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Research Article

Isolation of *Alcohol Dehydrogenase* cDNA and Basal Regulatory Region from *Metroxylon sagu*

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Alcohol dehydrogenase (Adh) is a versatile enzyme involved in many biochemical pathways in plants such as in germination and stress tolerance. Sago palm is plant with much importance to the state of Sarawak as one of the most important crops that bring revenue with the advantage of being able to withstand various biotic and abiotic stresses such as heat, pathogens, and water logging. Here we report the isolation of sago palm *Adh* cDNA and its putative promoter region via the use of rapid amplification of cDNA ends (RACE) and genomic walking. The isolated cDNA was characterized and determined to be 1464 bp long encoding for 380 amino acids. BLAST analysis showed that the *Adh* is similar to the *Adh*1 group with 91% and 85% homology with *Elaeis guineensis* and *Washingtonia robusta*, respectively. The putative basal *msAdh*1 regulatory region was further determined to contain promoter signals of TATA and AGGA boxes and predicted amino acids analyses showed several *Adh*-specific motifs such as the two zinc-binding domains that bind to the adenosine ribose of the coenzyme and binding to alcohol substrate. A phylogenetic tree was also constructed using the predicted amino acid showed clear separation of *Adh* from bacteria and clustered within the plant *Adh* group.

1. Introduction

Alcohol dehydrogenase (Adh) is an enzyme involved in various biological activities such as in the germination and abiotic stresses in plants [1–3]. Previous studies have shown that there are between two or three *Adh* loci in flowering plants with exception in *Arabidopsis* [4, 5]. Previous Adh protein work on sago palm, a flood-tolerant plant, by Roslan et al. [6] detected the presence of Adh in the leaf and roots. A higher Adh enzyme expression was observed in sago palm young shoots compared to the other part of *Metroxylon sagu* [6]. The finding was consistent with those of Padmanabhan and Sahi [7] that reported a greater increase in Adh activity in the leaves than the roots of sunflower that was treated with high phosphorus. In contrast, in flood-intolerant plants such as *Arabidopsis* and pea, increased Adh activity was determined in the roots than in the shoots under anaerobic condition [8, 9]. A higher expression level in different tissue and developmental stage may be because the cells are dividing and exposed to many stresses [10].

The discovery of Adh protein expression in young leaf prompted the work to isolate the *Adh* gene from sago palm. The isolation of the regulatory region was also conducted to further understand the regulation of *Adh* in sago palm. *Adh* gene have been isolated from several techniques from a number of plants such as in *Arabidopsis thaliana,* barley, maize [4, 5, 11], and including *Washingtonia robusta*, a member of same Arecaceae family with *Metroxylon sagu* [12, 13].

In this study, we report the isolation of full length *Adh* cDNA and the regulatory sequences from sago palm leaf using RACE and genomic walking methods. Full length cDNA was isolated using the RACE technique that is faster and less laborious compared to the screening of cDNA library by using gene-specific probe [14]. The *Adh* regulatory sequences of *M. sagu* was also isolated using the genomic walking.