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Highly efficient protocol for isolation of mitochondrial DNA and RNA using small scale plant tissues

Kyung-Min Kim[1], Yong Suk Lim[2], Ill Whan Sul[2*]

[1]Department of Environmental Horticulture, College of Life Science and Resources, Sangju National University, Sangju, 741-711, Korea.[2]Department of Biotechnology, Daegu University of Foreign Studies, KyungSan, 712-881, Korea

Objectives

We have tried to develop highly efficient protocol for isolation of mitochondrial DNA and RNA from small volume plant tissues through high ionic strength solutions.

Materials and Methods

Plant materials Leaves of tomato and tomato were produced by tissue culture at 261?.Culture medium tomato: MS + 0.1 mg/L BA + 0.35 mg/L IAA + 30 g/L sucrose + 8 g/L agar, Tobacco: MS + 0.1 mg/L BA + 30 g/L sucrose + 8 g/L agar. Solutions Homogenization buffer A: 50 mM Tris-HCl (pH8.0), 1.3 M NaCl, 25 mM EDTA (pH8.0), 0.2 % BSA. Add 0.05 % cysteine and 56mM -mercaptoethanol immediately before use. Homogenization buffer B: 100 mM Tris-HCl (pH8.0), 2.6 M NaCl, 50 mM EDTA (pH8.0), 0.4 % BSA. Add 0.1 % cysteine and 56 mM -mercaptoethanol immediately before use. Lysis buffer: 25 mM Tris-HCl (pH8.0), 20 mM EDTA (pH8.0), 0.5 % SDS. Ammonium acetate: 2 M. TE-saturated phenol/chloroform (50:50). Water-saturated phenol/chloroform /isoamyl alcohol (50:49:1). RNA extraction buffer: 4 M Guanidine thiocyanate, 25 mM Sodium citrate (pH7.0), 0.5 % sarcosyl. Add 0.1% -mercaptoethanol immediately before use.

Results and Discussion

We present a fast and simple protocol for purification of mitochondrial DNA and RNA from small amounts of tobacco and tomato leaves. This method uses a high ionic strength medium to isolate mitochondria and extract mitochondrial DNA and RNA from a single preparation and is easily adaptable to other plant species. The mitochondrial DNA was not contaminated by plastid DNA, was successfully used for PCR. Similarly, the isolated mitochondrial RNA was not contaminated only slightly contaminated (leaves) by plastid RNA. RNA prepared according to our method was acceptable for RT-PCR analysis. There has been an explosive interest in the field of plant molecular biology. Recent techniques have facilitated and simplified the procedure for DNA isolation so that they are easy to use and require minimal amount of time and with high quality. Many procedures have been developed to isolated plant mtDNA (Douce et al. 1987; Kemble 1987; Pehu 1991; Prez et al. 1990; Triboush et al. 1998; Wilson and Chourey, 1984), however most of them are either time consuming or ineffective when applied to low amounts of material. We present here a rapid procedure that provides mtDNA and mtRNA of sufficient purity from ultra amounts (>1g) of *in vitro* cultured tobacco and tomato leaves.

* *Corresponding author* : Ill Whan Sul, TEL: 053-810-7029, E-mail: iwsul@dufs.ac.kr