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Original Research Article

Isolation and *in silico* characterization of cinnamate 4-hydroxylase (C4H) gene controlling the early stage of phenylpropanoid biosynthetic pathway in Kelampayan (*Neolamarckia cadamba*, Rubiaceae) developing xylem tissues

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Abstract

Cinnamate 4-hydroxylase (C4H) is one of the enzymes involved at the starting point of the phenylpropanoid and lignin biosynthesis pathway. It involves in the hydroxylation of cinnamate to 4-coumarate. In this paper, we isolated and *in silico* characterized the complete sequence of cinnamate 4-hydroxylase (C4H) gene from *Neolamarckia cadamba* in Malaysia. The C4H singletons obtained from the NcdbEST were used to predict the hypothetical full-length of NcC4H through the contig mapping approach. RT-PCR was used to amplify the full-length C4H cDNA clone and subsequently the PCR amplicons were sequenced and analysed. The NcC4H cDNA was 1,651 bp long with a 505 amino acid sequence, a 18 bp 5'-UTR and a 115 bp 3'-UTR. The predicted NcC4H protein contains P450-featured motifs. These include the heme-binding domain, a threonine-containing binding pocket motif and the proline-rich region. Peptide sequence comparison and phylogenetic analyses revealed that NcC4H was clustered with class I C4H instead of class II C4H, which is preferentially involved in phenylpropanoid and lignin biosynthesis pathway. This full-length NcC4H cDNA can be used for developing genetic marker to identify economic trait loci (ETL) for wood quality traits via genomics-assisted selection (GAS) or candidate gene mapping approach.

Keywords: *Neolamarckia cadamba*, RT-PCR, Lignin biosynthesis, Cinnamate 4-hydroxylase (C4H), Expressed sequence tags (ESTs), Genomics-assisted selection

Introduction

Lignin represents about 20-30% of all the plant stem biomass. It is the second most abundant organic compound found in wood, especially in supporting and conducting tissue of the plants such as fibers and tracheary elements. Lignin is produced by dehydrogenative polymerization of monolignols known as coniferyl alcohol, coumaryl alcohol and sinapyl alcohol. The polymerization of these monolignols will give rise to guaiacyl (G) units, ρ-coumaryl units (H) and sinapyl (S) units of lignin