# *In-silico* mining and characterisation of TIFY genes in mango

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***Abstract***
*Mango (Mangifera indica L.) is an important commercial fruit worldwide. Dashehari cultivar of mango is one of the foremost varieties of North India and is liked due to its high pulp content, aroma and pleasant taste. Jelly seed formation is major disorder during Dashehari fruit ripening where it is commercially grown. However molecular mechanism behind this disorder is uncertain. We have mined 6 different TIFY transcription factors genes/transcripts (MiTIFY3B, MiTIFY4B, MiTIFY5A, MiTIFY6B, MiTIFY9, MiTIFY10A) in the fruit transcriptome data of mango jelly seed tissue. Except MiTIFY4B (CDS_17202) and MiTIFY6B (CDS_3945), all mined mango TIFY proteins contain the conserved TIFY (TIFY) and Jas domain ($FN_2KRK$).*

*To decipher role of TIFY factors in this disorder, the expression analysis through semi-quantative RT-PCR was analysed at 0 dpa, 10 dpa, ripe pulp and jelly seed, revealing that these TIFY genes play developmental and ripening roles. Expression patterns of the TIFY genes during fruit development and ripening indicated that they may be involved in development and ripening by the JA signaling pathway. Our findings provide for a further functional characterization of the TIFY family genes in mango and open the window of investigation of an important TIFY transcription factor family of genes and its role in mango fruit development and ripening.*

**Keywords:** Dashehari, Jelly seed, TIFY.

## Introduction

Mango (*Mangifera indica L.*) is an important commercial fruit worldwide. Mango fruits are highly perishable and have a limited shelf life, due to postharvest desiccation and senescence which limits their global distribution. The mango fruit varies in size, shape, color, fiber content, flavor and taste. Dashehari is one of the leading mango varieties of North India and area under this variety is increasing in other parts of the country also. The variety is relished due to its pleasant taste, aroma and high pulp content. In recent years during fruit ripening, jelly seed development has been experienced as one of the major disorder in some of the pockets where Dashehari is commercially grown. Fruit development and ripening are biochemical and genetically programmed process. After harvest and under native climatic conditions, Dashehari mango fruit ripes within 3 to 4 days, but reaches an overripe stage and spoils within 6 days.

TIFY transcription factors are plant-specific transcriptional regulators characterized by the presence of a highly conserved motif (TIF(F/Y)XG) in the TIFY domain with a length of approximately 36 amino acids[1]. According to their domain architectures, the TIFY proteins can be divided into four subfamilies including TIFY, ZIM-like, jasmonate ZIM-domain (JAZ) and PEAPOD (PPD). The JAZ subfamily proteins have a conserved jasmonic acid associated domain (Jas) with a SLX2FX2KRX2RX5PY consensus sequence, while the PPD subfamily proteins contain a typical PPD domain in the N-terminus and a truncated Jas motif lacking the conserved P and Y residues[2]. AtTIFY1/AtZIM is the first identified TIFY gene in plants and over-expression of

AtTIFY1/AtZIM promotes the elongation of the petiole and hypocotyl which is independent of gibberellin and brassinosteroids. In tomato, SlJAZ2 was described as an important regulator of the transition from vegetative growth to reproductive growth[3]. In addition, many JAZ genes were found to play key roles in jasmonic acid signal transduction and participate in the regulation of various developmental processes and biotic and abiotic stresses in plants.

Recently, genome-wide surveys of the TIFY gene family have been conducted in various plant species such as tomato[4], wheat[5] and pear[6]. The major difficulty in mango genetic research is the limited availability of genomic data. However, NGS methods and bioinformatic pipelines are facilitating the generation of genomic and transcriptomic analytical tools and resources. Though physiological studies have been done in jelly seed, no attempt has so far been made to characterize TIFY genes involved in the fruit development and Jelly seed of mango.

The objective of this study was to *in-silico* analyse TIFY in the jelly seed transcriptome data of mango and characterize their expression during fruit development and jelly seed in mango to decipher role in this disorder. Our findings lay foundation for further understanding the role of TIFY genes in the mango fruit development and jelly seed formation.

## Material and Methods

***In-silico* analysis:** Nucleptide CDS sequences were translated into aminoacid sequences using online sequence manipulation suite (https://www.bioinformatics.org/sms2/). Motif search analysis was done with MOTIF Search (https://www.genome.jp/tools/motif/). Multiple amino acid

sequence alignments of TIFY proteins from CDS of *transcriptome data* were completed with the MUSCLE program (https://www.ebi.ac.uk/Tools/msa/muscle/). Logo sequences for TIFY domains of MiTIFY proteins was prepared from weblogo tool (https://weblogo.berkeley.edu/).

**Semi-quantative RT-PCR analysis:** Frozen mango samples were ground to fine powder with liquid nitrogen using a mortar and a pestle. Total RNAwas isolated using Spectrum™ plant total RNA kit (Sigma, USA) according to the manufacturer's protocol. The purity and quantity of total RNA were monitored on NanoDrop. For each sample, 1 µg of total RNA was reverse transcribed using the Maxima first strand cDNA synthesis kit for RT-qPCR (Genetix) in a 20-µl reaction using Oligo dT and random primers according to the manufacturer instructions. The complementary DNAs (cDNAs) were diluted at 1:10 with nuclease-free water prior to the qRT-PCR analysis. The CDS obtained in fruit transcriptome data was used for primer designing using IDT PrimerQuest software with the following parameters: optimal length 25 base pairs, GC content 50–55%, melting temperature 57°C, amplicon length range 100–200 base pairs and then checked for the absence of stable hairpins and dimers using Oligo Analyzer.

The generated primer pair for each gene was then aligned against all mango CDS to confirm its specificity *in-silico*. PCR amplification was performed in a total volume of 10 µl by mixing 100 ng of cDNA, 0.5 µm conc. of each primer, 2.5 mM dNTPs and 1 unit of *Taq* DNA polymerase in 1x PCR buffer. The reaction were subjected to initial denaturation of $94^0$C for 5.0 min followed by 35 cycle of $94^0$C for 30 sec., $57^0$C for 30 sec., $72^0$C for 30 sec. with a final extension of $72^0$C for 5 min. in a Prima 96 thermal cycler (Hi-Media). PCR amplified products were analysed by running in 1.5% agarose gel, prepared in 1xTBE buffer and containing 0.5 µg ethidium bromide and photographed over a transilluminator.

## Results and Discussion

Jasmonic acid is considered a systemic signal transducer for many physiological processes in the plant such as vegetative growth, cell cycle, senescence, fruit ripening and biosynthesis of many plant secondary metabolites[7,8].The endogenous concentration of jasmonates increased transiently prior to the climacteric increase in ethylene biosynthesis during the onset of ripening of both apple and tomato fruit. Inhibition of ethylene action suppressed the jasmonate induced stimulation of ethylene biosynthesis indicating jasmonates act via ethylene action. These results suggest jasmonates may play a role together with ethylene in regulating the early steps of climacteric fruit ripening[9].

TIFY homologs are present in terrestrial plants and not in green algae or other non-photosynthetic eukaryotes[10]. This suggests that the *TIFY* family may have originated after aquatic plants evolved to survive on land. Multiple sequence alignment of *Arabidopsis* and mango TIFY sequences prepared from MUSCLE tool shows sequence conservation (Fig. 1). The *TIFY* gene family has been extensively studied in several plant species, however there has been a lack of information about its role in fruit development, ripening and jelly seed formation in mango.

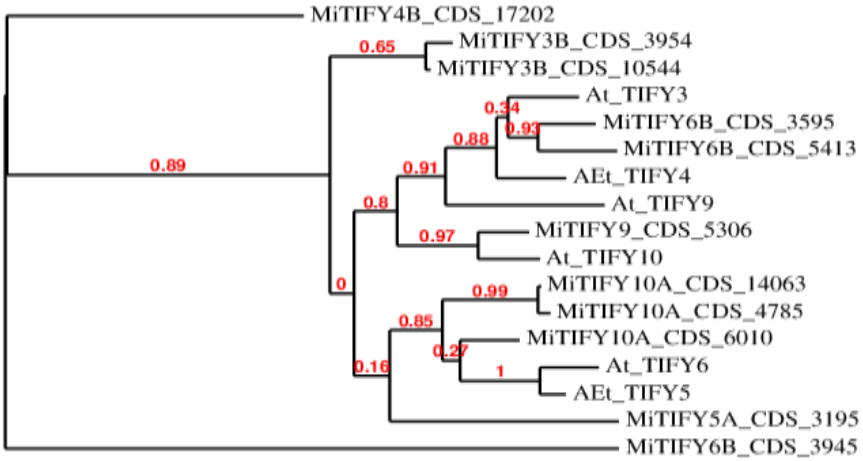


**Figure 1: Multiple sequence alignment of *Arabidopsis* and mango TIFY sequences prepared from MUSCLE tool**

**Table 1**
**Primers used in semi-quantative RT-PCR analysis**

| Gene | Forward primer (5'-3') | Reverse Primer (5'-3') |
|---|---|---|
| *MiTIFY3B* | *CAAGCTGCAAGCTGAATTACC* | *GTCCCAGATTGAGCAGGATATG* |
| *MiTIFY4B* | *GTCACTTCTCGACAAACCTCTC* | *GATGGCCTCCTCATACCTTTC* |
| *MiTIFY5A* | *GGCGAGTAGAGAAATGGAAGAG* | *AGACGACGGCCTGTATAATTG* |
| *MiTIFY9A* | *GGAAACAGCTCCTCTGACTATT* | *CTTCCTTCAACGGCAAGTTTC* |
| *MiTIFY10A* | *GAACCGCTTTCCTCAACAAAC* | *CAATCACTTGTCCGCCATAGA* |

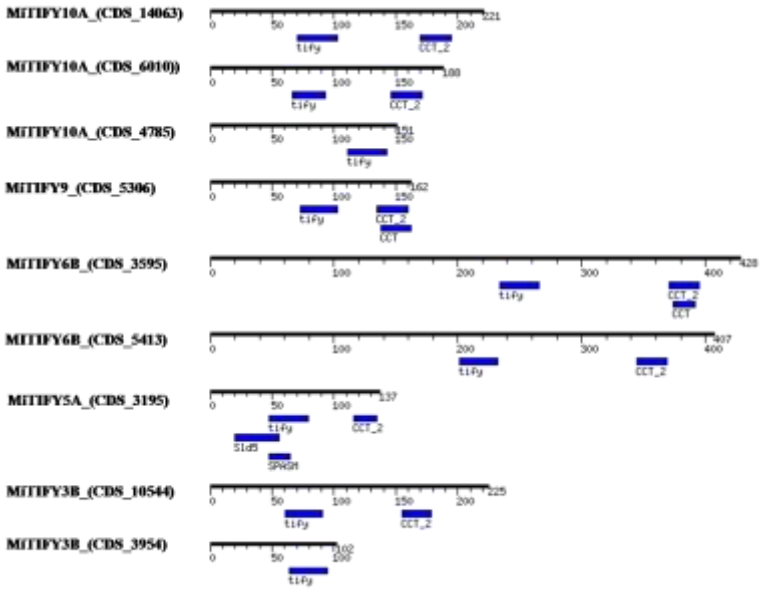


**Figure 2: Distribution of TIFY protein domains and motifs in different CDS of mango**

**a**

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MiTIFY4B_(CDS_17202)     .......... .......... .......... ..EATESPKK LNSVPSNPV.
MiTIFY3B_(CDS_10544)     .......... .......... .........P TSGANNNPSQ LTIFYGGNVC
MiTIFY3B_(CDS_3954)      .......... .......... ......MSMP TAGENTNPTQ LTIFYGGNVC
MiTIFY10A_(CDS_14063)    .........F PQQTCFAAKK ETPVDSSFNK SVAAEPQTAQ MTIFYGGQVI
MiTIFY10A_(CDS_4785)     .........F PQQTGFAAKK DTPVDSSFNK IVAAEPQIAQ MTIFYGGQVI
MiTIFY5A_(CDS_3195)      .......... .......... ......ESSS SQQSSQPSQQ LTIFYNGRVC
MITIFY6B_(CDS_5413)      IPTATSHSIL PTVGSV.... ...GITEPWN GVNTNGTSAQ MTIFYAGSVS
MiTIFY6B_(CDS_3595)      VSVISPVSVN PPTSSIIGTT SIIGTTDFRN ASKPNAAPAQ LTIFYNGCVC
MiTIFY6B_(CDS_3945)      .......... .......... .......... ..GQSVDGNT INSQPHGGVS
MiTIFY10A_(CDS_6010)     .......... .......... .......... .TMENGAGLQ MTIFYAGKVH
MiTIFY9_(CDS_5306)       .......... .......... ........RP GTETLPETAP LTIFYNGTVT
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**b**

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MiTIFY4B_(CDS_17202)     ------------------------------------------------------------
MiTIFY3B_(CDS_10544)     Q---------------AELPIARRHSLQRFFEKRRDRLAS-KNPYPAQSGTKNSDNAAA
MiTIFY3B_(CDS_3954)      ------------------------------------------------------------
MiTIFY10A_(CDS_14063)    RAV-------------TYLPIARRNSLHRFLQKRKDRITA-RVPYQPIKPAALPNKAAE
MiTIFY10A_(CDS_4785)     ------------------------------------------------------------
MiTIFY5A_(CDS_3195)      ----------------STSGLSMKRSLQRFLQKRKHRALA--SPYGH------------
MiTIFY6B_(CDS_5413)      GSVASTSVMPSGHSNFYAAIPQARKASLALFLEKRKQRSMN-AAPYPLHKKIPEGSTPES
MiTIFY6B_(CDS_3595)      GSAATSLIQT-------VAVPQARKASLARFLEKRKERVLS-TSPYNVNKKSLDCNSPGS
MiTIFY6B_(CDS_3945)      ------------------------------------------------------------
MiTIFY10A_(CDS_6010)     ----------------SDFPIKRRASLHRFFEKRKDRAVA-RAPYQINGGSVAPAKLEE
MiTIFY9_(CDS_5306)       ----------------GDFPIARKKSLQRFLEKRRERLTSVSLPYGC------------
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**c**


**d**


**Figure 3: Multiple sequence alignment of TIFY (a) Jas (b) motif of mango JAZ sequences prepared from MUSCLE tool. Logo sequences for TIFY[TIFYXG] (c) Jas [FN$_2$KRK] (d) motif of TIFY proteins prepared from weblogo**

In this study, we have mined 6 different TIFY transcription factors genes/transcripts (*MiTIFY3B, MiTIFY4B, MiTIFY5A, MiTIFY6B, MiTIFY9, MiTIFY10A*) in the fruit transcriptome data of mango jelly seed. Except *MiTIFY4B* (CDS_17202) and *MiTIFY6B* (CDS_3945) all mined mango TIFY CDS contain the conserved TIFY domain and conventional Jas motif (Fig. 2). Analysis of the deduced amino acid sequence of mango JAZ showed the conservation of the TIFY domains that characterize this family and a Jas domain which is specific of JAZ subfamily. The TIFY domain displayed conserved TIFY[F/Y]XG sequence (Fig. 3 a and b) and Jas domain displayed conserved $FX_2KKR$ like consensus sequence in mango. TIFY and Jas domain logo sequences also showed a highly residue conservation in MiTIFY proteins (Fig. 3 c and d).

Expression analysis (RT-PCR) of TIFY genes were analysed during fruit development and jelly seed of mango (Fig. 4). Results show that these genes are very important players throughout fruit development and jelly seed formation in mango. The signal molecule JA plays vital roles in plant growth, development and responses to environmental stresses.

Previous studies have revealed that the TIFY genes play vital roles in various biological processes of plants such as petiole and hypocotyl elongation[11], lamina size and curvature[12], flower development[13] and seed germination[14]. TIFYs may regulate plant development through the JA signaling pathway. TIFYs may regulate fruit development through the JA signaling pathway. For example, some JAZ proteins can interact with the bHLH/MYB complexes to suppress Jasmonic acid mediated anthocyanin accumulation in *Arabidopsis*[15].

In this study, mined mango TIFY genes were regulated by JA and exhibited differential expression, suggesting that these genes play important role in regulating the normal fruit development through the jasmonic acid signaling pathway. It is noteworthy that a majority of MiTIFY genes were highly expressed at 0 DPA and Jelly seed of mango (Fig. 4), suggesting that MiTIFY genes may function in the development of the flower and jelly seed development of mango. Stress can increase the jasmonic acid and TIFY genes were also shown to play vital roles in response to stresses through the jasmonic acid signaling pathway. During salt stress, *ClJAZ7* exhibited the highest expression in watermelon demonstrating that it may play a major role in salt stress response.

Similarly, over-expression of rice *OsTIFY11a/OsJAZ9* in rice resulted in significantly enhanced tolerance to salt and dehydration stresses and suppression of *OsJAZ9* resulted in reduced salt tolerance through the regulation of JA signaling[16]. In watermelon during flesh development, *ClZML1* showed low expression all the time, while *ClJAZ4* showed an observable accumulation of transcripts. During the development of rind, *ClJAZ4* and *ClJAZ8* exhibited specifically higher expression at some time points, while *ClJAZ5* showed lower transcripts at all stages of rind development. *ClJAZ1, ClJAZ4* and *ClJAZ7* were highly and preferentially expressed in flowers, leaves and fruits.

In addition, *ClJAZ3, ClZML1* and *ClZML2* had higher expression in fruits than in other tissues[17]. In strawbery, *FaJAZ* genes displayed a constant reduction pattern from flowering to ripe fruits and some genes like *FaJAZ1, FaJAZ5* and *FaJAZ8.1* showed a pronounced reduction at ripe stages[18].

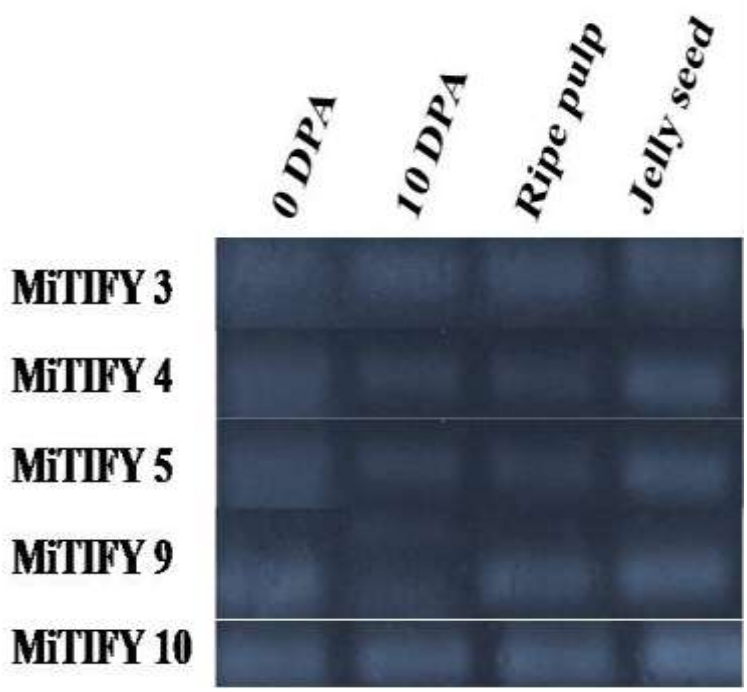


**Figure 4: RT-PCR analysis of different TIFY during fruit development and ripening in mango**

## Conclusion

In this study, a total of 6 TIFY genes were identified in the mango jelly seed transcriptome data. The analysis of semiquantative RT-PCR and RNA-seq data revealed that TIFY genes play developmental and ripening roles. Expression patterns of the TIFY genes during fruit development and jelly seed indicated that they may be involved in development and jelly seed formation by the JA signaling pathway.

Our findings lay a foundation for a further functional characterization of the TIFY family genes in mango and open the window of investigation of an important TIFY transcription factor family of genes and its role in mango fruit development and jelly seed.

## Acknowledgement

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