The Isolation of RNA from Raspberry *(Rubus idaeus)* Fruit

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Abstract

Previous attempts to extract high-quality, total RNA from raspberry *(Rubus idaeus)* fruits using published protocols have proven to be unsuccessful. Even the use of protocols developed for the extraction of RNA from other fruit tissue has resulted in low yields *(1)* or the isolation of degraded RNA *(2)*. Here, we report on the development of a quick and simple method of extracting total RNA from raspberry fruit. Using this method, high yields of good quality, undegraded RNA were obtained from fruit at all stages of ripening. The RNA is of sufficient quality for northern analysis and cDNA library construction.

Index Entries: RNA extraction; fruit tissue; raspberry; *Rubus idaeus*.

1. Introduction

The focus of many recent plant biotechnology programs has been the application of molecular techniques to processes involved in fruit ripening *(3)*. Research into improving the processing and postharvest characteristics of tomato fruit has been particularly successful *(4)*. Similar approaches are now being applied to a wide range of fruit species. The enzymic degradation of cell walls during ripening results in reduced postharvest quality and limited shelf-life for fresh raspberries *(5)*. The softening processes also render the fruit more susceptible to fungal pathogens *(6)*. The consequent reduction in the commercial potential of the crop for both processing and fresh fruit markets, makes the raspberry an attractive target for improvement using a molecular approach.

A requirement for good yields of high-quality RNA underpins any attempt to isolate genes from raspberry fruit by cDNA-based methodologies. Conventional methods of RNA extraction can be slow, laborious, and, when applied to fruit, low yielding *(7,8)*. Fruits are also a particular problem because they contain elevated levels of RNases and polysaccharides *(1)*. Protocols have been described for the isolation of RNA from several fruits *(1,2)*. When applied to raspberry fruit, however, these methods yielded either degraded or poor quality RNA (unpublished data).

Here we report on the development of a new method that can be used to isolate a reproducibly high yield of RNA from all stages of raspberry fruit development, as well as from leaf, stem, and root tissues. This high-quality RNA can be used for the construction of a cDNA library. We also report on some problems that may arise during the implementation of this technique.

2. Materials

2.1. Fruit Material

Raspberry (cv. Glen Clova) fruit material from glasshouse-grown plants, representing four ripening stages according to a subjective assessment of berry color (green, white, pink, and red) were harvested directly into liquid nitrogen and stored at –70°C until required.

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2.2. Extraction of RNA

1. Pestle and mortars, and glassware were baked for 2 h at 180°C.
2. 50-mL Centrifuge tubes (Oak-Ridge, Nalgene, Rochester, NY), were treated with a 0.1% solution of diethyl pyrocarbonate (DEPC) (Sigma, St. Louis, MO) overnight and autoclaved (120°C for 20 min).
3. Lysis buffer: 2% sodium dodecyl sulfate (SDS), 50 m*M* ethylenediamine tetra-acetic acid (EDTA), 300 m*M* Tris (adjusted to pH 8.0 with boric acid). Constituents were made up as concentrated stock solutions, autoclaved, and then combined.
4. Phenol (Sigma; phenol phase equilibrated to pH 7.9).
5. Insoluble polyvinylpolypyrrolidone ([PVPP]; Sigma).
6. Chloroform:isoamyl alcohol (24:1).
7. 12 *M* LiCl, autoclaved before use.
8. Ice-cold 70% (v/v) ethanol.
9. Sterile distilled water.

3. Method

1. Use 4 g fresh weight of frozen fruit for each extraction. Grind samples to a fine powder in liquid nitrogen in a precooled, pestle and mortar and allow to stand briefly at room temperature while excess liquid nitrogen volatilizes.
2. Add 20 mL lysis buffer:phenol (1:1[v:v]) preheated to 80°C (*see* **Note 3**).
3. Transfer the mixture to a DEPC-treated centrifuge tube containing insoluble PVPP to a concentration of 8.5% (w/v) and vortex for 1 min (*see* **Note 3**).
4. Centrifuge the mixture at 20,000*g* for 10 min at 4°C.
5. Remove approx 13 mL of the upper aqueous phase to a fresh tube and add an equal volume of 24:1 chloroform:isoamyl alcohol. Vortex the mixture and centrifuge as in **step 4**.
6. Remove approx 12 mL of the upper aqueous phase to a fresh tube and add one third volume of 12 *M* LiCl, mix thoroughly, and incubate at 4°C overnight.
7. Mark the tube to indicate the expected area of the RNA pellet and centrifuge at 20,000*g* for 90 min at 4°C.
8. Decant the supernatant and gently wash the pellet with 5 mL ice-cold 70% (v/v) ethanol. Decant the ethanol from the tube which should then be inverted to dry the pellet.
9. Resuspend the RNA pellet in 1 mL of sterile distilled water and transfer to a 2-mL Eppendorf tube. Following centrifugation at 8000*g* for 5 min at 4°C to remove any insoluble material, transfer the supernatant to a fresh 2-mL Eppendorf tube.
10. Add LiCl to a concentration of 0.8 *M* followed by 1 mL of propan-2-ol. Incubate the mixture at –20°C for at least 2 h.
11. Centrifuge the mixture at top speed in an Eppendorf centrifuge for 30 min at 4°C to pellet the RNA.
12. Wash the RNA pellet with 1 mL ice-cold 70% ethanol by dislodging and pipeting over the pellet approx 10 times. Centrifuge the Eppendorf tube briefly and decant the ethanol. Remove any residual ethanol by incubating the inverted Eppendorf tube at room temperature for 5 min.
13. Resuspend the RNA pellet in 50–100 µL of sterile distilled water.
14. Determine the total amount and purity of RNA by measurement of the A_{230}, A_{260}, and the A_{280} of the sample ***(9)*** (*see* **Note 2**).
15. Routinely, separate 10 µg of the RNA by electrophoresis on a denaturing agarose gel and visualize by ethidium bromide staining (*see* **Notes 1** and **4**).
16. The RNA may be stored at –70°C until required.

4. Notes

1. The average yield of total RNA is 50–100 µg/g fresh weight of fruit for each stage of fruit ripening investigated. This compares favorably with reported yields of between 12 and 20 µg/g fresh weight, using protocols developed for other fruit tissues ***(8)***. Following electrophoresis through denaturing agarose gels, intact ribosomal RNA bands were observed by ethidium bromide staining, providing an indication that the RNA is undegraded.
2. The extraction and precipitation conditions were critical to avoid the coprecipitation of polysaccharides and proteins. The $A_{260/280}$ and the $A_{260/230}$ ratios of the RNA were ≥2:1, approximating to that expected for a pure RNA sample ***(9)***. A spectrum of RNA extracted from raspberry fruit using this method indicates that there is minimal contamination with proteins and carbohydrates when compared with RNA extracted from raspberry fruit using another protocol published for the extraction of RNA from plant material (***10***; **Fig. 1**).

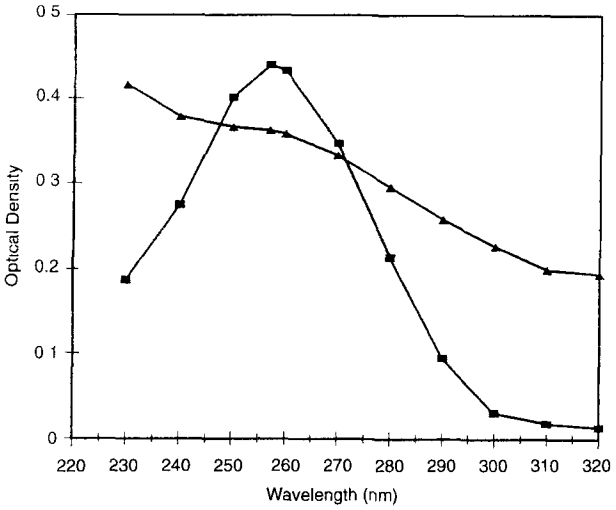

Fig. 1. Absorption spectra of RNA extracted from raspberry fruit employing the new method described in this article (■) and applying the protocol developed for the isolation of RNA from potato leaves (▲) *(9)*.

3. Protocols developed for the extraction of RNA from other plant species, such as tomato pericarp *(1)*, mango mesocarp *(2)*, and potato leaf *(10)*, could not be applied to raspberry fruit, emphasizing the requirement for the development of extraction methods for different plant tissues. Particular problems that had to be overcome with the raspberry was the high acidity of fruit extracts (measured as pH 3.5–4.0 for Glen Clova), combined with significant levels of phenolic compounds, polysaccharides, and RNases. Important features of this method, therefore, include the use of a lysis buffer with a high buffering capacity (300 m*M* Tris/Boric Acid). Initial attempts using a lower buffer concentration resulted in the isolation of poor quality RNA. In addition, the inclusion of phenol in the lysis buffer was essential for the rapid inactivation of RNases. The use of a high concentration of insoluble PVPP also appeared to prevent polyphenol oxidation and subsequent binding to nucleic acids and proteins *(8)*.
4. Total RNA was used for Northern blot analysis and mRNA purified from total RNA by oligo dT cellulose chromatography (Pharmacia Biotech, St. Albans, UK), was used as a template for cDNA synthesis. A cDNA library was constructed with an average insert size of 1 kb.

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