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Optimization of a genomic DNA extraction technique for genetic diversity studies of selected orchid cvs. with ornamental values

F. Farook, R. N. Attanayake* and **S. P. Senanayake**

Department of Botany, Faculty of Science,
University of Kelaniya. Sri Lanka
renuka@kln.ac.lk

Orchidaceae is one of the largest and most diverse families of flowering plants and a major export ornamental crop. Cultivar development is the key for the success of ornamental flower industry and therefore, it is vital to identify the genetic diversity of the cultivars. In such an attempt, the first step is to optimize basic molecular biological techniques involved in genetic diversity analysis. However, DNA extraction from orchids is challenging compared to the other plant species since orchids are rich in polysaccharides and secondary metabolites, which can act as inhibitors in downstream applications. Most of the standardized protocols require liquid nitrogen freezing step, which is not an affordable practice in the laboratories of developing countries. Therefore, optimization of a low cost protocol to obtain pure DNA is necessary. The objective of the current study was to optimize extraction of genomic DNA for molecular marker based genetic diversity studies of selected orchid cultivars with ornamental value. Leaf pieces and pestle and mortar were stored at -80°C for at least three days. DNA extraction was done from frozen orchid leaves (50-100 mg young leaves) using Promega Wizard genomic DNA purification kit (Promega Inc. USA), classical CTAB protocol and modified CTAB protocol. Except modified CTAB protocol, none of the other methods produced high quality DNA as determined by spectrophotometry and agarose gel electrophoresis. However, the method was successful only for the *Dendrobium* cultivars but not for the *Phalaenopsis* leaves tested. Successful amplification of orchid rDNA-ITS region confirmed that the quality of extracted DNA is suitable for other PCR based molecular marker studies such as Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeats (SSRs). The modified method was reliable and reproducible.

Keywords: DNA extraction, Orchid cultivars, Genetic diversity