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COMPARATIVE ASSESSMENT OF FIVE DNA EXTRACTION METHODS FOR GENOMIC DNA EXTRACTION OF *PTEROCARPUS MARSUPIUM* AND *LITSEA GLUTINOSA*

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ABSTRACT: Species tailored protocols are needed for extraction of high quality and quantity of DNA. Unfortunately, for most of the forest tree species which are generally rich in phenols, gums and other secondary metabolites tailored protocols are not available posing problems in DNA extraction. In present investigation, five DNA extraction methods were screened and evaluated for the ability of genomic DNA extraction from the leaves of two endangered tree species i.e. *Pterocarpus marsupium* and *Litsea glutinosa*. The DNA obtained from all procedures was quantified and checked on 0.8% agarose gel electrophoresis. Considering quantity and quality of the extracted DNA, it may be concluded that protocol of Doyle and Doyle (1990) with inclusion of washing steps of Deshmukh et al. (2007) would be suitable for *P. marsupium.* Whereas, for *L. glutinosa* protocol developed by the Michiels et al. (2003) for the latex-containing asteraceae species suited more compared to other screed methods.

Key words: *Pterocarpus marsupium*, *Litsea glutinosa,* genomic DNA

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Advances in biotechnology have generated new opportunities for genetic resources conservation and utilization which is very much essential to meet the demand for future food security. Molecular markers are increasingly used for screening of germplasm to study genetic diversity, identify redundancies in the collections, test accession stability and integrity, and resolve taxonomic relationships thus expanding the scope of genetic resources utilization (Rao, 2004). Application of the molecular markers has been further extended for the certification of the genetic fidelity of the clonally propagated plants, evolutionary and for genome stability studies (Gesteira et al., 2002, Nagh et al., 2008).

Success of the PCR/ molecular markers method depends on the quality and quantity of the DNA template (Coelho et al., 2004, von Wurmb-Schwark et al., 2006) which should be free of contaminants and DNA nucleases that impair the amplification process (Manuja et al., 2010). High quality and quantity of genomic DNA is primary and foremost requirement of any biotechnological study based on the DNA markers. The quality and quantity of DNA required depends upon the objective of the work. In population studies viz. structure, diversity etc., the number of PCR reactions to be performed are large and therefore considerable amount of purified DNA would be needed which can be stored for a longer duration. On the other hand in case of in case of clonal fidelity checking, marker assisted selection, DNA fingerprinting, etc., the numbers of samples are high but the number of PCR reactions to be performed are few and hence the amount of DNA needed is small but pure (McCarthy et al., 2002, Wang et al., 1993).

Numerous methodologies have been developed for high-throughput and cost-effective extraction of DNA from plant tissues (authors of different methods). Principle of DNA extraction involves disruption of the cell followed by lysis and extraction in suitable buffer, precipitation and purification. One of the most commonly used methods to extract DNA from plants uses the ionic detergent cetyl trimethyl ammonium bromide (CTAB) to disrupt membranes and a chloroform-isoamyl alcohol mixture that separates contaminants into the organic phase and nucleic acid into the aqueous phase (Doyle and Doyle, 1987). However, many plants contain very high levels of secondary metabolites, including lipids, phenolic