

Short Communication

A simple, rapid and efficient method for the extraction of genomic DNA from lychee (*Litchi chinensis* Sonn.)

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A simple, rapid and efficient method for isolating genomic DNA from lychee (*Litchi chinensis* Sonn.) was developed. This modified CTAB protocol include the use of 2 M NaCl, PVP, 5% mercaptoethanol and 80% ethanol in the extraction as well as reducing the centrifugation times during the separation and precipitation of the DNA. Isolated genomic DNA showed high purity and high quantity following restriction digestion analysis.

Key words: *Litchi chinensis*, genomic DNA isolation.

INTRODUCTION

Lychee (*Litchi chinensis* Sonn.), a member of the Sapindaceae family, has its origin in the low elevations of the provinces of Kwangtung and Fukien in Southern China. Cultivation spread over the years through neighbouring areas of southeastern Asia and offshore islands and is now one of the important fruit trees growing in the tropics and subtropics (Menzel, 1983). Isolation of plant nucleic acids for use for Southern blot analysis, polymerase chain reaction (PCR) amplifications, restriction fragment length polymorphisms (RFLPs), arbitrary primed DNA amplifications (RAPD, AP-PCR, DAF), and genomic library construction is one of the most important and time-consuming steps. The degree of purity and quantity varies between applications. A good extraction procedure for the isolation of DNA should yield adequate and intact DNA of reasonable purity. The procedure should also be quick, simple and cheap and, if possible, avoid the use of dangerous chemicals.

The extraction process involves, first of all, breaking or digesting away cell walls in order to release the cellular constituents. This is followed by disruption of the cell membranes to release the DNA into the extraction buffer. This is normally achieved by using detergents such as sodium dodecyl sulphate (SDS) or cetyl-methylammonium bromide (CTAB). The released DNA should be protected from endogenous nuclease. EDTA is often included in the extraction buffer to chelate magnesium ions, a necessary co-factor for nucleases, for this purpose. The initial DNA extracts often contain a large amount of RNA, proteins, polysaccharides, tannins

and pigments which may interfere with the extracted DNA and difficult to separate. Most proteins are removed by denaturation and precipitation from the extract using chloroform and/or phenol. RNAs on the other hand are normally removed by treatment of the extract with heat-treated RNase A. Polysaccharide-like contaminants are, however, more difficult to remove. They can inhibit the activity of certain DNA-modifying enzymes and may also interfere in the quantification of nucleic acids by spectrophotometric methods (Wilkie et al., 1993). NaCl at concentrations of more than 0.5 M, together with CTAB is known to remove polysaccharides (Murray and Thompson, 1980; Paterson et al., 1993). The concentration ranges mentioned in literature varies between 0.7 M (Clark, 1997) and 6 M (Aljanabi et al., 1999) and is dependent on the plant species under investigation. Some protocols replace NaCl by KCl (Thompson and Henry, 1995).

Lychee is considered to be a "difficult" plant for DNA isolation due to its high polyphenolic content, which may interfere with the DNA purity especially for subsequent manipulations. Antioxidants are commonly used to deal with problems related to phenolics. Examples include 2 β -mercaptoethanol, ascorbic acid, Bovine Serum Albumin, sodium azide and PVP amongst others (Dawson and Magee, 1995; Clark, 1997). Phenol extractions when coupled with SDS are also helpful. However, with plants having a high content of polyphenolics, SDS-phenol tends to produce low yields of DNA (Rezaian and Krake, 1987). Although there are

several published protocols on plant DNA isolation (Dellaporta et al., 1983; Rogers and Bendich, 1988; Draper and Scott, 1988), the production of large quantities of purified lychee genomic DNA is still difficult (Anuntalabhochia et al., 2002).

The optimised protocol described here is completely different from that proposed by Anuntalabhochia et al. (2002) and is specifically designed to isolate genomic DNA of lychee within a short period of time using small amounts of plant tissues, and yielding a high quantity of purified genomic DNA.

MATERIALS AND METHODS

Several experiments were carried out, however, only the optimised protocol is described here.

Plant material

Samples of young, tender, unbruised lychee (*Litchi chinensis* Sonn. var Tai So) leaves were collected early morning from the University farm. These were kept between moist tissue paper in a plastic bag and kept away from sunlight. The leaves were de-starched by covering them for 24 to 48 h before use.

Solutions

An extraction buffer consisting of 2% hexadecyltrimethyl-ammonium bromide (CTAB) (w/v), 100 mM Tris (pH 8.0), 20 mM EDTA (pH 8.0), 2 M NaCl, 2% polyvinylpyrrolidone (PVP - Mr 10,000, 5% β -mercaptoethanol (v/v), and 10 mM ammonium acetate was prepared. In addition, chloroform:octanol (24:1), 75% and 80% alcohol and a TE buffer consisting of 1 mM Tris (pH 8.0) and 1 mM EDTA (pH 8.0) were also needed.

DNA isolation and purification

Leaves were harvested and frozen immediately in liquid nitrogen. They were used immediately, or frozen at -80°C until required. A 4.0 g of leaf sample was ground in liquid nitrogen using a mortar and pestle pre-chilled to either -20°C or -80°C . Some liquid nitrogen was poured in just before adding the leaves. The pulverized leaves were quickly transferred to a liquid nitrogen pre-chilled, 50-mL Falcon tube. 2% of pre-heated (65°C) CTAB buffer (16 mL) containing 5% v/v β -mercaptoethanol and 2% PVP (Mr 10,000) was quickly added to the tube and stirred with a glass to mix. The tube was incubated at 65°C for 5 min with frequent swirling. An equal volume of chloroform:octanol (24:1) was added and the sample centrifuged for not more than 5 s in a bench-top centrifuge (Biofuge 13, Heraeus) at room temperature to separate the phases. The supernatant was carefully decanted and transferred to a new tube.

The above steps, beginning with the addition of chloroform/octanol (24:1) and ending with decanting of supernatant, were repeated twice. The supernatant was precipitated with 2/3 volume of isopropanol. The precipitated nucleic acids were collected and washed twice with the buffer (75% ethanol, 10 mM ammonium acetate, TE). The pellets were air dried and re-suspended in TE. The dissolved nucleic acids were brought to 2 M NaCl and re-precipitated using 2 volumes of 70% ethanol (If the pellet obtained was hard to re-suspend, this step was repeated one

more time). The pellets were washed twice using 80% ethanol, dried and re-suspended in 100 μL of TE buffer. The tube was incubated at 65°C for 5 min to dissolve genomic DNA, and RNase was then added.

Amount and purity of DNA

The yield of DNA per gram of leaf tissue extracted was measured using a UV-VIS Spectronic Genesys 5 (Milton Roy) spectrophotometer at 260 nm. The purity of DNA was determined by calculating the ratio of absorbance at 260 nm to that of 280 nm. DNA samples from the leaf tissues were digested with EcoRI and HindIII and electrophoresed on a 0.8% agarose gel.

RESULTS AND DISCUSSION

Experiments where the CTAB method of extraction without modification was used, gave a DNA yield of 335.02 μg per 1.0 g of leaf material. However, it was contaminated with polysaccharides and phenolics as shown by the spectrophotometer readings which gave a $A_{260}\text{ nm} / A_{230}\text{ nm}$ ratio of 1.11 and a $A_{260}\text{ nm} / A_{280}\text{ nm}$ ratio of 1.48. The sample was very viscous and pinkish in colour. Upon electrophoresis, fire type bands were obtained confirming the presence of polysaccharides. From the next set of experiments where the NaCl concentration was increased from 1.4 M to 2.0 M and use of PVP (Mr 40000) during extraction, the quality of DNA was improved. The bands following electrophoresis were sharper (Figure 1a). However, the yield was reduced to 240.12 μg DNA per 1.0 g tissue. Several modifications were made with one parameter tested at a time. Modifications included the ratio of buffer to tissue, use of 5% mercaptoethanol rather than 2%, reduction in the incubation time, washing in 80% ethanol and use of PVP (Mr 10000).

Isolation of genomic DNA using the described method was quite easy and did not take more than 15 min. The yield was 360 μg per 1.0 g of leaf material. The $A_{260}\text{ nm} / A_{230}\text{ nm}$ ratio was 1.80 while $A_{260}\text{ nm} / A_{280}\text{ nm}$ ratio was 1.90. From the different steps followed and modifications made, it was found that using younger leaves instead of older ones reduces nucleic acid contamination by plant metabolites that interfere with solubilisation of precipitated nucleic acids. In a set of experiments using different amount of starting material so that more reagent is present per amount of material, it was observed that the ratio of buffer to leaves should always be 4:1 v/w or greater to obtain sufficient amount of clean DNA.

During the addition of preheated CTAB containing β -mercaptoethanol, moving quickly at this stage was critical in getting good quality DNA. To aid in minimizing time spent doing this step, the 16 mL of 2% CTAB was measured in a 50 mL conical tube to which 836 μL of β -mercaptoethanol (5% v/v) was added and the tube placed in a 65°C water bath until ready for use. Addition of the

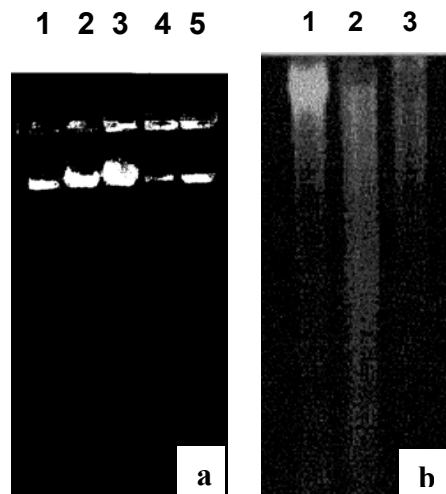


Figure 1. (a) Electrophoresis of lychee DNA on 0.8% agarose gel following RNase treatment. Lane 1: DNA standard; Lanes 2-5: Lychee DNA samples; 5 µg DNA was loaded per lane. (b) Restriction enzymes digestion of lychee genomic DNA. Lane 1: DNA digested with *EcoRI*; Lane 2: DNA digested with *EcoRI* and *HindIII*; Lane 3: DNA digested with *HindIII*.

pre-warmed, pre-measured CTAB buffer to the frozen leaf tissue contained in the pre-chilled conical tube saves precious time in bringing the tissue from -80°C to 65°C as rapidly as possible resulting in DNA of higher quality. Using 5% β -mercaptoethanol instead of the 2% used in the Doyle and Doyle (1987) procedure, produced nucleic acid pellets that were not nearly brown. Inclusion of PVP improved the colour of the nucleic acid obtained. From the spectrophotometer readings, PVP 10 (Mr 10000) gave better results than PVP 40 (Mr 40000). This may be explained by the fact that the high molecular weight PVP might have precipitated together with the nucleic acid, thereby being present as a contaminant.

During incubation at 65°C , it was found that there were no significant differences in the yield of DNA when comparing the incubation time of 4 h, an overnight incubation and an incubation of 5 min. The latter was adopted for convenience. Once the nucleic acids were collected, they were washed in the wash buffer. The purity of genomic DNA was dependent on the number of washes. A three-time wash combined with a short-run centrifugation was sufficient for DNA purification and removal of endogenous nucleases or other proteins. As CTAB is soluble in ethanol, residual amounts are removed in the subsequent wash. During ethanol precipitation of nucleic acids from 2 M NaCl, polysaccharides remain dissolved in the ethanol (Fang et

al., 1992). The freer the nucleic acids are from contaminants, the easier it is to re-suspend the pellet. If the pellet obtained from the first ethanol precipitation from 2 M NaCl was found to be hard to resuspend, two such precipitations were done and the pellet obtained from the second precipitation usually goes into solution very easily. It was found that washing in 80% ethanol gave better DNA as a result of the removal of any residual NaCl and/or CTAB. The DNA extracted can be digested with restriction enzymes such as *EcoRI* and *HindIII* (Figure 1b).

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