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TRANSFORMATION OF TOBACCO (*Nicotiana tabacum* L.) WITH THE OIL PALM *CINNAMYL ALCOHOL DEHYDROGENASE 2* GENE

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By

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Cinnamyl alcohol dehydrogenase (CAD) catalyses the committed step of monolignol biosynthetic pathway. Monolignols are monomers required in lignin synthesis. In plants, lignin is important for structural architecture and defence against pathogen attack. The CAD enzyme is encoded by a small multi-gene family whose members often differ in expression pattern. In oil palm, two copies of the *CAD* gene namely *EgCAD1* and *EgCAD2* have been identified and were equally expressed during oil palm development. Being a monocot with a relatively long life cycle, oil palm presents a great technical challenge for functional analysis of the gene in its native genome. Therefore, in this study, tobacco was chosen as a model plant to transform one of the CAD genes namely *EgCAD2*. In addition, the phylogeny of the CAD gene family from selected woody angiosperms was also constructed. Tobacco plants were transformed with two plasmids (pRI201-CAD2 and pMDC32-H2) based on the standard agrobacterium-mediated transformation protocol using leaf discs as target tissues. Antibiotic screening and PCR analysis of the transformed plants suggested that the transformation protocol was successfully implemented.

Fifty-eight putative transformed plants were obtained based on the amplified *EgCAD2* region of the transformation construct. The transformed plants exhibited normal phenotypes of wild tobacco plants based on the development of major organs at vegetative and reproductive stages. Phylogenetically, the *CAD* gene family was classified into four major groups with the oil palm CAD2 gene clustered together with members of group II. The gene shares the most recent common ancestor with *Eucalyptus grandis, Theobroma cocoa, Malus domestica, Citrus cinensis and Populus trichocarpa.* As a conclusion, the *EgCAD2* gene was successfully integrated into the tobacco genome without interrupting functionally genes involved in normal plant development. The transformed plants would serve as important biological materials for functional characterisation of the oil palm *CAD2* gene especially on its over-expressed effects on lignin deposition.

Keywords : *cinnamyl alcohol dehydrogenase,* lignin, oil palm

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TRANSFORMASI TEMBAKAU DENGAN GEN *CINNAMYL ALKOHOL DEHIDROGENASE* 2 (*CAD2*) DARIPADA KELAPA SAWIT

Oleh

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Cinnamyl alkohol dehydrogenase (CAD) memangkinkan langkah penghad kadar dalam tapakjalan biosintesis monolignol. Monolignol ialah monomer yang bertanggungjawap dalam pembentukan lignin. Dalam tumbuhan, lignin diperlukan untuk membentuk struktur arkitektur pokok dan pertahanan daripada serangan patogen. Enzim CAD dikodkan oleh gen famili yang ahlinya sering mempamerkan kepelbagiana dalam corak ekspresi. Dalam pokok kelapa sawit, dua salinan gen CAD iaitu *EgCAD1* dan *EgCAD2* telah dikenal pasti dan kedua-duanya mempunyai tahap ekspresi yang sama. Sebagai monokot dengan kitar hayat yang panjang, analisis kefungsian gen tersebut dalam kelapa sawit adalah amat mencabar dari segi teknikal. Oleh itu, dalam kajian ini, pokok tembakau telah dipilih sebagai tumbuhan model untuk transformasi salah satu gen tersebut iaitu *EgCAD2*. Di samping itu, filogeni bagi famili gen *CAD* daripada angiosperma berkayu juga telah dibina. Cakera daun tembakau telah ditransformasikan dengan dua plasmid (pRI201-CAD2 dan pMDC32-H2) menggunakan kaedah transformasi berperantara agrobacteria. Penyaringann antibiotik dan analisis PCR bagi tumbuhan yang telah

ditransfomasikan mengesahkan bahawa transformasi telah berjaya dilaksanakan. Lima puluh lapan tumbuhan transgenik telah diperolehi berdasarkan kejayaan untuk mengesan bahagian gen *EgCAD2* dalam genom tembakau. Kesemua pokok transgenik yang diperolehi menunjukkan fenotip normal pokok tembakau liar berdasarkan pemerhatian pada peringkat pertumbuhan vegetatif dan reproduktif. Secara filogenetik, famili gen CAD terbahagi kepada empat kumpulan utama dengan EgCAD2 berada dalam kumpulan II. Gen EgCAD2 didapati berkongsi moyang terkini dengan *Eucalyptus grandis, Theobroma cocoa, Malus domestica, Citrus cinensis* dan *Populus trichocarpa.* Kesimpulannya, gen EgCAD2 yang telah berjaya diintegrasikan ke dalam genom tembakau tanpa mengganggu fungsi gen lain dalam pokok tersebut. Pokok yang telah berjaya di transformasikan itu boleh digunakan dalam pencirian kefungsian gen EgCAD2 terutamanya untuk melihat kesan pengekspresan berlebihan gen tersebut ke atas pengumpulan lignin.

Kata kunci: *cinnamyl alkohol dehydronenase*, Transformasi, Lignin

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APPROVAL

This thesis was submitted to the Department of Cell & Molecular Biology, Faculty of Biotechnology & Biomolecular Science and has been accepted as fulfilment of the requirement for the degree of Bachelor of Science (Hons) Cell & Molecular biology. The member of the Supervisory Committe was as follows:

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DECLARATION

Declaration by undergraduate student

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LIST OF ABBREVIATION

NAA	1-Naphthaleneacetic acid
BAP	6-Benzylaminopurine
CAD	*Cinnamyl alcohol dehydrogenase*
No.	Number

CHAPTER 1

INTRODUCTION

The plant cell wall is made up of three main components namely cellulose, hemicellulose and lignin (Harris & Stone, 2008). The cell wall of all vascular plants contains lignin. Lignin provides mechanical support as well as defence in plants. Without lignin, plants become structurally weak and would not survive against strong wind and collapse. In addition, lignin acts as bulking agent that can resist compression and also acts as a hydrophobic chemical constituent in the cell. Because of the hydrophobic properties of lignin, it further strengthens the cell wall and helps to resist implosion due to rapid flow of water.

In plants, biosynthesis of the lignin is mediated by a series of enymes in the phenylpropanoid pathway. Cinnamyl-alcohol dehydrogenase (CAD) catalyses the committed step of monolignols formation . This committed step is the final step in the synthesis which involves the conversion of cinnamyl aldehyde to alcohols by *NADPH*.

In angiosperms such as oil palm (*Elaeis guineensis*), CAD is encoded by a multigene family. As agene family, the CAD genes are grouped into several classes based on their phylogenetic relationship. Different classes may represent different dedicated functions in plants.

The emergence of lignin in the vascular plant lineage is associated with the origin of the bona fide *CAD* gene. Members of the gene family that are involved in lignification are classed together such as in the case of the class I genes (*AtCAD5* and *AtCAD4*) from arabidopsis which were shown to be involved in lignification (Sibout *et al*., 2003). Homologs of the same class from other plants often exhibited the same function such as the class I *CAD* gene from rice (*OsCAD2*) which had been demonstrated to have a dominant role in lignification (Tobias & Chow, 2005). Other members of the CAD gene family may function differently in different plants but one for certain is that they normally carry other functions not related to lignification (Kiedrowski *et al*., 1992).

In oil palm, the CAD gene family has two genes that have not been functionally characterised previously. Preliminary analysis of the genes indicated that the two genes were expressed during oil palm development. To functionally analyse the function of the genes in oil palm by overexpression approach, the over-expressed CAD gene construct must be integrated into the oil palm genome by transformation. Unfortunately, the transformation protocol of oil palm was not well established and technically difficult based on a general technical agreement that the monocots are difficult to transform. In addition, being a woody monocot, the life cycle of oil palm is relatively long requiring a few years to complete which is not favourable for efficient gene functional analysis. Alternatively, different host plants may be used to study the gene function including Arabidopsis and tobacco. Tobacco may offer better alternative if one wants to carry out laboratory analyses that require massive biomass.

In this study, tobacco plants were chosen as a model plant to transform the oil palm *EgCAD2* gene, a member of the CAD gene family. Tobacco plants have a relatively short live cycle of approximately 30-50 days and the transformation protocol has been well established. Since this plant is not considered as food and feed crops, transforming the plant with oil palm gene would reduce the possibility of environmental contamination by cross-pollination of transgenes which is a major issue in oil palm transgenic studies. Therefore, the initial steps in studying the function of the oil palm *EgCAD2* gene were performed in this study with the following specific objectives:

i. to transform tobacco plants using an established Agrobacterium-mediated transformation protocol

ii. to relate the *EgCAD2* gene with other CAD genes in woody plants using phylogenetic methods

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