

Rapid Protocol for Isolation of PCR-Amplifiable DNA from Plants Containing High Level of Polysaccharides and Polyphenols

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Extraction of pure and high molecular weight genomic DNA from plants containing higher amount of proteins, polysaccharides, polyphenols and other secondary metabolites which interferes the isolation of pure DNA, is a prerequisite for PCR marker based molecular authentication methods such as DNA profiling. Medicinal plants contain high levels of polyphenols, polysaccharides, tannins and secondary metabolites. A medicinal plant *Cassia auriculata* was used as the study plant and an inexpensive and robust DNA extraction protocol that does not need liquid nitrogen was developed. The method is based on modified Cetyltrimethylammonium bromide (CTAB) extraction protocol. In this method, DNA was precipitated with salt based ethanol to reduce polysaccharides and salt contaminations by replacing isopropanol precipitation. The chilled incubation step at -20 °C for overnight was replaced with that particular condition at -80 °C for 2 hours to reduce protein and other co-precipitations. The use of increased β -mercaptoethanol concentration of 2% in CTAB extraction buffer removes phenolic and protein contaminations. With above modifications, average yield of extracted DNA increased to $740.00 \pm 47.55 \mu\text{g mL}^{-1}$. The mean $\text{OD}_{260/280}$ and $\text{OD}_{260/230}$ values were 1.82 ± 0.03 and 1.85 ± 0.07 respectively, indicating minimal levels of contamination. The optimized protocol was successfully tested for sensitivity and consistency using RAPD by arbitrary primer OP-U20 with 0.37 μg of DNA in 25 μL reaction volume. To our knowledge, this is the first attempt at isolating DNA profiling based PCR amenable genomic DNA from *C. auriculata* leaves. This protocol is suitable for extracting genomic DNA for DNA profiling and other downstream applications of medicinal plants.

Keywords: *Cassia auriculata*, CTAB protocol, RAPD, DNA profiling