

BRIEF COMMUNICATION

A method of DNA isolation from epiphytic CAM ferns for use in random amplified polymorphic DNA analysis

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A method for DNA extraction from *Pyrrosia piloselloides* (L.) Price and *Pyrrosia longifolia* (Burm.) Morton utilising hexadecyltrimethylammonium bromide is described. The genomic DNA isolated was found to be relatively pure and shown to be suitable for RAPD analysis.

Additional words: genomic DNA, *Pyrrosia longifolia*, *Pyrrosia piloselloides*.

Numerous workers have used random amplified polymorphic DNA (RAPD) markers to create DNA fingerprints (banding profiles) for the study of individual identity and taxonomic relationships in both prokaryotic and eukaryotic organisms. Despite this, there is a scarcity of such work carried out on ferns. Possible reasons include the difficulty faced in isolating DNA used for amplification. We therefore describe here a method of DNA isolation for the study of RAPD markers on sterile fronds of *Pyrrosia piloselloides* and *P. longifolia* (family *Polypodiaceae*), two epiphytic crassulacean acid metabolism (CAM) ferns commonly found on wayside trees.

The procedure of total DNA extraction from the epiphytic ferns was based on that of Van Coppenolle *et al.* (1993) but with several modifications. Sterile fronds of *P. piloselloides* and *P. longifolia* were collected and surface sterilized with 10 % (v/v) *Clorox*TM for 10 min before rinsing with copious amounts of distilled water. Following this, approximately 2 g fresh mass of frond tissue were ground to a fine powder in liquid nitrogen with a mortar and pestle. The frozen homogenate was then transferred to four 2.2 cm³ Eppendorf tubes containing 1 cm³ of DNA extraction buffer [1.875 % hexadecyltrimethylammonium bromide (CTAB); 37.5 mM Tris

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Abbreviations: CAM - crassulacean acid metabolism; CTAB - hexadecyltrimethylammonium bromide; EDTA - ethylenediaminetetraacetic acid; PVP - polyvinyl pyrrolidone; RAPD - random amplified polymorphic DNA; SDS - sodium dodecyl sulfate.

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buffer, pH 8; 7.5 mM ethylenediaminetetraacetic acid (EDTA); 1.3 M NaCl; 29 mM mercaptoethanol; 20 mg cm⁻³ polyvinyl pyrrolidone (PVP) and 2% sodium dodecyl sulfate (SDS)]. The preparation was mixed thoroughly and incubated at 65 °C for 30 min. The mixture was then vortexed with an equal volume of chloroform followed by centrifugation at 10 000 g at 4 °C. A second extraction was performed on the supernatant with 0.2 volumes of 10X CTAB extraction buffer (10 % CTAB, 0.7 M NaCl). The chloroform extraction step was repeated and DNA was precipitated from the supernatant with 2.5 volumes of absolute ethanol at -80 °C for 30 min (Sambrook *et al.* 1989). The mixture was centrifuged at 10 000 g for 15 min to pellet the DNA. The pellet was then resuspended in 0.1 cm³ of sterile distilled water. The preparation was subsequently treated with DNase-free RNase (0.1 g dm⁻³) to digest contaminating RNA. This was followed by a phenol:chloroform extraction (Sambrook *et al.* 1989). The DNA pellet was precipitated with absolute ethanol, air dried and resuspended in Tris-EDTA buffer (10 mM Tris; 1 mM EDTA, pH 8.0). The quality of the genomic DNA was then spectrophotometrically assayed, further verified by agarose gel electrophoresis and digested with restriction enzymes to confirm its purity. The DNA can be stored at 4 °C. The fronds can also be frozen in liquid nitrogen and kept at -80 °C before DNA extraction is carried out.

For the RAPD Assay of *Pyrrosia* DNA, all reagents used were obtained from Promega (Madison, USA) and Operon 10-mer Kit U primers were used (Operon Technologies, Alameda, USA). RAPD reactions were carried out in a total volume of 0.05 cm³ in thermophilic buffer (50 mM KCl; 10 mM Tris-HCl, pH 9 and 1 % Triton X-100), with 3 mg dm⁻³ of genomic DNA, 100 mM of each deoxyribonucleotide triphosphate (dNTP), 2 mM MgCl₂, 1 mM RAPD primer and 40 U cm⁻³ *Thermus aquaticus* DNA polymerase. The reaction tubes were heated to 95 °C for 10 min

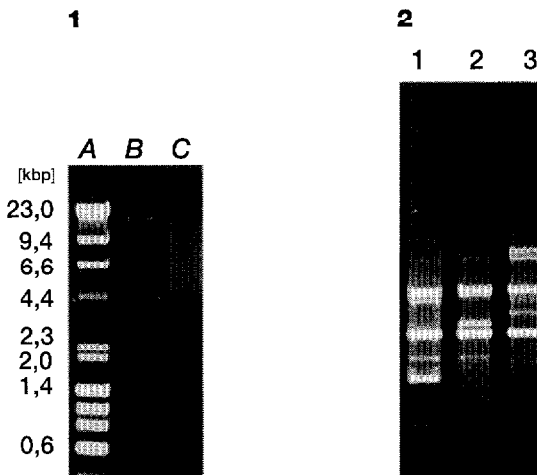


Fig. 1. Agarose gel electrophoresis of intact genomic DNA (B) and genomic DNA restricted with *Eco*RI showing complete digestion (C). Lambda *Hind* III and ϕ x174-*Hae* III DNA markers (A).

Fig. 2. RAPD markers amplified from different samples of *Pyrrosia piloselloides* obtained from 3 different locations in Singapore showing variations in banding patterns.

before undergoing 45 cycles of denaturation at 95 °C for 1 min, annealing at 32 °C for 3 min and extension at 72 °C for 2 min. The RAPD markers were then separated by electrophoresis through a 2 % agarose gel and DNA molecular mass markers (*Lambda Hind III* and *ϕx174 Hae III*) were used as molecular mass standards. The DNA fragments were visualised under UV and a polaroid photo was taken.

Several DNA extraction methods were employed to isolate DNA from *Pyrrrosia*. The method recommended by Honda and Hirai (1990) works well for orchids but not for ferns as a jello-like DNA pellet that could not be easily resuspended was obtained. The DNA extraction method suitable for plants rich in soluble polysaccharides (Li *et al.* 1994) did not yield high quality of DNA for the *Pyrrrosia* either. We used CTAB to form a complex with the polysaccharides, included PVP and mercaptoethanol in the extraction procedure and treated the DNA with DNase-free RNase to ultimately produce relatively clean DNA that can be digested with restriction enzymes (Fig. 1) and amplified with Taq DNA polymerase for RAPD reactions (Fig. 2). It is therefore recommended that this extraction protocol be employed in future genomic DNA isolation of epiphytic ferns and closely related fern species.

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