

Modulating endogenous levels and/or production of novel fatty acids of oils has gained significant attention in recent years due to the increasing awareness by consumers of the impact that dietary lipids have on health. Commodity palm oil is composed of four main fatty acids: palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1) and linoleic acid (18:2). The percentage of these fatty acids in palm oil averages 44%, 4%, 39% and 10%, respectively, with trace amounts of other fatty acids. Metabolic engineering may be used to produce oil crops with desired fatty acid composition.

GENE MANIPULATION

MPOB has isolated and characterised β -ketoacyl ACP-synthase II (KASII) cDNA from a high-oleic acid palm, *Jessenia bataua*. As shown in other crops, modification of fatty acids can also be done by introducing candidate genes from other crops such as *J. bataua*, which produces an oil high in oleic acid (~78% of the total fatty acid composition). *J. bataua* was introduced into Malaysia by MPOB as one of the exotic palm species after an expedition to collect exotic germplasm in South America (Rajanaidu *et al.*, 1991). *Jessenia* palms (Figure 1) are being grown in the form of open-pollinated family populations in MPOB Research Stations in an effort to evaluate their yield performance and agronomic traits. Realising the potential of *Jessenia* as a new crop that yields high quality edible oil, MPOB has attempted to isolate fatty acid biosynthetic genes from *Jessenia* that can be used for manipulating the oil composition of oil palm and other crops. Although *Jessenia* is very

valuable in terms of oil quality, and can become an immediate option as an acceptable substitute to olive oil, it has received minimal attention. 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 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 aim to enhance the value of *Jessenia* by using the limited plant materials available as the source of desirable genes, in particular those involved in fatty acid biosynthesis (*e.g.* stearyl ACP desaturase (SAD) and KAS II). The potential of the SAD gene from *Jessenia* in genetic manipulation for the synthesis of oleic acid has been previously described by Ramli *et al.* (2010).

ISOLATION OF KASII GENE FROM *Jessenia bataua*

Jessenia KASII (*Jb*KASII) encodes a 488-amino acid polypeptide that possesses conserved domains which are necessary for condensing activities (Figure 2). *Arabidopsis* plants expressing GFP-*Jb*-KASII fusions had elevated levels of arachidic acid (C20:0) and erucic acid (C22:1) at the expense of stearic acid (C18:0) and oleic acid (C18:1) (Figure 3a). Furthermore, *Jb*KASII failed to complement the *Arabidopsis* KASII mutant, *fab1-2* (Figure 3b). The results suggest that *Jb*KASII preferentially elongates stearic and oleic acids, and not palmitic acid. Our results suggest that *Jb*KASII may elongate C18:0 and C18:1 to yield C20:0 and C22:1, respectively (Teh and Ramli, 2010). *Jb*KASII may, therefore, be a candidate gene for the synthesis of very long chain fatty acids in oil crops.



Figure 1. *Jessenia* palm and cross-section of *Jessenia* (right) and oil palm (left) fruits.

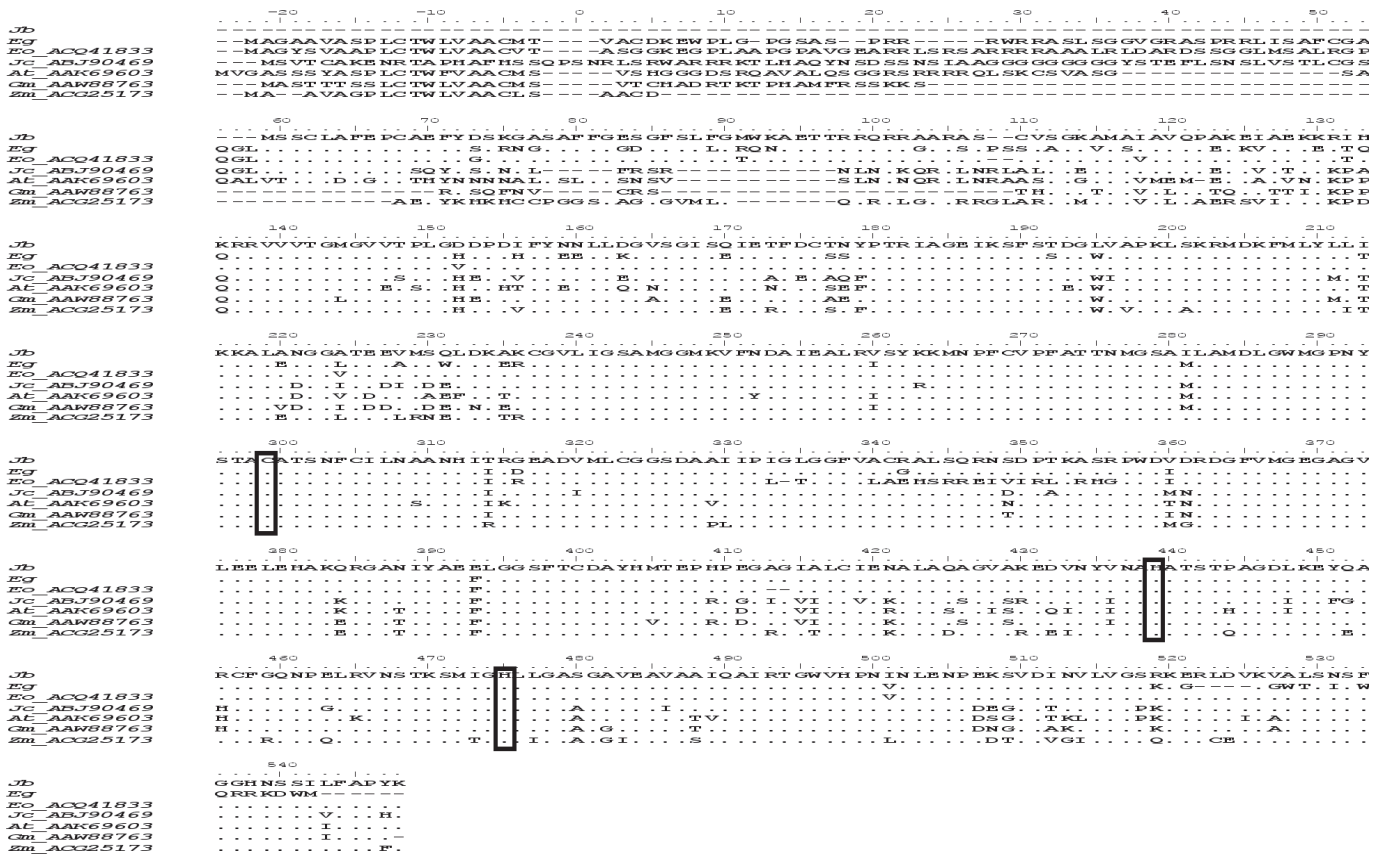


Figure 2. ClustalW multiple sequence alignment of KASII proteins from plants. The amino acid sequence of JbKASII was highly similar to that of the KASII homologs from other plant species, with the active site triad motif, Cys299-His439-His475 (in rectangular boxes), being present in all KASII proteins identified in plants. Using JbKASII as the reference sequence, identical amino acids are indicated as dots. The accession numbers for *Elaeis oleifera* (Eo, ACQ41833), *Jatropha curcas* (Jc, ABJ90469), *Arabidopsis thaliana* (At, AAK69603), *Glycine max* (Gm, AAW88763) and *Zea mays* (Zm, ACG25173) are indicated after the species names. The protein sequences of *Jessenia bataua* (Jb) and *Elaeis guineensis* (Eg) have yet to be submitted to GenBank. Dashed lines with open and oval arrows indicate the positions of the highly conserved N-terminal and C-terminal KASII domains, respectively.

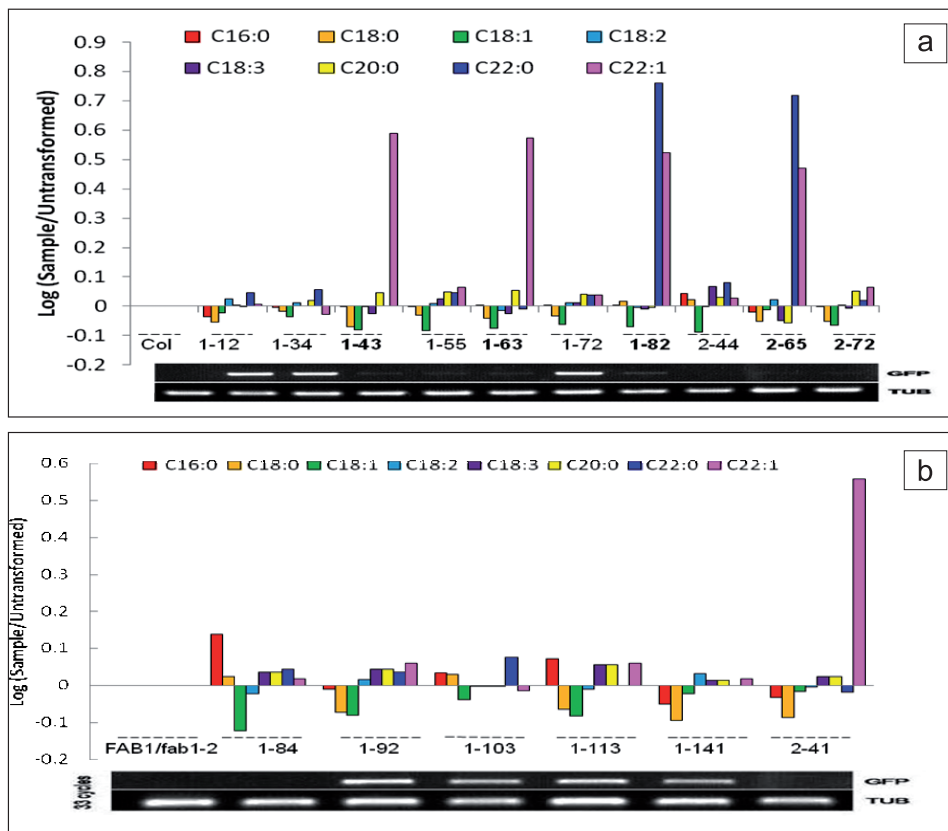


Figure 3. Fatty acid and real-time-PCR analysis of transgenic *Arabidopsis* plants expressing JbKASII. (a) Fatty acid profiles of 10 Col lines and (b) six FAB1/ fab1-2 lines, expressing JbKASII cDNA GFP fusion constructs. The numbers below the chart indicate independent T3 transgenic lines and their relative composition of major fatty acids expressed as log (sample/untransformed or parental line). Semi-quantitative real-time-PCR analysis of the level of GFP transcripts in the various transgenic lines was performed on RNA extracted from 12-day old seedlings. The *Arabidopsis tubulin* (TUB) gene was used as the loading control.

BENEFITS

Genetic engineering of JbKASII for modifying the fatty acid compositions of oils from oil palm and other crops towards producing very long chain fatty acids.

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