Rapid and Efficient RNA Isolation Protocol from Various Recalcitrant Tissues of Mango (*Mangifera indica* L.)

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Mango (*Mangifera indica* L.) is an economically important fruit crop of India. Based on its popularity in the masses, wide adaptability, high nutritive value, varietal diversity, delicious taste, excellent flavor and attractive appearance, it is appropriately titled as 'King of fruits' in India. It is grown in 2.5 million hectares with a production of 18 million tones ranking first in area and second in production among all other fruit crops cultivated in the country. Mango fruits are universally consumed as fresh fruits and the biochemical changes that occur during ripening impart fruit quality in terms of softening, carotenoid accumulation, and flavor volatile production. All these biochemical events are regulated at gene level and understanding these events is of utmost importance in improving the fruit quality and storage potential, which necessitates isolation of good quality RNA from the mango fruits during different stages of development and ripening.

Ribonucleic acid (RNA) isolation is a critical step in the molecular experiments involving reverse transcription polymerase chain reaction (RT-PCR), rapid amplification of cDNA ends (RACE), Northern hybridization, microarray analysis and transcriptome analysis for deciphering the mechanisms of gene expression, gene regulation, signal transduction and post translational studies. Mango is one of the most complex crop from which the RNA isolation was proved to be is very difficult due to significant difference in the chemical composition of various tissues at different stages of development and ripening, such as sudden shifts in pH, alterations in fatty acid, lipid, and protein concentrations, conversion of starch to sugars, and protopectins to pectin etc. Various protocols have been tried by the different researchers for isolation of good quality RNA from the mango tissues rich in polysaccharides and secondary metabolites, but most of them have failed. Though many tissue specific protocols have been developed, most of these conventional methods used for the extraction of RNA from different tissues of complex crops were successful only to some extent but they involve tedious procedural steps and require long

periods (1 or 2 days) for completion of extraction process. Hence, we attempted to develop a comprehensive and efficient protocol for the isolation of good quality RNA from different tissues of mango. The newly developed protocol has worked well for extracting RNA from mango tissues such as leaves, flowers, fruits, fruit peel and seed kernel. The quality (A260/A280 : 1.6-2.05 and A260/A230 : 1.6-2.2) as well as quantity (16-80 µg g^{-1} tissue) of the RNA was better in comparison to other methods. In addition, the shorter period of protocol allows us to simultaneously process many number of samples (10-12) in a single working day.

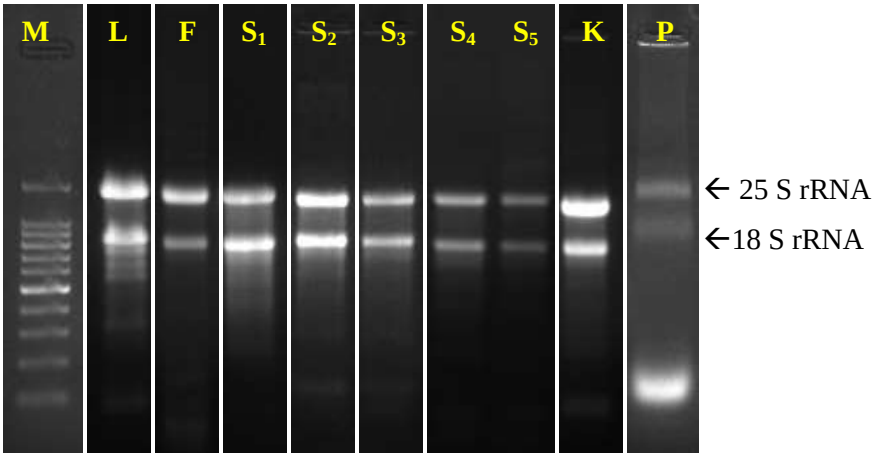
FLOW-CHART OF METHODOLOGY

1. The sample (leaf, flower, peel and kernel , fruit pulp) was frozen and ground thoroughly to a fine powder using pre-chilled mortar and pestle.
2. To this fine powder, 2ml of 1:1 pre-heated mixture of extraction buffer and saturated phenol was added and the mixture was homogenized thoroughly.
3. After complete thawing and intermittent grinding, 800 µl of DEPC treated water was added to it, mixed thoroughly and transferred to micro centrifuge tubes and incubated at room temperature for 5 min.
4. Later 200 µl of chloroform was added to each tube, vortexed thoroughly and incubated at room temperature for 10 minutes.
5. The incubated tubes were centrifuged at 13,000 rpm for 10 min. and the upper aqueous phase was transferred carefully into a 1.5 ml eppendorf tube without disturbing the equatorial plate formed in it.
6. To the aqueous phase, 0.6th volume of chilled isopropanol was added, vortexed thoroughly and incubated at room temperature for 10 min.
7. The mixture was centrifuged at 13,000 rpm for 10 min to collect the RNA pellet at the bottom of the tube.
8. The pellet was washed with 1 ml chilled ethanol (75 %) and air dried in an incubator at 27° C before eluting in 15-20 µl of microfiltered DEPC water
9. The eluted RNA was heated at 65°C and cooled immediately before storing at -80°C for future applications.

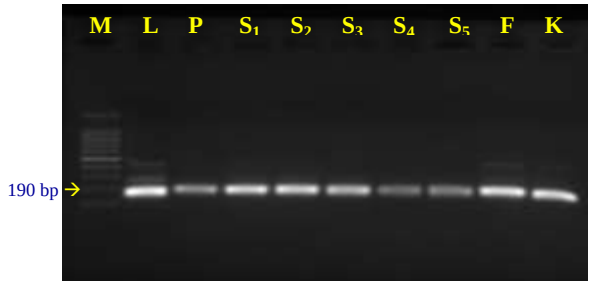
Mango sample	A_{260}/A_{280}	A_{260}/A_{230}	Concentration (µg g^{-1})

Quality and yield of RNA isolated from various tissues of mango using the newly developed protocol

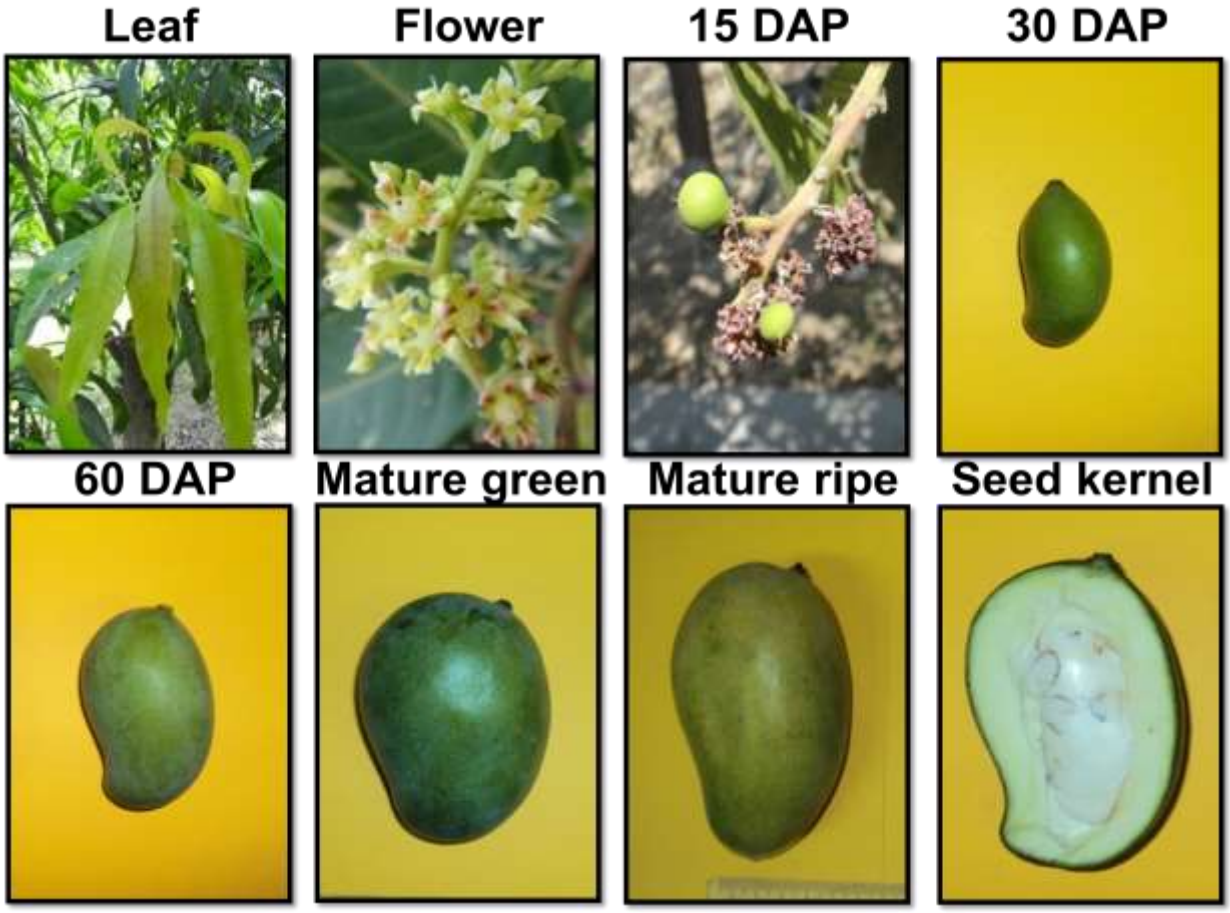
Mango sample	A_{260}/A_{280}	A_{260}/A_{230}	Concentration (µg g^{-1})
Young leaf	1.57	1.39	84.83
Flower	1.59	1.41	40.89
Fruit stage-S_1 (30 DAP)	2.00	2.20	41.68
Fruit stage-S_2 (60 DAP)	2.01	2.13	28.66
Fruit stage-S_3 (90 DAP)	2.03	2.14	21.48
Fruit stage-S_4 (mature unripe)	2.05	1.98	18.37
Fruit stage-S_5 (mature ripe)	2.03	1.68	16.57
Fruit peel	1.47	1.48	19.44
Seed kernel	1.78	1.61	52.94



1.2 % (w/v) Agarose gel electrophoresis of RNA isolated from different tissues of mango. RNA was visualised by staining with 0.1 µl ml^{-1}ethidium bromide and observed under a UV light. Lane M, 100 bp DNA ladder; lane L,leaf; lane F, flower; lane S_1, 30 DAP; lane S_2, 60 DAP; lane S_3,90 DAP; lane S_4, Mature unripe; lane S_5, Mature ripe; lane K, Seed kernel; lane P, fruit peel.



RT-PCR amplification of transcripts of the *actin* gene isolated using the protocol described in this paper from various mango tissues.Lane M, 100 bp molecular markers; lane L, leaf; lane F, flower; lanes S_1-S_5, fruit stages S_1-30 DAP; S_2 -60 DAP; S_3 -90 DAP; S_4- mature unripe; S_5- mature ripe; lane K, seed kernel; lane P,- fruit peel.



Different tissues of mango used for RNA isolation

Special advantages of this method

- This method is quite efficient for the isolation of good quality (i.e., high purity and integrity) and good quantity of RNA from various problematic tissues of mango.

- It has been developed to reduced the chemical usage and lower toxicity (CTAB- free, guanidine- free, and LiCl- free etc.) compared to other conventional protocols
- Through reduction in number of steps it takes the shortest time period of 1 - 2 h for RNA isolation.
- This method can be used for high- throughput sampling (10-12 samples in a day).
- The RNA isolated using this protocol was also suitable and highly competent for molecular downstream applications such as the construction of a cDNA library and RT-PCR.
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