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A Simple Procedure for RNA Isolation from Plants and Preservation of Plant Material for RNA Analysis

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簡便한 高等植物 RNA 分離 方法

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ABSTRACT

Total RNA was isolated from two months old wheat, rice, tobacco and sweet potato. The procedure used was simple and provided pure RNA preparation. Lysis of plant tissue in a buffer with guanidine thiocyanate and CsCl density gradient centrifugation separated RNA from the rest of the cellular components. Subsequent cholroform/1-butanol extraction and ethanol precipitation were necessary to ensure contaminant-free RNA preparation. Storage of the lysed plant tissue in the buffer with guanidine thiocyanate preserved the sample for two months without noticeable RNA degradation.

INTRODUCTION

Common methods for total RNA preparation in plants required freezing plant tissues in liquid nitrogen followed by fine powdering of the tissues and RNA extraction. Freezing plant tissues in liquid nitrogen served as a mean to prevent RNase activity and to store plant material for a latter RNA assay. Although RNA extraction procedures have been differ among investigators, usual steps are; 1)suspending powdered plant tissues in extraction buffer with detergents and/or RNase inhibitors, 2)fractionation by centrifugation, 3)extraction by solvents such as phenol, chloroform and isoamyl alcohol, 4)ethanol precipitation and 5)CsCl density gradient centrifugation. These steps have been modified in many ways, and some are simple but have contaminants such as DNA and proteins and many are rather lengthy to obtain contamination free RNA preparation(Lizzardi, 1983; Broglie *et al.*, 1984).

We though that enough RNase inhibitor in the RNA extraction buffer during RNA extraction and storage of plant material for latter RNA extractions will be good enough to prevent degradation of RNAs. We have designed a simple protocol for RNA isolation from well-

*Abbreviations: CsCl, cesium chloride. DNA, deoxyribonucleic acid. EDTA, ethylenediamine tetraacetate. kb, kilobase. RNA, ribonucleic acid. RNase, ribonuclease. SDS, sodium dodecyl sulfate.

developed plants and preservation of plant materials based on the procedure used for the materials without established cell wall(Lizzardi, 1983).

MATERIALS AND METHODS

Plant Materials. Field grown wheat(*Triticum aestivum* L.), rice(*Oryza sativa* L.), tobacco(*Nicotiana tabacum* L.)and sweet potato(*Ipomoea batatas*)were used. They were all about two months old.

Lysis of Plant Material. Each plant material, about 2g in fresh weight, was washed with tap water, cut into pieces and placed in a mortar chilled to 4℃. About five volumes of lysis buffer(50% guanidine thiocyanate, 0.5% N-lauroyl sarcosine, 0.1 % β-mercaptoethanol and 25mM EDTA, pH 7.5) was added, and the tissue was ground with a pestle at room temperature for five minutes. After filtering through one layer of mira cloth, the lysate was kept at 4℃ until further purification of RNA.

RNA Extraction. The lysed plant tissue was centrifuged at 6,000 x g for 10 min at 4℃. The supernatant was layered on the top of 5.7 M CsCl in 0.1 M EDTA(pH 7.5) cushion(2.3 ml of the lysate was layered on 2.5 ml cushion) and spinned for 22 hrs at 22,000 r.p.m. in SW 50.1 rotor at 15℃. The pellet was dissolved in 10 mM Tris · Cl(pH 7.5)and 0.1 % SDS at room temperature by sucking it up and down for several times. It was extracted with 4:1 mixture of chloroform and 1-butanol. The aqueous phase was collected and ethanol precipitated by adding 0.1 volume of 3 M sodium acetate(pH 5.2) and 2.2 volumes of absolute ethanol.

The amount of RNA was measured spectrophotometrically by checking absorbancies at 260 nm and 280nm. RNA was separated on formaldehyde gels and stained with methylene blue as Maniatis *et al.*(1982).

Chemicals and Glasswares. All chemicals were from Sigma Chemical Co., U.S.A. and chemicals and glasswares were treated to be RNase free as Maniatis *et al.*(1982).

RESULTS AND DISCUSSION

Methylene blue staining of the gel showed large and small ribosomal RNA bands. Tightness of the bands indicates that there has been no noticeable degradation of RNAs during the RNA extraction. Storage of the lysate at 4℃ for two months before RNA extraction did not cause any noticeable change in ribosomal RNA bands(Fig. 1).

Recovery of RNA in the range of 100 to 200 μg per gram of fresh weight is comparable to other reports(Delaigue *et al.*, 1984;Smith and Elllis, 1981). After CsCl density gradient centrifugation, separation of RNA from DNA, protein and others was welll establishedd. Staining of the gel with ethidium bromide also did not show any high molecular weight compounds which indicates the preparation being free from contaminants(data not shown).

We believe the procedure reported here can provide RNA good enough for Northern analysis and probably for *in vitro* translation assay also. Good recovery of RNA from all parts

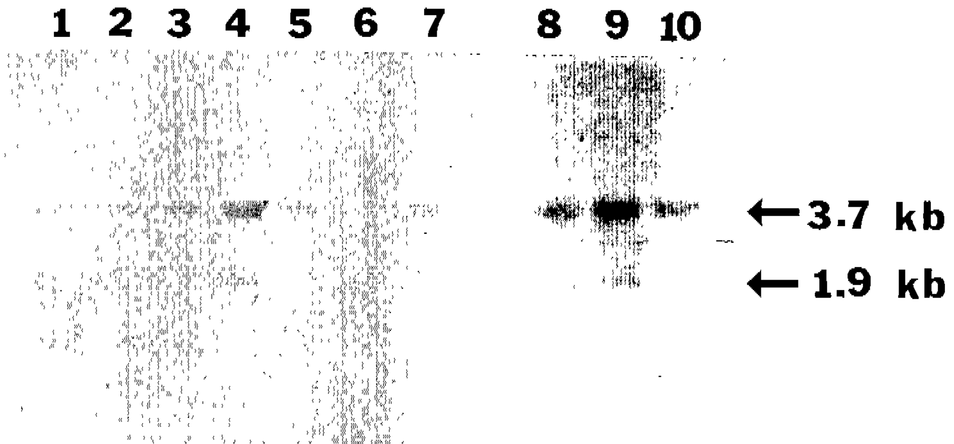

Fig 1. Total RNA was extracted from several plant species separated on a formaldehyde-agarose gel and stained with methylene blue. 1) RNA from wheat flag-leaf. 2) RNA from wheat leaves (not including flag-leaf). 3) RNA from wheat stem. 4) RNA from wheat root. 5) RNA from rice leaves. 6) RNA from tobacco leaves. 7) RNA from sweet potato leaves. 8) RNA from wheat leaves preserved in the lysis buffer for two months. 9) RNA from wheat stem preserved in the lysis buffer for two months. 10) RNA form wheat root preserved in the lysis buffer for two months. 3.7 kb represents the position of large ribosomal RNA. 1.9 kb represents the posit ion of small ribosomal RNA.

of wheat and from several species of plants indicate that this procedure should be usuable for all plant materials in RNA isolation.

摘　　要

두 달된 밀・벼・담배・고구마에서 總 RNA를 分離하였다. 植物體를 guanidine thiocyanate가 들어 있는 용해용 완충액에 녹이고 CsCl밀도 차 원심분리를 거친 후 1-butanol/chloroform 추출과 에틸알콜을 이용한 침전을 通해 純度높은 RNA를 얻을 수 있었다. 용해된 植物體를 guanidine thiocyanate가 들어 있는 용해용 완충액에 두 달 간 저장 후 RNA를 분리・분석해 본 결과 RNA가 원래 상태로 잘 보존되어 있음을 볼 수 있었다.

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