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Research Article

Improved RNA Extraction Technique for Tropical Plants

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Abstract: The study was aimed to compare the relative yield, purity and integrity of RNA extracted from leaf tissues of different plants using the modified CTAB method developed by Chang et al. 1993. Extraction of RNA is very crucial step in the gene expression analysis of plants. In this study, a large number of samples such as fresh leaves obtained from different plant species *Oryza sativa, Elusine korakkana, Zea mays, Azadirachta indica, Musa sapianatum, Sesbania grandiflora* were taken and modified CTAB method was applied for RNA extraction. Quality of the extracted RNA was tested by measuring the absorbance of RNA at 260 nm using Nanodrop® ND-1000 spectrophotometer and measuring the ratio of A260 / A280 respectively. Amplifiable quality of RNA with satisfactory concentration were tested by running a horizontal agarose gel electrophoresis using 1% agarose in TBE buffer at constant voltage of 60V confirms that the modified CTAB method could be recommended for the all the tested plant species except *Musa sapianatum*.
Keywords: CTAB method, RNA extraction, quality, spectrophotometer.

INTRODUCTION

High-quality RNA is required for many downstream applications in molecular Biology. Due to the high costs of many of these assays, RNA samples need to be analyzed properly to prevent the failures in downstream applications and eliminate costs associated with repeating the analysis. Properties of RNA such as quality, integrity and reproducibility among extractions of replicates from the same tissue, are critical for correct expression analysis and physiological and biochemical studies of plants. Partial degradation and loss during RNA preparation are important parameters to be examined before further analyses. Studies that involve screening of large number of samples require faster methods that reliably yield high-quality RNA [1]. Hence, there is demand for rapid, simplified and inexpensive RNA extraction/purification methods which can provide large amount of high quality RNA [2]. Extracted RNA samples are either contaminated with genomic DNA or degraded easily, which can cause false-positive outcomes in highly sensitive applications such as qrtPCR. The RNA concentration of a sample is commonly determined via measurement of absorbance at a wavelength of 260 nm. The purity of the RNA sample can be determined using theA260/A280 ratio as a reference (a value of 2.0 is considered "pure" RNA). However, the protein contamination can cause an overestimation of RNA content [3]. RNA is extremely susceptible to degradation due to the ubiquitous presence of RNases in the environment. This can result in shorter fragments of RNA and this decrease in RNA integrity might interfere with downstream applications [4]. However, purified nucleic acids, often required for many applications in molecular genetic studies, is much more difficult to obtain from trees than other plants [5]. Yield and quality of RNA often varied among species within same genera as well as among tissue types from the same plants. Since leaf and other tissues of plants often contain varying levels of tannins, polyphenols and polysaccharides, these impurities co-extract with RNA posing serious problems while obtaining RNA. Such impurities also interfere in further RNA analysis. Chemotypic heterogeneity among plant samples also would not allow optimal yield with a single protocol, and hence, specific protocols need to be followed for different plants. Among the RNA extraction methods available in practice, the method developed by Chang et al. [2] was very efficient in terms of quality and quantity [6]. The objective of the study was to compare and find out the relative yield, purity and integrity of RNA extracted from leaf tissues of different plants using the modified CTAB method.

MATERIALS AND METHODS

Plants material

Fresh leaves obtained from different plant species *Oryza sativa, Elusine korakkana, Zea mays, Azadirachta indica, Musa sapianatum, Sesbania grandiflora* were used as sources of DNA. All the above plants species are collected from local area of Northern Province, Jaffna District SriLanka.

RNA extraction

Total RNA was extracted using CTAB extraction protocol (Chang et al. 1993) with some modifications. Twenty mL of hexadecyl trimethyl ammonium bromide (CTAB) buffer (2% CTAB; 2% polyvinylpyrrolidone (PVP, MW 30000); 100 mM Tris-HCI pH 8.0; 25 mM EDTA; 2.0 M NaCI; 0.5 g L^{-1} spermidine) per sample were added to a labelled Falcon tube and kept in a water bath set at 65°C. Then, 400 µL of β-mercaptoethanol was added to each falcon tube and mixed just before the RNA extraction. Each ground leaf sample stored at -80°C was brought in liquid nitrogen and 600 µL of chloroform:isoamyl alcohol (24:1) was added to each ground sample containing tube and vortexed vigorously and the tubes were kept in the water bath at 65°C for 10 minutes with periodical vortexing. After 10 minutes, the samples were centrifuged for 15 min at 14000 rpm in a Beckman J2-21M/E refrigerated centrifuge equipped with a JA-20 rotor (Beckman Coulter, Brea, CA, USA). The supernatant was carefully transferred to a sterile eppendorf tube using a 10 mL sterile pipette. Chloroform:isoamyl alcohol addition, incubation at 65°C for 10 minutes, centrifugation, and transfer of the supernatant were repeated once again to the supernatant. One-third of the sample's recovered volume of chilled 10 M LiCI was added to the aqueous phase and mixed gently by inverting the tubes for five times and the tubes were incubated at -20°C for 30- 60 minutes in order to precipitate the RNA. Then the tubes were centrifuged at 14000 rpm for 15 minutes at 4°C in a Beckman JA-20 rotor to pellet the RNA and the supernatants were discarded. The pellets were washed by adding 800 µL of 80 % pre-chilled ethanol, briefly vortexed and centrifuged again at 14000 rpm at room temperature for 5 minutes. Supernatant was removed and the pellet was centrifuged again at full speed for 5 min. The pellet was air dried for 10 minutes and resuspended in sterile RNase-free baxter water. Total RNA was dissolved in 20 µL of sterile RNase-free water and quantified, at 260 nm wavelength with a NanoDrop ND-1000 spectrophotometer. The ratio between A260 and A280 was also recorded to determine the purity of the RNA sample to know whether it was contaminated with protein or not. The RNA quality was determined by running 1 µL of total RNA from each sample on a 1% agarose gel (0.5X TBE) for 45 minutes with 60 V current and with 0.5X TBE buffer, and visualized by SYBR safe. Total RNA was aliquoted and stored at -80°C.

RNA quantity and purity confirmation

RNA from the leaf samples were quantified by measuring the absorbance at 260 nm using Nanodrop® ND-1000 spectrophotometer. Absorbance was measured and the ratio (A260/280 nm) was calculated to determine the purity of the RNA sample to find out whether it was contaminated with protein or not.

Gel running

The size, purity and integrity of RNA were determined by running 200 ng of total RNA from each sample on a 1% agarose gel for 45 minutes with 60 V current and with 0.5X TBE buffer and visualized by SYBR safe.

RESULTS AND DISCUSSION

RNA quantity and quality

Fresh leaves of *Sesbania grandiflora* yielded maximum amount of RNA with overall mean of 2850.4 µg g^{-1} fresh leaf (Figure 1) and the minimum yield was obtained from the leaves of *Musa sapianatum* with overall mean of 1100.3 µg g^{-1} fresh leaf (Figure 1). Among the plants tested, *Sesbania grandiflora* and *Elusine korakkana* yielded RNA of the highest quality with the absorbance ratio (A_{260}:A_{280}) of 1.92 and 1.84 respectively. Among the plants tested, almost all the plants except *Musa sapianatum* yielded RNA of highest quality. *Musa sapianatum* resulted in the lowest quality of RNA with absorbance ratio of 1.12 (Figure 2). However, with this method, except for *Musa sapianatum*, all the other plants yielded RNA with satisfactory quality with absorbance ratio of about 1.7. Among the plant species, *Sesbania grandiflora* consistently yielded RNA with high purity ratio (A_{260}:A_{280} ≥ 1.8) with the method investigated. There are many characteristic features of RNA that can affect the results in downstream applications. RNA concentration, purity, integrity, DNA contamination or the presence of other contaminants often used in nucleic acid purifications may need to be determined to avoid repeating experiments and the associated high reagent, instrumentation and labor costs. RNA is extremely susceptible to degradation due to the ubiquitous presence of RNases in the environment. Therefore great care and consideration is required when working with RNA. Selection of proper quantification and analysis methods for the type of sample and applications is important. Quality (or purity) of RNA was examined by recording the absorbance of RNA preparations at 260 and 280 nm and computing A260:A280 ratio using Nanodrop® ND-1000 spectrophotometer. A260:A280 ratio of more than 1.8 indicates high quality whereas values less than 1.8 indicate protein contamination. Nucleic acid extraction methods and tree species were significant sources of variation for quality of extracted DNA / RNA [7, 8, 9, 5]. The quantity and quality of RNA extracted by modified Cheng et al method, was comparatively lower in *Musa sapianatum* and this may be due to the thick leaves and the time and complications involved in the grinding and extraction process. Variation in quality of RNA can be due to the genitical, structural and biochemical variation among leaf samples of different plant species, variation in types of buffers used for extraction and the difference in the extraction with varying parameters and chemicals [10].

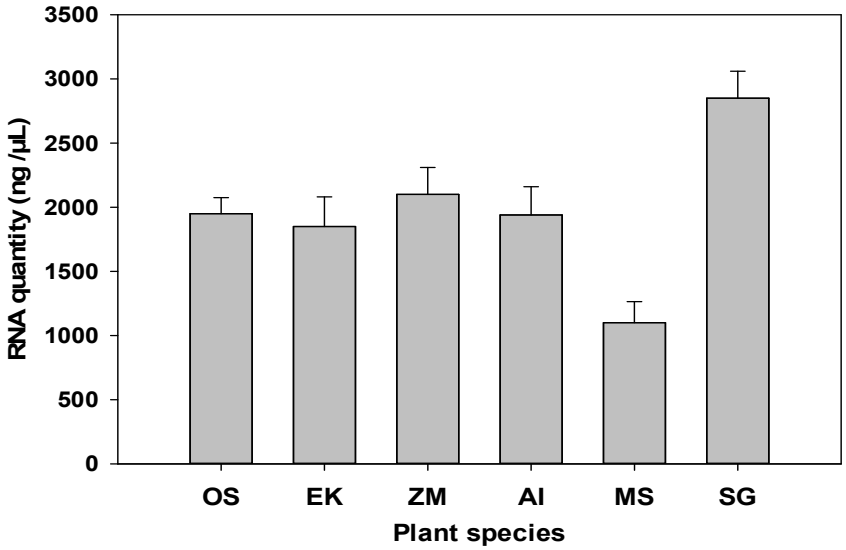

Fig-1: RNA quantity means of different plant species

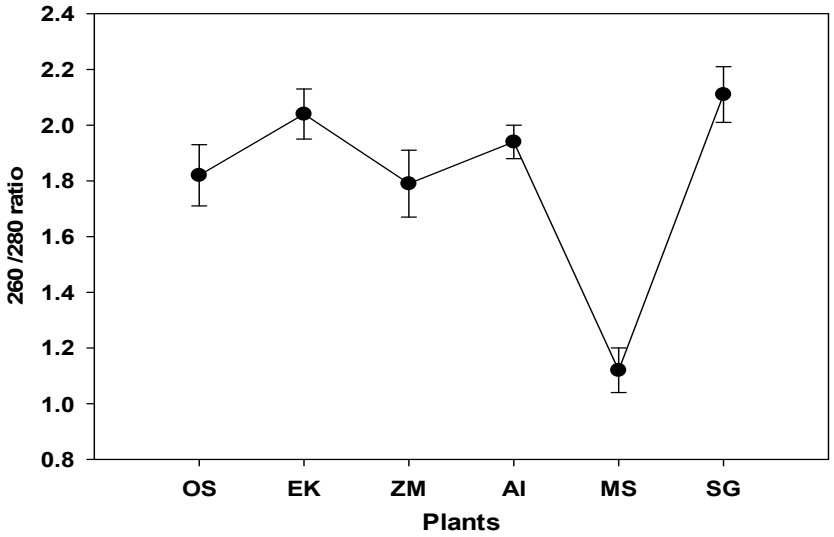

Fig-2: Means of the 260/280 ratio of RNA obtained for different plant species

Gel running of samples from all the plant species using this method showed considerable amount of amplifiable quality of RNA except in *Musa* (Figure 3). The method of Cheng et al. consisted of comparatively few steps for the completion of the entire extraction process and was the most rapid extraction method requiring less than seven hours. Total RNA run on a denaturing gel will have sharp, clear 28S and 18S rRNA bands (eukaryotic samples). The 28S rRNA band should be approximately twice as intense as the 18S rRNA band. This 2:1 ratio (28S:18S) is a good indication that the RNA is completely intact. Partially degraded RNA will have a smeared appearance, will lack the sharp rRNA bands, or will not exhibit the 2:1 ratio of high quality RNA. Completely degraded RNA will appear as a very low molecular weight smear. RNA extraction methods and plant species influenced PCR amplification of extracted RNA and gel running[5]. Production of good amplification from all the samples using this method may be demonstrated by the high purity ratio of these RNA samples indicating very low or no protein co-precipitation of extracted RNA.

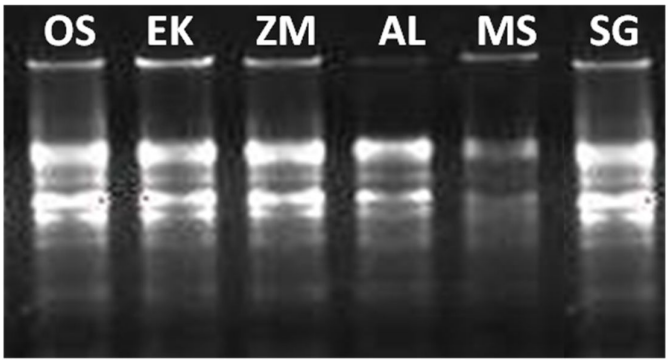

Fig-3: Bands of DNA on the 1% agarose gel with 0.5X TBE buffer after visualization with SYBR safe. M- Marker Other alphabets indicate the first letter of the generic and species names of the plants used.

CONCLUSION

In this study, a modified method of Chang et al,[2] was applied for RNA extraction from leaves of different selected plant species. This turned out to be a suitable method for extraction of RNA from leaves except *Musa*. While various plants leave have different chemicals contained molecules and hardness such as mucilage and phenolic compounds create difficulty in RNA extraction. Usage of this modified method of RNA extraction improved the quality and quanity of extracted RNA of *Sesbania grandiflora* than any other plants tested.

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