STEAROYL ACP DESATURASE GENE FROM *Jessenia bataua* FOR HIGH OLEATE OIL MANIPULATION

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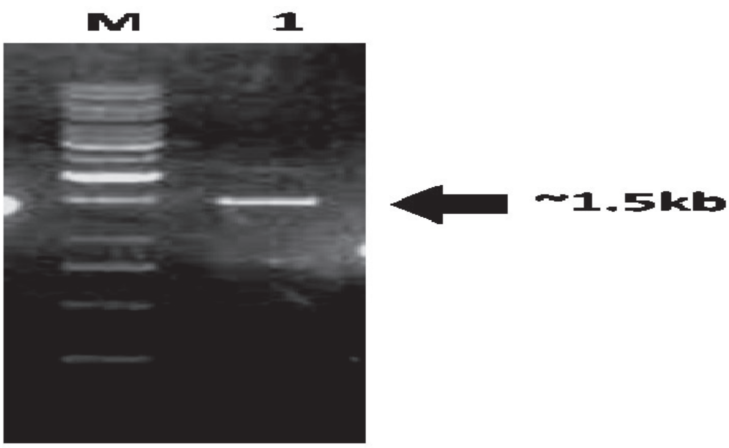
Numerous attempts have been made in the past to alter oil compositions in crops by introducing exogenous copies or anti-sense expression of endogenous candidate genes through genetic manipulation. In oil palm (*Elaeis guineensis*), strategies to manipulate palm oil composition involve manipulating the activity of key genes involved in fatty acid biosynthesis namely, β-ketoacyl-acyl-carrier-protein [ACP] synthase (KAS) II, stearoyl-ACP desaturase (SAD) and palmitoyl ACP thioesterase (FatB) (Parveez *et al*., 2010). As shown in other crops, modification of endogenous fatty acids could also be done by introducing candidate genes from other crops such as *Jessenia bataua* which produces an oil high in oleic acid (~78% of the total fatty acid composition). *J. bataua* was introduced into Malaysia as one of the exotic palm species during the initial expedition era by oil palm breeders to collect exotic germplasm in South America (Rajanaidu *et al*., 1991).

Jessenia palms (*Figure 1*) are being grown in the form of open-pollinated families mainly at MPOB Research Stations in an effort to evaluate their yield performance and agronomic traits. Today, MPOB houses the world's largest oil palm germplasm collection. Useful traits from this collection are being incorporated into the oil palm breeding programme. Realizing the potential of *Jessenia* as a new crop that yields high quality edible oil, we attempted to isolate fatty acid biosynthetic genes from *Jessenia* that can be used for manipulating palm oil and other oil crops. Although *Jessenia* is very valuable in terms of oil quality and could become an immediate option as an acceptable substitute to olive oil, it has received minimal attention, and efforts towards the goal of domesticating this species have scarcely begun. One of the reasons for this could be its very poor yield and slow growth rate compared to other species such as oil palm. As the properties of oils are determined by the fatty acid composition which in turn affects nutritional quality, we aimed to enhance the value of *Jessenia* by using the limited plant materials available as the source of genes, in particular those involved in fatty acid biosynthesis (*e.g.* SAD and KAS II).

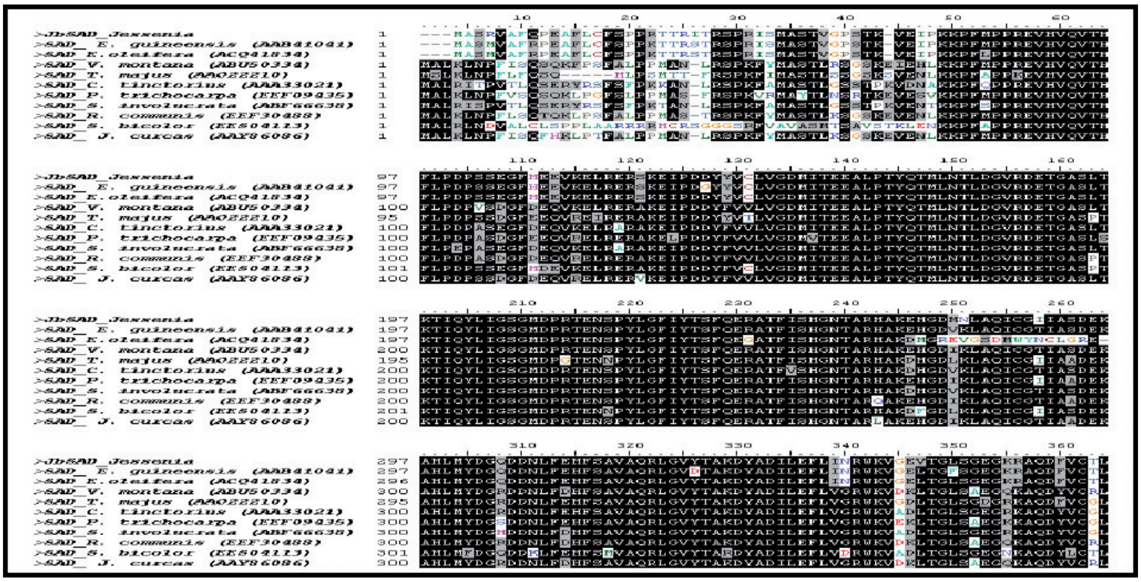
Figure 1. Jessenia *palm and the fruit. The thin mesocarp of* Jessenia *was used to prepare total RNA for isolating the JbSAD gene.*

ISOLATION OF STEAROYL ACP DESATURASE GENE FROM *Jessenia bataua*

Stearoyl-ACP desaturase (SAD) is one of the key enzymes involved in oleic acid biosynthesis. SAD is involved in the desaturation of stearoyl-ACP (C18:0-ACP) and introduces the first double bond to generate oleic acid (C18:1). The full length cDNA of SAD (named *JbSAD*) was isolated from the mesocarp of *J. bataua* (*Figure 2a*), and recombinant protein expression was obtained in *E. coli* to ensure the enzymatic activity of the protein. The full-length size of *Jb*SAD cDNA is 1540-bp with a 1182-bp open reading frame encoding a 30-amino acid signal peptide and a 363-amino acid mature peptide. The deduced amino acid sequence of *Jb*SAD shares approximately 90% sequence identity with SAD from other plants, and has acyl-ACP desaturase conserved domains



(a)



(b)

Figure 2 (a). The full-length JbSAD, and (b) homology search of JbSAD compared to SAD from other crops (dark area shows homology).

(*Figure 2b*). Application of the *Jb*SAD gene in the bacteria system *E. coli* as a His-tagged fusion protein was conducted. *Jb*SAD migrated as a 60kDa protein on SDS-PAGE, and detection of the recombinant protein was performed by Western blot analysis using His-tagged monoclonal antibody (*Figure 3*). Molecular masses of the purified peptide from the SDS-PAGE were confirmed by MALDI-TOF mass spectrometry (*Figure 4*). Analysis of protein homology revealed similarity to SAD from oil palm and other crops. In addition, there was a significant increase in oleic acid (~ 10%) from the transformed *E. coli*, thus confirming the functional activity of the *Jb*SAD gene.

BENEFITS

Application of *Jb*SAD in genetic modification for manipulating fatty acid compositions of oil palm and other crops towards producing higher oleic acid (over expression) or stearic acid (antisense expression).

INTELECTUAL PROPERTY

A patent will be filed for *Jb*SAD gene application in *E. coli*.

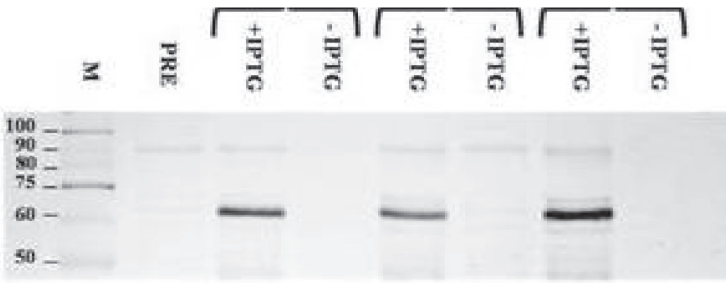


Figure 3. Western blot analysis of JbSAD expressed in E. coli.

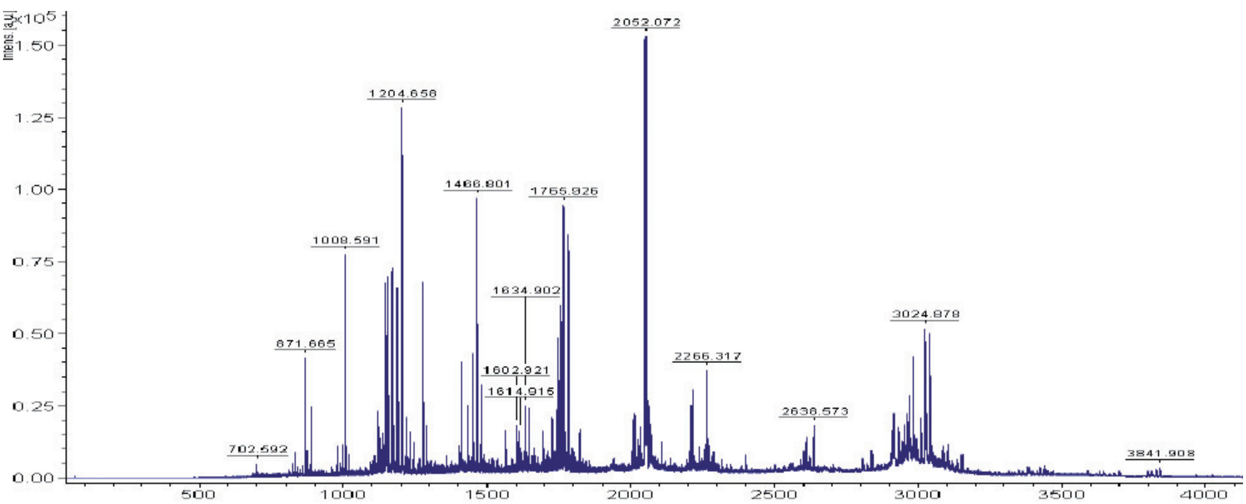


Figure 4. MALDI-TOF mass spectrometry analysis of JbSAD protein expressed in E. coli.

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