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A Rapid Method for High Quality RNA Isolation from Jute : *Corchorus capsularis* L. and *Corchorus olitorius* L.

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Abstract

The isolation of RNA from herbaceous plants is difficult when there is an abundance of polysaccharides in the plant material. *Corchorus capsularis* and *C. olitorius* are two jute species, where conventional isolation procedures gave poor results. The present paper describes a modified method, which yields a greater quantity of RNA compared to the use of conventional protocols in both jute species. This procedure yielded 500 - 600 µg of RNA per gram of fresh tissue and took only 3 h to complete. The RNA obtained was of high quality and proved suitable to Reverse Transcription-PCR.

Introduction

Many standard protocols (Schuler 1989) are available for isolation of RNA from plants but they do not work consistently well in plant tissues that are rich in polysaccharides and phenolics. These contaminants can directly affect the quality as well as the quantity of nucleic acids isolated. Polysaccharides often interfere with downstream applications thereby making the nucleic acid unusable. Since different plants contain different amounts of nucleic acids, a single nucleic acid isolation method is not likely to be suitable for all plants (Loomis 1974). Even closely related plant species can exhibit enormous variability in complexity of biochemical pathways. Therefore, the biochemical composition of plant tissues in different species is expected to vary considerably and a single isolation protocol is unlikely to be equally effective for all species (Weishing et al. 1995). Jute is the second most important fiber crop next to cotton on a global scale and yet very little information is available

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about its genome. To characterize the genome and gene expression in jute, a polysaccharide-rich plant, it is important to develop a suitable RNA isolation method. The present experiment was carried out for extraction of RNA from jute to be used in RT-PCR and the construction of a cDNA library. With key modifications in tissue growth, phenol chloroform extractions and precipitation reactions we have developed a simplified protocol for the isolation of high quality RNA from jute that is time saving and cost effective.

Materials and Methods

Plant material: Jute seeds were obtained from the University of Dhaka, Bangladesh and subjected to no prior treatment. The seeds were soaked in distilled water for 2 h, then placed on a moist filter paper in a Petri dish at 30 seeds/per plate. The seeds were germinated and grown for seven days at 28_C. The seed coats shed at the end of the seven-day period. The amount of tissue per Petri dish was approximately 0.6 g.

Solutions and reagents: Diethylpyrocarbonate (DEPC) treated water was prepared by adding 0.5 ml of DEPC to 1 liter of water and stirred for two hours. Monophasic Lysis Solution (MLS) was made by mixing 190 ml phenol (38 %), 59 g guanidine thiocyanate (0.8 M), 38 g ammonium thiocyanate (0.4 M), 16.7 ml of 3 M sodium acetate pH 5 (0.1 M), 25 ml glycerol (5 %) and the volume brought to 500 ml using DEPC treated water. Sodium chloride sodium citrate (SSC) was prepared by adding 0.8 M sodium citrate + 1.5 M NaCl (SSC) in DEPC-water. In addition chloroform without isoamyl alcohol, absolute isopropanol, 75% EtOH (in DEPC water) and 3 M sodium acetate pH 5.5 were used.

RNA isolation protocol: One g of fresh tissue was ground in liquid nitrogen to a fine powder. It was then divided into four quarters and each quarter of frozen powder was transferred to a microfuge tube containing 1 ml of MLS. The samples were mixed well by inversion, vortexed and incubated at room temperature for 10 min. They were then centrifuged at 12,000 x g for 10 min. The supernatant was transferred to a fresh centrifuge tube by pouring and 0.2 ml of chloroform was added to each tube and vortexed vigorously for 1 min. The tubes were left at room temperature for 2 - 3 min. After centrifuging again at 12,000 x g for 10 min, using a sterile transfer pipette, the aqueous phase was transferred to a fresh centrifuge tube. SSC (0.8 M Nacitrate/1.5 M NaCl) and isopropanol were added at half volume of aqueous phase each. The samples were mixed well by inversion and placed at room temperature for 10 min to allow the nucleic acids to precipitate. After the precipitation the samples were centrifuged at 12, 000 x g for 15 min and the supernatant was discarded. The

pellet was then dissolved in 0.4 mL DEPC-treated water. A mixture of 0.4 ml of phenol/chloroform was added, the tubes were vortexed vigorously for a minute and incubated at room temperature for 2 - 3 min. After incubation the samples were centrifuged at 12,000 x g for 5 min. Using a cut tip, the aqueous phase was transferred to a fresh centrifuge tube. SSC + isopropanol were added at half volume of aqueous phase each, the tubes were mixed well by inversion and placed at room temperature for 10 min to allow nucleic acids to precipitate. Once done the samples were centrifuged at 12, 000 x g for 15 min and the supernatant discarded. (It is to be noted that if the RNA does not form a tight pellet, it needs to be centrifuged again at 14, 000 x g for 10 min and then the supernatant removed). The pellet was rinsed with 0.5 ml of 75% ethanol and centrifuged at 12,000 x g for 10 min. The supernatant was discarded and the tubes inverted on an untouched area of a stack of 2 - 3 Kimwipes. The pellet was air dried for 5 - 10 min and re-suspended in 30 µl DEPC-H_2O for each tube.

Gel analysis: The RNA was size fractionated on a standard 1.2 % formaldehyde agarose gel (Sambrook et al.) and visualized using UV illumination.

RT-PCR analysis: Reverse Transcription-PCR was carried out using the Promega Access RT-PCR System in a single tube reaction. The reverse transcription was carried out at 48_C for 45 min, followed by heat inactivating the AMVRT at 94_C for 2 min. The PCR reaction was carried out for 32 cycles. The conditions for each cycle were: denaturation at 94_C for 30 sec, annealing at 42_C for 1 min and extension at 68_C for 2 min.

Results and Discussion

Various methods reported for the isolation of RNA from plants failed to yield high quality ribonucleic acids from jute. The presence of polysaccharides and other metabolites can hamper isolation procedures and reactions such as amplification and cloning. Using the above mentioned protocol specifically tailored for two species of the polysaccharide-rich jute plant such obstacles as low yield, degradation and poor PCR amplification were easily surmounted. The key steps in the protocol that makes it different from others are phenol chloroform extractions placed at critical steps as not to lower the yield. Also, an alternative method of growing the plants was devised. Initially the seeds were sown in soil with fertilizer and the plants were harvested at various stages of growth. The starch was depleted by keeping in the dark for two days and then used in isolation procedures. It was observed that the younger the plant, the lower the polysaccharide content. However, even the youngest

plants harvested had very high polysaccharides which made nucleic acid isolation difficult. The optimal growing method found was allowing the seeds to germinate on moist filter paper in Petri dishes at a controlled temperature of 28_C for seven days. This method was beneficial in overcoming polysaccharide as well as soil contamination by forcing the plants to only use the material stored in the endosperm for growth and preventing them from synthesizing and storing excessive polysaccharides and metabolites from external nutrients.

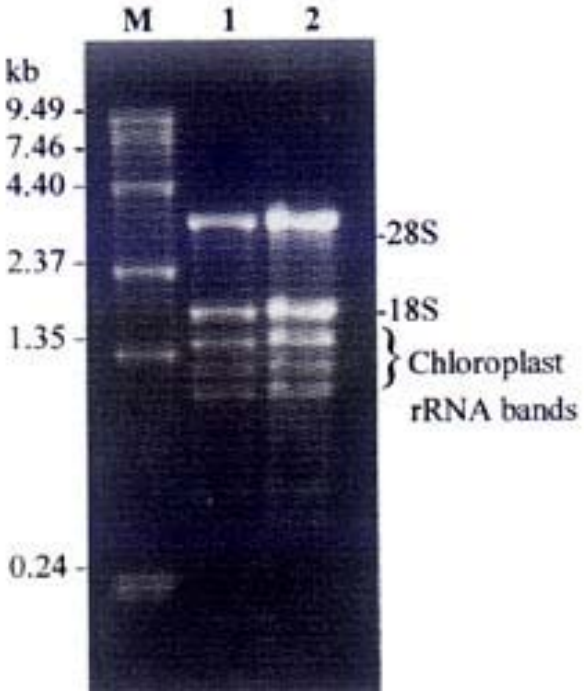

Fig. 1. Isolated total RNA from *Corchorous olitorius* and *C. capsularis* resolved on a 1.2% agarose gel. M indicates molecular weight marker, lane 1 shows RNA from *C. olitorius* and lane 2 from *C. capsularis*.

The RNA yield was about 600 µg per gram of tissue. The RNA quality was easily verified by visualization on an ethidium bromide agarose gel (Fig. 1), which showed no degradation. The intactness of the RNA was indicated by the integrity of the ribosomal bands. The RNA obtained was of high quality enabling us to use it in RT-PCR with various primers (Fig. 2), paving the way to clone genes for important agronomic characters. Thus, compared to conventional method, the present procedure was very effective in isolating RNA of the desired quality from the two polysaccharide rich jute species, *C. capsularis* and *C. olitorius*. This procedure yielded 500 - 600 µg of RNA per gram of fresh

tissue in both the species. The method is also rapid (~ 3 h to complete), simple, efficient, and it does not require long ultracentrifugation. Observing its high efficacy, the protocol is recommended for isolation of RNA from plant species belonging to other taxa that are rich in polysaccharides.

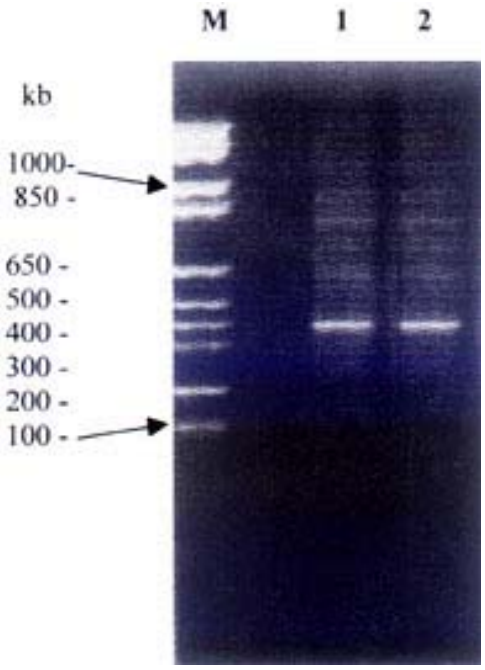

Fig. 2. RT-PCR from the RNA of *C. olitorius* and *C. capsularis* resolved on a 0.8% agarose gel containing ethidium bromide. M indicates the molecular weight marker, lane 1 and lane 2 show PCR products from *C. olitorius* and *C. capsularis,* respectively.

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