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Development of a highly efficient isolation protocol for mitochondrial DNA and RNA using small scale plant tissues

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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 26±1℃.

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 β-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 β-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% β-mercaptoethanol immediately before use.

Results and Discussion

We established a fast and simple protocol for purification of mitochondrial DNA and RNA from small amounts of tobacco and tomato leaves. This protocol uses a high ionic strength solution to isolate intact mitochondria and extract mitochondrial DNA and RNA from one step preparation and is easily adaptable to other plant species.

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