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An Optimized Cetyltrimethylammonium Bromide (CTAB)-Based Protocol for Extracting RNA from Young and Cassava Leaves

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Abstract: *RNA integrity, quality and quantity are critical in most plant molecular studies. Extracting high quality RNA from cassava leaves and other recalcitrant plant tissues are difficult due to the presence of polysaccharides, polyphenols and other secondary metabolites that often co-precipitate with the final RNA extract. This is an optimized a CTAB-based method that suitably extracts RNA from the polysaccharide-rich cassava leaves. The modifications were introduced into a version of the CTAB protocol as described by Gasic et al., (2004). The changes included an increased rate or use of Extraction Buffer (EB) for every gram ground leaf tissue (20ml EB per 1 gram tissue), incubation of the Tissue-EB and Chloroform: Isoamyl alcohol (24:1) mixture at a lower water-bath temperature of 50°C and all centrifugation steps carried out at 4°C. In addition, the EB contained a higher concentration of soluble polyvinylpyrrolidone (PVP-K-30). The pH of sodium acetate was lowered to 5.2 and a final two-step high molarity (10M) Lithium Chloride (LiCl) precipitation was applied. Ethyl alcohol concentration was raised to 100%. The modified CTAB method produced RNA of high concentration (> 1 µg), high A260:A280 and A260:A230 ratios (> 2.0) and high integrity (distinct and visible 28S and 18S rRNA bands) from young and old cassava leaves, compared to RNA (from the same leaf tissues) generated by several other published methods.*

Key words: *Polysaccharides, Polyphenols, RNA extraction protocol, cassava*

Introduction

Purification of RNA of high quality and quantity is a pre-requisite and an essential step for many molecular techniques (Gasic et al., 2004; Carra et al., 2007; Luoime et al., 2008; Gambino et al., 2008). However, isolating suitable RNA remains problematic especially from recalcitrant plant species or tissues with high levels of phenolic compounds and/or polysaccharides (Schneiderrbauer et al., 1991; Gehrig et al., 2000). The extracted RNA from these plant species are often of poor quality and too low for further downstream application (Gehrig et al., 2000; Alemzadeh et al., 2005). Cellular components that inhibit high quality and quantity RNA isolation include endogenous RNases, polysaccharides, polyphenols, proteins, lipids and other secondary metabolites (Azevedo et al 2003; Alemzadeh et al., 2005; Gambino et al., 2008). Phenolic compounds readily oxidize to form covalently linked quinones and easily bind proteins and nucleic acids resulting in high molecular weight complexes (Azevedo et al., 2003; Loomis, 1974).

Polysaccharides tend to co-purify and co-precipitate with the RNA in the presence of alcohols or low ionic strength buffers (Gehrig et al., 2000; Malnoy et al., 2001; Carra et al., 2007). Polysaccharide contamination hinders re-suspension of the precipitated RNA, interferes with absorbance-based RNA quantification, and may inhibit enzymatic manipulations, poly $(A)^+$-RNA isolation as well as electrophoretic migration (Wilkins and Smart, 1996). Endogenous ribonucleases reduce the integrity of the RNA, particularly when the amount increases, such as during senescence, wounding, or pathogen attack (Logemann et al., 1987; López-Gómes and Gómes-Lim, 1992; Green, 1994). Homogenization triggers inevitably the mixture of RNA and endogenous RNases (Wan and Wilkins, 1994). The above mentioned contaminants can occur at various concentrations depending on the plant species and organs that are considered for nucleic acid extraction. A number of CTAB-based methods have been developed for RNA extraction from tissues

containing high levels of polysaccharides and phenols (Chang et al., 1993; Jaakola et al., 2001; Hu et al., 2002).

The successes of yielding suitable RNA from cassava leaves using these methods have proved unreliable or have not been reported. The readily available commercial kits such as RNeasy plant kit (Qiagen), TRIzol Reagent (Sigma) and Concert Plant RNA Reagent (Invitrogen) have been successfully applied to extract RNA from cassava, but can be a costly option especially when a large number of RNA extractions are needed. The difficulty of obtaining RNA of high quality and quantity from cassava can be attributed to the high concentration of polysaccharides, phenolic compounds, proteins, and other secondary metabolites in the leaves. This paper describes an optimized protocol that provides high quantity and quality RNA from cassava leaves. This procedure is modified from a version of the CTAB-based method as described by Gasic et al. (2004).

Materials and Methods

Cassava leaves

Leaves of the cassava model genotype TMS 60444 were used in the extraction of RNA using a modified CTAB protocol as well as four other methods (obtained from literature) for comparison purposes. The genotype was first established and grown under greenhouse (Lindau-Eschikon, Zürich, Switzerland) conditions for four months before utilization of its leaves for RNA extraction. Young leaf tissues constituted the three top most fully expanded leaves, while three leaves at the mid-stem were considered old leaf tissues.

Extraction Buffer (EB)

Consisted of CTAB (2%), PVP K-30 (2%), Tris-HCl (100 mM; pH 8.0), EDTA (25 mM), NaCl (2 M), Spermidine (0.5g/l; free acid-HRS), 2% βmercaptoethanol (added just before use), and Sterile RNase-free H_2O. Other reagents included Chloroform: Isoamyl-alcohol (24:1), Lithium Chloride (LiCl; 10 M), Sodium acetate (3 M; pH 5.2), and Ethyl alcohol (100%).

Table 1: Main component of extraction buffers of the other four protocols (from literature review) that were also used to extract RNA and their final RNA extract compared with those of obtained from the modified CTAB-based method.

	Protocol	Main component of Extraction Buffer
1	RNeasy Plant Minikit Method	Guanidine Isothiocyanate
2	Total Nucleic Acid + DNase Treatment	Proteinase-K and RNase-free DNase 1 (Promega)
3	TRIzol Reagent Method	Phenol and Guanidine isothiocyanate
4	Reilly et al. (2001)	Sodium Lauryl-sarcosine

RNA Extraction Procedure

[1]. Cassava leaves were homogenized in liquid nitrogen with a pestle and mortar to a fine powder. Liquid N_2 was constantly added to the tissue during grinding to prevent thawing. The frozen powdered tissues were then quickly transferred to a pre-chilled 50 ml falcon tube containing EB at a rate of 20 ml of the extraction solution per 1g tissue.

[2]. The mixture was vortexed briefly and incubated on ice for 5 minutes. An equal volume of Chloroform: Isoamyl alcohol (24:1) was added. The sample was then heated in a water bath at 50°C for 15 minutes and then centrifuged at 5 000 rpm, RT, for 10 minutes.

[3]. The resulting supernatant was transferred to fresh a 50 ml falcon tube, where an equal volume of Chloroform: Isoamyl alcohol (24:1) was again added, mixed, vortexed and centrifuged as described above. The resultant supernatant was transferred to a fresh 50 ml tube, 0.25 volumes LiCl (10 M) were added and the mixture was incubated on ice overnight at 4°C. The sample was then centrifuged at 5 000 rpm, RT, for 20 minutes the resultant supernatant decanted and the precipitated pellet was dried by inverting the tubes for 10 minutes on a kimwipe.

[4]. The pellet was then re-suspended in 250 µl sterile RNase-free H_2O and transferred into a 2 ml micro-centrifuge tube where 250 µl LiCl (10 M) was added. The mixture was flicked to mix and the RNA precipitated by incubating on ice for 2 ½ hours. The sample was then centrifuged at 13 000 rpm, 4°C, for 10 minutes.

[5]. The resultant supernatant was decanted; the RNA pellet re-suspended in 250 µl sterile RNase-free H_2O and 25 µl sodium acetate (3 M; pH 5.2) and 1 ml 100% ethyl alcohol were added. The mixture was then incubated at -20°C for 60 minutes and then centrifuged as described above.

[6]. The resultant supernatant was decanted and the RNA pellet vacuum dried in a SpeedVac for 3 minutes. The dried RNA pellet was then re-suspended in 100 µl sterile RNase-free H_2O.

NB: The extraction of RNA using the modified CTAB method (as described in the procedure) and the other four protocols was repeated (replicated) five times i.e. on the three young and three old leaves of individual plants of cassava genotype TMS 60444.

RNA Analysis

The total quantity and purity of the final RNA extract was determined using a spectrophotometer (NanoDrop®ND-1000, Technologies Inc.). Analysis of Variance (ANOVA) was carried out on the concentration (ng/µl) and the spectrophotometric absorbance ratio (A_{260}:A_{280} and A_{260}:A_{230}) values and the means compared or separated using the Fisher's protected Least significant differences (Lsd) test at 5% probability level. The PROC ANOVA procedure of Genstat Discovery Edition 3 was (Lawes Agricultural Trust Rothamsted Experimental station, UK) was followed. The spectrophotometric ratios A_{260}:A_{280} and A_{260}:A_{230} are used to indicate protein and polysaccharide (+ polyphenols) contaminations respectively ((Iandolino et al., 2004). The integrity of the total RNA was verified by separating the fragments on 2% non-denaturing Agarose gels using electrophoresis (Sambrook et al. 2000).

Results

RNA purity via A_{260}:A_{280} ratio

Specific leaf tissue A_{260}:A_{280} ratio variation was significant. The modified CTAB protocol had a significantly higher ($P \leq 0.05$) A_{260}:A_{280} ratio in young leaves compared to other methods, while the method of Reilly et al. (2001) generated the least A_{260}:A_{280} ratio in young leaves. Total Nucleic Acid + DNase method generated significantly ($P \leq 0.05$) high RNA A_{260}:A_{280} ratio in young leaves, compared to the same ratio observed in RNeasy Kit and TRI®$_{ZOL}$ Reagent methods. The A_{260}:A_{280} ratio in TRI®$_{ZOL}$ Reagent and RNeasy Kit protocols did not differ significantly ($P > 0.05$) (Table 2).

Significant differences in the RNA's A_{260}:A_{280} ratios from older leaves were also observed. For instance, the A_{260}:A_{280} ratio from old leaves was significantly (P≤0.05) higher in modified CTAB protocol than in all the other methods, while the method of Reilly et al., (2001) generated the least A_{260}:A_{280} ratio from these leaf tissues. The A_{260}:A_{280} ratios obtained from TRI®$_{ZOL}$ Reagent and RNeasy Kit methods did not significantly differ (P≤0.05) from each other, although their means were significantly (P≤0.05) lower than that of Total Nucleic Acid + DNase method (Table 2).

RNA purity via A_{260}:A_{230} ratio

Significant variation in RNA A_{260}:A_{230} ratios of young leaves were observed across the protocols. The modified CTAB protocol produced significantly higher (P≤0.05) RNA A_{260}:A_{230} ratio from young leaves than all other methods, while the method of Reilly et al. (2001) generated the least A_{260}:A_{230} ratio from the same young leaf tissues. The A_{260}:A_{230} ratio was also significantly (P≤0.05) high in Total Nucleic Acid + DNase method, followed by TRI®$_{ZOL}$ Reagent and RNeasy Kit methods respectively (Table 2).

When the A_{260}:A_{230} ratios of older leaves were specifically analyzed across the protocols, the ratio observed in modified CTAB method was significantly (P≤0.05) higher when compared to the rest of the protocols. Although the A_{260}:A_{230} ratios of old leaves generated by Total Nucleic Acid + DNase and TRI®$_{ZOL}$ Reagent method were not significantly (P>0.05) different, the two protocols still produced significantly (P≤0.05) higher A_{260}:A_{230} ratios than those from the RNeasy Kit and Reilly et al. (2001) methods. No significant differences (P>0.05) were observed between the A_{260}:A_{230} ratios of RNeasy Kit and Reilly et al. (2001) procedures (Table 2).

RNA Yield (ng/µ)

RNA yield varied significantly between and within leaf tissues. For example, when RNA from specifically younger leaves was observed, the modified CTAB protocol generated significantly higher (P≤0.05) amounts of RNA compared to the other four protocols, while the RNeasy Kit produced the least concentrated RNA. Total Nucleic Acid + DNase method produced significantly (P≤0.05) higher concentrated RNA from young leaves compared to the TRI®$_{ZOL}$ Reagent and Reilly et al. (2001) methods (Table 2). Similarly, the modified CTAB method produced significantly (P≤0.05) more RNA from old leaves than the other four protocols. There was no significant (P>0.05) variation in the amount of RNA yielded from old leaves by TRI®$_{ZOL}$ Reagent, Total Nucleic Acid + DNase and Reilly et al., (2001) procedures. However, the RNA concentration from the three methods was significantly higher (P≤0.05) when compared to RNA from RNeasy Kit method (Table 2).

RNA purity and yield variation between protocols

The total RNA concentration between the five tested protocols varied significantly. The modified CTAB based method produced RNA of a significantly (P≤0.05) higher concentration compared to the RNA from the other four methods. RNeasy Kit method produced the least amount of total RNA. The amount of RNA from Total Nucleic Acid + DNase, TRI®$_{ZOL}$ Reagent, and Reilly et al. (2001) methods did not significantly (P>0.05) differ. However, observed trends indicated relatively high concentration of RNA from Total Nucleic Acid + DNase method, followed by TRI®$_{ZOL}$ Reagent and Reilly et al. (2001) respectively (Table 3).

The RNA A_{260}:A_{280} ratios also significantly varied between protocols. For example, the A_{260}:A_{280} ratio obtained from the modified CTAB method was significantly higher (P≤0.05) than similar A_{260}:A_{280} ratios from the other four protocols. The Reilly et al. (2001) method generated the least significant A_{260}:A_{280} ratio. The A_{260}:A_{280} ratio from the Total Nucleic Acid + DNase method was significantly (P≤0.05) higher than the

A_{260}:A_{280} ratio means of Reilly et al. (2001), RNeasy Kit and TRI®$_{ZOL}$ Reagent methods. The A_{260}:A_{280} ratio of TRI®$_{ZOL}$ Reagent and RNeasy Kit protocols did not significantly (P>0.05) differ from each other (Table 3).
Analysis of the total RNA A_{260}:A_{230} ratios showed significant variations between the studied protocols. The modified CTAB method produced significantly (P≤0.05) higher A_{260}:A_{230} ratio than similar ratios obtained from the other four protocols. The least A_{260}:A_{230} value was observed in Reilly et al. (2001) method. No significant (P>0.05) variation in the A_{260}:A_{230} ratios were observed between Total Nucleic Acid + DNase and TRI®$_{ZOL}$ Reagent methods. Similar observations were made between Reilly et al. (2001) and RNeasy kit methods (Table 3).

Table 2: The Purity and Yield (Mean ± P≤0.05) of Total RNA from Young and Old Cassava Leaf Tissues Isolated Using the Modified CTAB Method and Four other Protocols (obtained from literature). For each tissue, five samples were extracted and measured during three independent experiments.

Leaf Tissue	Protocols	A_{260}:A_{280}	A_{260}:A_{230}	RNA Yield (ng/µl)
Young leaves	TRI®$_{ZOL}$ Reagent	1.52 *s*	1.23 *k*	420 *c*
	Total Nucleic Acid + DNase	1.94 *r*	1.38 *j*	617 *b*
	Reilly et al., (2001)	1.18 *t*	0.68 *m*	284 *ce*
	RNeasy Kit	1.56 *s*	0.93 *l*	161 *de*
	Modified CTAB	2.19 *q*	2.21 *i*	1793 *a*
Old leaves	TRI®$_{ZOL}$ Reagent	1.42 *w*	1.24 *o*	248 *g*
	Total Nucleic Acid + DNase	1.83 *v*	1.26 *o*	212 *gh*
	Reilly et al., (2001)	1.18 *x*	0.40 *p*	100 *gh*
	RNeasy Kit	1.41 *w*	0.44 *p*	66 *hh*
	Modified CTAB	2.16 *u*	2.20 *n*	1078 *f*
	Lsd at P≤0.05 for leaf tissues	*0.11*	*0.14*	*160.2*

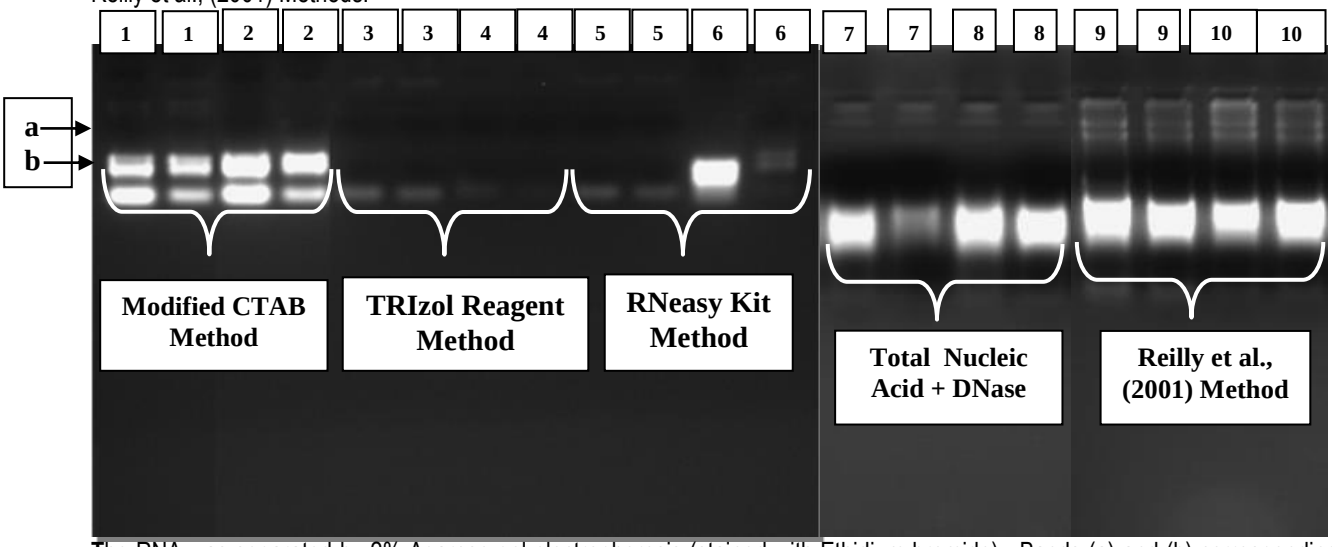
Means followed by the same letter in the A260:A280; A260:A230 and yield columns are not significantly varied (P>0.05)

Table 3: Comparison of the Total RNA Purity and Yield (Mean ± P≤0.05) between the Modified CTAB Method and Four other Protocols (obtained from literature).

Protocol	A_{260}:A_{280}	A_{260}:A_{230}	RNA Yield (ng/µl)
TRI®$_{ZOL}$ Reagent	1.47 *i*	1.24 *e*	334 *bc*
Total Nucleic Acid + DNase	1.88 *h*	1.32 *e*	414 *b*
Reilly et al., (2001)	1.18 *j*	0.54 *f*	192 *bc*
RNeasy Kit	1.49 *i*	0.69 *f*	114 *c*
Modified CTAB	2.18 *g*	2.21 *d*	1436 *a*
Lsd at P≤0.05 for Protocols	*0.17*	*0.23*	*253.3*

Means followed by the same letter in the A260:A280; A260:A230 and yield columns are not significantly varied (P>0.05)

Figure 1: Total RNA Extracted from Young Cassava Leaf Tissues (samples 2, 4, 6, 8 & 10) and Old Cassava Leaf Tissues (samples 1, 3, 5, 7, & 9) using Modified-CTAB, TRIzol Reagent, RNeasy Plant Kit, Total Nucleic Acid + DNase Treatment and Reilly et al., (2001) Methods.



The RNA was separated by 2% Agarose gel electrophoresis (stained with Ethidium bromide). Bands (a) and (b) corresponding to 28S rRNA and 18S rRNA respectively were more distinctly visible in lanes 1 and 2 of the modified CTAB method. This indicated that the modified CTAB method was effective in extracting high quality non-degraded RNA from the polysaccharide and polyphenol rich cassava leaves.

Discussion

The effectiveness of the modified CTAB-based method and four other protocols (from literature) in purifying RNA of high quality, quantity and integrity from polysaccharide rich cassava leaves was tested. The success of an RNA isolation procedure is judged by the quantity, quality and integrity of the isolated RNA (Suzuki et al., 2003). In this experiment, all the tested protocols permitted the extraction of RNA from both young and old leaf tissues. The RNA quality was measured by means of spectrophotometric ratios that relate differences in absorption spectra maxima of pure RNA, A_{max} = 260 nm, proteins, A_{max} = 280 nm, and polysaccharides, A_{max} = 230 nm (Iandolino et al., 2004). Pure RNA should have an A_{260}:A_{280} ratio between 1.9–2.1 and an A_{260}:A_{230} ratio of 1.8–2.3. These ratios varied in the five protocols that were tested therefore indicating differences in RNA purity levels from both young and old cassava leaves.

With the exception of the modified CTAB method, RNA samples from the other four protocols were significantly contaminated with polysaccharides, phenolic compounds and proteins as shown by the low A_{260}:A_{280} and A_{260}:A_{230} ratios (all < 1.8; Table 2 and Table 3). The A_{260}:A_{280} and A_{260}:A_{230} ratios from modified CTAB method were more than 2.0 (Table 2 and Table 3). Although RNA produced by the Total nucleic acid + DNase method from young leaf tissues was protein-free (A_{260}:A_{280} > 1.9, Table 2), the sample was still significantly contaminated with phenolic compounds (A_{260}:A_{230} < 1.8; Table 2).

The modified CTAB method produced RNA of high concentration from both young and old cassava leaf tissues (more than 1000 ng/µl; Table 2 and Table 3). All the other four protocols generated RNA of low concentration (less than 500 ng/µl) in both young and old leaf tissues, except Total Nucleic Acid + DNase method that yielded more than 500 ng/µl of RNA in young leaves (Table 2 and Table 3). These results showed that the RNA from the modified CTAB method was not only sufficient in concentration, but was also free from contamination by polysaccharides, phenolic compounds or proteins. Using a closely related (but not a replica) CTAB based method, Gasic et al., (2004) obtained similarly high quantity and quality RNA

from various apple tissues rich in polyphenols and polysaccharides. The successful extraction of RNA from cassava leaves using the modified CTAB-based method can be attributed to modifications introduced on the original procedure.

The changes included using 20 ml EB (instead of 10 ml) for every 1 gram ground tissue. This not only improved the 'capture' of the RNA from the starting material, but also the efficiency of separating organic and aqueous phase after centrifugation process. The Tissue-EB and chlorophyll: Isoamyl alcohol mixture was incubated in a water-bath pre-warmed at 50°C instead of 60°C. The lowered incubation temperature reduced the chances of RNA degradation, as shown by the distinctly visible 28S and 18S rRNA bands (Figure 1). Similar findings have been reported by Alemzadeh et al. (2005) who observed that a lower temperature during RNA extraction was effective in isolating high-quality non-degraded RNA from phenolic-rich tissues of eelgrass.

Finally a two-step (overnight and $2^1/_2$ hrs) precipitation (instead of single) with LiCl (2.5 M) was introduced into the protocol. This further eliminated polysaccharides and thus improved and increased the purity and yield of final RNA (Carra et al., 2007; Iandolino et al., 2004). In addition the reagents used to constitute the EB of the modified CTAB method also contributed to the extraction of a high quantity and quality of RNA from both cassava leaf tissues. Iandolino et al. (2004) also reported that an improved EB, precipitation procedure and a final clean-up step differentially remove contaminating metabolites.

CTAB is a detergent that preserves the integrity of nuclear and organelle membranes yielding total RNA with lower concentrations of un-spliced heteronuclear transcripts, as well as an increased RNA-to-DNA ratio (Mejjad et al., 1994; Dellaporta et al., 1984). The (PVP) K-30 (soluble) in the EB improved sequestration and elimination of phenolic compounds (Gambino et al., 2008; Salzman et al., 1999) and inclusion of low spermidine concentration deterred co-isolation of the RNA with polysaccharides and phenolics (Chang et al., 1993).

The high molarity of NaCl (5 M) and the strong reductant βME in the EB increased the solubility of polysaccharides, reducing their co-precipitation with RNA in later steps of the protocol and denatured ribonucleases and other contaminating proteins that are released during tissue disruption and homogenization (Iandolino et al., 2004; Fang et al., 1992; Lodhi et al., 1994). The high molarity LiCl not only differentially precipitated RNA from admixture with DNA, but it also increased RNA yield and favored precipitation of larger transcripts over smaller ones (Carra et al., 2007).

The centrifugation steps were carried out at 4°C because the low temperature reduced RNA degradation and decreased the rate of chemical reactions between nucleic acid and phenolic compounds hence improving elimination of phenolic compounds (Gambino et al., 2008). RNA purification protocols with CTAB in the EB have been adapted to extract RNA from other polysaccharides and polyphenol-rich plant tissues such as *Arabidopsis siliques*, sweet potatoes, grape berries and other woody plants (Gambino et al., 2008; Carra et al., 2006; Jaakola et al., 2001).

In two studies; isolation and characterization of cassava catalase expressed during post-harvest physiological deterioration and towards identifying the full set of genes expressed during cassava post-harvest physiological deterioration (Reilly et al., 2001; Reilly et al., 2007), Sodium Lauryl-sarcosine in the EB was used to extract total RNA from storage roots of cassava cultivar CM2177-2 or leaves for northern hybridizations. However, when the EB containing Lauryl-sarcosine was tested and used to extract RNA from young and old leaf tissues of cassava genotype TMS 60444, the final RNA extract was of low quantity

and purity (Tables 2 and 3). This suggests a possible cassava genotypic (or leaf-tissue-age) difference in RNA extraction. Here, the RNeasy Plant Kit EB containing guanidinium isothiocyanate, guanidinium hydrochloride and Tris-Hcl NaCl, was used (following the manufacturer's instructions (Qiagen, Cat. #. 74903) to extract RNA, but resulted in RNA of low yield and of poor-quality from both young and old cassava leaf tissue. Although Mackenzie et al. (1997) successfully extracted RNA from grapevines with the RNeasy kit, they used a different extraction buffer (Gambino et al., 2008). Nassuth et al. (2000) used the same protocol and obtained similar results but reported problems in extraction from old grapevine tissues. RNeasy Kit protocol has also been used to extract RNA from apple, citrus, olive, pear, and plum (Bertolini et al., 2001; Ragozzino et al., 2004) for virus detection by RT-PCR, but little information was provided on the quality and quantity of the isolated RNA (Gambino et al., 2008).

Purification of RNA using TRIzol Reagent according to manufacturer's (Invitrogen, Cat #. 15596-018) instructions is based upon a guanidinium thiocyanate-phenol-chloroform extraction method originally described by Chomczynski and Sacchi (1987). The addition of acidic guanidinium thiocyanate is widely employed to inhibit RNase activity (Chomczynski and Sacchi, 1987; Valenzuela-Avendaño et al. 2005). Total nucleic acid + DNase method constituted SDS, Proteinase K, phenol: chloroform: isoamyl alcohol extraction and isopropanol precipitation procedure as describe by Soni and Murray (1994). The addition and incubation with Proteinase K decreased RNase activity during the extraction process (Azevedo et al., 2003).

Here, the main modification to the protocol was the treatment of re-dissolved total nucleic acid with DNase buffer (Promega, Cat. #. M198A), RNase inhibitor (Invitrogen, Cat. #. 10777-019) and RQ1 RNase-Free DNase 1 (Promega, Cat. # M6101), after the first phenol: chloroform: isoamyl alcohol (25:24:1) centrifugation and precipitation with Ammonium acetate (10 M; + 99% Ethyl alcohol) step. The purpose of treatment with RQ1 RNase-Free DNase was to degrade the contaminating genomic DNA.

The RNA samples (young and old cassava leaf tissues) from the two protocols (TRIzol Reagent method and the Total nucleic acid + DNase method) were of low quantity and poor quality i.e. the samples contained significantly high levels of polysaccharides, polyphenolic compounds, and protein contaminants as determined by $A_{260:230}$ and $A_{260:280}$, ratios (Logemann et al., 1987; Manning 1990).

Conclusion

The success of most molecular techniques depends on RNA of high quality, quantity, and integrity. RNA of high A_{260}:A_{280} and A_{260}:A_{230} ratios (more than 1.8), high concentration (more than 1 µg) and integrity (distinct and visible 28S and 18S rRNA bands) is preferred. Based on these requirements, we recommend the adoption and employment of the optimized CTAB-based method to extract RNA of sufficient quantity and quality from metabolite-rich cassava leaves. The protocol is efficient, simple, and reproducible.

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