

SINGLE NUCLEOTIDE POLYMORPHISMS DISCOVERY IN *Cinnamate 4-Hydroxylase* AND *Cinnamyl Alcohol Dehydrogenase* GENES OF *Neolamarckia Cadamba* (KELAMPAYAN)

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ABSTRACT: Cinnamate 4-hydroxylase (C4H) and cinnamyl alcohol dehydrogenase (CAD) are two key enzymes involved in the lignin biosynthesis pathway. The sequence variations within these two genes are found to be associated with certain wood property traits. Hence, the objectives of this study were to discover the full-length cDNA of *C4H* and *CAD* genes from *Neolamarckia cadamba* and to identify the single nucleotide polymorphism (SNPs) in both partial genomic sequences of *C4H* and *CAD*. The full-length cDNA of *C4H* (1.6 kb) and *CAD* (1.2 kb) were isolated from *N. cadamba* through RT-PCR approach. Then, overlapping primers were designed to flank the *C4H* and *CAD* genomic DNA sequences from 12 *N. cadamba* samples. The amplified DNA fragments were cloned into pGEM-T Easy Vector and sent for sequencing. The sequence variations analysis revealed that there were 61 and 32 SNPs detected in *C4H* and *CAD* genomic DNA sequences, respectively. The SNPs detected were distributed throughout the exon, intron, 5'-UTR and 3'-UTR regions. Among the SNPs detected in exon regions of *C4H*, 16 were synonymous mutations and 8 were nonsynonymous mutation. For *CAD*, 6 SNPs had lead to synonymous mutations and one SNP had lead to nonsynonymous mutation in the translated amino acid sequences. The SNPs detected in the present study can become marker of choice in association genetic study to identify the genotype that is correlated with traits of interest. SNP markers are very useful in tree breeding programme as the plus tree can be selected in shortest time and under lowest-cost routing through gene-assisted selection (GAS) approach.

Keywords: *Neolamarckia cadamba*, *Cinnamate 4-hydroxylase (C4H)*, *Cinnamyl alcohol dehydrogenase (CAD)*, Single nucleotide polymorphism (SNP)

INTRODUCTION

Wood is one of the most complex materials which composed of polymers of lignin and carbohydrates that are physically and chemically bond together. Lignin is the second most abundant organic compound after cellulose, represents approximately 20-30% of the plant biomass (Brett & Waldron, 1990). The functions of lignin are to provide mechanical and structural support to the plants, as well as to furnish a significant protective function against pathogen or decaying fungi (Higuchi, 1997).

The genes within the lignin biosynthesis pathway are varied and of crucial. Abreu *et al.* (2009) had proposed that the high β -O-4 (Alkyl Aril Ether) bonds in lignin of angiosperms may possibly affect the wood properties. Moreover, candidate gene based association genetic study on lignin biosynthesis genes, for example *cinnamate 4-hydroxylase (C4H)* and *cinnamyl alcohol dehydrogenase (CAD)*, have showed that the sequence variations or single nucleotide polymorphism (SNP) in these two genes are able to affect the growth, lignin content and some aspects of the wood properties (Yu *et al.*, 2006; Gonzalez-Martinez *et al.*, 2007; Schillmiller *et al.*, 2009; Bjurhager *et al.*, 2010; Wegrzyn *et al.*, 2010; Tchin *et al.*, 2011). These have reflected