Extraction of Total RNA from Melon Mesocarp Tissue

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Analysis of fruit ripening at the molecular level requires extraction of intact ribonucleic acid (RNA) from fruit tissue. Many fruits contain substances, such as polyphenols and polysaccharides, that can interfere with the isolation of RNA. Unique RNA extraction methods have been developed for diverse fruit tissues and sometimes for different maturity stages of the same fruit tissue (Franke et al., 1995). RNA isolated from mature melon tissue using the procedure of Lopez-Gomez et al. (1992) failed to present two ribosomal RNA bands upon gel electrophoresis. To our knowledge, no published method is currently available for extracting total RNA from melon (*Cucumis melo* L.) mesocarp tissue, nor have optimum sampling and handling procedures been identified for such tissue. Extraction of RNA from mature, pre- and postclimacteric tissue is likely to be more difficult than from immature tissue because biosynthesis of potentially interfering substances is known to increase during maturation and ripening. Whether a similar method can be used to extract total RNA from ripe and unripe muskmelon (*Cucumis melo* var. *reticulatus* Naud.) and honeydew (*Cucumis melo* var. *inodorus* Naud.) type melons is unknown. We isolated intact, total RNA from mature, pre- and postclimacteric melon tissue by modifying a procedure for plant tissue (Pawlowski et al., 1994).

Mesocarp tissue was obtained from three mature, preclimacteric (≈ 30 d old), recently harvested and three postclimacteric, retail-purchased muskmelons and honeydew melons. A 2.2-cm-diameter cork borer was used to section equatorial mesocarp tissue into 3-g samples of inner (near cavity), middle, and outer (near peel) tissue. Sectioned tissue was immediately frozen in liquid N, placed into sterile 50-mL tubes, and stored at -80°C . The quantity and purity of extracted RNA was estimated by dissolving the pellet in 0.5 to 2 mL sterile, deionized water and scanning an aliquot (20 μL in 980 μL deionized water) at

A230, A260, A280, and A320. RNA concentration was calculated in $\mu\text{g}\cdot\text{mL}^{-1}$ as (A260:A320)/(40 $\mu\text{g}\cdot\text{mL}^{-1}$) (dilution factor) (Kaufman et al., 1995). Protein and carbohydrate contamination was estimated from the A260:A280 and A260:A230 ratio, respectively. Yield and purity data were subjected to a factorial analysis of variance with melon type, mesocarp tissue location, and melon maturity as main effects and individual fruit considered replications.

RNA extraction protocol. Frozen, sectioned mesocarp tissue (≈ 3 g) was ground to a fine powder in liquid N, added to a 1:1:1 mixture of RNA extraction buffer (100 mM LiCl, 100 μM Tris pH=8, 10 mM EDTA, 1% sodium dodecyl sulfate), tris-saturated phenol (5 mL), and chloroform: isoamyl alcohol (25:1); vortexed for 30 s, and chilled on ice or (preclimacteric muskmelon samples only) heated in 80°C water for 20, 25, or 30 min. The samples were centrifuged (10,000 g , 15 min, 4°C), and 80% to 90% of the aqueous phase was decanted. The interface and remaining supernatant were reextracted with 5 mL RNA extraction buffer, and the combined supernatants were extracted with 5 mL chloroform: isoamyl alcohol (25:1). The aqueous phase was centrifuged and decanted. We precipitated with ethanol before precipitation with LiCl by adding 0.1 volume of 3 M sodium acetate (pH 5.2 adjusted with acetic acid) and three volumes of pure ethanol, vortexing for 30 s, storing at -20°C for 2 h, centrifuging (10,000 g , 15 min, 4°C), dissolving the pellet in 10 mL of water, and precipitating with an equal volume of 4 M LiCl for at least 12 h at 4°C . Following centrifugation, the pellet was washed with 10 mL of 70% ethanol at 4°C , centrifuged, and left to dry in air.

Heating for 20 min degraded but enhanced the purity of RNA extracted from preclimacteric muskmelon. RNA isolated from heated samples (Fig. 1, lanes 1–3) did not separate into two distinct, ribosomal bands as did samples that were not heated (lanes 4–6). Since heating helped remove carbohydrate (A260:A230 = 2.08 vs. 1.23 for nonheated extractions) and protein (A260:A280 = 1.91 vs. 1.68 for nonheated extractions), we tried adding heated (60°C) phenol/extraction buffer to the ground tissue (Speirs and Longhurst, 1993) and then immediately chilling on ice. Adding hot phenol/extraction buffer eliminated RNA degradation but did not en-

hance removal of protein (data not shown).

A similar amount of total RNA was recovered from post- (20 $\mu\text{g}\cdot\text{g}^{-1}$) as from preclimacteric (19 $\mu\text{g}\cdot\text{g}^{-1}$) melons. Statistically similar amounts of total RNA were recovered from inner (21 $\mu\text{g}\cdot\text{g}^{-1}$), outer (16 $\mu\text{g}\cdot\text{g}^{-1}$), and middle (21 $\mu\text{g}\cdot\text{g}^{-1}$) mesocarp sections. However, significantly more total RNA was recovered from muskmelon (27 $\mu\text{g}\cdot\text{g}^{-1}$) than from honeydew (12 $\mu\text{g}\cdot\text{g}^{-1}$). The RNA extracted from inner, middle, and outer mesocarp tissue was of statistically similar purity.

Extraction of intact, total RNA from melons as diverse as muskmelon and honeydew suggests that our method may also be suitable for other melon types. Results from this study suggest that ripening events occurring 30 d after flowering do not significantly reduce the quantity nor the quality of extractable total RNA. We have also used our method to isolate total RNA from immature (20 d old) muskmelon and honeydew mesocarp and recovered more (35 $\mu\text{g}\cdot\text{g}^{-1}$) RNA of higher purity (A260:A280 ratio = 1.84, A260:A230 ratio = 2.62) than that reported herein for mature mesocarp tissue.

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Fig. 1. Total RNAs isolated from ≈ 3 g of recently harvested, preclimacteric muskmelon outer (lanes 1 and 4), middle (lanes 2 and 5), and inner (lanes 3 and 6) mesocarp tissue and subjected to electrophoresis on a 1% agarose gel containing 15% formaldehyde. Samples in lanes 1–3 were heated during initial extraction in 80°C water for 30, 25, and 20 min, respectively, and samples in lanes 4–6 were chilled on ice. Total RNA (5 μg) was loaded into each lane. Only lanes 4–6 show two sharp bands characteristic of rRNA. Smears in lanes 1–3 indicate degradation of extracted RNA.

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