A simple and rapid method for RNA isolation from plant tissues with high phenolic compounds and polysaccharides

Kam-Lock Chan
Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM-Serdang, Selangor, Malaysia.

Chai-Ling Ho
Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM-Serdang, Selangor, Malaysia.

Parameswari Namasivayam
Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM-Serdang, Selangor, Malaysia.

Suhaimi Napis
Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM-Serdang, Selangor, Malaysia.

Method Article

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Abstract

Introduction

Isolation of good quality RNA from plant tissues such as mangosteen is troublesome and challenging because they are rich in secondary metabolites such as phenolic compounds and polysaccharides that coprecipitate with nucleic acids. The phenolic substances interact irreversibly with nucleic acids and proteins[1], leading to their oxidation and degradation[2] and finally render RNA unsuitable for downstream purposes. Previously, two RNA extraction protocols have been tried on mangosteen tissues i.e. the methods described by Rochester _et al._ [3] and Matsumura _et al._ [4], respectively; however, the RNA isolated were partially degraded, brown in color and difficult to dissolve \(Figure 1 a-b, Table 1). This may due to the browning effect[1], whereby a brown color supernatant is developed upon oxidation of the homogenate. The poor quality of RNA may attribute to coprecipitation of polysaccharides and oxidation of phenolic compounds that interact irreversibly with nucleic acids[1,2]. In this study, a method was developed to isolate good quality RNA from leaves and flowers of mangosteen. In this method, polyvinylpyrrolidone \(PVP) was added in the extraction buffer, so that it can bind to the phenolic compounds which are then eliminated by ethanol precipitation. The protocol described here is simple, fast and does not require ultracentrifugation.

Reagents

•Liquid nitrogen •Extraction buffer: 0.25 M NaCl, 0.05 M Tris-HCl \(pH 7.5), 20 mM EDTA, 1% \(w/v) sodium dodecyl sulphate \(SDS) and 4% \(w/v) PVP \(M.W. 360,000) •Chloroform : Isoamyl alcohol \(CI, 24:1 v/v) •Phenol:Chloroform isoamyl alcohol \(PCI, 1:1 v/v) •3 M sodium acetate \(adjusted to pH 5.2 with acetic acid) •Cold 70 % \(v/v) ethanol •Cold absolute ethanol •0.1 % \(v/v) diethyl pyrocarbonate \(DEPC)-treated-autoclaved water •10 M LiCl

Equipment

•Pre-cooled pestle and mortar CRITICAL This is important to avoid the thawing of frozen tissues. •High speed centrifuge \(Beckman JS-HS, USA) •Spectrophotometer \(Pharmacia Biotech, UK) •Agorose gel electrophoresis equipment •Power supply •Vortex mixer

Procedure

RNA extraction 1 Add 7.5 ml of extraction buffer and 7.5 ml of CI to a 30 ml-round bottom Nalgene tube. **Critical Step** 2 Grind 1 g frozen mangosteen sample to fine powder with a mortar and pestle in liquid nitrogen. 3 Transfer 1 g of ground sample to a tube containing the extraction buffer and CI and vortex vigorously. 4 Centrifuge at 12,857 _g_ for 2 min at room temperature. 5 Transfer the supernatant to a new 30 ml-round bottom Nalgene tube and purify with an equal volume of PCI. Centrifuge at 12,857 _g_ for 2 min at room temperature. Repeat this step until there is a clean interface observed. **Critical Step**

6 Transfer the supernatant to a new 30 ml-round bottom Nalgene tube and add one tenth volume of 3 M sodium acetate pH 5.2 and 2.5 volume of cold absolute ethanol, mix well, and incubate at 4 °C for 30 min. **Critical Step** 7 Recover the nucleic acids by centrifugation at 12,857 _g_ for 20 min at 4 °C. 8 Wash the pellet with 70% \(v/v) ethanol, air-dry, and add 200 µl DEPC-treated water to dissolve the pellet. 9 Transfer the supernatant to a 1.5 ml tube and add 10 M LiCl to a final concentration of 2 M and keep on ice for 1 h. Centrifuge at 18,514 _g_ for 20 min at 4 °C. 10 Wash the pellet with 70% \(v/v) ethanol, air-dry, and add 20 µl DEPC-treated water to dissolve the pellet. Centrifuge at 18,514 _g_ for 10 min at 4 °C. 11 Add 0.1 volume of 3 M sodium acetate pH 5.2 and 2.5 volume of cold absolute ethanol, mix well, and store at -80 °C. **PAUSE POINT** RNA can be stored in ethanol at -80 °C for at least 2 months. **Analysis of RNA quality** 12. The total RNA was quantified with a spectrophotometer at 230, 260, 280 nm. The integrity of total RNA was verified by analyzing approximately 1 µg RNA sample on 1% \(w/v) formaldehyde denaturing agarose gel \(Sambrook _et al._, 1989).

Timing

Steps 1-11, 3-4 h Step 12, 2-3 h

Critical Steps

Step 1. This should be done before grinding. Ground sample should be added into the extraction buffer in the shortest time possible to avoid it from thawing. The presence of water will hasten the RNA degradation process. Step 5. It is important to ensure that a clean interphase is obtained to avoid contamination of protein. Step 6. Incubation at -80 °C for 30 min usually causes browning.

Anticipated Results

PVP is an inhibitor of polyphenol oxidase which can prevent browning effect[6,7] and its use in the removal of secondary metabolites from nucleic acid has been widely reported[8,9,10]. In this study, the use of PVP in the extraction buffer renders a significant improvement in both yield and quality of RNA as compared to that obtained from other methods \(Figure 1, Table 1). The RNA extracted by the modified method showed two distinct rRNAs with no degradation. The protocol developed in this study also gave the best quality of RNA for mangosteen, the $A_{260/280}$ and $A_{260/230}$ obtained were 1.79 and 2.75, respectively \(Table 1, Figure 2), indicating minimum contamination from proteins and polysaccharides in the RNA isolated. There was no severe browning of RNA observed during LiCl precipitation. Furthermore, with this modification, this improved method can be completed within three to four hours as compared to the six to seven hours of the original protocol[4]. Table 2 shows that the mature leaf, flower of more than 2 cm, and fully open flower gave lower yield and quality of RNA compared to the other tissues. Mature leaves may possess higher levels of secondary cell wall materials that contribute to fresh weight, thus reducing the cell number per g of tissue and decreasing the amount of RNA per gram fresh weight. Similarly, the low yield of total RNA from flower more than 2 cm and fully open flower is likely because these stages have a

bigger average cell size and lower cell number as compared to the same fresh weight of close floral buds. We also showed that the modified protocol described herein produced total RNA of sufficient quality for RT-PCR of a housekeeping gene, cyclophilin with the expected size of approximately 320 bp \(Figure 2). A cDNA library of 9 X 108 pfu/mL and a subtractive cDNA library were also successfully constructed by using the RNA extracted by using this protocol \(data not shown). In conclusion, the modified method developed in this study allowed the isolation of intact, high yield and quality RNA from mangosteen leaves and flowers and may be other plant tissues with high phenolic compounds and polysaccharides, for RT-PCR and library constructions. This method is effective, simple and can be completed within four hours and does not require ultracentrifugation.

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Figures

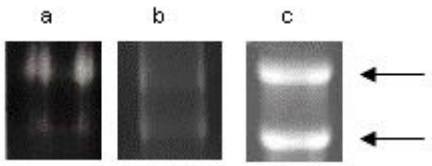

Figure 1

Comparison of total RNA isolated by four different methods. Comparison of total RNA isolated by four different methods. RNA extracted from young shoot by using (a) Rochester _et al._ ^3^ method; (b) Matsumura _et al._ ^4^ method; (c) NSTEP method (this protocol). RNA samples (approximately 1 μg each) were electrophoresed in a 1 % (w/v) formaldehyde denaturing agarose gel. The 28 S (upper) and 18 S (lower) rRNAs are indicated by arrows.

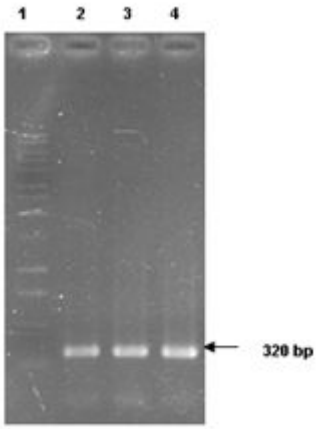

Figure 2

RT-PCR analysis of a housekeeping gene, cyclophilin. RT-PCR analysis of a housekeeping gene, cyclophilin. Lane 1: 1kb marker (Promega, USA); Lanes 2-4, RT-PCR amplicons from young shoot (1); flower of >1.0-1.5 cm (2) and flower of >1.5-2.0 cm (3), respectively. Samples were electrophoresed in a 1 % (w/v) agarose gel.

Method	Tissue	Purity		RNA Yield (µg/g)
		$A_{260/280}$	$A_{260/230}$	
Rochester *et al.*[3]	Young shoot	1.69	0.89	154.4
Matsumura *et al.*[4]	Young shoot	1.11	Negative	7.56
NSTEP (This protocol)	Young shoot	1.79	2.75	80
	Flower 0.5-1.0 cm	1.72	2.21	139.68
	Flower > 1-1.5 cm	1.72	2.17	110.27
	Flower 1.5-2.0 cm	1.71	1.21	120
	Flower > 2.0 cm	1.68	2.27	38.07
	Fully open flower	1.60	1.73	26.67
	Mature leaves	1.73	2.46	8.72

Figure 3

Table 1 Purity and yields of RNA from mangosteen extracted using different extraction methods.