described by Qu et al. [18].

Nuclear acid extraction and analysis

DNA and RNA were isolated and analyzed according to previously described methods [18]. For quantitative PCR analysis, two primers were used for the *JcFT* gene

(F: GTTACTTATAATCACAGAGAGGT, R: TCTCATAG CACACTATCTCTTGC). The *Jatropha UBQ* transcript served as an internal control for RNA samples with primers (F: GAGGTGGAAAGCTCAGATACAATT, R: AAAGTG ATGGTCTTTCCGGTCAATG). For Southern blot analysis, nylon Hybond-N^+ membranes were hybridized with DNA probes encoding the *HPT* or *JcFT* open reading frame [18].

Phylogenetic analysis

Multiple alignments were generated using Clustal W. The Neighbor-Joining method in MEGA4 was used to reconstruct a phylogeny tree. Bootstrap analysis was performed to estimate nodal support on the basis of 1,000 resamplings. The phylogenetic tree was constructed with proteins including sugar beet (*Beta vulgaris – BvFT1*, No. HM448910; *BvFT2*, No.HM448912; *BvBFT1*, No.HM448916; *BvCEN1*, No. HM448914; and *BvMFT1*, No. HM448918), apple (*Malus x domestica– MdFT1*, No.AB161112; *MdFT2*, No. AB458504; *MdCENa*, No.AB366641; *MdCENb*, No.AB3 66642; *MdTFL1*, No.AB052994; *BFTL1*, No.EB138045; and *MFTL1*, No. EB134193), black cottonwood (*Populustrichocarpa– PtFT1*, No.XM_002311228; *PtFT2*, No.XM_002316 137; *PtCENL1*, No.XM_002328224; *PtCENL2*, No. XM_ 002312775; and *PtMFT*, No. XM_002321471), morning glory (*Ipomoea nil – PnFT1*, No. EU178859; and *PnFT2*, No. EU178860), orange (*Citrus sinensis– CiFT*, No. CK9 39149; and *CsTFL*, No. AY344244), rice (*Oryza sativa – Hd3a*, No. NM_001063395; *RFT1*, No.NM_001063394; *RCN1*, Os11g05470), snapdragon (*Antirrhinum majus– CEN*, No.AJ251994), arabidopsis (*Arabidopsis thaliana– FT*, No.NM_105222; *TFL1*, No.NM_120465; *MFT*, No. NM_101672; *ATC*, No.NM_128315; *TSF*, No. NM_118156; and *BFT*, No. NM_125597), and tomato (*Solanumlycopersicum– SP*, No.U84140; *SP3D*, No.AY186735; *SP2G*, No. AY186734; and *SP9D*, No. AY186738). The amino acid sequences encoded by *JcFT* (*Jatropha curcas FT), RcFT* (*Ricinuscommunis FT)* and *Me FT (Manihotesculenta)* are listed in Additional file 6 and [20].

Additional files

Additional file 1: Figure S1. Southern blot analysis of transgenic *Jatropha* lines overexpressing *JcFT*. Four independent transgenic lines were analyzed using wild-type (WT) *Jatropha* as a negative control. Southern blots were hybridized to a *JcFT* probe (left) or a *hygromycinphosphotransferase* (*hgy*) probe (right). Molecular size markers (kb) are given on the right.

Additional file 2: Figure S2. RT-PCR analysis of *JcFT* and *AtFT* transcripts in transgenic *Arabidopsis* plants expressing *35S:JcFT*. Total RNAs were extracted from leaves of 25 day-old plants. Nine independent transgenic lines were analyzed. WT *Arabidopsis thaliana* (Col-0) was used as a negative control. *JcFT: Jatropha curcas Flowering locus T, AtFT: Arabidopsis thaliana Flowering locus T, AtUBQ: Arabidopsis thaliana ubiquitin 10.*

Additional file 3: Figure S3. Schematic diagrams of transgene before and after marker excision and location of probes used for Southern blot analysis.

Additional file 4: Figure S4. RT-PCR analysis of *JcFT* transcripts from transgenic *Jatropha* plants overexpressing *JcFT*. Total RNAs were extracted from leaves of 30 day-old plants of WT *Jatropha* (*Jc*-MD) and transgenic lines (#8 and #33) overexpressing *JcFT*.

Additional file 5: Figure S5. Heritable early flowering phenotypes in T1 *Jatropha* plant overexpressing *JcFT*. (A) (#33) and (B) (#19 right plant) show two independent T1 lines. WT plant in (B)is negative control. Bar: 1 cm in (A) and 10 cm for (B). Red arrows indicate fruits.

Additional file 6: The amino acid sequences encoded by *JcFT* (*Jatropha curcas FT), RcFT (Ricinuscommunis FT)* and *MeFT (Manihotesculenta FT).*

Abbreviations

AP1: *APETALA1*; *BFT*: *Brother of FT*; *FT*: Flowering locus T; *LFY*: *LEAFY*; *MFT*: *Mother of FT*; *TFL1*: *TERMINAL FLOWER1*; GM: genetic modification; bp: base pairs; *Jc*-MD: *Jatropha curcas* MD isolate; RT: reverse transcriptase; VIGS: virus-induced gene silencing.

Competing interests

Patents relating to the *JcFT* and its usage have been filed by the Temasek Life Sciences Laboratory.

Authors' contributions

JY and NHC designed the experiments, analyzed the data and drafted the manuscript. JY, YFG, BPZ, and JQ performed vector construction, genotyping, molecular analysis, flowering traits collection and analysis. HZM did the *Jatropha* transformation. All authors read and approved the final manuscript.

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