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Protocol

DNA Extraction from a Previously Recalcitrant Plant Genus

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Abstract: Numerous DNA extraction methods failed to remove contaminants that interfere with restriction digests of *Cuphea* DNA. The method described here removes those contaminants and maintains relatively high DNA yields. The primary purification process consists of washing the DNA with phenol while it is complexed with CTAB and dissolved in 1 M NaCl.

DNA extraction from tissue needs to be simple, rapid, inexpensive, and effective when many samples are used, such as in population studies. Numerous methods for extracting plant DNA (Dellaporta et al., 1983; Keim et al., 1988; Rogers and Bendich, 1985; Saghai-Maroof et al., 1984; and Wagner et al., 1987), including modifications, were tried with *Cuphea lanceolata* Ait. and *Cuphea viscosissima* Jacq., new seed oil crops high in capric acid (Graham, 1989). These methods failed because contaminants were not removed and the DNA was unusable. *Cuphea* tissue contains copious amounts of a sticky, resinous material (substance unknown), which was probably the primary contaminant of the DNA.

We modified a method by Liechtenstein and Draper (1985), which is based on that of Murray and Thompson (1980), and obtained DNA from greenhouse-grown *C. lanceolata* and *C. viscosissima* plants suitable for

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Abbreviations: CTAB, hexadecyltrimethylammonium bromide; RFLP, restriction fragment length polymorphism.

complete restriction digestion and RFLP analysis. The essential modifications are as follows: The concentration of CTAB in the extraction and precipitation buffers is reduced from 1% to 0.5%; tissue is mixed in the extraction buffer using a Polytron instead of a mortar and pestle; the second CTAB-chloroform wash is deleted; centrifugation speed to pellet the CTAB-DNA precipitant is increased from 1500 g to 4500 g; and a phenol-chloroform wash is incorporated before dissolving the DNA in TE buffer.

Procedure

Solutions

Extraction buffer: 0.5% (w/v) CTAB 50 mM Tris-HCl pH 8.0, 0.7 M NaCl, 10 mM sodium EDTA pH 8.0, 1% (v/v) 2-mercaptoethanol

Precipitation buffer: 0.5% (w/v) CTAB, 50 mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0

Chloroform: chloroform:octanol 24:1 (v/v)

Phenol: equilibrated to pH > 7.8 with equal volume 1 M Tris-HCl pH 8.0, then equal volume 0.1 M Tris-HCl pH 8.0

TE: 10 mM Tris-HCl pH 8.0, 0.1 mM EDTA pH 8.0

Tissue preparation

- Collect tissue on ice, lyophilize, and grind it to a fine powder using a coffee grinder. Store the tissue at -20°C.

Cell disruption

- Add 900 mg ground tissue and 15 mL extraction buffer to a 50-mL disposable centrifuge tube.
- Mix the tissue and buffer with a Polytron or other tissue homogenizer at a moderate speed for 45 to 60 seconds.
- Pour the mixture into a 50-mL teflon-coated Oak-Ridge tube. Rinse the first tube and Polytron head with 9 mL extraction buffer and add this to the first mixture.
- Incubate the mixture at 60°C for 60 minutes.
- Mix the sample occasionally during this incubation.

Chloroform wash

- Add 15 mL chloroform and mix by gently inverting the tube for 4 minutes or until a nearly complete emulsion forms.
- Spin at 14,500 g for 10 minutes to separate the phases.

CTAB-DNA precipitation

- Add 19 mL precipitation buffer to a 50-mL polysulfone Oak-Ridge tube. Transfer the supernatant to the same tube using a 25-mL pipette.
- Mix the sample by several gentle inversions and set it at room temperature for 20 minutes.
- Spin at 4500 g for 10 minutes in a swinging bucket rotor.
- Pour off the supernatant and drain the tubes for several minutes on a paper towel.

CTAB-DNA dissolving

- Add 400 µL 1 M NaCl to the pellet and set the sample at 45° to 65°C with gentle shaking for 10 to 30 minutes or at room temperature overnight without shaking. Dissolve as much material as possible.
- Pour the solution into a 2.0-mL microfuge tube.
- Rinse the 50-mL tube with 200 µL 1 M NaCl and add this to the same microfuge tube.

Phenol wash

- Add 700 µL phenol to the sample and mix by gentle inversions.
- Spin at high speed for 2 minutes to separate phases.
- Transfer the aqueous (top) phase to a 2.0-mL tube. Do not discard the phenol phase or the interface.

Back extraction

- Add 300 µL TE to the phenol phase and interface and mix by gentle inversions.
- Spin at high speed for 2 minutes.
- Add this aqueous phase to the 2.0-mL tube with the first aqueous phase of that sample.
- Discard the interface and phenol.

Chloroform wash

- Add 900 µL of chloroform to the sample and mix by gentle shaking.
- Spin at high speed for 2 minutes.
- Transfer the aqueous phase to a 2.0-mL tube.

DNA precipitation

- Add 950 µL cold isopropanol to the sample and mix by gentle inversions.
- Place the sample at -20°C for 30 minutes or overnight.
- Spin at high speed for 4 minutes.
- Pour off the supernatant and drain the tube on a paper towel.

Pellet washing

- Wash the salt from the DNA by adding 500 µL of 65% (v/v) ethanol.
- Invert the tube several times and pour off the ethanol.
- Add 500 µL 85% (v/v) ethanol and pour off.
- Drain the tube on a paper towel, remove residual alcohol from the tube bottom using a micropipettor, then vacuum or air dry the sample.
- Add 200 to 300 µL TE to dissolve the DNA.

Results and Discussion

Typical yields range from 40 to 70 µg DNA per gram of fresh tissue. Sufficient quantities of DNA were obtained from six grams of fresh leaf tissue for large-scale RFLP analysis. DNA obtained by this method dissolves easily and has been completely digested with Eco RI (Fig. 1), *Hind* III, *Bam* HI, and *Pst* I.

The Polytron effectively disrupts cells without significant DNA shearing (Fig. 1), possibly because the highly viscous contaminants cushion the DNA against shearing forces. DNA yields are four to six times higher when the tissue and buffer are mixed in the Polytron rather than by hand.

The effectiveness of CTAB as a detergent and precipitant is not diminished by lowering its concentration from 1% to 0.5%. The benefits are that the troublesome foaming that occurs in the Polytron is eliminated and the viscosity of the solution is reduced. Having less viscosity, the speed and time of centrifugation required to pellet the precipitated CTAB-DNA is reduced by half that otherwise needed for *Cuphea*.

A moderately tight CTAB-DNA pellet is made to minimize DNA loss when the supernatant is removed and to minimize supernatant carryover into the 1 M NaCl. Thorough drainage of the supernatant from the pellet improves solubilization in 1 M NaCl and keeps the subsequent volumes low for using microfuge tubes.

We deleted the second CTAB and chloroform wash used in the methods by Murray and Thompson (1980) and Liechtenstein and Draper (1985) because it was ineffective with *Cuphea*. We added a phenol-chloroform wash to remove most contaminants. Usually a chloroform wash occurs after the DNA is dissolved in TE. By incorporating the phenol-chloroform wash earlier, the DNA dissolves in TE much easier and substantial time is saved because a second alcohol precipitation and solubilization in TE is eliminated.

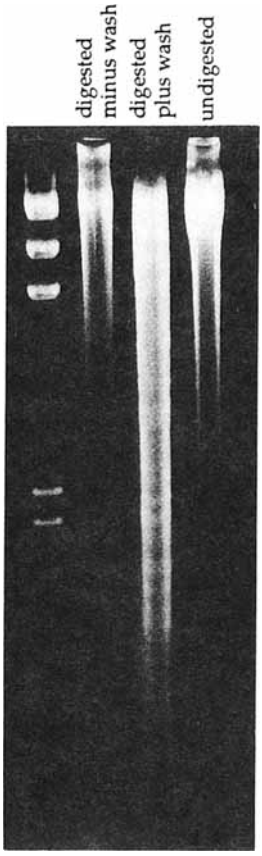

Fig. 1. Electrophoresis of *Cuphea* DNA. *Cuphea* DNA on a 0.8% (w/v) agarose gel in Tris-borate-EDTA buffer after 15 hours at 21 volts. Lanes: λ, *Hin*dIII-digested λ phage; **digested minus wash,** *C. lanceolata* DNA was extracted without a phenol-chloroform wash and digested with *Eco*RI; **digested plus wash,** *C. lanceolata* DNA was extracted with a phenol-chloroform wash and digested with *Eco*RI; **undigested,** *C. lanceolata* DNA extracted with a phenol-chloroform wash and not digested.

Phenol is most effective in removing contaminants from *Cuphea* tissue when not mixed with chloroform and when equilibrated successively with 1.0 M and 0.1 M Tris buffer. For unexplained reasons, more extensive equilibration methods substantially reduce DNA yields. Back extracting the phenol phase and interface with TE almost doubles the DNA yield.

Using this protocol, we extracted *Cuphea* DNA that is clean enough for complete digestion with restriction enzymes. Other methods and their modifications yielded much less DNA or DNA that is highly contaminated and unusable. This protocol is relatively simple and inexpensive. Up to 24 samples can be processed within one normal workday. The DNA dissolves quickly and can be used the next day. This protocol may be useful with other plant species where DNA extraction is made difficult by contaminants.

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