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A SCIENCE AND TECHNOLOGY NEWSLETTER

RESEARCH UPDATE

PROMISING TECHNOLOGIES

Rapid- and- efficient RNA isolation protocol for recalcitrant tissues of mango

Mango (*Mangifera indica*) is an economically important fruit-crop of India. Biochemical changes occurring in the fruit during its ripening impart it softening, carotenoid accumulation and flavour-production qualities. And all the biochemical events are regulated at the gene level, and thus understanding them is of paramount importance in improving fruit quality and its storage potential. Thus, there is a need to isolate good quality RNA from fruits at different stages of development and ripening.

Ribonucleic acid (RNA) isolation is a critical step for molecular experiments involving reverse transcription polymerase chain reaction (RT-PCR), rapid amplification of cDNA ends (RACE), Northern hybridization, and microarray analysis and transcriptome analysis for deciphering mechanisms of gene expression, gene regulation, signal transduction and in post-translational studies.

Mango is one of the most complex crops from which RNA isolation was found very difficult due to significant differences in chemical composition of its tissues at varied stages of development, such as sudden shift in *p*H, changes in fatty acid, lipid and protein

Special Advantages of the Protocol

- This method is quite efficient for isolation of good quality (i.e., high purity and integrity) and quantity of RNA from problematic tissues of mango.
- It has been developed to reduce chemical usage and to lower toxicity (CTAB-free, guanidine-free and LiCl-free), compared to conventional protocols.
- Through reduction in number of steps, it takes lesser time 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 has been found suitable and highly competent for molecular downstream applications such as construction of a cDNA library and for RT-PCR.

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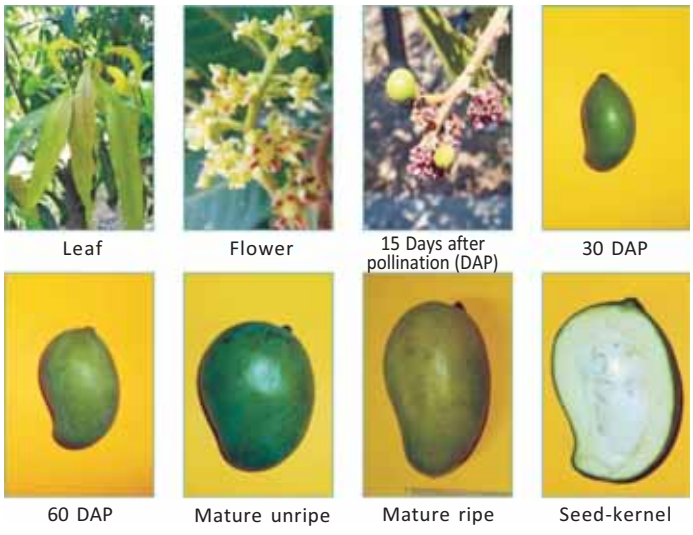
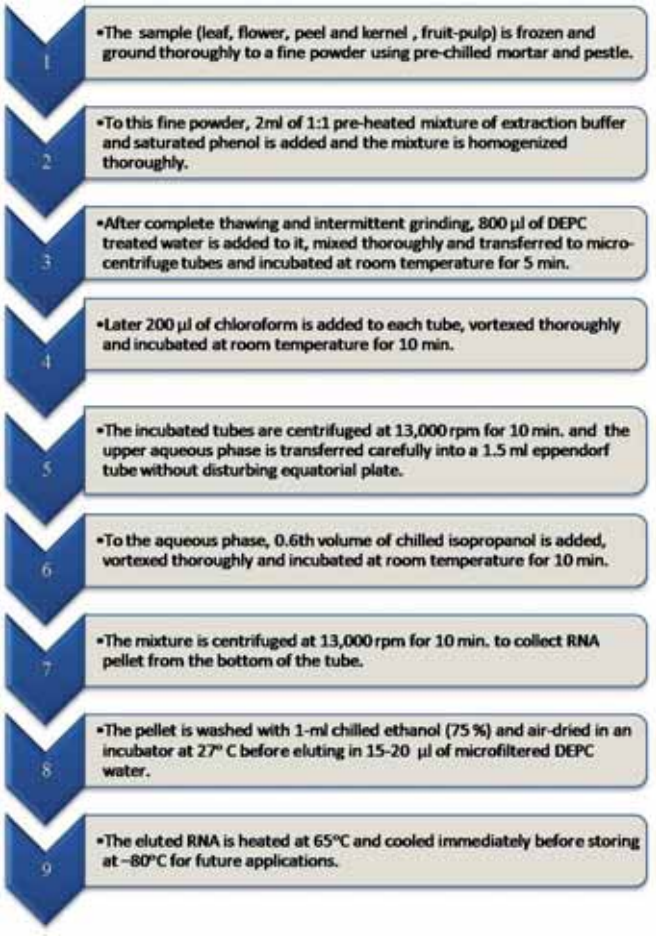


Different tissues of mango used for RNA isolation

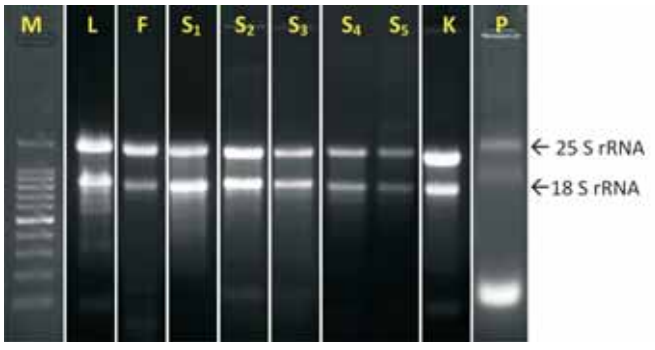
concentrations, and conversion of starch into sugars and protopectins into pectin. Many protocols were tried for isolation of good quality RNA from the tissues rich in polysaccharides and secondary metabolites, but most of them failed.

This newly developed protocol has worked well for extracting RNA from tissues of leaf, flower, fruit, fruit-peel and seed- kernel of mango. The quality (A260/A280: 1.6-2.05 and A260/A230: 1.6-2.2) as well as the quantity (16-80 µg/ g tissue) of the RNA were better with this in comparison to other methods. In addition, the shorter period of the protocol allows simultaneous processing of 10-12 samples in a single working day.

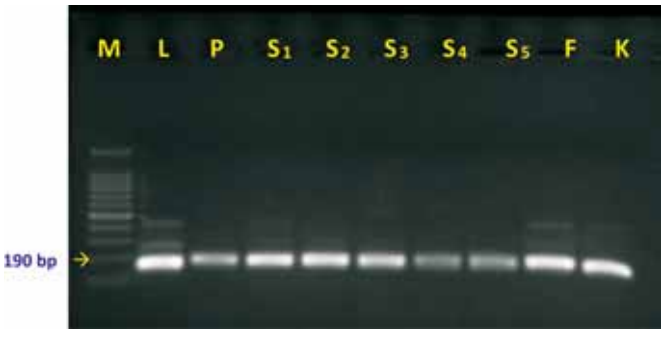


Quality and yield of RNA isolated from mango tissues with the protocol

Mango sample	A_{260}/A_{280}	A_{260}/A_{230}	Concentration (µg/g tissue)
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



Agarose gel 1.2% (w/v) electrophoresis of RNA isolated from different tissues of mango. RNA was stained with 0.1 µl/ml ethidium bromide and observed under UV light. (Lane M, 100 bp DNA ladder; lane L,leaf; lane F, flower; lanes S_1-S_5 fruit stages: 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 from various mango tissues using the protocol. (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)

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