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REGULAR ARTICLE

A SIMPLE AND RAPID METHOD FOR DNA EXTRACTION FROM LEAVES OF TOMATO, TOBACCO AND RAPE SEED

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SUMMARY

We have optimized a simple and rapid method for isolation of high quality genomic DNA from leaves of tomato, tobacco and rape seed. This protocol significantly minimizes time and the use of laboratory materials. The extracted DNA was suitable for PCR analysis. This method requires less than 1 mg of leaf tissue and is useful for transgene detection, genetic maps and other DNA based molecular analyses.

Keywords: Tomato, Tobacco, Rape Seed, DNA extraction, PCR analysis.

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1. Introduction

Extraction of DNA from plants is prerequisite when hundreds of samples need to be analyzed rapidly for PCR analysis, genome mapping and molecular markers development. However the extraction of high-quality DNA from plants is tedious, time consuming and expensive because of different manipulation steps (1-5). In order to overcome these problems, we have optimized a simple and rapid method for high-quality DNA isolation from leaf tissues. This protocol was used as part of molecular cloning of antiporter gene conferring resistance to salt stress to minimize the time and the use of laboratory materials.

2. Materials and Methods

Plant Material

Transgenic plants were developed through *A.tumefaciens* mediated genetic transformation harboring the vacuolar antiporter (AtNHX1) gene in tomato, tobacco and rape seed (6-8). The leaves from these plants growing in the green house were used for DNA extraction to assay transgene possession.

Reagents and Chemicals:

- DEB (DNA Extraction Buffer : 1.0M Tris HCl, pH 8.0 and 0.5 M EDTA, pH 8.0;10% (w/v) SDS; 5M NaCl and 0.2% Mercaptoethanol)

- 5M Potassium acetate

DNA Isolation Protocol

- A piece of fresh leaf (approx 3-5 mg) was placed in a centrifuge tube/eppendorf
- Add 400µL of DEB and leaf tissue was crushed with glass rod against the tube walls.
- To this add 50µL of 5M Potassium acetate
- Centrifuge at 16K rpm for 30 seconds
- Carefully transfer 10µL of supernatant to an eppendorf.
- To this add 990µL of sterile nanopure water

Directly this DNA was used as template for Polymerase Chain Reaction (PCR) amplification.

PCR amplification and DNA analysis

0.5µL of DNA extraction solution was added to 24.5µL of PCR mixture containing 1.5 µL 50mM $MgCl_2$, 2.5 µL of FP and RP (10 pmol/ µL), 2.5 µL PCR-buffer (Invitrogen), 0.5 µL 10mM dNTPs, 0.2 µL Taq Polymerase (Invitrogen), and 14.8 µL sterile double distilled water. PCR aliquot (25 µL) was centrifuged for a short spin. A thermocycler gradient (MJ Research Gradient Cycler) was used for thermal cycling as follows: 4 min at 94^0C, followed by 3 cycles (6 times) of 50 Seconds at 94^0C, 45 min at 50^0C, 1 min at 72^0C; Step II for 29 min; 10 min at 72^0C; 10 min at 4^0C. Primer sequences for AtNHX1 gene were as follows:

FP: 5′ TTGGGTGATTATCTTGCTATTGG 3′

RP: 5′ CGGCCCTTGTAAACTTGTTGTA 3′

PCR products (5-20 µL) were analysed with 1.7% agarose gel electrophoresis, stained with ethiddium bromide, and visualized with UV transilluminator.

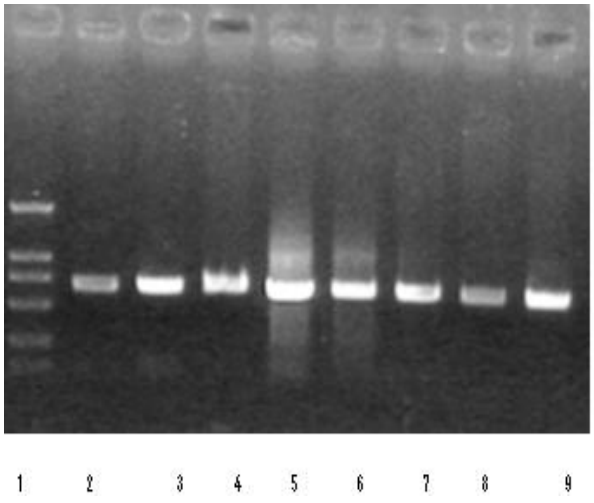
3. Results and Discussion

DNA isolated from fresh leaves was evaluated by NanoDrop ND-1000 UV-Vis Spectrophotometer. This procedure yielded 392ng/µl of high molecular weight DNA. A ratio of absorbance at 260nm to that of 280nm of 1.80-1.81 indicated the purity of DNA obtained (Table 1). PCR products were electrophoresed on agarose and amplified fragment of 800 bp was observed (Fig. 1). Unlike other high quality plant genomic DNA extraction protocols (1,3,4,5), this method does not include the use of liquid nitrogen or freeze drying for initial grinding of the leaf tissue. This protocol reduces the times of chloroform extraction by directly using Potassium acetate to remove contaminating proteins in the same step.

Table 1. Quantified DNA after isolation from leaves of transgenic plants using simple and rapid DNA extraction method

Transgenic Plant	DNA yield (ng/µlg of sample)	A260/A280
Tomato	354	1.81
Tobacco	392	1.82
Brassica napus	348	1.80

Fig. 1: A representative picture showing the amplified products of AtNHX1 (800bp) in DNA extracted samples of transgenic plants. Lane 1 (DNA Ladder); Lane 2&9 (positive control – plasmid carrying AtNHXI); Lane 3-4 (Tomato), Lane 5-6 (Brassica napus), Lane 7-8 (Tobacco)



These alterations significantly minimize the time and the use of laboratory materials. One person can process as many as hundred DNA samples within one hour. The extracted DNA could be amplified by means of PCR both before and after storage at 4^0C. The present protocol for DNA extraction is stable and suitable for PCR and other molecular techniques.

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