Postprint: Screening of Total RNA Extraction Methods for Xinjiang Wild Apple Fruits

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Abstract

Due to the richness of polysaccharides, polyphenols, soluble pectin, and other secondary metabolites in Xinjiang wild apple WTBX fruits, total RNA extraction from these fruits is relatively difficult and yields products of poor quality. Therefore, this study utilized fruits of Xinjiang wild apple at different developmental stages as material to explore effective methods for extracting total RNA from wild apple fruits using five approaches: TaKaRa kit, Quanshijin kit, CTAB method modified by Tian Wei et al., CTAB method modified in this study (I), and CTAB method (II). The results demonstrated that both kits failed to extract RNA from wild apple fruits; RNA extracted via the three modified CTAB methods displayed clear 28S and 18S bands on agarose gels with high purity, exhibiting OD260/OD280 ratios between 1.8 and 2.2 and OD260/OD230 ratios greater than 2.0. However, only the CTAB method modified in this study (I) achieved higher RNA yields, with average RNA quantities extracted from wild apple fruits at five different developmental stages ranging from 116.79–474.76 $g \cdot g^{1}$. The modified CTAB method (I) effectively eliminated contaminating impurities from RNA and enabled complete RNA precipitation by extending the LiCl precipitation time, specifically by changing the condition from -20 °C for 15 min to 4 °C overnight (not exceeding 16 h). Although this modified method is relatively time-consuming, requiring 2 days, it is straightforward to perform and involves low extraction costs. Consequently, the modified CTAB method (I) represents an effective approach suitable for extracting total RNA from wild apple fruits (particularly at late developmental stages), fully satisfying the requirements for subsequent molecular biology experiments.

Full Text

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Choosing of Methods for Total RNA Extraction from *Malus sieversii* Fruits in Xinjiang

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Abstract: *Malus sieversii* fruits are rich in polysaccharides, polyphenols, soluble pectin and other secondary metabolites. Therefore, it is relatively difficult to extract high-quality total RNA from *M. sieversii* fruits. In order to identify an effective extraction method, five RNA extraction methods were compared using fruits at five different developmental stages: the TaKaRa Kit, TransKit, improved CTAB method by Tian Wei, modified CTAB method I, and CTAB method II. Results showed that neither kit-based method could successfully extract RNA from *M. sieversii* fruits. While the three modified CTAB methods yielded good quality total RNA with two distinct electrophoresis bands of 28S and 18S rRNA, OD /OD values ranging from 1.8 to 2.2, and OD /OD values higher than 2.0, only modified CTAB method I proved effective for obtaining high quantities of total RNA. The average extraction yields from fruits at five developmental stages ranged from 116.79 g · g ^{1} to 474.76 g · g ^{1}. For modified CTAB method I, impurities were effectively removed by extending LiCl precipitation time and incubating the extraction at 4°C overnight (not exceeding 16 hours) instead of at -20°C for 15 minutes. Although this method required 2 days, it was easy to operate and had low extraction costs. Therefore, modified CTAB method I is an effective approach for total RNA extraction from *M. sieversii* fruits (especially ripe fruits) and is suitable for further molecular biology research.

Keywords: *Malus sieversii* Roeml; total RNA; extraction method; improved CTAB method

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Note: Figure translations are in progress. See original paper for figures.