Plant Omics Journal POJ

POJ 5(5):453-457 (2012) ISSN:1836-3644

Research Note

Primers to amplify *flowering locus T* (*FT*) transcript in mango (*Mangifera indica*) and their potential use in other angiosperms

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Abstract

Mango (*Mangifera indica*) is a commercially important fruit crop around the world. So far, *Flowering Locus T* (*FT*), a floral integrator gene, has not been identified in this plant as well as in other economically important angiosperms. Two pairs of primers to amplify fragments of *FT* transcripts from *M. indica* were designed using an alignment of forty-one amino acid sequences of this transcript belonging to fifteen angiosperm species. Designed primers, FTf1/FTr2 and FTf2/FTr2, amplified fragments of approximately 210 and 150 bp, respectively, which were sequenced by Sanger platform. Sequences obtained were analyzed and compared, using BLAST, with those of *FT* deposited in the NCBI GenBank database, *FT* transcripts of 207 bp (Accession No. JX316911) and 147 bp (Accession No. JX316912) from *M. indica* showed high identity with *FT* of *Populus nigra* (86% and 84%, respectively). In addition, FTf2/FTr2 was able to amplify 150 bp fragments from *Duranta dombeyana* (Accession No. JX316913), *Gazania linearis* (Accession No. JX316915), and *Lantana camara* (Accession No. JX316914), with significant identity to *FT* of *Xanthium strumarium* (82%), *Helianthus annuus* (91%), and *Ficus carica* (79%), respectively. Furthermore, phylogenetic relationship analysis showed that the fragment of 147 bp from *M. indica* *FT* transcript has more similarity to those belonging to the subclass Rosidae, while *FT* from *D. dombeyana*, *G. linearis* and *L. camara* are more related to the subclass Asteridae. Overall, these primers may be useful to amplify fragments of *FT* transcript from other angiosperm species for a variety of downstream applications, such as monitoring their expression profiles under certain conditions, isolation of *FT* full-length transcripts, etc. This will lead to propose more precise models and alternatives to control the flowering in plants of interest.

Keywords: *Duranta dombeyana;* Flowering; *Gazania linearis; Lantana camara;* Primers; RNA messenger.
Abbreviations: NCBI-National Center for Biotechnology Information; cDNA- complementary DNA; BLAST-Basic Local Alignment Search Tool; RT-PCR-reverse transcriptase PCR; M-MuLV RT RNase H-Moloney Murine Leukemia Virus (M-MuLV) Reverse Transcriptase RNase H- (minus).

Introduction

Mango (*Mangifera indica*), a member of the Anacardiaceae family, is an important fruit crop around the world. Typical production of mango is approximately 30 x 10^6 tons per year (FAO, 2009). The potential for increased productivity is constrained by the limited knowledge of the molecular and biochemical mechanisms that control flowering, an essential precursor to mango production (de los Santos-Villalobos et al., 2011). Although, several models of mango flowering have been developed, much is based on the wide information generated from agricultural practices around the world rather than genetic information (Tiwari and Rajput, 1976; Oslund and Davenport, 1987; Salazar et al., 2000). At present, it has been reported a potential relationship between a temperature-dependent florigen promoter (FP) (potentiated at low temperatures) and an age-dependent vegetative promoter (VP) (potentiated at early ages) involved in mango flowering (Davenport and Núñez, 1997, Davenport, 2000, 2003, 2008). In addition, there is only one published report on the molecular basis of mango floral pathway (Davenport et al., 2006), leading to the isolation of a gene called *MiCOL* (*CONSTANS*-like) from leaf of mango tree. *CONSTANS* is a circadian-regulated gene in the photoperiod pathway of the model plant, *Arabidopsis thaliana* (Putterill et al., 2004). Interestingly, mango flowering time is photoperiod-independent and therefore the role of this gene remains uncertain (Núñez-Elisea and Davenport, 1995). In addition, efforts have been made, without success, to isolate *Flowering Locus T* (*FT*) gene in mango, likely responsible for the synthesis of the protein FP (Davenport, 2007). Molecular studies in *Arabidopsis thaliana* have reported that the sequence length of *FT* gene and transcript has 2507 bp and 528 bp, respectively, and a protein sequence length of 175 aa (http://www.arabidopsis.org/servlets/TairObject?id=30541&type=locus). *FT* and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS* (*SOC1*), floral integrator genes, are involved in the integration of environmental and endogenous signals such as gibberellins, autonomous, vernalization, thermosensorial, and photoperiod response (Liu et al., 2009; Castro et al., 2011). These floral pathway integrator genes have as targets genes that

Table 1. Amino acid and nucleotide sequences of primers corresponding to highly conserved amino acid sequences obtained of FT protein alignment.

Name	Amino acid sequence	Nucleotide sequence	Tm, ^{o}C
FTf1	FYTLVMV	5`-TTCTACACTCTGGTTATGGTGG-3`	53.0
FTf2	EYLHWLV	5`-GAATACTTGCATTGGTTGGTGAC-3`	53.5
FTr2	QLGRQTV	5`-TACACTGTTTGCCTACCCAGTTG-3`	55.3

Note: FTf1: FT forward primer 1; FTf2, FT forward primer 2; FTr2: FT reverse primer 2.

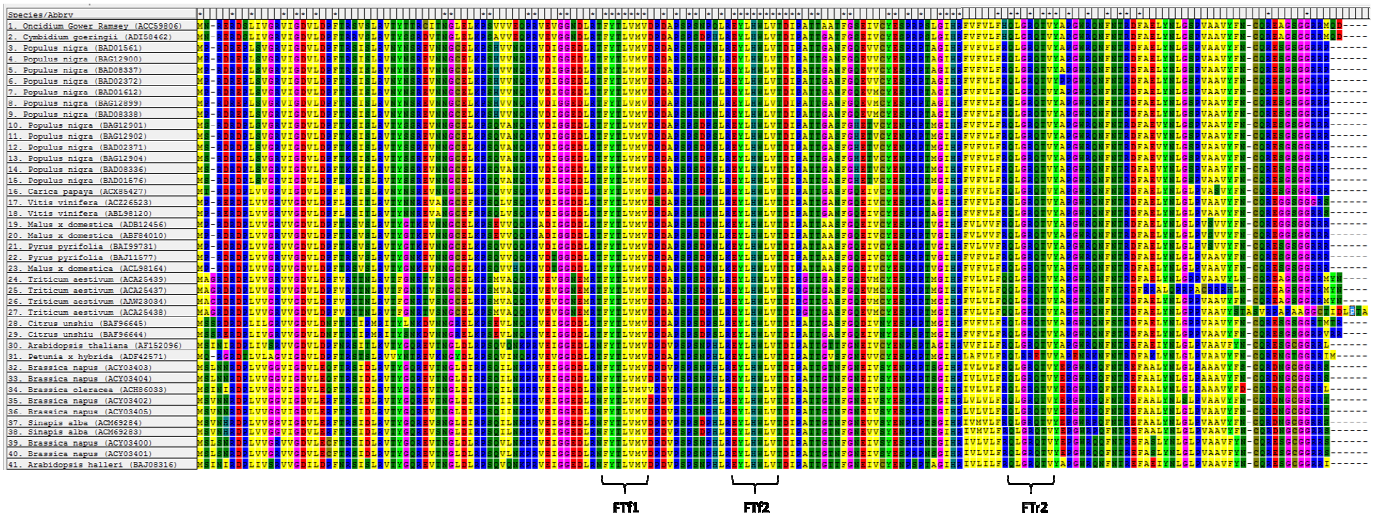

Fig 1. Clustal X amino acid alignment of forty-one FT sequences belonging to fifteen angiosperm species. The highly conserved regions identified (FTf1, FTf2 and FTr2) were reverse translated *in silico* to design primers for this study.

confer floral identity to new emergent meristems called "floral meristem identity genes", which subsequently activate the "floral organ identity genes" leading to an appropriate development of floral organs (Blázquez, 2000; Liu et al., 2009). In the present paper, we report the design and implementation of primer sets successfully used to amplify *FT* transcripts from mango, as well as for three additional agriculturally important plant species (*Duranta dombeyana, Gazania linearis* and *Lantana camara*). This new tool will allow researchers to further elucidate the molecular basis for flowering in mango as well gain insight into the role of *FT* in other additional angiosperm clades.

Results

The primers were designed based on alignments of forty-one FT amino acid sequences from fifteen different plant species (Wei et al., 2003), which were obtained from NCBI GenBank, searching for highly conserved regions (Fig. 1): *Oncidium Gower Ramsey* (ACC59806), *Cymbidium goeringii* (ADI58462), *Triticum aestivum* (ACA25439), *Triticum aestivum* (ACA25437), *Triticum aestivum* (AAW23034), *Triticum aestivum* (ACA25438), *Citrus unshiu* (BAF96645), *Citrus unshiu* (BAF96644), *Malus x domestica* (ADB12456), *Malus x domestica* (ABF84010), *Pyrus pyrifolia* (BAI99731), *Pyrus pyrifolia* (BAJ11577), *Malus x domestica* (ACL98164), *Vitis vinifera* (ACZ26523), *Vitis vinifera* (ABL98120), *Populus nigra* (BAD02371), *Populus nigra* (BAG12904), *Populus nigra* (BAG12902), *Populus nigra* (BAD08336), *Populus nigra* (BAG12901), *Populus nigra* (BAD01576), *Populus nigra* (BAG12900), *Populus nigra* (BAD08337), *Populus nigra* (BAD01561), *Populus nigra* (BAD02372), *Populus nigra* (BAD08338), *Populus nigra* (BAD01612), *Populus nigra* (BAG12899), *Carica papaya* (ACX85427), *Petunia x hybrida* (ADF42571), *Brassica napus* (ACY03403), *Brassica napus* (ACY03404), *Brassica oleracea* (ACH86033), *Brassica napus* (ACY03402), *Brassica napus* (ACY03405), *Sinapis alba* (ACM69284), *Sinapis alba* (ACM69283), *Brassica napus* (ACY03400), *Brassica napus* (ACY03401), *Arabidopsis halleri* (BAJ08316), and *Arabidopsis thaliana* (AF152096).

Three highly conserved regions were identified in our FT amino acid alignment (Fig. 1), hereafter FTf1, FTf2 and FTr2, which were reverse translated to nucleotide, obtaining PCR primers sequences (Table 1). Once the primers were designed, total RNA was extracted from leaves of *Mangifera indica, Duranta dombeyana, Gazania linearis* and *Lantana camara* presenting floral transition in the field. Total RNA from each species was used for subsequent cDNA synthesis and PCR. Expected amplicons (approximately 210 bp and 150 bp using FTf1/FTr2 and FTf2/FTr2, respectively) were purified, cloned and Sanger-sequenced (Fig. 2). Sequences obtained were analyzed and queried against those of *FT* deposited in the NCBI GenBank database using the BLAST algorithm (Table 2). All amplicons obtained had high identity percentage (79 to 91%) to known *FT* gene sequences reported from other plants (Table 3). In addition, a neighbor-joining phylogenetic tree was constructed for the newly-identified *FT* amplicons (Saitou and Nei, 1987), observing a clear subclass-specific clustering (Fig. 3): The *FT*

Table 2. Nucleotide sequences of amplicons obtained corresponding to *FT* from *Mangifera indica*, *D. dombeyana*, *G. linearis* and *L. camara* using set designed primers.

Analyzed plant species/ Nucleotide length (bp) number (Accession No.)	Nucleotide sequences
Mangifera indica /207 (JX316911)	5'-TACACTCTGGTTATGGTGGATCCTGATGCACCTAGC CCTAGTAATCCAAGTCTCAGAGAATACTTGCATTGGTT GGTGACTGATATTCCCGGATCTACAGGGGCACCCTTCG GACAAGAGATTGTGAATTATGAGAGCCCAAGACCAAC AGTGGGGATTCACAGGTTTGTATTTGTGTTGTTTCGTCAACTGGGTAGGCAA ACAGTG-3´
Mangifera indica /147 (JX316912)	5'-TACTTGCATTGGTTGGTGACTGATATTCCCGGATCTA CAGGGGCACCCTTCGGACAAGAGATTGTGAATTATGAGAGCCCAAGACCAA CAGTGGGGATTCACAGGTTTGTATTTGTGTTGTTTCGTCAACTGGGTAGGCA AACAGTG-3´
Duranta dombeyana /150 (JX316913)	5'-GAATACTTGCATTGGTTGGTGACAGATATTCCAGCTA CCACTGGATCAAACTTTGGGCAAGAGATTGTGTGCTACGAGAGTCCACGGC CATCGATGGGGATCCACCGGCTTGTTTTCGTGCTGTTCCGCCAACTGGGTAG GCAAACAGTG- 3´
Gazania linearis /150 (JX316915)	5'-GAATACTTGCATTGGTTGGTGACTGATATTCCAGCGA CCACAGGAGCACGATTTGGCCAAGAAATCGTGTGCTACGAAAGTCCAAGAC CATCAATGGGAATTCATCGGATGGTTTTCGTGTTGTTCCGACAACTGGGTAG GCAAACAGTG-3´
Lantana camara /150 (JX316914)	5´-GAATACTTGCATTGGTTGGTGACTGACATTCCAGCAG CCACTGGAACAACATTTGGACAAGAAATTATTTGCTACGAGAGCCCACGAC CATCGATGGGCATTCCCCGGGTCTTATTCGTGTTGTTCCAGCAACTGGGTAG GGAAACAGTG-3´

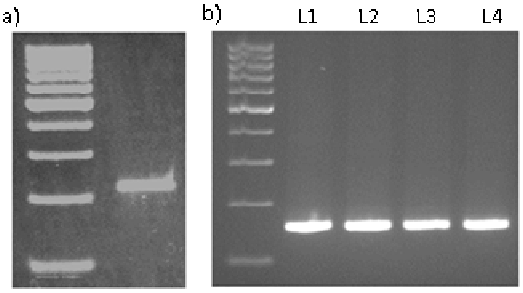

Fig 2. Agarose gel (2%) electrophoresis of PCR amplicons of *FT* transcripts in studied plants. (a) the amplified fragment of 210 bp obtained using FTf1 and FTr2 primer from *Mangifera indica* RNA, and (b) the amplified fragments of 150 bp obtained using FTf2 and FTr2 primer from L1: *Mangifera indica*, L2: *Duranta dombeyana,* L3:*Gazania linearis* and L4:*Lantana camara* RNAs. DNA ladder of 100 bp.

sequence from *Mangifera indica* is more similar to those from *Pyrus pyrifolia*, *Malus x domestica*, *Prunus persica*, *Vitis vinifera* and *Citrus unshiu*; all species, including *Mangifera indica*, belong to the subclass Rosidae. *FT* amplicons from *Duranta dombeyana*, *Gazania linearis* and *Lantana camara* are more related to the subclass Asteridae (*Chrysanthemum lavandulifolium*, *Solanum tuberosum* and *Petunia x hybrida*).

Discussion

Floral induction, a necessary process in angiosperm reproduction, has been extensively studied by scientists and growers aiming to control the onset floral induction and/or increase floral abundance in important ornamental and higher plants into the global market (Carmona et al., 2008). Flowering is an extremely complex event. Molecular studies in *A. thaliana* report that *FT* is a floral integrator gene, which is involved in to sense endogenous and exogenous signals to induce flowering through systemic movement of FT protein from companion cells in donor leaves to receptor buds (Notaguchi et al., 2008; Kong et al., 2010). *FT* function has not been reported in many angiosperm species of economic importance, which limit the generation of efficient models and alternatives to enhance flowers or crop production when they are demanded for global market (Lopez and Runkle, 2005). In the present work, primer sets were designed and successfully implemented to identify novel *FT* transcript fragments in four angiosperm species: *Mangifera indica, Duranta dombeyana*, *Gazania linearis,* and *Lantana camara*. Our results suggest the potential of these primers to identify *FT* transcripts in other angiosperms of interest. The amino acid alignment of forty-one FT amino acid sequences (from fifteen different plant species), more than nucleotide sequence, reveals three highly conserved regions (Fig. 1), suggesting a conserved role for *FT* in angiosperms and potential functional motifs (Corbesier et al., 2007; Zhang et al., 2010; Oda et al., 2012). Primer sets generated to target the conserved motifs identified from the amino acid alignment (Table 1) led to the identification of *FT* transcript fragments in

Table 3. Identity of amplicons obtained in this study compared with *FT* nucleotide sequences present in the NCBI GenBank database.

Analyzed plant species- Nucleotide length (bp) number	Phylogenetic related species and NCBI accession number	Identity, %
M. indica-207	*Populus nigra*, Flowering Locus T RNA – AB110613	86
M. indica-147	*Populus nigra*, Flowering Locus T RNA – AB110613	84
D. dombeyana-150	*Xanthium strumarium*, Flowering Locus T RNA – JF434701	82
G. linearis-150	*Helianthus annuus*, Flowering Locus T RNA – GQ884987	91
L. camara-150	*Ficus carica,* Flowering Locus T RNA – AB457620	79

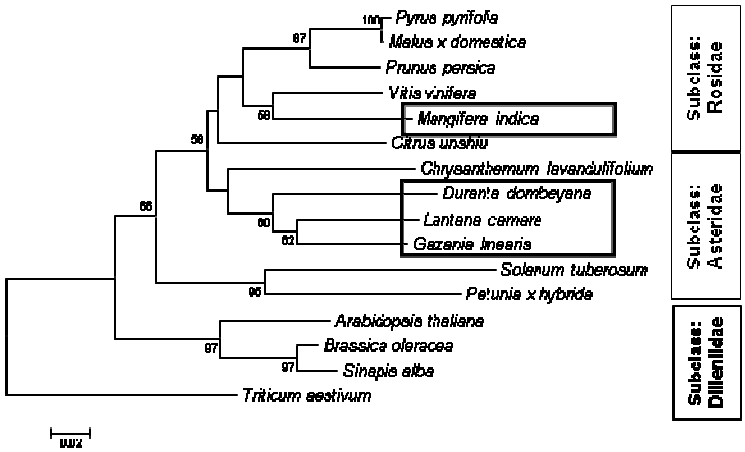

Fig 3. Phylogenetic relationship of *FT* nucleotide sequences (150 bp) from different plant species. *FT* sequences identified in this study are indicated by rectangles. *Triticum aestivum FT* (Accession No. AY705794) was used as the outgroup. The numbers displayed at the nodes indicate confidence based on the percentage of 1000 bootstrap re-sampled data sets (>50). Accessions numbers of sequences are: *Pyrus pyrifolia*- AB524587, *Malus x domestica*- DQ535887, *Prunus persica*- JF806623, *Vitis vinifera*- DQ871590, *Citrus unshiu*- AB027456, *Chrysanthemum lavandulifolium*- GU120195, *Solanum tuberosum*- GU223211, *Petunia x hybrida*- GU939627, *Arabidopsis thaliana*- AB027504, *Brassica oleracea*- HM030997, *Sinapis alba*- FJ613328.

plants studied (Fig. 2) with high identity percentage (79 to 91%) and predictable taxonomy to known *FT* transcript sequences reported from other plants (Table 2, Table 3 and Fig. 3). The novel *FT* fragments identified in this study can be proposed as a putative flowering signal in *Mangifera indica*, *Duranta dombeyana*, *Gazania linearis* and *Lantana camara* based on homology and expression level in leaves under floral induction (Xue et al., 2012). Unreported *FT* transcript isolation will led to elucidate regulation mechanisms and develop models to generate successfully alternatives to induce flowering in interest plants when growers need it.

Materials and methods

Software

Clustal X software version 2.0.12 (Thompson et al., 1997) was used to align amino acid sequences belonging to FT proteins, obtained from NCBI GenBank, and later to identify highly conserved regions. Those regions were reverse translated to obtain nucleotide sequences of primers using Sequence Manipulation Suite: Reverse Translate (http://www.bioinformatics.org/sms2/rev_trans.html). Sequence Scanner (version 1.0) and MEGA (version 5.0) were used to analyze electropherograms obtained from Sanger sequencing and to determine phylogenetic relationships using the Neighbor-Joining method, respectively.

Plant RNA extraction

Total RNA was extracted from *Mangifera indica, Duranta dombeyana, Gazania linearis* and *Lantana camara* using Invitrogen's Concert™ Plant RNA Reagent (Invitrogen). Prior to RNA extraction, all leaves were collected and immediately frozen using liquid nitrogen and stored at -70°C until processed for RNA extraction.

RT-PCR

Total RNA obtained was used for cDNA synthesis and PCR in the same tube using the RobusT II RT-PCR Kit (Finnzymes): [10x RobusT Reaction Buffer, 5 µl; 50 mM $MgCl_2$, 1.5 µl; dNTP mix (10 mM each), 1 µl; template RNA, 2.5 µg; FTf1 or FTf2, 10 pmol; FTr2, 10 pmol; M-MuLV RT RNase H^- 5 U/µl, 2 µl; DyNAzyme EXT DNA Polymerase 1 U/µl, 2 µl; RNase free H_2O add to 50 µl]. The reaction was performed using the following amplification conditions in a Master Gradient Eppendorf Thermocycler: 30 min at 40°C (1 cycle); 2 min at 94°C (1 cycle), 45 s at 94°C, 30 s at 53°C, 1 min at 72°C (30 cycles) and a final elongation step at 72°C for 5 min. PCR products were analyzed by electrophoresis in a 2% agarose gel in TAE buffer containing 0.5 µg/mL ethidium bromide.

Expected amplicons were purified using the GFX PCR DNA kit and Gel Band Purification Illustra, and cloned using the TOPO TA Cloning® kit (Invitrogen), and sequenced by Sanger platform.

Conclusion

Designed primers in this work were able to amplify novel *FT* transcript fragments from four angiosperm species of economic and agricultural importance (*Mangifera indica, Duranta dombeyana*, *Gazania linearis* and *Lantana camara*). The ability to amplify regions of *FT* transcripts for these diverse angiosperms suggests a broad utility for our primer sets, including the potential to discover novel *FT* transcripts in other interesting angiosperms, enabling researchers to perform evolutionary studies of floral regulation. In addition, this primer suite enables researches to study expression profiles of *FT* under different growth conditions impacting the reproduction of economically important plants and can aid in the isolation of *FT* full-length transcripts for more comprehensive studies of the molecular regulation of flowering, allowing the generation of efficient tools to control the flowering time in interest plants through "environmentally friendly" alternatives when they are need for the global market.

Acknowledgments

We express our thanks to James M. Kremer for his suggestions, which improved the manuscript.

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