**Jatropha curcas** ortholog of tomato MADS-box gene 6 (**JcTM6**) promoter exhibits floral-specific activity in *Arabidopsis thaliana*

**Jing-Xian Wang**, **Xin Ming**, **Yan-Bin Tao** Corresp., **Zeng-Fu Xu** Corresp.

1 School of Life Sciences, University of Science and Technology of China, Hefei, Anhui, China  
2 CAS Key Laboratory of Tropical Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Innovation Academy for Seed Design, Chinese Academy of Sciences, Xishuangbanna, Menglun, Mengla, Yunnan, China  
3 Center of Economic Botany, Core Botanical Gardens, Chinese Academy of Sciences, Menglun, Mengla, Yunnan, China

Corresponding Authors: Yan-Bin Tao, Zeng-Fu Xu  
Email address: taoyanbin@xtbg.ac.cn, zfxu@xtbg.ac.cn

**Background.** *Jatropha curcas* L., a perennial oilseed plant, is considered as a promising feedstock for biodiesel production. Genetic modification of flowering characteristics is critical for *Jatropha* breeding. However, analysis floral-specific promoters in *Jatropha* is limited.

**Methods.** In this study, we isolated the *Jatropha* ortholog of **TM6** (**JcTM6**) gene from *Jatropha* flower cDNA library and detect the expression pattern of **JcTM6** gene by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). We isolated a 1.8-kb fragment from the 5' region of the **JcTM6** gene and evaluated its spatiotemporal expression pattern in *Arabidopsis* using the **β-glucuronidase** (**GUS**) reporter gene and *Arabidopsis* **ATP/ADP isopentenyltransferase 4** (**AtIPT4**) gene, respectively.

**Results.** **JcTM6** was identified as a flower-specific gene in *Jatropha*. As expected, **JcTM6** promoter was only active in transgenic *Arabidopsis* flowers with the strongest activity in stamens. Moreover, **JcTM6**:**AtIPT4** transgenic *Arabidopsis* showed a phenotype of large flowers without any alterations in other organs. Furthermore, deletion of the region from -1,717 to -876 bp resulted in the disappearance of promoter activity in stamens but an increase in promoter activity in young leaves, sepals, and petals. Deletion analysis suggests that the -1,717- to -876-bp promoter fragment contains regulatory elements that confer promoter activity in stamens and inhibit activity in young leaves, sepals, and petals.
Jatropha curcas TOMATO MADS-BOX GENE 6 (JcTM6) promoter exhibits floral-specific activity in Arabidopsis thaliana

Jing-Xian Wang¹,², Xin Ming¹,², Yan-Bin Tao²,³,*, and Zeng-Fu Xu²,³,*

¹School of Life Sciences, University of Science and Technology of China, Hefei, Anhui, China
²CAS Key Laboratory of Tropical Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Innovation Academy for Seed Design, Chinese Academy of Sciences, Menglun, Mengla, Yunnan, China
³Center of Economic Botany, Core Botanical Gardens, Chinese Academy of Sciences, Menglun, Mengla, Yunnan, China

Corresponding authors:
Yan-Bin Tao
88 Xuefu road, Kunming, Yunnan, 666303, China
Email address: taoyanbin@xtbg.ac.cn
Zeng-Fu Xu
88 Xuefu road, Kunming, Yunnan, 666303, China
Email address: zfxu@xtbg.ac.cn

Abstract
Background. Jatropha curcas L., a perennial oilseed plant, is considered as a promising feedstock for biodiesel production. Genetic modification of flowering characteristics is critical for Jatropha breeding. However, analysis floral-specific promoters in Jatropha is limited.

Methods. In this study, we isolated the Jatropha ortholog of TM6 (JcTM6) from Jatropha flower cDNA library and detect the expression pattern of JcTM6 gene by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). We isolated a 1.8-kb fragment from the 5' region of the JcTM6 gene and evaluated its spatiotemporal expression pattern in Arabidopsis using the β-glucuronidase (GUS) reporter gene and Arabidopsis ATP/ADP isopentenyltransferase 4 (AtIPT4) gene, respectively.

Results. JcTM6 was identified as a flower-specific gene in Jatropha. As expected, JcTM6 promoter was only active in transgenic Arabidopsis flowers with the strongest activity in stamens. Moreover, JcTM6:AtIPT4 transgenic Arabidopsis showed a phenotype of large flowers without any alterations in other organs. Furthermore, deletion of the region from –1,717 to –876 bp resulted in the disappearance of promoter activity in stamens but an increase in promoter activity in young leaves, sepals, and petals. Deletion analysis suggests that the –1,717 to –876-bp promoter fragment contains regulatory elements that confer promoter activity in stamens and inhibit activity in young leaves, sepals, and petals.
Introduction

Promoter plays a significant role in gene expression regulation. Three types of promoters are currently employed in plant genetic engineering, constitutive, tissue-specific, and inducible promoters (Muthusamy et al. 2017; Potenza et al. 2004). Tissue-specific promoters drive transgene expression in a specific spatiotemporal pattern, which is effective in the modification of agronomic traits of crop plants. For example, the rice (Oryza sativa L.) gene OsGA2ox1 encodes a gibberellin (GA) catabolic enzyme, GA 2-oxidase (Lester et al. 1999; Martin et al. 1999; Thomas et al. 1999). When the expression of OsGA2ox1 was driven by the constitutive Actin promoter, transgenic rice plants failed to set grains. To prevent sterility, the promoter of a GA biosynthesis gene, OsGA3ox2, which encodes GA 3-oxidase and is specifically active in shoots, was used to control the expression of OsGA2ox1. As expected, transgenic rice exhibited a semi-dwarf phenotype with normal yield (Sakamoto et al. 2003). GA 20-oxidase is a GA biosynthetic enzyme in plants (Coles et al. 1999). In poplar (Populus spp.), overexpression of the Pinus densiflora GA 20-oxidase gene (PdGA20ox) under the control of the constitutive 35S promoter increased GA levels, thereby accelerating stem growth and plant biomass; however, transgenic poplar plants showed poor leaf development and root growth. When the PdGA20ox gene was driven by a xylem-specific promoter DX15 from poplar, the undesirable phenotypes were reduced (Jeon et al. 2016).

Physic nut (Jatropha curcas L.) is an oilseed plant belonging to the Euphorbiaceae family. The seed oil of Jatropha is a promising feedstock for biodiesel production (Kumar & Sharma 2008). However, low seed yield, which is mainly caused by low female: male ratio, is a long-standing problem in Jatropha (Raju & Ezradanam 2002; Rao et al. 2008). Jatropha is a monoecious plant species with male and female flowers on the same inflorescence, and the average ratio of female to male flowers is 1:13–1:29 (Raju & Ezradanam 2002; Tewari et al. 2007). There are 100–300 flowers in each inflorescence of Jatropha, which only produce approximately 10 fruits (Kumar & Sharma 2008; Pan & Xu 2011). Hence, genetic modification of flowering characteristics is critical for Jatropha breeding. Floral-specific promoters play crucial roles in this modification because they can drive efficient expression of functional genes in flowers without affecting the vegetative growth of plants. In pea (Pisum sativum), the PsEND1 promoter exhibits anther-specific activity. Expression of the ribonuclease gene barnase (Gardner et al. 2009) in Arabidopsis and Brassica napus under the control of the PsEND1 promoter causes anther ablation at an early developmental stage, leading to male sterility (Roque et al. 2007). Arabidopsis APETALA3 (AP3) promoter was identified as a floral-specific promoter in petunia (Petunia x hybrida). Expression of the Agrobacterium tumefaciens isopentenyltransferase (ipt) gene under the control of the AtAP3 promoter in petunia increased the flower size, without affecting vegetative development (Verdonk et al. 2008). However, analysis of promoters, especially floral-specific promoters, in Jatropha is limited. Although the Jatropha APETALA1 (JcAP1) promoter was recently identified as a reproductive tissue-specific promoter showing high activity in inflorescence buds and seeds (Tao et al. 2016), it is not sufficient to address transgene expression analysis in Jatropha.
In this study, we isolated the promoter of the *Jatropha* ortholog of *TOMATO MADS-BOX GENE 6* (*JcTM6*), a floral-specific gene. The activity of *JcTM6* promoter was evaluated in *Arabidopsis* using the β-glucuronidase (*GUS*) reporter gene. The results of GUS staining showed that the *JcTM6* promoter was active only in flowers, with the highest activity in stamens. By using this promoter directed a cytokinin biosynthesis gene, *Arabidopsis ATP/ADP isopentenyltransferase 4* (*AtIPT4*) gene (*Li et al. 2010*), only flower phenotype was changed in transgenic *Arabidopsis*. Furthermore, deletion analysis showed that an approximately 0.85-kb fragment of the *JcTM6* promoter (–1717 to –876 bp) is critical for maintaining its floral-specific expression pattern.

**Materials & Methods**

**Plant materials**

Plants of *Jatropha curcas* and *Arabidopsis thaliana* ecotype Columbia (Col-0) were used in this study. *Jatropha* plants were cultivated in Xishuangbanna, Yunnan Province, China, as described previously (*Pan & Xu 2011*). *Arabidopsis* plants were grown in an environmentally controlled room at 22°C under 16-h light/8-h dark photoperiod.

**JcTM6 expression analysis**

The *JcTM6* gene (GenBank accession no. MN820724) was identified in the *Jatropha* flower cDNA library (*Chen et al. 2014*). Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed to examine the expression level of *JcTM6* in different organs of *Jatropha* (roots, stems, young leaves, mature leaves, inflorescence buds, female flowers, male flowers, pericarps and seeds at 42 days after pollination (DAP), male sepal and petals, stamens, female sepal and petals, and pistils) and *Arabidopsis* (leaves and flowers). Total RNA from each organ was isolated using the silica particle extraction method (*Ding et al. 2008*). Then, qRT-PCR was performed as previously described in Tao (2015). The *JcGAPDH* and *AtActin* were used as an internal control for data normalization. Primers used for qRT-PCR are listed in Table 1. The results of qRT-PCR were obtained from three biological replicates and three technical replicates.

**Cloning of the upstream region of JcTM6**

The 5′ region of *JcTM6* was isolated from *Jatropha* genomic DNA by genome walking (*Siebert et al. 1995*) according to the Genome Walker™ Kit Universal User Manual (Clontech). Then, the full-length *JcTM6* promoter was amplified using the primers, XT405 and XT408. The PCR product was cloned into the pGEM-T Easy vector. Putative cis-acting elements in the *JcTM6* promoter were analyzed using the PLACE database (*Higo et al. 1999*). The transcriptional start site of *JcTM6* was identified as previously described in Tao (2016). Primers employed for genome walking and 5′-RACE are listed in Table 1.

**Construction of JcTM6 promoter-GUS fusion and Arabidopsis transformation**
To generate the JcTM6:GUS plasmid, XbaI and BamHI were used to digested pBI101 (Jefferson et al. 1987), and the pGEM-T Easy vector containing the JcTM6 promoter, respectively. The resulting fragments were ligated using the T4 DNA Ligase (Promega) to generate the JcTM6:GUS fusion construct. Then, the JcTM6:GUS plasmid was introduced into Agrobacterium tumefaciens EHA105 by electroporation (GenePulser Xcell; Bio-Rad), and the transformed A. tumefaciens cells were used to transform Arabidopsis plants by the floral dip method (Clough & Bent 1998).

Histochemical GUS staining assay
To perform GUS staining, various tissues of transgenic Arabidopsis were submerged in the GUS assay buffer (50 mM sodium phosphate [pH 7.0], 0.5 mM K₃Fe(CN)₆, 0.5 mM K₄Fe(CN)₆·3H₂O, 0.5% Triton X-100, and 1 mM X-Gluc) and vacuum-infiltrated for 15 min. Then, tissues were incubated overnight at 37°C, cleared in 70% ethanol (Jefferson et al. 1987), and examined under a stereomicroscope (Leica M80). The results of GUS staining were obtained from five biological replicates and three technical replicates.

Results
JcTM6 expression in Jatropha
We identified the JcTM6 cDNA (GenBank accession no. MN820724) from our Jatropha flower cDNA library constructed previously (Chen et al. 2014). JcTM6 encodes a 230-amino acid protein, which shows high similarity to TM6 homologs from other plant species (Fig. 1A). Phylogenetic analyses showed that JcTM6, which contains the paleoAP3 motif, belongs to the TM6 group, rather than the euAP3 group (Fig. 1B).

To analyze the expression pattern of JcTM6 in Jatropha, qRT-PCR was performed using total RNA extracted from various tissues including roots, stems, leaves, inflorescences, female and male flowers, and pericarps and seeds at 42 DAP. The JcTM6 gene was predominantly expressed in female and male flowers (Fig. 2), indicating that JcTM6 is a flower-specific gene. Furthermore, JcTM6 showed high expression in the stamens of male flowers and petals of male and female flowers but low expression in sepals and pistils (Fig. 2). Thus, the expression pattern of JcTM6 in floral organs is consistent with that of class B genes (Weigel & Meyerowitz 1994).

Isolation and sequence analysis of JcTM6 promoter
A 1.8-kb fragment of the JcTM6 promoter (Fig. 3A, –1717 to +103 bp; GenBank accession no. MN044579) was isolated from Jatropha genomic DNA by genome walking (Siebert et al. 1995). The transcription start site of JcTM6 was located 103 nt upstream of the translation start codon (Fig. 3A). Analysis of the JcTM6 promoter using the PLACE database (Higo et al. 1999) revealed various putative cis-elements in the 1.8-kb JcTM6 promoter fragment (Fig. 3A) including two CArG boxes, which act as binding sites for MADS-box transcription factors (Irish & Yamamoto 1995), some pollen-specific elements, including five GTGANTG10 motifs (GTGA) and eight POLLEN1LELAT52 motifs (AGAAA) (Musciotti et al. 1994; Rogers et al.
160 and a Q element (TGACCT), which shows enhancer-like activity for the pollen-specific expression of maize (Zea mays L.) ZM13 gene (Hamilton et al., 1998).

**Activity of the JcTM6 promoter in Arabidopsis**

To detect the activity of JcTM6 promoter, a JcTM6 promoter-GUS fusion construct (Fig. 3B) was expressed in Arabidopsis, and GUS staining was monitored in homozygous T3 plants (Fig. 4). No GUS staining was observed in 10-day-old Arabidopsis seedlings (Fig. 4A). Among the five tissues of adult plants examined (including roots, stems, leaves, flowers, and green siliques) GUS staining was detected only in flowers (Fig. 4B–G). Among all floral organs, GUS staining intensity was the strongest in stamens, followed by sepals and petals, with faint staining in carpels (Fig. S1). Based on the results of GUS staining, we conclude that the JcTM6 promoter functions as a flower-specific promoter in Arabidopsis.

**Deletion analysis of the JcTM6 promoter**

To analyze the region essential for flower-specific activity of the JcTM6 promoter, we carried out a deletion analysis. A deletion variant of the JcTM6 promoter lacking the region from –1,717 to –876 bp was fused to the GUS gene and transformed into Arabidopsis (Fig. 5A). Compared with the full-length JcTM6 promoter, the deletion was not only active in flowers but also in young leaves (Fig. 5B). Moreover, the deletion showed no promoter activity in stamens but increased activity in sepals and petals (Fig. 5C and D). These results indicate that the region from –1,717 to –876 bp is critical for JcTM6 promoter activity in stamens and inhibition of promoter activity in young leaves, sepals, and petals.

**JcTM6:AtIPT4 transgenic Arabidopsis produced large flowers**

To further verify the floral specificity of JcTM6 promoter, a cytokinin biosynthetic gene (AtIPT4) was expressed under the control of JcTM6 promoter in Arabidopsis. JcTM6:AtIPT4 vector was constructed and was transformed into Arabidopsis plants. A total of 25 independent JcTM6:AtIPT4 lines were obtained. As expected, all transgenic lines showed no vegetative difference from the wild type and most of them produced larger flowers (Fig. 6). Furthermore, the development of siliques was also unaffected. To verify the morphological alteration in flowers that is caused by the transgene, we examined the expression levels of AtIPT4 and the cytokinin signaling genes Arabidopsis histidine kinase 2 (AHK2) (Nishimura et al., 2004) and Arabidopsis response regulator 5 (ARR5) (D'Agostino et al., 2000) in wild type and JcTM6:AtIPT4 transgenic plants. The expression level of AtIPT4 in flowers of transgenic lines is significantly higher than that in wild type, whereas the AtIPT4 expression in the leaves of transgenic plants was not different from that in leaves of wile-type plants (Fig. 7A). As expected, higher expression levels of AHK2 and ARR5 were detected in the flowers of transgenic lines (Fig. 7B). These results indicate that the morphological alteration in flowers of JcTM6:AtIPT4 transgenic plants is caused by the flower-specific expression of the transgene driven by the JcTM6 promoter. JcTM6 promoter is indeed a flower-specific promoter.
Discussion

TM6 is a member of the MADS-box gene family, which belongs to the paleoAP3 lineage (Pnueli et al. 1991; Rijpkema et al. 2006; Wu et al. 2011). In tomato (Solanum lycopersicum) and petunia, TM6 functions as a class B gene that play an essential role in stamen development, although it is mainly expressed in whorls 3 and 4, similar to a class C gene (de Martino et al. 2006; Rijpkema et al. 2006). In tomato (Solanum lycopersicum) and petunia, TM6 functions as a class B gene that play an essential role in stamen development, although it is mainly expressed in whorls 3 and 4, similar to a class C gene (de Martino et al. 2006; Rijpkema et al. 2006). In trioecious papaya (Carica papaya) plants, which produce male, female, and hermaphrodite flowers, two TM6 genes were isolated previously (CpTM6-1 and CpTM6-2). Both genes are predominantly expressed in the petals of all sex types and stamens of hermaphroditic and male flowers, although CpTM6-2 is also expressed in leaves (Ackerman et al. 2008). In this study, we identified JcTM6 as a flower-specific gene in Jatropha, with high expression in female and male flowers (Fig. 2). Similar to CpTM6-1, the JcTM6 gene showed high expression in the petals of female and male flowers and stamens of male flowers. Because JcTM6 showed flower-specific expression, we isolated its upstream region from Jatropha genomic DNA and analyzed its activity in Arabidopsis by GUS staining.

In transgenic Arabidopsis, GUS staining showed that the JcTM6 promoter was active only in flowers (Fig. 4), suggesting that the JcTM6 promoter is a flower-specific promoter. AtIPT4 is a cytokinin biosynthesis gene encoding ATP/ADP isopentenyltransferase. The expression of this gene under the control of AP1 promoter results in the alterations in flower number and organs (Li et al. 2010). However, the AtIPT4 driven by JcTM6 promoter only gave rise to the changes in flower organs (Fig. 6), indicating that JcTM6 promoter is active at the late stage of flower development rather than floral meristem. This activity is consistent with the expression pattern of the JcTM6 gene in Jatropha. Recently, Ming et al. (2020) showed that JcTM6 promoter has a high activity in female flowers of Jatropha, suggesting that JcTM6 promoter can drive flower-specific expression of transgenes in different plant species.

When the 842-bp fragment of the JcTM6 promoter (–1,717 to –876 bp) was deleted, the promoter was not only active in flowers but also in young leaves (Fig. 5B). We found that the deleted region contained one of the two CArG box motifs, which are very important for mediating the regulatory effect of MADS-box transcription factors (Dolan & Fields 1991; RichardTreisman 1992). In Jatropha, a fragment of the JcAP1 promoter (from –1,313 to –1,057 bp), which contains a CArG box motif, is required for promoter activity in inflorescence buds (Tao et al. 2016). The Arabidopsis AP3 promoter contains three CArG boxes: CArG1 is essential for AP3 promoter activity at all stages of flowering; CArG2 is critical for AP3 expression in petals, and CArG3 represents the binding site of a transcription factor that represses the activity of AP3 promoter during early floral stages (Tilly et al. 1998). Therefore, we propose that the CArG box motif in JcTM6 promoter plays an important role in conferring floral-specific activity in transgenic plants.

Among the floral organs, stamens exhibited the highest activity of JcTM6 promoter (Fig. 4F). This expression pattern could be regulated by pollen-specific elements contained in this promoter, including five GTGA and eight AGAAA motifs. The GTGA motif is critical for the
expression of g10 promoter in tobacco pollen because mutation of the GTGA motif reduced g10 promoter activity in pollen (Rogers et al. 2001). The AGAAA motif, which was identified in the tomato late-stage pollen-specific LAT52 promoter, is necessary for promoter activity during pollen maturation (Bate & Twell 1998). In potato (Solanum tuberosum L.), the GTGA and AGAAA motifs present in the promoter of SBgLR, a pollen-specific gene, are critical for high-level gene expression in pollen (Lang et al. 2008). In the current study, deletion of an 84283-bp fragment of the JcTM6 promoter, containing four GTGA and two AGAAA motifs, abolished promoter activity in stamens (Fig. 5D). We assumed that these motifs are essential for the activity of the JcTM6 promoter in stamens. Given the importance of CArG box motifs, it is possible that the GTGA and AGAAA motifs cooperate with the CArG box to regulate JcTM6 promoter activity in stamens. In addition, although the deleted region contained six AGAAA motifs, these motifs do not seem to be required for JcTM6 promoter activity in stamens. Furthermore, the deleted region also contained a 6-bp quantitative element (Q-element), which plays an enhancer-like role (Hamilton et al. 1998). In maize, deletion of the Q-element from the pollen-specific ZM13 promoter reduced the promoter activity by 10-fold (Hamilton et al. 2000). Deletion of the Q-element probably also contributed to the loss of JcTM6 promoter activity in stamens in this study (Fig. 5D). In addition, the deletion variant of the JcTM6 promoter exhibited increased activity in sepals and petals (Fig. 5C and D), indicating the presence of potential negative elements in the deleted region, which inhibit promoter activity in sepals and petals. By the deletion analysis of the JcTM6 promoter, we demonstrate the combination of these elements are of great importance to the promoter activity in the flowers, and detailed studies of the functions of these elements will be conducted in the future.

**Conclusions**

Floral-specific promoters play crucial roles in genetic modification of flowering characteristics. In this study, a 1.8-kb JcTM6 promoter fragment was isolated from Jatropha and characterized as a flower-specific promoter in transgenic Arabidopsis plants. When the region from –1,717 to –876 bp in the JcTM6 promoter was deleted, the promoter lost its flower-specific activity and gained activity in young leaves. Our results suggest that the JcTM6 promoter could be used to drive flower-specific expression of transgenes in plants.

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Table 1 (on next page)

Sequences of the primers used in this study
## Table 1. Sequences of the primers used in this study

| Name    | Sequence (5’ to 3’) | Feature                                      |
|---------|---------------------|----------------------------------------------|
| GSP1    | CTCTTGGAAATAACCTGTCTGG | *JcTM6* gene-specific primer for genome walking |
| GSP2    | CAAAACACTACTACAAAAACCGAAG | *JcTM6* gene-specific primer for genome walking |
| XT95    | GCTGCTAAGGCTGGGAA | *JcGAPDH* gene primer for qRT-PCR |
| XT96    | GACATAGCCCAAATATCCCTCGA | *JcGAPDH* gene primer for qRT-PCR |
| XK712   | TATCTCTCGTCTGTTTGTAGTAGTGGA | *JcTM6* gene primer for qRT-PCR |
| XK713   | TCTCTTGGAAATACTACAAAAACCGAAG | *JcTM6* gene primer for qRT-PCR |
| XT405   | TGCTCTAGAAATAGCTATAAAATT | For cloning the full-length promoter and construction of *JcTM6:GUS* |
| XT408   | CGCGGATCCTTTTTCCTTCTGATA | For cloning the full-length promoter and construction of *JcTM6:GUS* |
| XD548   | GCTCTAGACGCTTACAGAATTTGCGA | For construction of *D:GUS* |
| XB994   | CAATCTTTTCCACGACCCCAATTTTTTCTTT | *JcTM6* gene-specific primer for 5’-RACE |
| XK718   | TGTTGCAATCTACGAGGTTT | *Atactin* gene primer for qRT-PCR |
| XK719   | TTTCCCAGCTCTCTGTGTTT | *Atactin* gene primer for qRT-PCR |
| XK984   | TCGCTAGTTCCACGCTCTAAG | *AtIPT4* gene primer for qRT-PCR |
| XK985   | AGGGTCCTTATCCTCAGTCEATT | *AtIPT4* gene primer for qRT-PCR |
| XE815   | CCGTGCTCAATGGCAAGGAGGAAGCA | *AHK2* gene primer for qRT-PCR (Nishimura et al, 2004) |
| XE816   | CACCTTCTCGAATCTCGTCTGT | *AHK2* gene primer for qRT-PCR |
| XE819   | TCGAGAACAATCTCGTCTCGT | *ARR5* gene primer for qRT-PCR |
| XE820   | AGCTGCGAGTAGATATCATTAGCTT | *ARR5* gene primer for qRT-PCR |
Figure 1

A comparison of JcTM6 and its homologs.

(A) The alignment of the deduced amino acid sequences of JcTM6 with that of Vitis vinifera VvTM6 (accession No.DQ979341), Carica papaya CpTM6-1 (accession No.ABQ51321), and CpTM6-2 (accession No.ABQ51322), Populus trichocarpa PTD (accession No.AAC13695), Gossypium hirsutum GhTM6 (accession No.ADX60056), Petunia x hybrida PhTM6 (accession No.AF230704) and Solanum lycopersicum SlTM6 (accession No.CAA43171). Identically and partially conserved amino acid sequences are shown in black and gray, respectively. The conserved regions, MADS domain and K domain and paleoAP3 C-terminal motif in JcTM6 are underlined. (B) A phylogenetic analysis of JcTM6 and other homologs. Jatropha curcas JcDEF (accession No. XP_012071964), Solanum lycopersicum TAP3 (accession No. ABG73412), Vitis vinifera VvAP3 (accession No. NP_001267960), Arabidopsis thaliana AP3 (accession No. BAA04665), Petunia hybrida PMADS1 (accession No. Q07472). The tree was constructed using MEGA 7.0 software and the neighbor-joining (N-J) method. The N-J unrooted dendrogram was generated from an alignment of the deduced amino acids with the ClustalW program. One thousand replicates were used for the Bootstrap test. The scale bar indicates the average number of substitutions per site.
Figure 2

Expression pattern of JcTM6 in Jatropha.

Samples from adult plants: roots (R), stems (S), young leaves (YL), mature leaves (ML), inflorescence buds (If), female flowers (FF), male flowers (MF), pericarps at 42 days after pollination (DAP) (Pp 42d), seeds at 42 DAP (Sd 42d), male sepals (MS), male petals (MP), stamens (St), female sepals (FS), female petals (FP), and pistils (Pi). qRT-PCR results were obtained from three biological replicates. The errors denote the SD. The values were normalized to the expression of JcGAPDH (Zhang et al. 2013). The relative expression level of young leaves was set as the standard value of 1.
Figure 3

*JcTM6* promoter sequence and promoter-reporter gene construct.

(A) The nucleotide sequence of the *JcTM6* promoter. The transcription start site (+1) is in red. The start codon ATG is in bold and boxed. Putative regulatory elements on both strands are shown in bold and underlined. (B) A schematic of the T-DNA regions of the *JcTM6:GUS* binary vector used for transformation.
Figure 4

Histochemical GUS staining of transgenic *Arabidopsis* harboring the *JcTM6:GUS* fusion.

(A) Ten-day-old seedlings, (B) roots, (C) stems, (D) leaves, (E) inflorescence buds, (F) open flowers, (G) green siliques. Pe, petals; Se, sepals; St, stamens. Red bars = 1 mm, black bars = 2 mm.
Figure 5

Histochemical GUS staining of transgenic Arabidopsis harboring the \textit{JcTM6} deletion.

(A) Schematic representation of \textit{JcTM6} promoter deletion. FL, full length \textit{JcTM6} promoter, Del, deletion. GTGA: GTGANTG10 motif (gray vertical bars), AGAAA: POLLEN1LELAT52 motif (black vertical bars), CArG box: CWWWWW (yellow vertical bars), Q-element: TGACCT (red vertical bar). (B) young leaves, (C) flower buds, (D) flowers. Pe, petals; Se, sepals; St, stamens. Red bar = 1 mm, black bars = 0.5 mm.
Figure 6

Flower size is increased in transgenic \textit{JcTM6:AtIPT4 Arabidopsis}.

Inflorescences of wild-type (A) and transgenic L1 (B) and L22 (C) lines. Flowers of wild-type and transgenic L1 and L22 lines (D). Dissected flowers of WT and transgenic L1 and L22 lines (E). Se, sepals; Pe, petals; St, stamens; Ca, carpels; WT, wild-type. White bars = 3 mm, yellow bar = 2mm.
Figure 7

The expression analysis of \textit{AtlPT4}, \textit{AHK2} and \textit{ARR5} in \textit{JcTM6:AtlPT4} transgenic \textit{Arabidopsis}.

(A) The expression levels of \textit{AtlPT4} in the leaves and flowers of wild type (WT) plants and transgenic lines (L1 and L22). (B) The expression levels of \textit{AHK2} and \textit{ARR5} in the flowers of wild type (WT) plants and transgenic lines (L1 and L22). The values represent the means ± standard deviation (n =3). Student’s t-test was used to determine significant differences. * p ≤0.05, ** p ≤0.01.
