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SINGLE STEP ISOLATION OF PLANT RNA

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Key Word Index—*Cicer arietinum*; Fabaceae; chickpea; RNA; sec-butanol.

Abstract—A rapid method for preparation of plant RNA by single step extraction and instantaneous precipitation using *sec*-butanol is standardised. © 1997 Elsevier Science Ltd

INTRODUCTION

A rapid method for the preparation of pure samples of RNA is a critical step in RNA stability studies. Because of the wide distribution of nucleases and the potential damage that they can inflict on RNA during isolation, it is important to ensure that nuclease activity is fully inhibited. The most commonly used procedure for this purpose is the single step acid guanidinium thiocyanate method developed by Chomczynski and Sacchi [1] for animal tissues, which allows the preparation of RNA within 4 hr. Due to the presence of high level of ribonucleases and cell wall components, isolation of pure RNA from plant tissues is complicated. Wadsworth *et al.*, [2] modified the single step method [1], using LiCl instead of *iso*-propanol for precipitation of RNA from plant cell extract and the complete procedure takes about 5 hr. However, they have reported that small M_r RNAs may not be precipitated with LiCl.

Here we report an improvement of the original Chomczynski method for the rapid small scale isolation of total RNA from plants. This method involves a single step extraction and instantaneous precipitation of RNA with *sec*-butanol. The whole process can be completed in less than 2 hr. Not only is a high yield of purified RNA obtained, but simultaneous processing of a limited quantity of a large number of samples can also be undertaken.

RESULTS AND DISCUSSION

The basic protocol in our method is adapted from [1]. Two 1 hr incubation steps with *iso*-propanol in the original method have been eliminated by using *sec*-butanol for RNA precipitation. *sec*-Butanol is sparingly soluble in water–buffer, but the presence of guanidinium thiocyanate and *N*-lauroylsarcosine as chaotrophs increased the miscibility of *sec*-butanol and simultaneously rendered the RNA insoluble.

The ratio of absorbance at 260 nm and 280 nm ($A260/A280$) was taken as a measure of purity, with a value of 2.0 for pure RNA [3]. In various plant tissues tested in the present study, the *sec*-butanol precipitation method resulted in purer RNA (A_{260}/A_{280}—1.75 to 1.93) than *iso*-propanol precipitation (A_{260}/A_{280}—1.66 to 1.80) (Table 1). The purity of RNA was further confirmed by studying the UV absorbance spectrum of the hypocotyl RNA isolated by using the two methods. Absorption spectra for the two samples, from 200–350 nm, were virtually identical at pH 7.0. Wadsworth *et al.* [2] observed an additional major peak of A at 270 nm with their *iso*-propanol precipitated total RNA preparation. However, they did not give any specific reason for this. In the present study we did not observe any such A peak at 270 nm in both *iso*-propanol as well as *sec*-butanol precipitated RNA preparations. This A peak observed by Wadsworth *et al.* [2] might be due to the contamination by guanidinium thiocyanate in their preparations. The two 75% ethanol washings followed in the present method might be of help to remove the contaminants.

The efficacy of *sec*-butanol to precipitate the total RNA was compared with that of *iso*-propanol (Table 1). The data show that the yield of total RNA obtained by *sec*-butanol precipitation is 7–21% more, depending on the tissue used.

To check the integrity of isolated RNA, 6 μg of RNA from two methods was analysed on denaturing HCHO/glyoxal agarose electrophoresis (Fig. 1). The patterns and levels of ethidium bromide fluorescence in both the preparations were similar. No detectable degradation of RNA was observed in both samples, as shown by intact ribosomal RNA bands. The fluorescence in the 4S region was observed in both *iso*-propanol and *sec*-butanol precipitations, indicating that they were equally good in precipitating low M_r

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Table 1. A comparison of the purity and yield of RNA isolated by the *iso*-PrOH precipitation and *sec*-BuOH precipitation method*

		sec-BuOH Method†		*iso*-PrOH Method†	
Tissue		*A*260/*A*280‡	Yield§ (μg mg^{-1})	*A*260/*A*280‡	Yield§ (μg mg^{-1})
Hypocotyl	Expt. 1	1.84 ± 0.03	0.34 ± 0.04	1.66 ± 0.04	0.30 ± 0.06
	Expt. 2	1.93 ± 0.05	0.40 ± 0.04	1.71 ± 0.03	0.33 ± 0.04
Epicotyl	Expt. 1	1.86 ± 0.05	0.21 ± 0.06	1.70 ± 0.04	0.21 ± 0.03
	Expt. 2	1.80 ± 0.04	0.28 ± 0.05	1.75 ± 0.05	0.26 ± 0.04
Leaf	Expt. 1	1.88 ± 0.03	0.33 ± 0.04	1.80 ± 0.02	0.30 ± 0.06
	Expt. 2	1.75 ± 0.02	0.40 ± 0.06	1.70 ± 0.04	0.37 ± 0.07

* Results represents the mean ± standard deviation of 4 separate RNA preparations in each experiment.

† RNA was isolated by Chomczynski method [1] by using *iso*-PrOH precipitation of RNA and by the described rapid method using *sec*-BuOH as precipitating agent.

‡ The ratio of *A* at 260 and 280 nm.

§ Yield was determined spectrophotometrically and is presented as μg RNA mg^{-1} fresh weight of tissues.

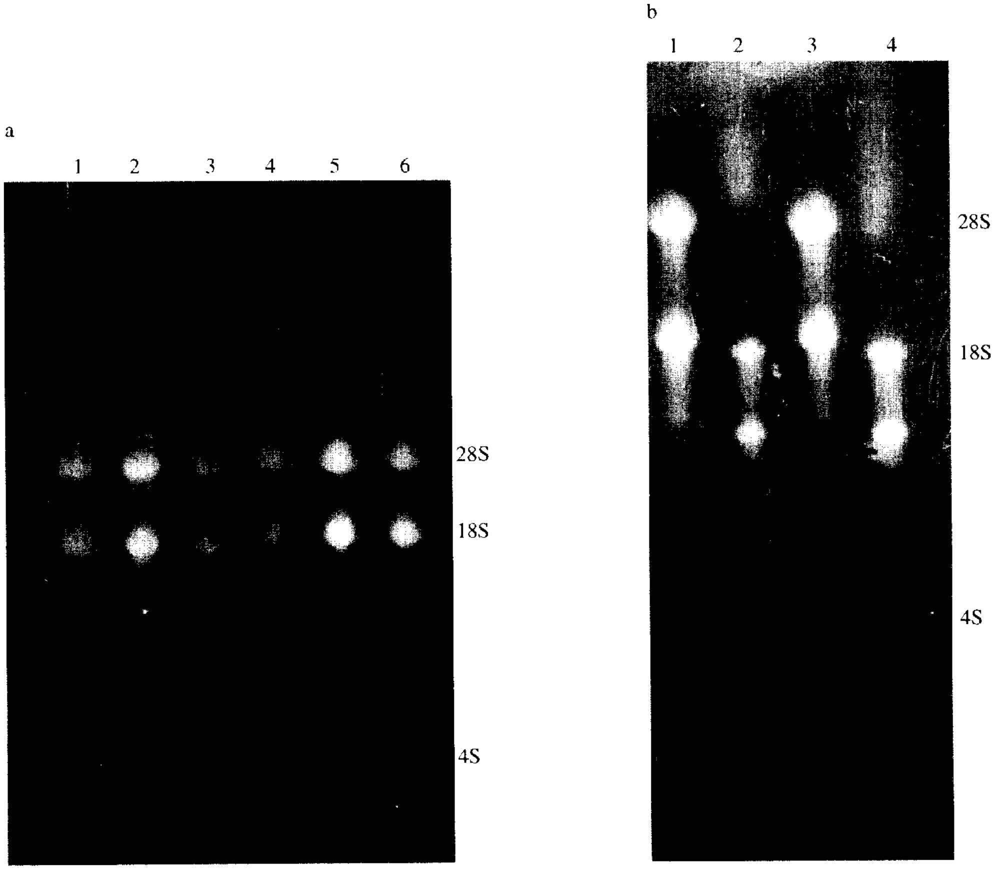

Fig. 1. (a) HCHO agarose gel electrophoresis of RNA isolated by *iso*-PrOH precipitation (lanes 1, 3, 5) and by *sec*-BuOH precipitation (lanes 2, 4, 6) from chick pea hypocotyl (lanes 1, 2), epicotyl (lanes 3, 4) and leaves (lanes 5, 6). (b) Glyoxal agarose gel electrophoresis of hypocotyl RNA isolated by *iso*-PrOH precipitation (lanes 1, 2) and *sec*-BuOH precipitation (lanes 3, 4). Lanes 1 and 3 were with glyoxal while lanes 2 and 4 were without glyoxal denaturation. The RNA samples (6 μg) were electrophoresed on 1.2% formaldehyde–glyoxal agarose gel. The RNA was stained with ethidium bromide (50 μg ml^{-1}) and photographed under UV light.

RNA, which is not possible with LiCl precipitation [4]. The *sec*-butanol as a precipitating agent along with the denaturing buffer will not only save time by instantaneous precipitation of RNA but is suitable for precipitating low M_r RNAs too.

EXPERIMENTAL

Ultrapure guanidinium thiocyanate and *N*-lauroylsarcosine were purchased from Sigma, PhOH (analytical grade from BDH, redistilled and satd with sterile H_2O and kept at 4°); *sec*-BuOH from Koch-Light, England: $CHCl_3$ and *iso*-PrOH from Glaxo. Seedings of chick pea (*Cicer arietinum*) var. C-235 were grown at 23 ± 0.5°, in Petri plates containing sterilized acid washed quartz sand. Hypocytyls and epicotyls were harvested from 4- and 6-day-old dark grown seedlings, respectively. Green leaves were harvested from 14-day-old light grown plants.

Tissues (0.2 g) were ground to a fine powder after freezing in liquid N_2 with a small mortar and pestle. The samples were transferred to 1.5 ml microfuge tubes, vortexed for 15 sec in 0.5 ml of denaturing soln (4 M guanidinium thiocyanate, 25 mM Na citrate pH 7, 0.5% sarkosyl, 0.1 M $HS(CH_2)_2OH$. A stock soln of denaturing soln minus $HS(CH_2)_2OH$ was prepd according to [1]. $HS(CH_2)_2OH$ was added just prior to extraction. Then 50 μl of 2 M NaOAc, 0.5 ml of H_2O satd PhOH, 0.1 ml of $CHCl_3$–*iso*-AmylOH (49:1) were added sequentially to the homogenate, mixing thoroughly after each addition. The final suspension was shaken vigorously for 30 sec and incubated on ice for 15 min. Samples were centrifuged at 9000 *g* for 10 min at 4° in Sorvall RC 5C with SS-34 rotor. The aq. phase was re-extracted with 0.1 ml of $CHCl_3$–*iso*-AmylOH. After final extraction, aq. phase was carefully transferred to a fresh tube, mixed with 0.6 vol of *sec*-BuOH and centrifuged at 4° for 10 min at 9000 *g*. The supernatant was discarded, the RNA pellet was dissolved in 0.3 ml of denaturing soln and reprecipitated with 0.6 vol. of *sec*-BuOH. After centrifugation at 9000 *g* for 10 min at 4°, the RNA pellet was washed twice with 75% EtOH, sedimented and dried for 15 min under vacuum. The dried pellet was dissolved in 50 μl of 0.5% SDS in DEPC treated water at 65°. Samples were stored at −70° until use.

RNA concn was determined spectrophotometrically [3]. Denaturing HCHO and glyoxal agarose electrophoresis of RNA was done according to [5] and [4], respectively.

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