UNIVERSITI TEKNOLOGI MARA

FUNCTIONAL CHARACTERIZATION OF *ELAEIS GUINEENSIS CINNAMYL ALCOHOL DEHYDROGENASE 2*

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ABSTRACT

The phenylpropanoid pathway produces numerous essential compounds that contribute to structural support, defense, and adaptation. Among the genes in this pathway, *cinnamyl alcohol dehydrogenase (CAD)* is one of the most crucial. Previous studies have shown that the oil palm genome contains seven *CAD* genes, designated as *EgCAD1-EgCAD7*. Among them, *EgCAD1* and *EgCAD2* were classified as *bona fide CADs*. Since the function of the *EgCAD2* remains unclear, this study aimed to clarify its role. This study aimed to investigate the role of *EgCAD2* in relation to lignin biosynthesis. Transgenic tobacco plants carrying the *EgCAD2* and GUS genes were generated by the *Agrobacterium*-mediated transformation method. The transgenic plants were verified by Polymerase Chain Reaction (PCR), and the PCR-positive lines were subjected to segregation analysis to identify the homozygous T_2 lines. The expression of the transgenes was then evaluated using semi-quantitative Reverse Transcription PCR (RT-PCR). Next, the CAD enzyme activity in the selected transgenic lines was analyzed and compared with that of wild-type tobacco. The transgenic lines were then assessed for visible morphological changes, total lignin, and flavonoid contents. Additionally, the lignin composition was analyzed using High-Performance Liquid Chromatography (HPLC), and the cell wall surface structure was examined using Field Emission Scanning Electron Microscopy (FESEM). The transgenic plants were also evaluated for their response to salt and drought stress. A total of 22 CAD2-OE lines and nine GUS-OE lines were successfully generated and verified. Three CAD2-OE lines (P1, U1, W5) and one GUS-OE line (GUS1), which contain a single transgene integration locus, were selected to produce homozygous T_2 generation. RT-PCR analysis indicated that the *EgCAD2* or GUS genes was present in the T_2 generation and was abundantly expressed in the selected transgenic lines. Furthermore, the increase in CAD activity was accompanied by notable morphological changes, including reduced height, shorter internodes, altered flower coloration, and delays in germination and flowering. Moreover, histochemical staining showed enhanced lignin deposition in the transgenic lines compared to the wild-type. Additionally, an increase in xylem cell wall thickness was observed in the transgenic lines. Elevated lignin accumulation (70-120% increment) in the transgenic lines was confirmed by the acetyl bromide assay. HPLC analysis revealed that the transgenic plants have a higher level of S- and H-units of lignin. Surprisingly, overexpression of *EgCAD2* resulted in a reduction in total flavonoid content by ~41-51%. Additionally, the CAD-OE transgenic lines exhibited higher sensitivity to salt stress but responded similarly to the control plants under drought conditions. In this study, the thickening of the xylem cell wall likely reduced the efficiency of phytohormone diffusion, leading to a delay in flowering time. Meanwhile, the delayed germination time may result from increased lignin accumulation in the seed coat, which inhibits the imbibition process. Furthermore, the reduction in flavonoid content is likely caused by the overexpression of the lignin biosynthetic gene, which leads to a shift in metabolic flux, directing more resources toward lignin production rather than flavonoid production. As flavonoid levels decrease, osmoprotectant levels also decline, making the plant more sensitive to salt stress. In conclusion, the functional analysis of *EgCAD2* suggests that it plays a key role in lignin biosynthesis, affecting plant growth and development, as well as osmotic stress tolerance. These findings provide a valuable basis for targeted manipulation of lignin content in crop improvement and industrial applications.

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INTRODUCTION

1.1 Research Background

Oil palm (*Elaeis guineensis*), which originated in West Africa, has become one of the most important crops in Malaysia. Oil palm was introduced to Malaysia in the early 1870s as an ornamental plant, and the first commercial oil palm planting estate was established at the Tennamaram Estate in Selangor in 1917 (Nambiappan, 2018). As of 2024, the total planted area in Malaysia had reached approximately 5.65 million hectares (Ghulam Kadir et al., 2024). Over the past few decades, the Malaysian oil palm industry has experienced significant expansion, positioning Malaysia as the world's second-largest palm oil producer, after Indonesia. Due to its high yield per hectare, oil palm provides a higher income than other cash crops (Ghulam Kadir et al., 2024).

Despite its widespread cultivation, the oil palm industry in Malaysia faces multiple challenges, such as environmental stress, aging trees, and plant diseases, which hinder the plant's growth (Alam et al., 2015). According to Barcelos et al. (2015), by the end of 2050, global demand for vegetable oils is estimated to reach approximately 240 million tons. However, over the past two decades, the average yield of Malaysian oil palm has remained stagnant, raising concerns about its ability to meet future consumer demand (Yaakub & Husri, 2018). Malaysia's oil palm industry must find solutions to mitigate these issues and ensure the sustainability of the oil palm sector. With advancements in science and technology, biotechnology has been extensively studied, and various methods, including genetic transformation, bioinformatics, and breeding technologies, have been employed to further our understanding of the field. One notable achievement in the oil palm study was the release of a 1.8-gigabase (Gb) *Elaeis guineensis* genome sequence, which provides valuable genetic information for identifying genes of interest in oil palm (Singh et al., 2013). The release of this genome sequence accelerates research and development efforts to address challenges in the oil palm industry by facilitating the production of improved oil palm varieties with higher yields, better oil quality, greater environmental adaptability, and enhanced disease resistance (Yaakub & Husri, 2018).