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The best method for isolated total RNA from durian tissues

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Abstract: Plant tissues, especially durian tissues contain high content of polysaccharides, polyphenols and other secondary metabolites which can co-precipitate with RNA causing problem in further transcriptomic study. In this experiment, three basic chaotic agents, CTAB, SDS and guanidine are used in three basic protocols for RNA isolation. The effectiveness of each method was determined by spectrophotometer, denaturing agarose gels analysis and northern blot hybridization. CTAB combining with additional sodium acetate precipitation step showed highest yield and best quality of isolated RNA which was free from contaminations of polysaccharides, polyphenols and other secondary metabolites. Furthermore, the total RNA from 4-month old durian flesh of clone D24 was successfully used to construct a cDNA library. In conclusion, CTAB method is effective to isolate total RNA on various types of durian tissues for further gene expression analysis.

Keywords: ***Durio zibethinus*** **Murr., CTAB, SDS, guanidine, total RNA**

Introduction

Durio zibethinus Murr., or durian, is a cultural and economical important fruit in South East Asia (Zappala *et al*., 2002). Until now, little researches related to the molecular aspect of durian have been carried out. Thus, construction of cDNA library from durian flesh can play an important role in the future durian breeding and improvement.

To obtain good cDNA library and gene expression profile, it is crucial to extract high quality and quantity of total RNA. However, most plant material contains relatively high levels of RNase activity, high levels of proteins, polysaccharides and polyphenols, which decrease RNA yield and quality (Tai *et al.*, 2004; Wang *et al*., 2008). Like tissue from other plants, durian tissues also contain high levels of starches (fruit), polysaccharides, polyphenols (leaf) and other secondary metabolites (Brown, 1997). All these compounds may bind or co-precipitate with RNA causing the viscosity, and resulting in the difficulty to isolate pure RNA. The purposed of this study is to evaluated the efficiency of three basic detergents including cetyltrimethyl ammonium bromide (CTAB) (Porebski *et al.*, 1997), sodium dodecyl sulfate (SDS) with hot phenol (Gao *et al*., 2001) and guanidine thiocyanate (GTC) (Rochester *et al*., 1986) to isolate high yield and quality of RNA from different types of durian tissue.

Materials and Methods

Plant materials, total RNA isolation, Northern blot hybridization and cDNA library construction

Five difference types of durian tissue that used in this study were 1-month old durian flesh, 4-month old durian flesh, ripening durian flesh, leaves and young flower buds. Samples were freshly collected at Department of Agriculture Serdang, Universiti Putra Malaysia and immediately frozen in liquid nitrogen. Subsequently, samples were ground into fine powders in liquid nitrogen using pre-cold mortar and pestle. The resulting tissue powders were stored at -80^{o}C until used. Total RNA was isolated from difference types of durian tissues using SDS with hot phenol (Gao *et al*., 2001), GTC chaotropic reagent (Rochester *et al*., 1986) or CTAB method (Porebski *et al*., 1997). An additional carbohydrate precipitation step (1/30 volume of 3 M sodium acetate and 1/10 volume of absolute ethanol) was performed after the PCI precipitation step 25:24:1 for the CTAB method. Quality and quantity of total RNA were determined by monitoring A_{260}/A_{280} and A_{260}/A_{320} absorbance ratio using NanoDrop spectrophotometer (Implen, Germany). Total RNA in the amount of 20 µg was subjected to electrophoresis on a 1% agarose gel that contained formaldehyde (Sambrook *et al*., 1989) and blotted onto hybond-N^{+} nylon membranes

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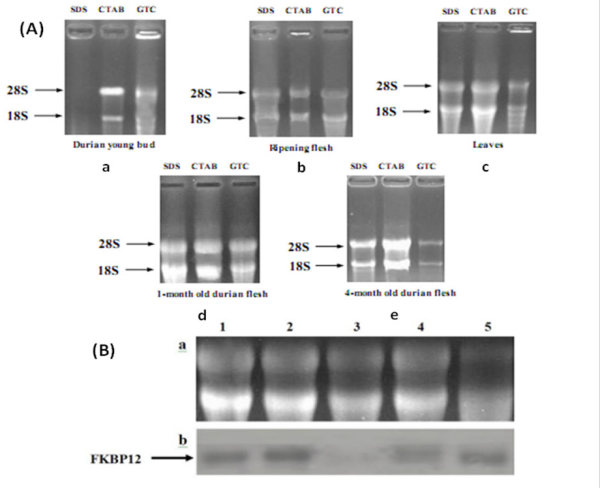

Figure 1. (A) Formaldehyde denaturing agarose gels (1% (w/v)) were used to separate total RNA of five types of tissues by different RNA extraction procedures. (a) Durian young bud; (b) Ripening flesh; (c) Leaf; (d) 1-month old durian flesh; (e) 4-month old durian flesh (e); lane 1, SDS method; lane 2, CTAB method; lane 3, GTTC method. (B) Northern blot with 20 mg of total RNA from five different types of durian tissues; a). Total RNA (20 mg) were separated on 1.2% (w/v) formaldehyde denaturing agarose gel and transffered onto nylon mambrane. b.). The transferred total RNA were hybrdized with biotin labeled DNA probe for durian FKBP12. 1) lane 1-1-month old flesh; 2-4-month old flesh; 3- ripening flesh; 4- leave; 5- young bud.

(Amersham Biotech, USA). The transferred RNA was fixed to the membrane by UV crosslinking at a wavelength of 254 nm using a UV-crosslinker (Ultra-Violet Products, UK). The blot were probed by a random-primer labeled with immunophilin FKBP12 cDNA in the presence of biotin 14-dCTP (Invitrogen, CA, USA). Prehybridization, hybridization, and washing of the filters were performed based on Sambrook *et al.* (1989). Data from spectrophotometry measurement were subjected to statistical analaysis with randomized complete block design (RCBD) (three replication for each treatment). Statistical significance was determined by one-way analysis of variance (ANOVA) using the MSTAT-C program (Michigan State University 1986). The significant difference of mean of RNA yield were test by DMRT at 5%. mRNA was isolated from approximately 450 µg of the total RNA from 4-month old durian flesh using PolyATtract® mRNA Isolation Systems (Promega, USA). Five micrograms of the poly (A^+) RNA isolated from 4-month old durian flesh tissue were used for cDNA library construction. The cDNA library was prepared according to the user manual of Uni-Zap™ II cDNA synthesis kit (Catalog No. 200400, Stratagene, CA, USA).

Results and Discussion

Many molecular studies in fruit trees, especially durian require high quality and quantity of intact RNA. However, isolation of large quantity and quality of RNA from fruit trees can be technically complicate due to the present of large amount of polysaccharides, polyphenolic and other compounds that accumulate during ripening. These polysaccharides and polyphenolic compounds often co-precipitate and contaminate the RNA during extraction, thereby affecting the quality and quantity of isolated RNA (Logemann *et al*., 1987; Asif *et al*., 2000).

In this study, evaluation of quality and quantity of total RNA isolated from three different protocols for each kind of tissue was done spectrometer (Figure 1b) and formaldehyde denatured gel (Figure 1a). As shown in Figure 1b, the interaction between each kind of tissue and method gave a significant difference on the yield of total RNA ($P \leq 0.05$). CTAB method gave the highest yield for all kind of durian tissues ranging from 75.83 to 272.50 µg/g of frozen tissue. On the contrary, two other methods produced lower yields than CTAB method. Among all types of durian tissue, yield for total RNA ripening fruit flesh tissues was the lowest in all method compared to four other types of tissues. CTAB method gave A_{260}/A_{280} range of 1.73 to 1.80 and abundant band for 28S rRNA than 18S rRNA, indicating that the RNA was none degraded and free from protein or polysaccharides contaminant (Figure 1a, 1b). The intactness of the RNA could be seen during Northern blot analysis and cDNA library construction. Expression of the putative FKBP 12 was monitored during fruit development, in the leaf and young flower bud (Figure 1c). Expressions of this gene was represented as a single band without any smearing; indicating that the RNA was intact and can be monitored even at low expression. For cDNA library construction, 5 µg of mRNA obtained from the total RNA of 4-month old durian flesh was used for first strand and second strand synthesis. On a preliminary screening of recombinant clones from the library, there were approximately 5.1x106 pfu/mL in the primary library, indicating that the isolated RNA or mRNA was sufficient quality for cDNA library contruction. Similar to CTAB method, SDS method was able to produce high quality RNA and could be used for RNA isolation from 1-month old durian flesh and 4-month old durian flesh (Figure 1b) because the starches, polysaccharides and protein were effectively removed by PVP and BSA treatment. However, RNA from young durian flesh and old durian flesh were partly degraded, as indicated by some smearing of two major 28S and 18S bands and the A_{260}/A_{230} ratio (Figure 1a, 1b). The reason for the observed degradation may be due to RNase contamination during incubation at 56°C for 30 minutes. This maybe due to cross contamination of RNase present

in the water bath. Based on the above results, we can conclude that SDS method may not suitable for the other durian tissue types (ripening flesh, leave and young flower buds). This may be due to the fact that PVP and BSA could not form a complex with starches or polyphenol in these tissues (Sue and Grant, 1997). As the result, RNA can not be separated from these components during ethanol precipitation step. GTC procedure could not sufficiently purify RNA from polyphenols, carbohydrates and protein from young bud, 4-month old durian flesh, and ripening flesh (Figure 1b). Added to this, smears below the bands were observed after formaldehyde gel electrophoresis (Figure 1a). Likewise, this method also failed to yield useable for cDNA library contruction (result not shown). Thus, we can conclude that the GTC method was not suitable for RNA isolation from all types of durian tissues. Unsuitability of SDS and GTC methods to extract RNA from durian tissues could be due to the high levels of insoluble starch containing in the unripe fruit which dramatically changed to soluble starches during fruit ripening. The ripening fruit contains high amount of sugar and organic acids. Two other types of durian tissues, the leaf and young flower buds, contain high amount of polyphenol, chlorophyll and other metabolites (Brown 1997). These substances can interact with nucleic acids to form insoluble complexes (Pawlowski *et al*., 1994).

Conclusion

CTAB method extracted the highest quality and quantity of RNA from all the tested durian (*Durio zinberthinus Murr.*) tissues. The use of carbohydrate precipitation step (1/30 volume of 3 M sodium acetate and 1/10 volume of absolute ethanol) significantly improved the purity of isolated total RNA from durian tissues.

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