An Optimized DNA Extraction Protocol For Isolation Of High Quality Genomic DNA From Camphor Containing Timber Tree Species, *Dryobalanops Beccarii* Dyer

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Abstract: Isolation of high-quality genomic DNA from Dryobalanops beccarii is obviously difficult due to the existence of large amounts of camphor and other secondary metabolites. These contaminants will co-precipitate with DNA during DNA isolation and purification processes, and therefore, resulting in a brownish DNA pellet that is unsuitable for downstream applications. Many DNA isolation protocols are available for various plant tissues; however these protocols are inefficient in yielding high-quality amplifiable genomic DNA especially from camphor containing timber tree species. A CTAB based protocol has been optimized for isolating genomic DNA from camphor containing timber tree species. Key steps include: 1) using 1% β-mercaptoethanol and 2% PVP 40 (Mr 40,000) in the extraction buffer; 2) sample incubation time, 40 minutes at 65°C, and 3) DNA precipitation at room temperature (25°C). The isolated DNA pellet was transparent colour and the purified genomic DNA is suitable for PCR amplification.

Key words: Genomic DNA, CTAB, Drynobanalops beccarii, Camphor, Secondary metabolites, PCR

The most essential principle in the modern molecular biology is the isolation of high-quality DNA in a reasonable amount. The presence of DNA degrading endonucleases, polysaccharides, polyphenolics and other secondary metabolites in the plant tissue makes the isolation of high-quality intact nucleic acids problematic [1, 2]. In general, specific reagents are required for removing secondary compounds during DNA isolation as plants produce different types of secondary compounds [3]. To date, most standard methods and technologies are available for genomic DNA isolation from various plant tissues. However, these protocols are inefficient in yielding high-quality amplifiable genomic DNA especially from camphor containing timber tree species, *Dryobalanops beccarii* Dyer. Camphor is a white crystalline bicyclic saturated terpene ketone compound with chemical formula $C_{10}H_{16}O$ with formal chemical name (IUPAC) 1,7,7-trimethyl-bicyclo(2,2,1)heptan-2-one. Other names such as 2-camphanone, bornan-2-one, caladryl and 2-bornanone also exists [4].

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The physical-chemical properties of camphor includes having a pungent odour and taste that is flammable and volatile; melting at 176°C - 180°C, boiling at 204°C, and specific gravity 0.99. It is insoluble in water but soluble in ethanol, ethylether, turpentine, and essential oils [5]. The biosynthesis of camphor involved cyclisation of linaloyl pyrophosphate from geranyl pyrophosphate to becoming bornyl pyrophosphate, followed by hydrolysis to borneol and oxidation to camphor. *Dryobalanops beccarii* or locally known as Kapur Bukit is a moderately heavy timber species of the Dipterocarpaceae family (Fig. 1). It is mainly found in South East Asia, Sumatra and Borneo including Sarawak, Brunei, Sabah and East Kalimantan [6]. It produces large amounts of camphor, one of the polyphenolics found naturally in cavities or fissures in the wood or leaves of the camphor trees either in the form of solid camphor or a light fluid called camphor oil [7]. In oxidized form, phenolic compounds irreversibly bind to protein and nucleic acids [8] and, the isolated DNA becomes unsuitable for downstream applications [9]. Camphor has been medicinally used against coughs, asthma, headache, pains in the stomach or liver and diseases in the urino-generative system as well as against ulcers in mouth and nose, rheumatism, burns and wounded eyes. Kapur Bukit is an important source of quality wood for construction particularly in plywood production, furniture, joinery, beams, toys and decking. In addition, it also can be used for bridges, ship building, vehicle bodies, and railway sleepers [6]. Recently, this species has suffered a massive population reduction due to the human activities and natural catastrophe such as deforestation, pollution, industrial and urban development, and global greenhouse effects [10]. Conservation of forest tree genetic resources is important as it provides a means for breeding, reintroduction programmes or as insurance against possible extinction of species in the wild. According to PROSEA [6], conservation of genetic diversity of forest tree species should be realized *in situ* but occasionally *ex situ* conservation also can play an important role [10, 11]. However, it is difficult to conserve tree species as long as logging is done at trade group level and little attention is paid to inventorying stands of individual species.