

Confirmation of soybean plastid rRNAs by formaldehyde denaturing agarose gel electrophoresis

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ABSTRACT. Owing to their prokaryotic origin, plastid rRNAs are mainly 23s/16s/5s rRNAs. We present a novel plant RNA isolation method in this paper. Also, not only the eukaryotic 28s (26s, 25s)/18s rRNAs but the prokaryotic 26s/23s rRNAs as well were demonstrated in a single sample for the first time by formaldehyde denaturing agarose gel electrophoresis.

Key words: Plant; Soybean; Plastid rRNAs; Gel electrophoresis

INTRODUCTION

According to the endosymbiosis theory, plastids originated from eubacteria-like cells that had invaded and then lived within primitive eukaryotic hosts (Bonen and Doolittle, 1975; Schwarz and Kössel, 1980). Gradually, they became an important group of plant cellular organelles with essential functions (McFadden, 2001). All plastids such as the chloroplast, amyloplast, chromoplast, and leucoplast were initially originated from proplastids during cell differentiation (Pyke, 1999).

Different methods, including denaturing salts such as guanidinium (guanidine salts) (Chomczynski and Sacchi, 1987)/urea (Almarza et al., 2006), acid phenol (Geuna and Hartings, 1998), detergents such as cetyltrimethylammonium bromide (CTAB) (Chang et al., 1993; Békésiova et al., 1999; Kiefer et al., 2000)/sodium dodecyl sulfate (SDS) (Newbury and Possingham, 1977; Wan and Wilkins, 1994) and hot borate (Manning, 1991), have been developed to purify plant RNA. Formaldehyde denaturing agarose gel electrophoresis (Sambrook et al., 1989) is usually utilized to check RNA integrity. A high-quality plant RNA electrophoresis pattern always comprises two main 26s (25s) and 18s rRNA bands, plus a 5s rRNA band depending on the RNA isolation procedure. Here, we report a plant RNA isolation method, where not only the 26s/18s rRNAs but the 23s/16s rRNAs of a single sample were demonstrated by formaldehyde denaturing agarose gel electrophoresis. We also showed some degraded RNA electrophoresis patterns, which are usually considered as patterns of intact RNA samples.

MATERIAL AND METHODS

Approximately 500 mg soybean seeds (at mature green stage) was thoroughly ground into fine powder in the presence of liquid nitrogen with a mortar and pestle. The frozen powder was quickly transferred to a 10-mL tube containing 2.5 mL preheated (at 65°C) homogenization solution (2% CTAB, 1.5 M NaCl, 100 mmol Tris, 20 mmol EDTA, pH 8.0, 2% β -mercaptoethanol). The tube was vigorously shaken and then incubated at 65°C for 5 min. The tube was spun at 14,000 rpm for 2 min at room temperature. The supernatant was transferred to 2 new microfuge tubes. A 0.1 volume of 10 M LiCl was added to the supernatant. The tubes were inverted several times and then centrifuged at 14,000 rpm for 30 min at 4°C to pellet RNA. The supernatant was completely decanted and the pellet resuspended with 100-200 μ L denaturing solution (4.0 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7.0, 0.5% sodium N-lauroyl sarcosine and 2% β -mercaptoethanol). The supernatant was equally transferred to 2 fresh microfuge tubes labeled A and B. Two volumes of 100% ethanol were added to tube A followed by centrifugation at 14,000 rpm for 15 min at 4°C. A 0.2 volume of 10 M LiCl was added to tube B, which was then inverted several times and spun at 14,000 rpm for 30 min at 22°C. The supernatant of tube B was transferred to a new microfuge tube labeled C. Two volumes of 100% ethanol were added to tube C, followed by centrifugation at maximum speed for 15 min at 4°C. The supernatant was discarded and the RNA pellets in the 3 labeled tubes washed with 0.5 mL 75% ethanol twice. The supernatant was discarded, the tubes spun briefly, and the residual supernatant aspirated. The pellets were air-dried at room temperature and dissolved in 20 μ L DEPC-treated water. The total RNAs of cultivated *Escherichia coli* DH5 α and *Saccharomyces cerevisiae* Y190 were also extracted as previously reported (Zhu et al., 2013). RNA samples of 3-12 μ L were subjected to 1% (w/v) formaldehyde denaturing 1.2% agarose gel electrophoresis to determine RNA integrity.

RESULTS AND DISCUSSION

Because of excessive loading of samples, the rRNA bands of lanes 1 and 3 were ambiguous (Figure 1). When we reduced the RNA samples, the main rRNA bands turned out to be distinct (Figure 2A).

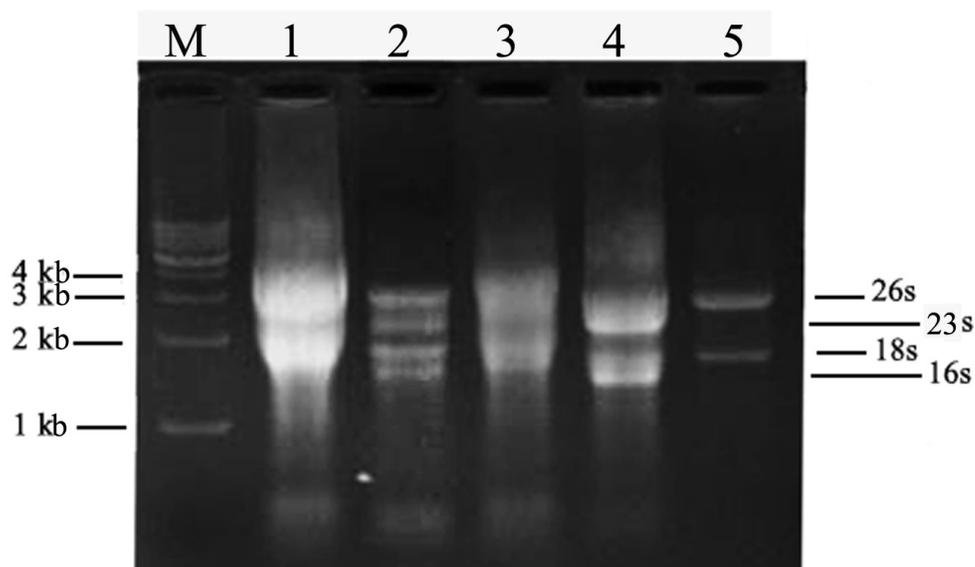


Figure 1. RNA integrity judged by formaldehyde denaturing agarose gel electrophoresis. Lane M = 1-kb DNA ladder marker (TaKaRa, China); lane 1 = RNA sample A; lane 2 = RNA sample B; lane 3 = RNA sample C; lane 4 = RNA extracts of *E. coli* DH5 α ; lane 5 = RNA extracts of *S. cerevisiae* Y190.

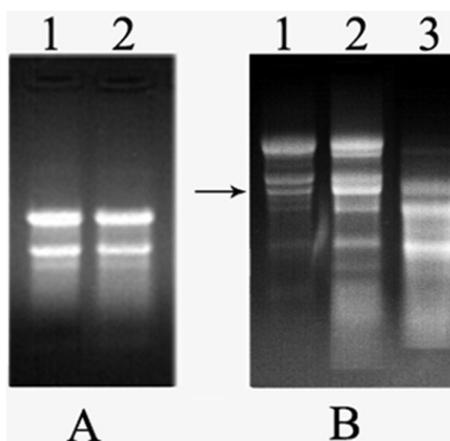


Figure 2. Intact and partially degraded RNA electrophoresis patterns. **A.** Lane 1 = RNA sample A; lane 2 = RNA sample C. **B.** Lane 1 = RNA extracts of young *Litchi chinensis* leaf purified by a modified hot borate method (Wu and Liu, 2004); lane 2 = RNA extracts of young cotton leaf purified by a modified hot borate method (Wu and Liu, 2004); lane 3 = extracts of young cotton leaf purified by a modified hot borate method (Wan and Wilkins, 1994).

The rRNA ratio (26s/18s, 23s/16s) is considered an essential measure of RNA intactness of the denaturing agarose gel electrophoresis test. Figure 1 (lane 4/lane 5) and Figure 2A showed that the respective 26s/23s ribosomal RNA band intensity was approximately 1.5-2 times that of the corresponding 18s or 16s rRNA band. It indicated high RNA integrity. RNA sample B displayed 4 main sharp bands (Figure 1, lane 2). The sizes of the 4 bands corresponded to 26s, 23s, 18s, and 16s rRNAs, and there were no other smaller bands appearing in the lane. Besides, RNA of sample C, which was obtained from the supernatant of the precipitated RNA of sample B, was highly intact. Thus, we believe that RNA of sample B was intact, rather than degraded RNA fragments. Why were there 4 distinct rRNA bands in lane 2 (Figure 1)? We consider that the 26s/18s rRNAs are much more abundant than the 23s/16s rRNAs in plant cells. When the total RNAs prepared by the commonly used methods (as mentioned above) are analyzed by denaturing agarose gel electrophoresis, the 23s/16s rRNAs are concealed by the 26s/18s rRNAs, or the quantity of the 23s/16s rRNAs is too little to be evident. Since we loaded 3 μ L RNA sample in lanes 1 and 3 and 12 μ L in lane 2 (Figure 1), the band intensity of lane 2 was a little weaker than that of lane 3. Accordingly, it can be calculated that approximately 20% of the total RNAs were precipitated. The total RNAs including 26s/23s/18s/16s rRNA may be selectively precipitated by LiCl under the strong denaturing condition, which may eventually result in the comparative enrichment of the 23s/16s rRNAs. This may be the possible reason that there were four distinct rRNA bands in lane 2 (Figure 1). According to their sizes, we believe that the four rRNA bands corresponded to 26s, 23s, 18s, and 16s rRNAs. This corroborates the presence of both eukaryotic and prokaryotic rRNAs in *Glycine max* L. soybean cells, which in turn may serve as evidence that plastids originated from prokaryotic cells.

In Figure 2B (lane 1), the band indicated by the arrow is usually considered 16s rRNA of an intact RNA sample. The bands were sharp and the 26s rRNA band intensity was twice that of the 18s rRNA band. But there were several weaker and smaller bands other than the band indicated by the arrow in the lane, and the 23s rRNA band was not detected. Therefore, the band indicated by the arrow as well as the smaller bands actually came from partially degraded 26s/18s rRNAs. This circumstance was more evident in lane 2. Lane 3 showed an electrophoresis pattern of RNAs that had undergone severe degradation (Figure 2B).

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