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Isolation and nucleotide variation of COBRA gene in a tropical timber tree *Neolamarckia cadamba*

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Abstract

COBRA gene is involved in the regulation of cellulose deposition and orientation of cell expansion. Any single nucleotide differences may influence the functions of this gene. Hence, this study was carried out to isolate the partial COBRA genomic sequence and subsequently, to determine the nucleotide variation of COBRA gene in *Neolamarckia cadamba*. *N. cadamba*, also locally known as kelampayan is one of the fast-growing deciduous and indigenous tree species with great commercial values in Malaysia. The targeted DNA sequence of COBRA gene was amplified with the designed primer pair by using Polymerase Chain Reaction (PCR) technique. The partial COBRA genomic sequence (517 bp) was subjected to BLASTn analysis to search for homology sequence and validate the identity of the sequence through NCBI. Multiple alignment was carried out by ClustalW for manual detection of single nucleotide polymorphisms (SNPs). Five SNPs were detected in the exon region and two SNPs in the intron region of COBRA partial genomic sequences. Of these five detected SNPs, four non-synonymous mutations and one synonymous mutation were discovered in the COBRA amino acid sequences. Based on the in silico restriction analysis, one possible restriction enzyme, HpyCH4III was detected to restrict at a SNP site (384 bp) which could be useful for genetic marker development, such as CAPS marker development in the efforts of genotyping project.

Keywords: *Neolamarckia cadamba*, polymerase chain reaction (PCR), COBRA gene, single nucleotide polymorphisms (SNPs), molecular markers