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Evaluation Of Two Methods For Genomic DNA Extraction From Silica Gel Preserved Pinnae Of Several Common Fern Species In Peninsular Malaysia

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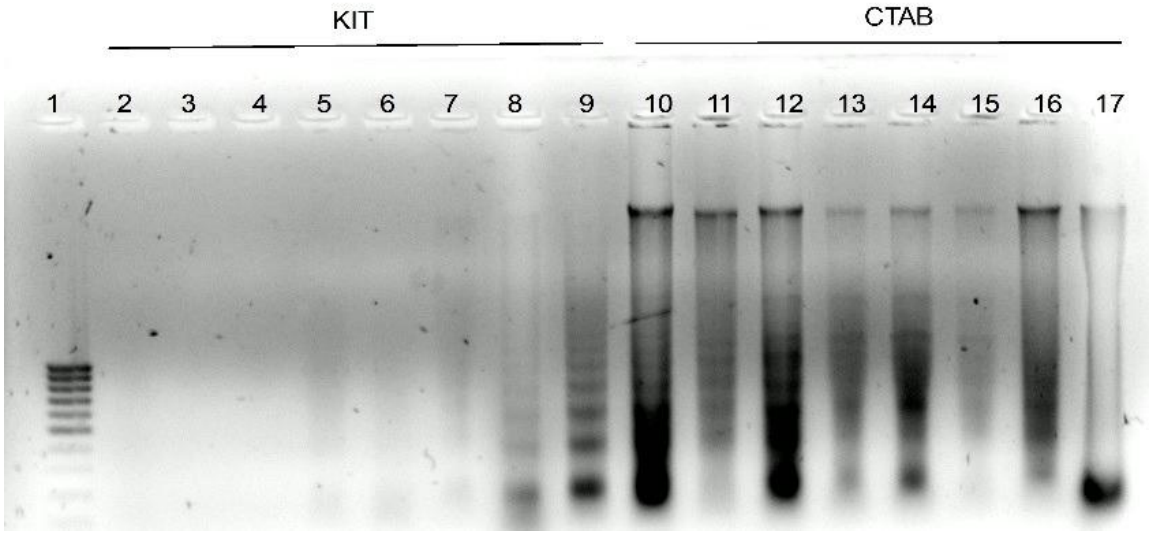
Abstract (141 words)
Several extraction methods of genomic DNA with the purpose of species identification and characterization of genetic diversity in numerous fern species are being used currently. However, it is important to identify which extraction method is the best in removing the presence of undesirable compounds in fern sample mainly polysaccharides and polyphenols. High contents of polysaccharides and polyphenols increase the sample viscosity and decrease the DNA quality, which will interfere with PCR performance. Therefore, this study evaluated the quality of genomic DNA extracted using commercial plant extraction kit and modified CTAB extraction method to compare consistency of both methods for several common fern species in Peninsular Malaysia. Results showed the efficiency of the modified CTAB extraction method compared to kit extraction method. The genomic DNA isolated was found to be relatively pure and suitable for phylogenetic as well as conservation genetic studies.

Keywords: pinnae DNA extraction, common fern, silica-gel preservation, modified CTAB

DNA extraction has always been crucial as the first step in plant phylogeny research and has been the focus in several recent studies (Schuettpelz and Pryer, 2007; Rothfels *et al.*, 2012; Christenhusz and Chase, 2014). Nevertheless, the DNA extraction method for fern samples still lack robustness (Yi *et al.*, 2018). Here, we test two methods: genomic DNA extraction using Hi-Yield Genomic DNA Plant Mini Kit and modified CTAB protocol from Doyle and Doyle (1987). The aim was to identify an accurate and consistent, yet time and money-efficient extraction method for fern species. Thirty three fern samples from eleven species (*Nephrolepis biserrata, Dicranopteris linearis, Diplazium esculentum, Cyathea latebrosa, Belvisia callifolia, Adiantum latifolium, Lygodium flexuosum, Aglaomorpha heraclea, Asplenium nidus, Odontosoria chineensis, Cyathea contaminans*) were sampled for pinnae and preserved in silica gel immediately after collection. Each pinnae were analysed in triplicate for each species. For all extractions, silica dried fern samples were grounded using mortar and pestle with the aid of very fine sand to produce the powdery structure of sample weighing 25mg. As for extraction using kit, standard procedures from Hi-Yield Genomic DNA Plant Mini Kit were followed. A protocol for extracting plant genomic DNA using CTAB by Doyle and Doyle (1987) were adjusted and modified as the following:

Polyvinylpyrrolidone (1%) and β-mercaptoethanol (2%) was added to the CTAB Buffer 2% CTAB, 1.4M NaCl, 100mM Tris pH8.0, 20mM EDTA pH8.0) and dissolved in 65°C water bath before starting experiment. Samples weighing 25mg each were then transferred into a 1.5mL microcentrifuge tube and added with the pre-warmed (65°C) CTAB Buffer. The samples were incubated for 1 hour at 65°C and mixed by inverting for every 5-10 minutes interval. The tubes were then centrifuged at 13000rpm for 3 minutes and the aqueous phase were transferred to a clean 1.5mL microcentrifuge tubes. An equal volume of chloroform/iso-amyl alcohol (24:1) was added and mixed well to form an emulsion. The tubes were inverted for 1 minute before centrifuged at 13000 rpm for 5 minutes to form 3 layers (top = aqueous phase, middle = debris, bottom = chloroform) and the aqueous phase was pipetted immediately to a clean 1.5mL microcentrifuge tube. The chloroform/iso-amyl alcohol extraction was repeated. Then, ice-cold isopropanol with the volume of 2/3 was added and then mixed well before left in the freezer for 1 hour to precipitate. The tubes were then centrifuged at 13000 rpm for 5 minutes to form pellet and the liquid was carefully pipetted out until only pellet was left. Wash buffer (76% EtOH, 10mM Ammonium Acetate) with the volume of 1000uL was added and was left at the stand for at least 20 minutes. The tubes were centrifuged again at 3000rpm for 5 minutes and the liquid was pipetted out carefully without disturbing the pellet. The pellet was dried on a heat block at 65°C for 10 minutes before finally adding the TE buffer (10mM Tris HCl, 1mM EDTA (pH7.4)) with the volume of 30uL to dissolve the pellet and heated on a heat block at 65°C for 10 minutes.
High contents of polysaccharides and polyphenols commonly found in reseach samples (pinnae of ferns) were successfully reduced using the modified CTAB extraction. Figure 1



shows comparison gel results between genomic DNA extracted using Hi-Yield Genomic DNA Mini Kit and modified CTAB method.
Figure 1 Agarose gel (1%) results:100bp marker (lane 1), extraction using Hi-Yield Genomic DNA Mini Kit (Plant)(lanes 2-9) and CTAB extraction (lanes 10-17).

Using kit, such molecules might not be eliminated and inhibit PCR reactions (refer result in Figure 2).

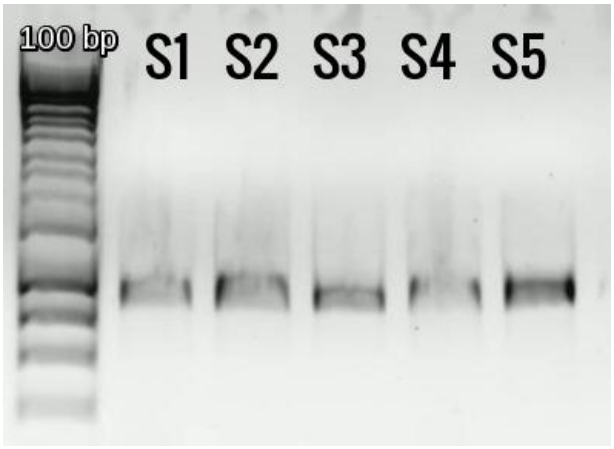


Figure 2 Agarose gel (2%) results: Sample S1- Cyathea contaminans, S2 - Cyathea latebrosa, S3 - Diplazium esculentum, S4 - Asplenium nidus, S5 - Adiantum latifolium amplified using trnL-trnF region.

Genomic DNA extracted were also found to be nearly pure with purity analysis showing absorbance range of (A280/260) of 1.8-1.9. This modified method offers promising and cheaper alternative for studies using silica dried pinnae samples as the origin for fern DNA extraction. As reliability, feasibility and reproducibility of molecular genetics studies depend on high molecular weight and high quality genomic DNA, this method has potential for applications in critical molecular biology techniques for plant phylogeny research especially for fern species.

Compliance with Ethical Standards:

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Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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