Improved and convenient method of RNA isolation from polyphenols and polysaccharide rich plant tissues

Rekha Kansal, Kalika Kuhar#, Isha Verma, Ram Niwas Gupta, Vijay Kumar Gupta# & Kirpa Ram Koundal

National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi 110 012, India
# Department of Biochemistry, Kurukshetra University, Kurukshetra 136 119, India

Received 14 August 2008; revised 27 October 2008

It has been difficult to extract a good quality total RNA from the plant parts (such as seeds) which contain high levels of phenolic compounds, carbohydrates and other compounds that bind and/or co-precipitate with RNA. A simple, rapid and efficient method for isolating total RNA from polyphenols and polysaccharide rich plant tissues has been developed. Seeds of leguminosae family were chosen for the study. The good quality and high yield of total RNA was achieved with $A_{260}/A_{280}$ ratio of 1.9. Seeds of three different crops (Cajanus cajan, Dolichos biflorus and Vigna mungo) at different developmental stages were evaluated for total RNA extraction using standardized protocol. Seeds at 21 days after flowering (DAF) gave the best results among others (7 DAF and dry seeds). Quality of isolated RNA from all the three crops was further checked by cDNA synthesis. The extracted RNA was found suitable for further molecular applications such as reverse transcription and cDNA library construction.

Keywords: cDNA synthesis, Leguminous seeds, RNA isolation

Successful isolation of RNA is essential for studying gene expression, regulation and function. RNA isolation from usual herbaceous model plants like Arabidopsis, tobacco, tomato, potato or maize is usually achieved by classical phenol/lithium chloride and guanidinium based methods1-3. Various protocols for RNA isolation from plant species rich in polyphenols or polysaccharides have been reported4-10. Presence of these compounds prevents the extraction of high quality RNA from tissues. It has been observed that phenolic compounds are readily oxidized to form covalently linked quinones11 and avidly bind nucleic acids, which renders RNA unusable for fundamental procedures such as reverse transcription and cDNA library construction. In addition, contamination with polysaccharides causes RNA samples to nearly impossible to be loaded equally during Northern blotting and hence preventing different fractions of each sample entering the gel. Although, some methods have been reported for RNA isolation from specific plant tissues, but that necessarily cannot be applied for other plant tissues and thus ultimately put up the need of exploring different RNA extraction protocols.

Efficient extraction procedure for isolation of RNA should yield adequate and intact RNA of reasonable purity. Qualitative and quantitative differences in composition of plant phenolics and polysaccharides in different plant tissues significantly affect the efficiency of nucleic acid extraction and purification procedures. Therefore, in order to isolate RNA from each new plant material examined, several methods need to be investigated to select the one resulting in high yield and high quality RNA. The procedure should also be rapid, simple, economical and devoid of use of toxic chemicals. Therefore, the method that showed best results was further modified to fulfill the above requirements.

In the present investigation, a simple, rapid and convenient method to extract consistently good quality and high yield RNA from the ecologically and biochemically important plant (legume) species for further analysis was developed. Seeds at different developmental stages (7 Day after flowering (DAF), 21 DAF and dry) were used for total RNA extraction by using the standardized protocol also. The chemotypic heterogeneity among species may not
allow optimal RNA yield from one isolation protocol and, perhaps, even closely related species may require different isolation protocols. The method thus proved best was used for other species of legumes (Cajanus cajan, Dolichos biflorus) also.

Materials and Methods

Seed material—Dry seeds of Vigna mungo and Dolichos biflorus were obtained from National Seed Corporation, Indian Agricultural Research Institute, New Delhi, India. Cajanus cajan seeds were obtained from Division of Seed Science and Technology, Indian Agricultural Research Institute, New Delhi, India. They were sown under field conditions. Seeds of 7 and 21 DAF were collected and their testa was removed. They were frozen in liquid nitrogen and finally stored at -80°C until the RNA extraction was done.

Solutions—Urea-LiCl lysis buffer (8 M, urea; and 4 M, lithium chloride) and resuspension buffer (2%, SDS; 0.1 M, NaCl; 0.025 M, EDTA; 0.01 M, Tris (pH 7.5), 2.5%, PVP) were prepared. In addition, 3 M, sodium acetate (pH 5.2); and phenol:chloroform:isoamylalcohol (25:24:1) were also used.

RNA isolation—Different protocols available for RNA isolation were tried, however, only the optimized protocol from the developing and dry seeds of three different crops (Cajanus cajan, Dolichos biflorus and Vigna mungo) was followed for the study. Seed samples (200 mg) were ground to fine powder in liquid nitrogen using a mortar and pestle pre-chilled to -20°C or -80°C. Some liquid nitrogen was poured in just before adding the sample. The pulverized seeds were quickly transferred to microfuge tube containing 1.0 ml of ice-cold urea-LiCl lysis buffer and mixed vigorously by vortexing. The homogenate was kept at -20°C for 2 hr. The homogenate was thawed in ice for 30 min and then added to the top of QIAshredder column (Qiagen, Inc., Hilden, Germany) and centrifuged at 10,000 rpm for 30 min at 4°C. The pellet obtained was resuspended in 1.0 ml of resuspension buffer and centrifuged at 13,000 rpm at 4°C for 10 min. Now, the supernatant was transferred to a fresh microfuge tube and extracted twice with phenol: chloroform: isoamylalcohol (25:24:1). The total RNA was precipitated by adding 1/10 volume of 3 M of sodium acetate and equal volume of absolute isopropanol to the separated aqueous layer at -20°C for 2 hr. RNA was spun at 13000 rpm for 12 min. at 4°C and the pellet was washed with chilled ethanol (70%) and finally dissolved in 15 µl of RNase-free water.

RNA analysis—The quantity and quality of isolated RNA was assessed by observing the absorbance at 260 and 280 nm. The A260/A280 ratio was calculated to determine purity of RNA sample. The RNA was run on a standard 1.0% of formaldehyde agarose gel and visualized using UV illuminator. cDNA from the isolated total RNA was synthesized using Qiagen Omniscript cDNA synthesis kit (Qiagen, Inc., Hilden, Germany).

Results and Discussion

Attempts were made to isolate RNA from seeds of legumes by using available different RNA isolation protocols. Although, most of the protocols for plant RNA extraction had failed to give good quality and high yield RNA from seeds. By modifying a method that was previously described for isolating RNA from Picea mariana dry seeds, we have successfully isolated total RNA from leguminous seeds at different developmental stages.

In the modified method, urea (8 M) was used as a denaturant and lithium chloride (4 M) for selective precipitation of RNA. Polysaccharide and polyphenolic contaminations were removed by adding steps such as QIAshredder columns (it reduced tissue viscosity and removed cell debris) and the use of PVP (2.5%) in resuspension buffer. Proteins, carbohydrates and lipids were removed by phenol extraction and centrifugation. Several modifications were made with one parameter tested at a time. The method described by Tai et al. for RNA isolation took much more time for RNA isolation, whereas in the modified protocol the time was reduced appreciably by reducing the times of centrifugation during separation and precipitation of RNA. Samples of seed (100, 200 and 300 mg) were drawn to optimize the amount of seed required for successful isolation of total RNA. Up to 200 mg of the sample was processable, whereas 300 mg sample was not processable due to formation of highly viscous suspension. Concentrations of LiCl, SDS and PVP were also increased to 4 M, 2% and 2.5%, respectively which further helped to increase the yield and quality of RNA. Instead of using ammonium acetate
and ethanol, sodium acetate and iso-propanol were used because of its easy availability. The precipitated RNA obtained was free from contaminating DNA and therefore eliminated the need for deoxyribonucleases (DNAses). The method described was better because urea posed fewer health hazards than 2-mercaptoethanol as used in Vicient and Delseny\textsuperscript{14} and also easier to use in the laboratory. The typical yield for this method was 5.4 μg of total RNA from 200 mg of seeds used. It was observed that for all types of seed samples (Cajanus cajan, Dolichos biflorus and Vigna mungo) examined, A$_{260}$/A$_{280}$ was 1.9 by using improved method, which indicated good quality RNA\textsuperscript{15}, whereas other methods yielded RNA with A$_{260}$/A$_{280}$ ranging from 1.05-1.3 indicating poor yield and quality. The quality of RNA was also checked by agarose gel electrophoresis using standard 1% of formaldehyde agarose gel. Clear bands with no smearing or smudging proved that this method can be applied for isolating total RNA from the leguminous seeds (Fig. 1a) rich in polysaccharides and polyphenols compared to the RNA isolated using different methods (Fig. 1b). When the modified protocol was tried using seeds of different developmental stages (7 DAF, 21 DAF and dry seeds), the best results were obtained when seeds of 21 DAF from Vigna mungo (Fig. 2a) were used. High yield and better quality of extracted RNA was also spectrophotometrically observed and the ratio of 260 and 280 nm was calculated for different developmental stages of seeds (Table 1). Similar results were observed for the two other crops (Cajanus cajan and Dolichos biflorus); (Fig. 2b, c). Also, it was observed that the freshly harvested dry seeds yielded good quality total RNA from all the three crops under study (Fig. 3a, b, c). The above results indicated the high efficiency of the modified protocol for RNA isolation. Hence, it can be recommended for isolation of RNA from plant species belonging to other taxa that are rich in polysaccharides.

Isolated RNA from all the three crops was further reverse transcribed and the size range of the synthesized cDNA indicated intactness of mRNA and minimal degradation (Fig. 4a, b, c). Thus, compared to conventional method, the present procedure was simple, rapid and effective in isolating RNA of the desired quality from legume seeds.
Acknowledgement

The authors are thankful to the financial assistance received from ICAR-Network Project on Transgenic Crops, New Delhi, India to carry out the work.

References


Fig. 3—Total RNA isolated from freshly harvested dry seeds of three different crops (a) Vigna mungo (b) Dolichos biflorus (c) Cajanus cajan [1,2 – Sample run in duplicate]

Fig. 4—Size range of cDNA synthesized from RNA isolated from three different crops (a) Vigna mungo (b) Dolichos biflorus (c) Cajanus cajan Lane M-Ladder, Lane 1-cDNA synthesized