PHYLOGENETIC OF *Aquilaria microcarpa, Aquilaria malaccensis* AND *Aquilaria beccariana* BASED ON *rbcL* GENE SEQUENCES

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INTRODUCTION

Aquilaria microcarpa, Aquilaria malaccensis and *Aquilaria beccariana* from the family Thymelaeaceae are species of tropical trees that have been found to produce an aromatic resin, one of the most highly valuable forest products and currently traded internationally. The resinous wood has been traded for more than 2000 years for its use in medicinal, religious and aromatic preparations (Burkill, 1966; Denovan & Puri, 2004). Due to the increasing demand, high prices, and easy access to wild stocks, gaharu is over-harvested in Central and Southeast Asia (LaFrankie, 1994; Chakrabarty *et al.*, 1994; Soehartono & Newton, 2001). As a result, all species of *Aquilaria* were placed on the Appendix II list of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in 1994 (Eurlings & Gravendeel, 2005).

In order to further understand the relationship between the three Aquilaria spp., we undertook molecular study in order to establish the relatedness between the spp. Presented here is a phylogenetic analysis of *A. microcarpa, A. malaccensis* and *A. beccariana* based on chloroplast DNA (cpDNA) sequence data. cpDNA region, *rbcL*, from 30 individuals of *Aquilaria* species were amplified by the polymerase chain reaction (PCR). Variation in cpDNA sequences were used to construct phylogenetic relationships. The *rbcL* gene, which encodes the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO), has been widely sequenced from many plants and the sequenced data obtained has been widely used in the study of plant phylogeny. cpDNA been used in this study because it is considered to be conservative in its evolution in terms of nucleotide substitution, making it suitable to study phylogenetic relationships among species.

MATERIAL AND METHODS

Plant materials

In this study, 10 individuals of each *A. microcarpa, A. malaccensis* and *A. beccariana* were included. *A. microcarpa* and *A. beccariana* samples were obtained from the UNIMAS green house while *A. malaccensis* samples were obtained from Tasik Kenyir. Fresh leaves samples were used for DNA extraction.

DNA extraction, PCR amplification and sequencing

Leaf material was ground with a mortar and pestle in with liquid nitrogen. Total genomic DNA was extracted using modified methods of Doyle & Doyle (1987). Purification of extracted DNA was done using Wizard® Genomic DNA Purification Kit from PROMEGA. The *rbcL* region was amplified using a pair of universal