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An optimized preparation method to obtain high-quality RNA from dry sunflower seeds

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ABSTRACT. In an attempt to isolate high-quality, intact total RNA from sunflower (*Helianthus annuus*) seeds for investigation of the molecular mechanisms of mutations, we tested various procedures, using kits, including RNAiso Plus, RNAiso Plus+RNAiso-mate for Plant Tissue, Trizol, and the Qi method, but no high-quality total RNA of high integrity was obtained with any of these methods, probably due to the high content of polyphenols, polysaccharides, and secondary metabolites in mature sunflower seeds. Modifications were made to the Qi method. To avoid polyphenol oxidation, frozen dry seeds free of the seedcase were ground in a mortar with an equal amount of PVP30, and the fine ground powder was transferred to an extraction buffer with 2% PVP30 (w/v), 5% β-mercaptoethanol (v/v) and LiCl (8 M). A sample homogenate was extracted with chloroform prior to acidic phenol-chloroform extraction. The total RNA was precipitated with 1/4 volume of NaAc and 2 volumes of absolute ethanol to prevent contamination by polysaccharides. The yield of total RNA was 29.95 μg/100 mg husked dry seeds; the ratios of A260/A230 and A260/A280 were 2.44 and 2.09, respectively. Electrophoretic analysis clearly showed 28S and

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18S ribosomal RNA bands. Using the extracted RNA, a fragment of the actin gene was successfully expressed by RT-PCR. This modified protocol is suitable for isolating high-quality total RNA from sunflower seeds for molecular research.

Key words: Sunflower seeds; RNA isolation; PVP; RT-PCR; β-mercaptoethanol; Ethanol

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