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Isolation and Characterization of the GA 20-Oxidase cDNA from Sago Palm (*Metroxylon sagu* Rottb.)

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Abstract. GA 20-oxidase is involved in controlling stem elongation, maturity and flowering. Based on published conserved amino acid sequences of plant GA 20-oxidase cDNA clones, oligonucleotide primers were constructed and used to amplify partial sequence of the GA 20-oxidase gene of *Metroxylon sagu*. A 500 bp PCR product was obtained. BLAST analysis showed that the PCR product was homologous with the GA 20-oxidase gene from other plant species. The PCR product was labelled using the Digoxenin (DIG)-labelling system and used as a probe to obtain the full-length GA 20-oxidase gene sequence of a genomic fragment using the genome walking method. The genomic sequence was used for primer construction which were subsequently used to amplify a full-length cDNA copy of the GA 20-oxidase gene. The cDNA obtained was cloned into a pPCR-Script Amp SK (+) vector for sequence confirmation. The 1161 bp sequence obtained from the cDNA copy was compared with the genomic sequence of GA 20-oxidase. Comparison between genomic and cDNA fragments indicated that the GA 20-oxidase gene from sago palm is comprised of two introns and three exons. Heterologous expression of the GA 20-oxidase cDNA in *Escherichia coli* showed a similar expression pattern as the endogenous GA 20-oxidase of sago palm.

Keywords: DNA sequencing, GA 20-Oxidase, Genome Walking, Sago palm, Southern hybridization, Western hybridization.

INTRODUCTION

Sago palm (*Metroxylon sagu*) is one of the few tropical crops that can tolerate wet growing conditions such as peat swamps. In Sarawak, sago is grown as a starch crop by rural communities living along the coastal areas of certain districts. The total acreage of sago in Sarawak is about 65,000 hectares (ha) of which 45,000 ha are held by smallholders and 20,000 ha consist of plantations. About 75% of the sago planting growing area is located in the Mukah, Igan and Oya-Dalat districts of Sibu Division and Balingian (Tie *et al.*, 1991). There is also a substantial acreage of sago in the Pusa and Saratok districts of Sarawak. The total export of Sarawak sago starch in the year 2010 was 44,192 tons.

Research on sago has begun to gain momentum since the 1970's (Stanton, 1972). Initially R&D focused on agronomic practices to improve growth and yield but later emphasis was placed on downstream applications of sago starch. One of the key issues for sago which needs to be critically addressed is that the palm takes about 10-12 years to reach maturity depending on soil type, while other starch producing crops, potato and cassava, take only 3 and 6 months, respectively (Chulavatnatol, 2002). This reduces the competitiveness of sago as compared to other starch producing crops.

In most quickly maturing plant species, conventional plant breeding techniques are very successful in generating new elite varieties. This technique is unsuitable however for slowly maturing plants such as sago palm. Sago is a hapaxanthic palm flowering only once at the end of its lifetime. Moreover quality palms are harvested prior to flowering for maximum starch yield. Therefore, an alternative breeding approach needs to be explored and developed.

Molecular breeding techniques appear to be the best option to obtain new improved varieties of sago palm. This technique allows researchers to identify and manipulate the potential genes encoding for desired traits, and transform it into the host plant within a shorter time frame. A number of genes involved in the starch biosynthetic pathway have been studied in sago palm (Salleh *et al.*, 2000; Salleh & Lau, 2003; Salleh *et al.*, 2004), however no reports have been published to date concerning the GA 20-oxidase gene of sago. GA 20-oxidase plays an important role in the biosynthetic pathway of growth regulators that control various aspects of plant development, such as seed germination, stem elongation, flower formation and fruit production (Hooley *et al.*, 1994; Swain & Olszewski, 1996; Weiss *et al.*, 1992). This gene was found to be expressed at a high level in leaves compared to expression in the internodes (Garcia-Martinez *et al.*, 1997). A study of GA 20-oxidase in the rice variety IR8 demonstrated that the mutant form of this gene (*sd*-1)

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