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A simple and convenient approach for isolating RNA from highly viscous plant tissue rich in polysaccharides

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Abstract

RNA isolation is a prerequisite to the study of gene expression of herbaceous plant *Dendrobium nobile* under an environmental stress. However, RNA isolation is difficult in the plant of genus *Dendrobium*, which is relatively viscous, due to being rich in polysaccharides. The common protocols for RNA isolation are tedious and usually result in poor yields when applied to *D. nobile*. Here, we describe a simple and convenient method for high quality total RNA extraction from *D. nobile*. Main procedures are as follows: smashing plant tissue and cell wall by means of pre-cooled citrate homogenization, cleaving cell membrane by using guanidine hydrochloride lysis buffer, removing proteins, polyphenols and polysaccharides by acidic phenol/chloroform. It is particularly useful for processing large numbers of plant samples. The whole process can be completed within 2.5 h. The extracted RNA is suitable for applications such as RT-PCR, Northern analysis and screening of differential expression genes.

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Keywords: RNA extraction; *Dendrobium nobile*; Reverse transcription-polymerase chain reaction (RT-PCR); Northern dot hybridization; Polysaccharides

1. Introduction

The Chinese crude drug “Shihu” is derived from the dried or fresh stems of *Dendrobium loddigesii* Rolfe, *D. fimbriatum* Hook. var. oculatum Hook., *D. chrysanthum* Wall., *D. officinale* Wall. ex Lindle. and *D. nobile* Lindle. It can be used to benefit the stomach, to promote the production of body fluid, to nourish *Yin*, and to remove heat [1]. The investigation into drug sources of Shihu showed that *D. nobile* is abundant in the source and commonly used in Southwest China, but the sources are drastically reduced owing to the increasing demand and destructive collection [2].

With the development of molecular biology, it has been widely studied about genes on the officinal plant. As a rare Chinese herb, *D. nobile* has been extensively studied by the technique of molecular biology [3]. However, the plants of *Dendrobium* genus contain considerable amounts of polysaccharides [4], which make the isolation of RNA difficult and reduce the quality of RNA. Many problems in current method occurred when total RNA was to be isolated from the callus of *D. nobile*. RNA produced by the current method for polysaccharidic- and/or phenolic-rich plant tissues appeared good quality upon gel analysis with intact 25S and 18S ribosomal RNA. Whereas, the spectral analysis of the purified RNA revealed contaminating molecules with maximum absorbencies at 235, 268 and 275 nm, suggesting that the RNA preparation contained polysaccharides. Thus quantification of isolated RNA by UV absorption spectrophotometry and its use in RT-PCR experiments were not possible. Moreover, Northern blot experiments were not successful, possibly due to such contamination [5].

However, extraction of high-quality RNA is necessary for making cDNA libraries, isolating genes by RT-PCR, or investigating gene expression profiles, which is also be great importance to molecular biological and genomic study of these plants. Several methods are commonly used for isolation of total RNA [6–8] and are being developed because plants contain high amounts of many different substances [9]. Therefore, just one nucleic acid isolation method suitable for all plants can never exist [10]. Even plant species belonging to the same genus or related genera can exhibit enormous variability in the complexity of pathways of dispensable functions. Thus, the biochemical

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compositions in plant tissues of different species are expected to vary considerably. 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 [11].

As our aim has been to study sound-wave-induced differential expression of genes and to make knowledge of the genes expression pattern on different culture conditions in *D. nobile*, we need a protocol that not only can give the same quality and quantity of RNA at each condition but also can be high yielding in order to detect the rarely expressed genes. Our previous RNA isolation protocols [12,13] were not suitable for this species. In this report, a simple procedure was developed and applied to *D. nobile*, in which need not to grind in the liquid nitrogen but use citrate acid tissue mashing method to stave plant tissue. Further, the quality of the isolated RNA was consistently high as indicated by spectrophotometer readings and its separation on denaturing agarose gels. The yield and quality were suitable for RT-PCR and Northern dot hybridization.

2. Materials and methods

2.1. Plant materials

2.1.1. Plant tissue culture

Stems from *Dendrobium nobile* Lindl. seedlings were inoculated in conical flasks with 20 ml MS solid medium each and were cultured in illumination incubator at 26 °C.

2.1.2. Plant material stimulation

Sound generator in our laboratory achieved alternating stress. Inoculated stems were stimulated by sound wave with a certain intensity (100 db) and frequency (1000 Hz) for 9 days, respectively, and each day for 60 min. The control groups were placed in the same environment with the stimulated groups. The strong sound field apparatus refers to Liu et al. [14].

2.2. Treatment of reagents and experimental things

2.2.1. Reagents

Citrate homogenization buffer: 1.5% citrate, 2% 2-mercaptoethanol, 50 mM EDTA, pH 3.5. Lysis buffer: 8 M guanidine hydrochloride, 0.1% SDS, 20 mM sodium acetate, pH 5.8. Phenol/chloroform/isoamyl alcohol (CI) mix (25/24/1, v/v) saturated with 0.1 M Tris–HCl (pH 8.0). Two molars of sodium acetate pH 5.4. Seventy-five percent ethanol prepared from DEPC-treated water. Twenty millimolars of EDTA pH 8.0 DEPC-treated and autoclaved distilled water.

2.2.2. Experimental things

Glassware and mortar pestles (baked overnight at 180 °C), pipette tips (DEPC treated and autoclaved), gel running apparatus (treated with 3% H_2O_2 and incubated overnight with DEPC-treated water), 50-ml polypropylene centrifuge tubes (washed and incubated overnight at room temperature with DEPC-treated water).

2.3. RNA extraction protocol

Weigh up 500 mg fresh material and cut it up, put it into a High Speed Tissue Masher (made in Yancheng, Jiangsu, China). Add 15 ml citrate homogenization buffer (pre-cooled on the ice), homogenate at 10,000 g for 5 s and repeat five times. Filtrate it by using a layer of gauze and obtain the filtrate. Centrifuge it at 30,000 × *g* for 10 min at 4 °C. Remove and discard as much supernatant as possible and capture cell precipitate.

Add 1–2 ml lysis buffer to the tube, mix thoroughly by inverting the tube. Then add 0.2 ml 2 M sodium acetate to the tube, shake vigorously for 10 s to cut DNA long strands into short slices. Quickly add an equal volume of phenol/CI, shake vigorously for 15 s and place it on the ice, store exactly for 15 min with the ice together at 4 °C. Centrifuge at 30,000 × *g* for 15 min at 4 °C. Transfer the supernatant to a fresh tube. Repeat the phenol/CI extraction until the interface is clear.

Add one volume of isoamylalcohol (pre-cooled at −20 °C) to the clear supernatant. Mix well and place for 30 min at −20 °C. Recover the rough RNA by means of centrifugation at 30,000 × *g* for 15 min at 4 °C.

After isoamylalcohol volatilizing completely, add 2 ml 20 mM EDTA to the pellet to dissolve RNA. Add 2.5–3 volumes of absolute ethanol (pre-cooled at −20 °C), store for 5 min at least at −20 °C. Centrifuge at 30,000 × *g* for 15 min at 4 °C and discard the supernatant. Wash the pellet with 75% ethanol two to three times the remaining mucilage, and air-dry it for 10 min. Dissolve the RNA in DEPC-treated water, store the RNA at −80 °C until use.

Using this protocol, 500 mg fresh weight (FW) of *D. nobile* were extracted respectively on the stimulated groups and the control groups (S1 and C1, S2 and C2, S3 and C3). Three samples of every group were tested.

2.4. RNA analysis

The quality and the integrality of the total RNA were analyzed using the standard methods for UV spectrophotometer and agarose formaldehyde denaturing gel electrophoresis [11].

2.5. RT-PCR analysis

RT-PCR was performed according to our lab method [11,12].

2.6. Re-amplification and Northern dot hybridization

The gel bands of differentially displayed cDNA fragments were cut down with a scalpel and added to an Eppendorf tube to boil for 15 min. The extract served as the template to re-amplify and the primes were same with RT-PCR. The products of re-amplification were detected on agarose gel. The fragments were reclaimed for preparing probes.

Two micrograms of total RNA from the stimulated and the control materials were denatured at 70 °C for 5 min, and spotted onto a nylon membrane (Hybond-N^+; Amersham,

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