



RESEARCH ARTICLE

# Unlocking medicinal potential: Comparative transcriptome profiling of *Aegle marmelos* fruit and leaf tissues reveals key functional pathways

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## Abstract

The study aims to provide a comprehensive comparative transcriptome analysis of *Aegle marmelos* (L.) Correa, focusing on its fruit and leaf tissues, with the goal of expanding genomic knowledge and identifying tissue-specific gene expression related to its therapeutic properties. High-throughput RNA sequencing (RNA-seq) was employed to generate transcriptomic data from both fruit (47 million clean reads) and leaf (34 million clean reads) tissues of *A. marmelos*. The resulting sequences were assembled into 61860 unigenes, with 83 % and 89 % of the fruit and leaf-derived transcripts, respectively, mapping to the de novo assembly. Gene Ontology (GO) annotations were performed to categorize the molecular functions of the identified genes. The analysis revealed distinct tissue-specific expression profiles, with 14578 genes exclusive to the leaf and 11086 exclusive to the fruit. The predominant molecular functions were associated with binding, catalytic activity and transporter processes. The data demonstrated significant differences in gene expression between the two tissues, highlighting their divergent roles in the plant's metabolic and therapeutic functions. This study provides the first in-depth genomic resources for *A. marmelos*, enhancing the understanding of its biological and pharmacological potential. The identification of tissue-specific genes offers insights into the molecular mechanisms behind its therapeutic properties, supporting further investigation into its biosynthetic pathways. RNA sequencing emerges as a critical tool for exploring neglected horticultural species, paving the way for targeted research and the discovery of novel bioactive compounds.

**Keywords:** *A. marmelos*; de novo assembly; gene ontology; transcriptome

## Introduction

*Aegle marmelos* (L.) Correa, commonly referred to as bael, is a member of the Rutaceae family and holds significant cultural, medicinal and horticultural value in South and Southeast Asia. Indigenous to India, it is often cultivated in temple gardens and dry deciduous forests across regions such as Sri Lanka, Myanmar, Thailand and Java (1). Its adaptability has led to widespread cultivation in tropical Africa and Southeast Asia (2). The fruit of *A. marmelos* is characterized by a hard, woody rind enclosing a fragrant, orange pulp. Traditionally, the pulp is consumed for its cooling properties and is used as a remedy for gastrointestinal disorders, respiratory ailments and inflammatory conditions (3). Modern analyses confirm its nutritional richness, including

carbohydrates, proteins, dietary fiber, vitamins (A and C) and minerals like calcium and phosphorus, positioning it as a functional food. The fruit pulp has various pharmacological applications and used in treatments of diarrhoea, dysentery, ulcers and even cardiovascular issues (4).

Beyond its dietary uses, *A. marmelos* fruits are processed into value-added products such as jams, syrups and juices, leveraging their high content of bioactive compounds. These include marmelosin, phenolic acids like chlorogenic and ellagic acid and alkaloids like angeline, which contribute to its therapeutic potential (5). Agronomically, selective breeding has prioritized large-fruited varieties (up to 1 kg per fruit) with desirable traits such as thin, easily breakable rind and sweet, fine-

textured pulp for commercial processing (6). Under Indian climatic conditions, peak fruiting occurs from May to June, yielding 50-140 kg per tree annually (4, 7).

Despite its prominence, genomic studies on *A. marmelos* remain limited. Early genetic characterization using RAPD markers (7) provided preliminary insights into its diversity in India's Western Ghats. However, advanced transcriptomic resources are critical to unravel the biosynthetic pathways underlying its medicinal compounds. Previous studies emphasized the need for genomic tools to validate its ethano-pharmacological claims and optimize nutraceutical production (8). To address this gap, the present study conducts a comparative transcriptome analysis of *A. marmelos* leaf and fruit tissues, marking the first report of its fruit and leaf tissue specific transcriptome. RNA sequencing generated 47 million clean reads from fruit and 34 million from leaf tissues, assembled into 61860 unigenes. Tissue-specific mapping efficiencies (83 % for fruit, 89 % for leaf) and GO annotations highlighted functional roles in binding, catalytic activity and transport processes. Distinct expression profiles identified 14578 leaf-exclusive and 11086 fruit-exclusive genes, offering a foundation to explore pathways linked to secondary metabolite synthesis.

This work advances genomic resources for *A. marmelos*, enabling targeted breeding for enhanced bioactive compound production. By elucidating tissue-specific gene expression, it supports efforts to harness its full pharmacological potential while preserving its traditional significance as a medicinal tree.

## Materials and Methods

### Plant material

Bael leaf samples of the Kagzi variety was procured from the Government Garden Nursery in Kurukshetra, Haryana, India (29°58' 06.9" N 76°52'50.8" E). For reference, we downloaded leaf transcriptome data from our earlier study from the NCBI Bio project (PRJNA433585). Horticulturally ripened fruits along with leaf samples were collected in mid-April and collected samples were stored in liquid nitrogen used for extraction of the RNA.

### RNA extraction and sequencing

The fruits were cut open to extract the pulp and accordingly pulp and fruit tissue were used for RNA sequencing. The method outlined by previous researchers (9) was used for extracting total RNA and DNase used to eliminate DNA contamination from the RNA samples. Subsequently, agarose (1 %) gel electrophoresis was executed to verify the reliability of the RNA samples. Later on, RNA obtained from the fruit samples were combined to form a single sample for the development of cDNA libraries with the TruSeq RNA Library Prep Kit v2 (Illumina, Inc., USA). RNA quantification was accomplished through the Agilent 2100 Bioanalyzer (Agilent, USA) and a 1 % agarose gel. The HiSeq 2500 (2 x 150 bp chemistry) platform was used to produce the paired-end reads. To produce mRNA libraries from every sample, 10 g of total RNA was utilized (10). The mRNA library went through qualitative and quantitative validation with an Agilent Bioanalyzer Chip DNA 1000 series II (Santa Clara, CA, USA) and was sequenced via an Illumina HiSeq™ 2000 to produce single-end 100-base pair (bp) sequences. The RNA sequencing data were provided to the NCBI (National Centre for Biotechnology Information, USA) under the BioProject accession number PRJNA433585 (National Centre for Biotechnology

Information). Trinity assembler version 6-8-2012 was utilised to assemble the reads, setting a minimum contig length of 200 bp and an ideal k-mer length of 25 for de novo assembly (11).

### Classification and annotation

BlastX used to compare all unigenes against the non-redundant sequence (nr) and Swiss-Prot databases, applying an e-value cut-off of 1e-5 to differentiate between the two databases. Unigenes from the transcriptome of bael were analysed alongside comparisons to existing EST assemblies, coding sequences from leaves and fruit, all with a cut-off of 1e-5.

## Results and Discussion

Findings of the present investigation is based on fruit pulp and leaf tissues of bael and the cDNA library was constructed utilizing the TruSeq RNA Library Prep Kit. However, due to the absence of a de novo genome assembly in bael, the transcripts was conducted using the procedures outlined by previous researchers (12, 13). In general, Bael genotypes vary in fruit weight, yield, sugar, pulp, seed and fibre content (14-16). Using modern tools, numerous bioactive compounds from nearly every part of bael can be recognized. A total of 49 million and 35 million as raw reads were observed in fruit and leaf samples of bael respectively. However, after trimming, there were 47 million and 34 million clean reads for fruit and leaf tissues, respectively (Table 1). Additionally, GC % for all the unigenes was 46.33 % for fruit pulp while for leaves it was 44.57 %. There were 86 million nucleotides which had a median length 1077 and N50 of 2058 unigenes (Table 2). However, unigenes reported from *Sorbus pohuashanensis*, had 770 bp (13) while *Platyclusus orientalis* had 534 bp (9) are lower to those of our researched unigenes. In order to uncover transcriptionally active genes in Bael tissues, which are critical for its medicinal value, this unique transcriptome survey provides a foundation for future in-depth studies on several vital metabolic pathways.

**Table 1.** Reads obtained in the leaf and fruit sample of bael

Lengths (bp)	Unigenes
Minimum	201
Mean	1405
Median	1077
Maximum	16700
N 50	2058
N 90	661
Total	86898704

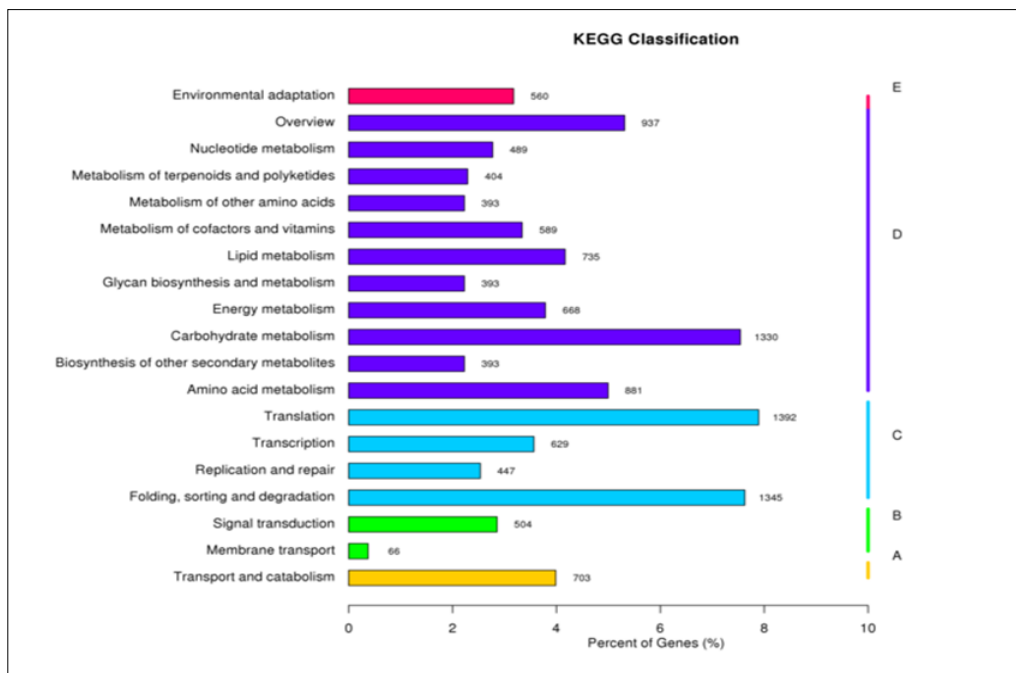
**Table 2.** Length (base pair) of transcripts and unigenes

Name of sample	Raw reads	Clean reads	Error (%)	Q20 (%)	Q30 (%)	GC (%)
Fruit	49587436	47718736	0.02	96.86	92.22	46.33
Leaf	35489094	34841868	0.03	96.82	88.7	44.57

### Gene functional annotation

The information regarding the annotation of genes using different databases was performed to understand annotated and matched genes (Table 3). Out of 61860 unigenes, 71.49 % of genes were annotated with the NR database and 52.20 % were annotated with the GO database. Though 75.55 % unigenes were annotated with the NT database while minor 15.68 % unigenes were annotated with all the databases. The areas of matching and similarity between the genes can be easily understood by depicting the Venn diagram. The Venn diagram mapped with the database annotation is presented in Fig. 1.



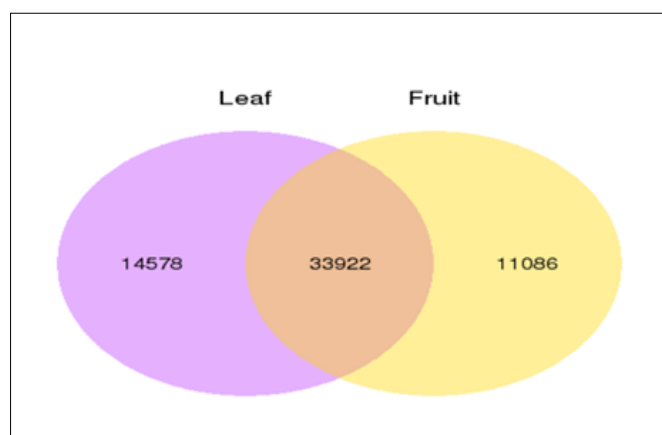


**Fig. 3.** KEGG classification where Y-axis denotes for type of pathway and X-axis for the ratio of genes annotated in the pathway versus total genes.

petroleum ether, arabinose, n-hexane, oleic acid,  $\beta$ -caryophyllene, linolenic acid, oleic palmitic acid, cryptone and humulene which are known to act against several groups of bacteria (23-24). In previous studies aegeline, lupeol, citronella, skimmianine, marmesinine, eugenol, cineol and cuminaldehyde were extracted from leaves (25) whereas marmelide was extracted from fruit (7).

### Expression profiling of unigenes

In order to investigate the differences, reads of leaf and fruit tissue libraries were mapped into the assembly mapped to the special database using the RSEM program. The results revealed that out of a total of 82560604 reads generated for leaf and fruit tissues, 83.35 % of fruits reads were mapped to the database and 89.23 % of the



**Fig. 4.** Venn diagram of expression gene between leaf and fruit tissues of bael.

**Table 4.** Reference alignment of the leaf and fruit sample of bael

Sample	Total reads	Total mapped
Fruit	47718736	39771516(83.35 %)
Leaf	34841868	31090806(89.23 %)

leaf reads were mapped to the database (Table 4).

### Filtering the differential gene expression

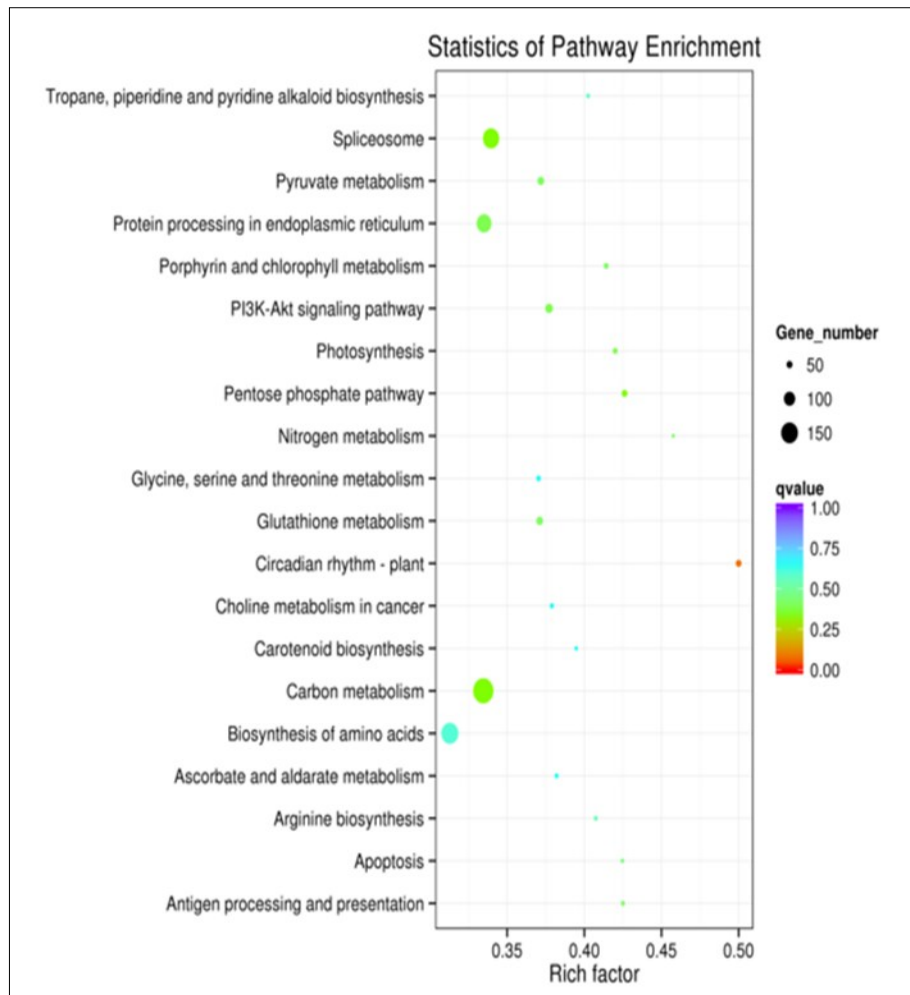
The Venn diagram of expression genes are plotted when only two samples or groups are used. Individually in each circle within a group and the overlap represents the genes expressed in common between groups. Based on the FPKM > 0.3 criteria, the number of genes expressed in leaf was 14578 while in fruit it was 11086, whereas the genes common to both the samples was around 33922 (Fig. 4).

### KEGG pathway enrichment analysis

The KEG plot unveiled the DEG profile enriched in the KEGG pathway which displayed top 20 enriched pathways. The degree of KEG enrichment was determined by richness element, q-value and number of genes. The findings of present study were related to the carbon metabolism, spliceosome and protein processing in the endoplasmic reticulum (Fig. 5). From the KEGG pathway, few mechanisms and functions were determined, wherein the p-value is

the same for three mechanisms i.e., for carbon metabolism, pentose phosphate pathway and spliceosome, which is 0.33, whereas the corrected p-value for circadian rhythm plant was 0.079932.

Various studies have demonstrated that the bael tree possess many coumarins viz., marmenol, marmelosin, scoparone, imperatorin, methyl ether, marmin, xanthotoxol, marmesin, scopoletin, marmelid, psoralen and umbelliferone (26-27). Additionally, other alkaloids like fragrine, aegelenine, aeglin, dictamine, etc., are reported in bael (12, 28). Similarly, the potential molecular mechanisms of flavonoids and carotenoids in guava pulp with different colours were reported earlier (29). Additionally, comparative transcriptome analysis revealed molecular response of salt-tolerant and sensitive polyembryonic mango genotypes to salinity stress at seedling stage (30) and citrate accumulation in sweet orange (31). The transcriptome analysis of *Aegle marmelos* provides crucial insights into its genetic makeup and bioactive compound production, highlighting its significant potential in improving human health. The identification of key genes involved in secondary metabolite biosynthesis, particularly those responsible for terpenoid and alkaloid production, underlines Bael's medicinal value. As a source of vital phytochemicals, bael can play an essential role in addressing health challenges, reinforcing its



**Fig. 5.** Statistics of pathway enrichment in Bael. Dot size denotes the number of the genes and the colour shows the q value.

importance in future research and biotechnological applications for human benefit (7).

## Conclusion

Bael fruits are well known for their nutritional and medicinal values. Bael is not well studied on the lines of molecular biology and genomics and information regarding the transcriptome of bael fruit mRNA is lacking. To fill this gap, we tried in our study to assemble and compare the leaf and fruit transcriptome of *A. marmelos*. For the first time, we have compared the fruit transcriptome with the leaf transcriptome of bael and here the fruit transcriptome was inferior to the leaf. This study has generated the necessary genomic resources for *A. marmelos*.

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## Authors' contributions

PK<sup>1</sup> and KS conceptualised the work, carried out the systematic literature review and drafted the manuscript. MS, AY, and PSD carried out the graphical presentation. VY, PR, AI and DSM wrote the manuscript, edited the manuscript and Literature survey. PK<sup>2</sup>, DSM and MS attended the editing work and coordination.

PK<sup>1</sup>, MS, AY, PSD and KS participated in editing the manuscript and sample analysis. All authors read and approved the final manuscript (PK<sup>1</sup>- Prashant Kaushik; PK<sup>2</sup>- Prabhat Kumar).

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None

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