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RESEARCH ARTICLE

Isolation of high-quality RNA from a wide range of woody plants

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ABSTRACT: Isolation of high-quality RNA is one of the most crucial methods in molecular biology. RNA extraction from woody plants has been problematic due to the presence of rigid and woody tissues, large amounts of polysaccharides, polyphenols and other secondary metabolites. Here we present a suitable protocol for RNA isolation from a wide range of woody plants that includes eight gymnosperms and four angiosperms. The method is based on the CTAB protocol which was modified by adding sodium citrate and two helper buffers. Agarose gel electrophoresis showed a good RNA integrity and total RNA profile that includes all expected RNA bands. Also, DNA and protein contaminations were not observed. Spectrophotometric quantification of RNA samples by NanoDrop showed that the average RNA yields ranged from 35.68 to 216.98 µg per gram fresh weight, that is enough to proceed into cDNA synthesis and other RNA-related works. Both the A260/A280 and A260/A230 ratios were in the desired ranges, indicating that RNA was of high purity and without protein, polyphenol, and polysaccharide contamination. The efficiency of isolated RNA for downstream applications was verified by real-time PCR and successful amplification of a long cDNA. Finally, some advantages and possible applications of the method are also mentioned.

KEYWORDS: RNA isolation, Woody plants, Contaminations, CTAB, Sodium citrate, Helper buffers

ABBREVIATIONS: CTAB: cetyl trimethylammonium bromide, DEPC: diethyl pyrocarbonate, LiCl: lithium chloride, Na_2EDTA: disodium ethylenediaminetetraacetate, PVP: Polyvinylpyrrolidone, RT-PCR: reverse transcription polymerase chain reaction, SDS: sodium dodecyl sulfate

INTRODUCTION

Woody plants are enormously important and beneficial to mankind. They are sources of essential products including lumber, pulp, food, fuel, waxes, oils, gums, and resins. The environmental impacts of woody plants are significant, they ameliorate climate, reduce consumption of energy for heating and air conditioning of buildings, serve as sinks and long-term storage sites for greenhouse gases, and abate the harmful effects of pollution, flooding, and noise. They protect land from erosion and wind, and provide habitats for wildlife [24]. Woody plants also have a wide range of therapeutic properties, including anticancer (*Taxus* spp.) [29], antimicrobial (*Azadirachta indica*) [31], neuroprotective (*Gingko biloba*) [25], antidiabetic (*Aegle marmelos*) [8], anti-inflammatory (*Eriobotrya japonica*) [33] and other medicinal effects. Despite these advantages, woody plants research is less advanced compared to herbaceous crops [30]. Isolation of high-quality RNA is a prerequisite to success in several molecular biology studies such as reverse transcription polymerase chain reaction (RT–PCR), rapid

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amplification of complementary DNA ends (RACE), Northern hybridization, and microarray analysis [21, 22]. However, RNA extraction from woody plants is often difficult due to the presence of the rigid cell walls, large amounts of tannins, pigments, polysaccharides, polyquinones, and other secondary metabolites [22].

Polyphenols and polysaccharides are the compounds that interfere the most in the extraction process [4, 15, 28]. The phenolics are readily oxidized to form covalently linked quinones, that vigorously bind proteins and nucleic acids and cause to insoluble high molecular weight complexes, whereas polysaccharides tend to co-precipitate with nucleic acids in buffers with low ionic strength [14]. Moreover, phenolics and polysaccharides severely interfere with RNA-dependent works including Northern blotting, reverse transcription and absorbance-based quantification [13, 14]. So far, a number of methods have been described for isolating RNA from plants including guanidinium thiocyanate [6], SDS-phenol [34], hot-borate [40], citrate [23] and CTAB [5] methods, that vast majority are not completely satisfying for woody plants due to the presence of large amounts of contaminants [20].

In this study, we describe an efficient and versatile method for high-quality RNA isolation from a wide range of woody plants rich in polysaccharides, polyphenols, and other interfering substances. The purity, integrity, and suitability of isolated RNA for molecular studies such as gene expression and cloning were demonstrated by real-time PCR and RT-PCR.

MATERIALS AND METHODS

Plant materials

The fresh leaves and stems of a wide variety of woody plants which include Yew (*Taxus baccata*), Cephalotaxus (*Cephalotaxus* spp.), Cypress (*Cupressus sempervirens*), Pine (*Pinus sylvestris*), Cedar (*Cedrus* spp.), Blue spruce (*Picea pungens*), Ginkgo (*Ginkgo biloba*), Cycas (*Cycas* spp.), Walnut (*Juglans regia*), Pistachio (*Pistacia vera*), Fig (*Ficus carica*) and Apple (*Malus domestica*) were collected in July 2017, from the plants were grown outdoor or greenhouse. These plants were selected based on their medicinal, ornamental and nutritional values. All samples were immediately frozen in liquid nitrogen and then stored at -70°C until use.

Buffers and reagents

Buffers and reagents were prepared using DEPC treated water (0.1%) as below:

Lysis buffer (CTAB buffer): 100 mM Tris-HCl (pH=8), 25 mM Na_2EDTA (pH=8), 3% CTAB (w/v), 2 M NaCl, 3% PVP (MW: 40000).

Helper buffer 1: 25 mM Tris-HCl (pH=8), 10 mM Na_2EDTA (pH=8), 50 mM glucose.

Helper buffer 2: 60 ml 5 M potassium acetate, 11.5 ml glacial acetic acid, 28.5 ml diethyl pyrocarbonate (DEPC) treated water (0.1%).

1 M sodium citrate, chloroform-isoamyl alcohol (24:1, v/v), 10 M LiCl, 0.5% SDS, 70% ethanol, and isopropanol also were prepared in this study. It should be noted that, all solutions were autoclaved at 121°C for 20 min except chloroform-isoamyl alcohol, 70% ethanol and isopropanol. Then buffers and reagents were stored at room temperature and away from light.

Pestles, mortars, glassware and plastic ware should be sterilized by autoclaving.

RNA isolation

This protocol is a modification of previously described methods [1, 12, 17]. The method includes the following steps:

1- Pre-warm extraction buffer to 65°C for 15 min (in a water bath), Then add 1 ml preheated lysis buffer and 50 µl sodium citrate (1 M) to each 2 ml microtube. Vortex tubes for 5 s and place them in a water bath.

2- Grind 100 mg of plant materials to a fine powder in liquid nitrogen with a pestle and mortar. Then immediately remove tube containing lysis buffer from the water bath, add 50 µl β-mercaptoethanol and transfer the powdered plant materials to the tube. Shake the mixture vigorously by vortexing for 20 s. Incubate tubes at 65°C for 30 min and vortex samples every 10 min.

3- Remove the samples from the water bath and sequentially add helper buffers 1 and 2 (each 50 µl), vortex tubes for 20 s, then cool the tubes at room temperature for 5 minutes.

4- Add 600 µl chloroform-isoamyl alcohol (24:1, ice cold), vortex tubes for 20 s, then centrifuge at 12,000 rpm for 10 min at 4°C.

5- Transfer the upper phase (aqueous phase) into a new 2 ml microtube, add 1000 µl chloroform-isoamyl alcohol, mix by inversion and then centrifuge as before.

6- Transfer supernatant into a 1.5 ml tube, add 400 µl of 10 M LiCl (ice cold), mix by inversion and place on ice and store in the refrigerator (4°C) overnight.

7- Centrifuge samples at 12,000 rpm for 30 min (4°C) to form pellets.

8- Invert the tubes to discard supernatant, wash the pellet with 70% ethanol and air-dry. Then add 20 µl DEPC treated water to the pellet and dissolve it by 5 min incubation at 65°C.

9- Treat samples with *DNase* I (Thermo Fisher Scientific, USA) according to the manufacturer's instructions.

10- Add 200 µl 0.5% SDS, 300 µl DEPC treated water and 500 µl chloroform-isoamyl alcohol, and mix thoroughly by inverting the tube several times, then centrifuge for 10 min (12000 rpm and 4°C).

11- Transfer supernatant into new 1.5 ml tube, add 500 µl isopropanol (-20°C), mix by inverting and then place at -20°C for 1 hour.

12- Centrifuge samples at 12,000 rpm for 30 min (4°C) to form pellets.

13- Discard supernatant, wash RNA pellet with 70% ethanol and air-dry. Then add 20 µl DEPC treated water to the pellet and dissolve it by 5 min incubation at 65°C. Store the RNA at -20°C (for short-term storage) or -70°C (for long-term storage).

Quantification and qualification of isolated RNA

The purity and quantity of the isolated RNA was evaluated by determining the spectrophotometric absorbance of the samples at 230, 260 and 280 nm using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific). RNA integrity was evaluated by electrophoresis on 1% (w/v) agarose gel after staining with ethidium bromide and visualization under UV light.

cDNA Synthesis

First-strand cDNA was synthesized from 1 µg total RNA using a cDNA synthesis kit (RevertAid First Strand cDNA Synthesis Kit, Thermo Fisher Scientific) with Oligo (dT) primer. All cDNA samples were stored at -20°C until use.

Real-time PCR

Real-time PCR was performed to assess the RNA quality for molecular biology applications. The expression pattern of two genes of *Taxus baccata* including *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*), as a housekeeping gene and *phenylalanine aminomutase* (*PAM*), as a gene involved in Taxol biosynthesis (with low expression levels) was investigated [7]. Primers were designed (Table 1) using the Primer Premier 5.0 software (PREMIER Biosoft International, USA) and validated by Primer-BLAST (www.ncbi.nlm.nih.gov/tools/primer-blast/).

PCR reactions included 1× Maxima^{TM} SYBR Green qPCR Master Mix (Thermo Fisher Scientific), 250 nm primers and 1 µl (50 ng) of template (cDNA) in a 20 µl reaction volume. Also, negative controls containing RNA (as minus reverse transcription control or -RT control) and water (as no template control or NTC) were subjected to the same procedures to detect any possible contamination. Real-time PCR was conducted using a C1000 thermal cycler (Bio-Rad, USA).

The amplification conditions were: 5 min at 95°C, followed by 40 cycles of 30 s at 95°C, 30 s at 61°C and 30 s at 72°C. The specificity of amplicons was verified by melting curve analysis (60 to 95°C) after 40 cycles and agarose gel electrophoresis. Furthermore, to compare different RNA transcription levels, the cycle threshold (Ct) values were compared. Two biological replicates for each sample were used for real-time PCR analysis and

Table 1. Primers used for real-time PCR analysis

Gene	Primer sequence	Accession number	Amplicons (bp)
GAPDH	5'-ATCAAGGCTGCCATCAAGGAG-3' 5'-CTGTAACCCCATTCGTTGTCG-3'	L26922.1	191
PAM	5'-GCTCCTCTGGTGCAGACAATCAG-3' 5'-TATGAAGTCGGCAGATTGCTTAACG-3'	GU214709.1	141

three technical replicates were analyzed for each biological replicate. The data were analyzed by using Bio-Rad CFX manager software version 1.6 (Bio-Rad Laboratories Inc.).

Amplification of *TXS* full-length cDNA

To evaluate the RNA integrity, it is better to amplify long RNAs (cDNAs) which are often much degraded than the shorter RNAs [11]. To this aim, a set of primers (Forward: 5'-ATGGCTCAGCTCTCATTTAATGC-3', Reverse: 5'-TGCCAATACAATAATAAGTCAATTTATT-3') used for full-length (2643 bp) amplification of *taxadiene synthase* (*TXS*) cDNA (GenBank accession: AY424738). Like *PAM*, *TXS* also involved in Taxol biosynthesis pathway with low expression levels [7]. PCR was performed in a total volume of 20 µL containing 50 ng cDNA (*Taxus baccata*), 250 nM primers, 1 mM $MgCl_2$, 200 µM dNTPs (each) and 1 unit *Taq* DNA Polymerase (Cinnagen, Iran). Reactions were carried out on a T-personal thermocycler (Biometra, Germany). The thermal cycling conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles 94°C for 60 s, 55°C for 45 s and 72°C for 3 min, with a final extension at 72°C for 10 min. PCR products were analyzed by gel electrophoresis with 1% agarose, followed by ethidium bromide staining and UV visualization.

RESULTS AND DISCUSSION

Our preliminary results showed that RNA extraction with CTAB method [5] has better quality and quantity than the other methods that also commonly used for RNA extraction such as guanidinium thiocyanate [6], SDS-phenol [34], citrate [23], RNX plus kit (Cinnagen), GeneJET™ Plant RNA Purification Mini Kit (Thermo Fisher Scientific) and FastPure RNA Kit (Takara, Japan). Non-CTAB-based methods produced low yield RNA with high amounts of contaminating proteins, polysaccharides and polyphenols. RNA pellets were viscous and water-insoluble and therefore unusable for further studies. Other researchers have reported similar results using the guanidinium thiocyanate, SDS/phenol and TRIZOL methods [17, 32, 35].

Although, the quality of RNA samples using CTAB method was much higher than the other methods, however The quality of RNA due to the interfering materials were not optimal, thus we still need to improve the protocol to achieve high-quality RNA. Optimization of the method for the removal of polyphenols and polysaccharides was carried out by the addition of some substances from other nucleic acid isolation methods. Experiments showed that the addition of sodium citrate and helper buffers 1 and 2 to the CTAB method, removed the pollutants and resulted in high-quality RNA. RNAs of twelve selected woody

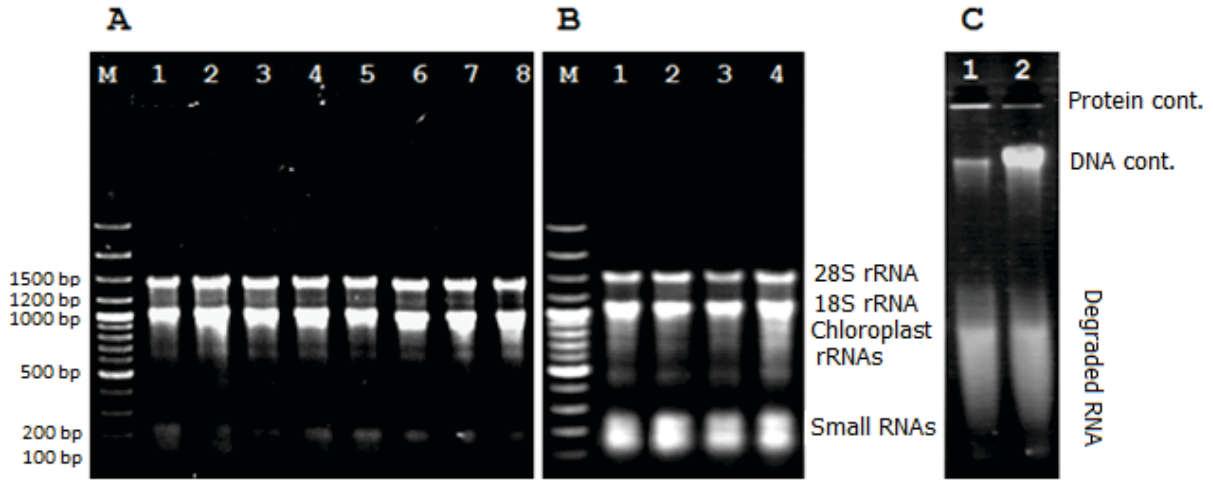

Figure 1. Gel electrophoresis of the total RNA extracted from different plant species. (A) Analysis of total RNA isolated from eight different gymnosperm species including 1: *Taxus baccata*, 2: *Cephalotaxus* spp., 3: *Cupressus sempervirens*, 4: *Pinus sylvestris*, 5: *Cedrus* spp., 6: *Picea pungens*, 7: *Ginkgo biloba* and 8: *Cycas* spp. M: DNA size marker (GeneRuler 100 bp Plus DNA Ladder, Thermo Fisher Scientific). (B) Analysis of total RNA isolated from four different angiosperm species including 1: *Juglans regia*, 2: *Pistacia vera*, 3: *Ficus carica* and 4: *Malus domestica*. The presence of sharp and distinct 28S and 18S rRNA bands and the lack of smear among these bands, verifying the integrity of RNA. Samples were free of protein and DNA contaminations. (C) RNAs with undesirable characteristics (isolated by other methods), including the lack of sharp rRNA bands, sample degradation and the presence of protein and DNA contaminations. 2 µg RNA was loaded per lane

Table 2. RNA yields and quality from different woody plant species using NanoDrop spectrophotometer

Species	RNA yield (µg/g fresh weight)	A260/A280	A260/A230
Taxus baccata	136.82±17.94	2.06±0.03	2.13±0.05
Cephalotaxus spp.	114.82±12.96	2.11±0.04	2.03±0.23
Cupressus sempervirens	43.95±6.70	2.03±0.07	1.92±0.04
Pinus sylvestris	87.61±5.37	2.07±0.02	2.02±0.03
Cedrus spp.	35.68±4.82	2.05±0.05	2.14±0.13
Picea pungens	97.93±9.47	2.07±0.03	2.24±0.09
Ginkgo biloba	69.86±5.14	2.06±0.05	2.19±0.01
Cycas spp.	54.91±6.70	2.03±0.06	2.00±0.06
Juglans regia	124.70±12.37	2.08±0.02	2.20±0.02
Pistacia vera	93.92±8.75	2.04±0.04	1.96±0.12
Ficus carica	71.58±10.09	2.06±004	2.09±0.07
Malus domestica	216.98±11.21	2.09±0.03	2.20±0.02

Values are the mean of four samples (± standard error)

plant species were successfully isolated by our improved method.

Agarose gel electrophoresis showed sharp and distinct 28S and 18S ribosomal RNA bands and the lack of RNA degradation, indicating high quality and integrity of isolated RNA (Fig. 1). Furthermore, chloroplast ribosomal RNAs and small RNAs were also detected. Visualization of all expected RNA bands on agarose gel in this study, demonstrate that our (optimized) RNA extraction protocol is suitable for further RNA based experiments such as cDNA synthesis and real-time PCR. There were no additional bands of other sizes detected in gel electrophoresis, demonstrating the lack of DNA and protein contaminations in RNA samples. These results confirm that the isolated RNAs were of high quality and integrity. Spectrophotometric data (Table 2 and Fig. 2) were in good agreement with those obtained by electrophoresis.

The average RNA yields ranged from 35.68 to 216.98 µg per gram fresh weight (g FW) tissue, which is sufficient for further analysis including RNA quantification, RNA qualification, and cDNA synthesis [23]. Small volume extractions (into microfuge tube) are more efficient than large volume extractions especially when enough sample quantities are difficult to obtain. Although there are some reports of the RNA isolation in large scale volumes (with more than 5 ml extraction buffer) from woody plants [17, 20, 21, 35], these procedures have some limitations, because they require the more organic solvent use and also more labor and equipment [23]. The 260/280 ratio for all RNA samples ranged from 2.03 to 2.11 and the 260/230 ratios ranged from 1.92 to 2.24 (table 2). These values indicated that the higher quality of RNA samples and no protein, polyphenol and polysaccharide contaminations [14, 22, 23]. Gene-specific amplification (in real-time PCR) was confirmed by a single peak in the melt-curve analysis (Fig. 3) and a single band with the expected size in agarose gel (1%) electrophoresis (Fig. 4). The negative controls showed no amplification, indicating that there was no DNA contamination in isolated RNAs, so the high

quality of extracted RNA was confirmed [21]. Moreover, the Ct values showed that *GAPDH* was the most abundant transcript with a mean Ct value of 20.45, whereas *PAM* was the least abundantly transcribed (mean Ct=26.71).

Full-length cDNA of *taxadiene synthase* was successfully amplified and PCR products of expected size (2643 bp) with intense bands on the agarose gel (1%) were generated (Fig. 5). Amplification of such long genes is usually considered an acceptable indicator of RNA integrity because of long RNA fragments are very sensitive to degradation [11]. According to real-time PCR and long cDNA amplification results, this new procedure has permitted us to obtain sufficient RNA with high purity and integrity to perform sensitive molecular biology experiments including gene expression and amplification of the full length of low expressed genes.

CTAB-based methods are most widely used in plant RNA extraction that are rich in polyphenolic and polysaccharide compounds [12, 17, 22, 27, 36]. CTAB is a detergent which can lyse the cell membrane. It promotes separation of polysaccharides from nucleic acids and

nucleic acids are selectively precipitated with CTAB [12, 16, 38]. PVP plays an important role in removing phenolics and secondary metabolites from nucleic acid extracts [17]. β-mercaptoethanol has a strong reducing capacity that prevents oxidative cross-linking of nucleic acids by phenolics, and inhibition of nucleases activity by disrupting disulphide bond formation [37]. Chloroform is a de-proteinization solvent employed to protect RNA from *RNase* degradation [1]. High ionic strength buffers combined with LiCl precipitation effectively prevent the co-precipitation of polysaccharides along with RNA [10, 39].

Our results showed that the addition of sodium citrate into lysis buffer, increased RNA yields and purity. It seems that sodium citrate involves cell lysis and separation of RNA from DNA and proteins [23]. Moreover, sodium citrate acts both as a pH buffer and a chelating agent that minimize alkaline hydrolysis of RNA [2, 9, 23].

We have also found that the addition of helper buffers into the isolation procedure significantly removed phenolic compounds and other secondary metabolites. It seems that, the helper buffers affect by lowering the pH that leads to moving the phenolic compounds from the aqueous phase (containing RNA) into the chloroform phase [26]. However, the use of helper buffers for RNA isolation has not been reported so far. The idea of using these helper buffers was based on our observations in the plasmid extraction by alkaline lysis method [3]. It has been frequently observed that the 260/280 and 260/230 ratios of plasmid samples were always desirable, so we

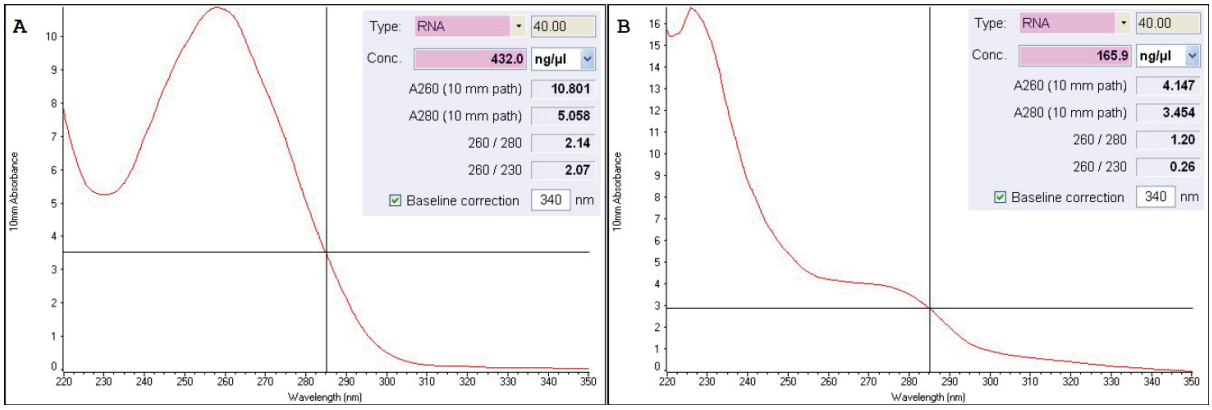

Figure 2. NanoDrop measurement profiles of RNA extractions. (A) A profile of RNA of good quality. It shows a strong single absorbance peak at 260 nm, and both 260/280 and 260/230 ratios are more than 2, indicating high purity RNA. (B) A profile of RNA of bad quality. It exhibits an absorption peak at 230 nm. The 260/280 and 260/230 ratios are very far from the desirable ranges, indicating the presence of large amounts of protein, polysaccharide and polyphenol contaminations. Absorbance at 320 nm that is more than several percent of the absorbance at 260 nm, indicates the presence of some other undesirable foreign materials

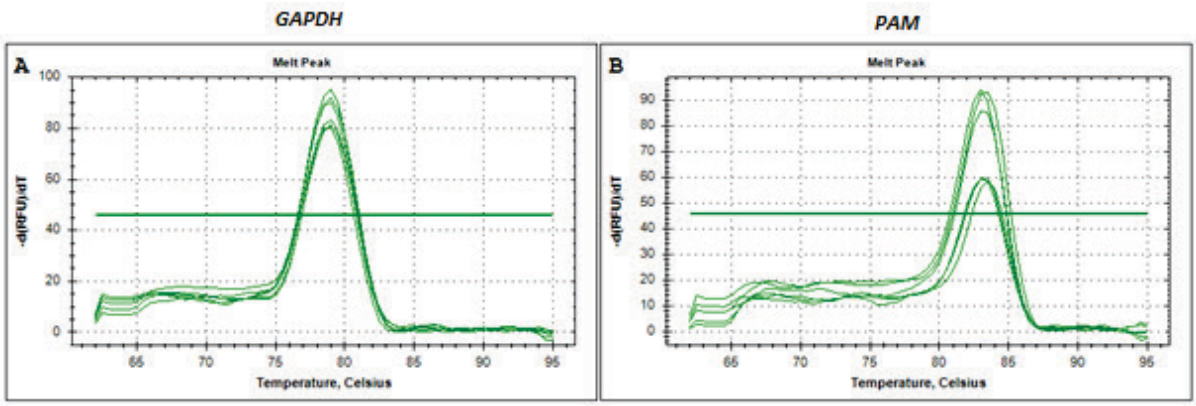

Figure 3. Melting curve analysis of *GAPDH* (A) and *PAM* (B) genes by real-time PCR. The presence of a single sharp peak confirms the specific amplification of the selected genes

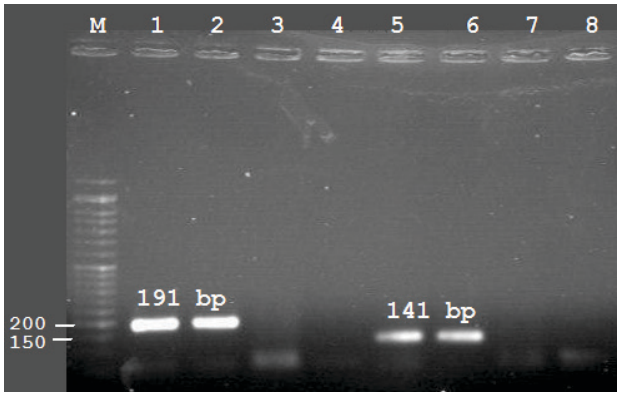

Figure 4. Agarose gel electrophoresis of the real-time PCR products. Lanes 1-4 amplified by *GAPDH* primers. 1 and 2 amplified with cDNA template, 3 with RNA template (as minus reverse transcription control or -RT control) and 4 with no template (as no template control or NTC). Lanes 5-8 amplified by *PAM* primers. 5 and 6 amplified with cDNA template, 7 with RNA template and 8 with no template. Only the samples with cDNA templates resulted in amplification products of the expected size, whereas the negative controls resulted in no amplification products, indicating that RNA samples are DNA-free and have enough quality to be used in sensitive assays such as real-time PCR. M: DNA size marker (Orange ruler 50 bp DNA ladder, CinnaGen, Iran).

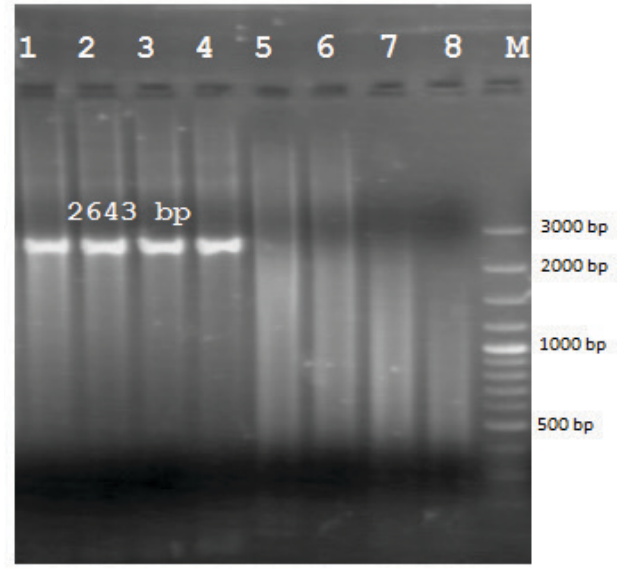

Figure 5. Agarose gel electrophoresis of full length amplification of *TXS* gene. Lanes 1-4 are the reactions with cDNA templates, lanes 4 and 5 with RNA templates and 7 and 8 with no templates. Only the samples with cDNA templates showing a single band of the expected size (2643 bp) and the negative controls resulted in no amplification products. M: DNA size marker (GeneRuler 100 bp Plus DNA Ladder, Thermo Fisher Scientific).

concluded that using some components of alkaline lysis method into the RNA isolation procedure, will help in removal of contaminations. This idea was verified by experimental results.

Moreover, *DNase* treatment was carried out before the final RNA precipitation step, considerably improved RNA yield and quality. *DNase* digestion after the final RNA precipitation step involves adding extra salts and protein to the sample and, since they can affect the efficiency of the cDNA synthesis, so additional purification steps are required [1, 23].

Another significant advantage of the method is the high stability of RNA over long term-storage, as repetitive (n = 5) freeze-thaw cycles caused only slight degradation of RNA samples. The high stability of extracted RNA by this method is largely attributed to the supplementary substances. These substances have some good characteristics including: pH lowering, chelating and protein denaturant, which effectively improves the quality and stability of RNA samples [2, 9, 23, 26].

According to our another research [18], we showed that, RNA from other tissues of woody plants including bark, root, callus and suspension cell cultures can be isolated by the method described in this paper (in some tissues there are some modifications needed, such as decreasing the plant materials and/or increasing amounts of sodium citrate and helper buffers). It also demonstrated that, RNA obtained from this method is applicable for the gene cloning of rare transcripts (*DBAT* gene from *Taxuus baccata*). [19].

CONCLUSION

The protocol developed and described in this paper proved to be completely suitable for isolating RNA from a wide range of woody plants and their different tissues. RNA purity and integrity were validated and the RNA showed excellent results in downstream applications such as RT-PCR, real-time PCR, and gene cloning. The sensitivity of the method was proved by amplifying the genes with low expression levels. The method has some prominent advantages including; only small amounts of plant materials are required, RNA samples have long-term stability and the method does not use dangerous chemicals such as phenol. In conclusion we recommend the use of this method for successful RNA isolation from a wider range of woody and other recalcitrant plants, and it is expected that the extracted RNA will be suitable for

more RNA-related investigations including Northern blotting, RACE, microarray, and RNA-seq.

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جداسازی RNA با کیفیت بالا از طیف وسیعی از گیاهان چوبی

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چکیده

جداسازی RNA با کیفیت بالا یکی از اساسی‌ترین روش‌ها در بیولوژی مولکولی می‌باشد. استخراج RNA از گیاهان چوبی به دلیل حضور بافت‌های سخت و چوبی و مقادیر بالای ترکیبات پلی‌ساکاریدی، پلی‌فنلی و دیگر متابولیت‌های ثانویه با مشکلات فراوانی همراه است. در تحقیق حاضر، یک روش مناسب برای جداسازی RNA از طیف وسیعی از گیاهان چوبی مشتمل بر هشت گونه بازدانه و چهار گونه نهاندانه معرفی شده است. روش مذکور مبتنی بر CTAB است که با افزودن سدیم سیترات و بافرهای کمکی، تغییراتی در آن اعمال شده است. ارزیابی نمونه‌های استخراج شده با الکتروفورز ژل آگارز نشان داد که RNAهای استخراج شده از سلامت بالایی برخوردار بوده و پروفایل RNA دارای تمامی باندهای مورد انتظار RNA می‌باشد. همچنین آلودگی‌های DNA و پروتئینی نیز مشاهده نشدند. ارزیابی‌های اسپکتروفتومتری نمونه‌های RNA با استفاده از نانودراپ نشان داد که غلظت RNAهای استخراجی در دامنه‌ای بین ۳۵,۶۸ تا ۲۱۶,۹۸ میکروگرم بر گرم وزن تر بافت قرار دارند که RNA کافی برای سنتز cDNA و سایر آزمایشات مبتنی بر RNA را فراهم می‌آورد. هر دو نسبت جذبی ۲۶۰ به ۲۸۰ و ۲۶۰ به ۲۳۰ نانومتر نیز در دامنه مطلوب قرار داشتند که بیانگر خلوص بالا و فقدان آلودگی‌های پروتئینی، پلی‌فنلی و پلی‌ساکاریدی در RNA استخراجی می‌باشد. کارایی RNA استخراج شده در کاربردهای پایین‌دستی، توسط real-time PCR و همچنین تکثیر یک cDNA طویل مورد تأیید قرار گرفت. در پایان نیز به برخی از مزایا و همچنین کاربردهای احتمالی روش مذکور اشاره شده است.

کلمات کلیدی: جداسازی RNA، گیاهان چوبی، آلودگی‌ها، CTAB، سدیم سیترات، بافرهای کمکی