ISO 9001:2008 Organization



Agrésearch with a Buman touch



1

3

5

6

9

10



RESEARCH UPDATE

Promising Technologies

- Rapid- and- efficient RNA isolation protocol for recalcitrant tissues of mango
- Magnetic field application catalyzes growth of sunflower-crop
- Biomaterials for corneal grafting in animals

New Initiatives

- Leaf blotch on walnut in Uttarakhand
- Cashewnut a valuable nutritional 7 package

Natural Resource Management

- New chemotypes of sweet basil –
 Rich source of methyl chavicol
- SSR markers identified in *Chitala* chitala through third generation sequencer

Profile

 ICAR-Indian Institute of Soil Science, 11 Bhopal

Spectrum

- Genetic diversity of chayote 21 (Sechium edule (Jacq.) Sw.) in Sikkim Hills
- Technology for remote and degraded 22 coastal lands of Andaman Islands
- Knocking down *myostatin* gene in chicken for enhancing body weight
- Population stock structure of narrow-barred Spanish mackerel in Indian waters

Way Forward

23

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 flavourproduction 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 pH, 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.
 - Indian Council of Agricultural Research Krishi Bhavan, New Delhi 110 001, India

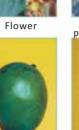
www.icar.org.in

PROMISING TECHNOLOGIES











30 DAP



60 DAPMature unripeMature ripeSeed-kernelDifferent 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.

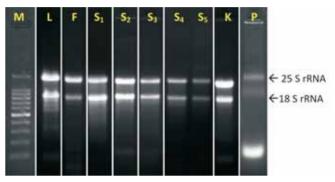
This newly developed protocol has worked well for extracting RNA from tissues of leaf, flower, fruit, fruit-peel and seed- kernel of mango. The quality (A260/A280: 1.6-2.05 and A260/A230: 1.6-2.2) as well as the quantity (16-80 μ g/g tissue) of the RNA were better with this in

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

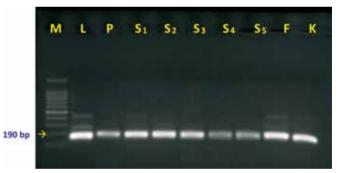
2

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 μ l/ml ethidium bromide and observed under UV light. (Lane M, 100 bp DNA ladder; lane L,leaf; lane F, flower; lanes S₁-S₅ fruit stages: lane S₁, 30 DAP; lane S₂, 60 DAP; lane S₃, 90 DAP; lane S₄, mature unripe; lane S₅, 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)

comparison to other methods. In addition, the shorter period of the protocol allows simultaneous processing of 10-12 samples in a single working day.

S.V.R. Reddy¹, R.R. Sharma¹, S. Barthakur² and M. Srivastav³

¹Division of Food Science and Post-harvest Technology ICAR-IARI, New Delhi 110 012; ²ICAR-National Research Centre on Plant Biotechnology, New Delhi 110 012; ³Division of Fruits and Horticultural Technology ICAR-IARI, New Delhi 110 012 *e-mail*: rrs_fht@rediffmail.com

ICAR NEWS