UNIVERSITI PUTRA MALAYSIA

CLONING, EXPRESSION AND CHARACTERIZATION OF A STEAROYL-ACP DESATURASE GENE FROM Jessenia bataua mart, var. bataua

MUHAMAD SYAFIQ BIN SHARIAN

FBSB 2012 41

CLONING, EXPRESSION AND CHARACTERIZATION OF STEAROYL-ACP DESATURASE GENE FROM *Jessenia bataua* Mart. *var. bataua*

By

MUHAMAD SYAFIQ BIN SHARIAN

© COPYRIGHT UPM

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Science

July 2012

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master in Science

CLONING, EXPRESSION AND CHARACTERIZATION OF STEAROYL-ACP DESATURASE GENE FROM *Jessenia bataua* Mart. *var. bataua*

By

MUHAMAD SYAFIQ BIN SHARIAN

July 2012

Chair: Parameswari Namasivayam, PhD

Faculty: Faculty of Biotechnology and Biomolecular Sciences

Jessenia bataua is one of the exotic palms species planted mainly at the Malaysian Palm Oil Board research stations for assessment of yield and agronomic traits. *J. bataua* was introduced to Malaysia by the oil palm breeders in the eighties and it was brought from South America. *J. bataua* produces relatively low oil yield despite the high value of the oil which contains 78% of oleic acid and 13% of palmitic acid. The oil resembles the olive oil in taste and chemical composition which makes this crop an exciting prospects in meeting the needs of highly unsaturated oil. To date, little is known about the biology and particularly the fatty acid biosynthesis in *J. bataua* palm. Stearoyl-ACP desaturase (SAD) is an important fatty acid biosynthetic enzyme responsible for the production of oleic acid. It is a soluble enzyme in the plastid which introduces a *cis* double into saturated stearoyl-ACP (18:0-ACP) at the Δ^9 position to produce monounsaturated oleoyl-ACP (18:1-ACP). It has an important housekeeping role for producing unsaturated fatty acids for membrane lipid biosynthesis. In oil accumulating tissues like anthers, seeds and mesocarp, it is

© COPYRIGHT UPM

involved in the developmentally regulated process of storage lipid biosynthesis. Oleic acid is an omega-nine fatty acid, and considered as one of the healthier sources of fat in the diet. In this study, the full-length of SAD cDNA namely *Jb*SAD has been cloned and characterized from the mesocarp of *J. bataua* by RT-PCR and RACE techniques, respectively. The objectives of this study were; to isolate and characterize SAD cDNA from the mesocarp tissue of *J. bataua* and to express SAD recombinant cDNA of *J. bataua* in *Escherichia coli* system. The full-length of *Jb*SAD cDNA clone is ≈ 1540 bp with 1182 bp open reading frame encoding for a 30-amino acid signal peptide and a 363-amino acid mature peptide. The predicted molecular mass and isoelectric point of *Jb*SAD is ≈ 45.04 kDa and 5.91, respectively. The deduced amino acid sequence of the *Jb*SAD shares approximately 81% sequence identity to SAD from the other plants, with the highest homology to that of oil palm (≈ 96%). *Jb*SAD also contains an acyl-acyl carrier protein-desaturase (acyl-ACP-desaturase) conserved domain which is a mu-oxo-bridged diiron-carboxylate enzyme that belongs to a broad superfamily of ferritin-like proteins. Southern blot analysis using acyl-ACP-desat-specific probe revealed that the *Jb*SAD is a multiple-copy genes. Functional studies were also performed by expressing the *Jb*SAD as a His-tagged cDNA fusion protein in *E. coli*. Upon induction by 1 mM IPTG and incubation at a different temperatures, *Jb*SAD migrated as a ≈ 60 kDa protein on SDS-PAGE, in agreement with the predicted of 59.61 kDa molecular mass. Identification of protein band corresponds to His-tagged-*Jb*SAD protein fusion (≈ 60 kDA) was achieved by using the Western blot experiment. The fatty acid composition of the transformed *E. coli* was identified by the Gas Chromatography. The result showed that there was an increased in the relative proportion of oleic acid

© COPYRIGHT UPM

(by ≈ 15%) by overexpressing *Jb*SAD in *E. coli*. The data suggested that *Jb*SAD cDNA expressed in *E .coli* was in its active form.

© COPYRIGHT UPM

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

PENGKLONAN, PENGEKSPRESAN DAN PENCIRIAN GEN STEAROYL-ACP DESATURASE DARI *Jessenia bataua* Mart. *Var. bataua*

Oleh

MUHAMAD SYAFIQ BIN SHARIAN

Julai 2012

Pengerusi: Parameswari Namasivayam, PhD

Fakulti: Fakulti Bioteknologi and Sains Biomolekular

Jessenia bataua adalah salah satu tumbuhan palma eksotik yang ditanam di stesen penyelidikan Lembaga Minyak Sawit Malaysia dengan tujuan untuk pemantauan prestasi pengeluaran hasil buah dan ciri-ciri agronominya. Ia telah dibawa ke Malaysia oleh ahli pembiak bakaan kelapa sawit pada tahun 80-an dari Amerika Selatan. *J. bataua* menghasilkan minyak yang agak sedikit walaupun bernilai tinggi. Dengan lebih kurang 78% asid oleik dan 13% asid palmitik. Dari aspek rasa dan komposisi kimia, minyak *J. bataua* sangat hampir kepada minyak zaitun. Oleh yang demikian, tumbuhan ini mempunyai prospek yang menarik dalam usaha meningkatkan pengeluaran dan memenuhi pemintaan pasaran minyak tidak tepu. Sehingga kini, tidak banyak yang diketahui mengenai biologi dan biosintesis asid lemak *J. bataua*. Menyedari potensi tersebut, kajian telah dijalankan untuk memencilkan cDNA Stearoyl-ACP Desaturase (SAD) yang terlibat di dalam tapak jalan penghasilan asid lemak *J. bataua* dan selanjutnya mengekspreskan cDNA rekombinasi SAD di dalam sistem bakteria *Escherichia. coli*. Jujukan penuh cDNA

© COPYRIGHT UPM

SAD *J. bataua* (*JbSAD*) telah berjaya dipencilkan dari tisu mesocarpa *J. bataua* menggunakan teknik RT-PCR dan RACE. Saiz klon tersebut adalah ≈ 1540 bp, dengan 1182 bp bingkai bacaan terbuka, 30 asid amino isyarat peptida dan 363 asid amino peptida matang. Berat molekular protein tersebut adalah lebih kurang 45.04 kDa dan titik isoelektrik jangkaannya adalah 5.91. Jujukan asid amino *Jb*SAD yang diperolehi menunjukkan homologi yang tinggi (purata 81%) dengan SAD dari tumbuhan-tumbuhan lain dengan homologi yang paling tinggi (lebih kurang 96%) dengan SAD dari kelapa sawit. *Jb*SAD juga mengandungi 2 jujukan terpelihara iaitu jujukan terpelihara "Acyl-Acyl Carrier Protein Desaturase" (Acyl-ACP-Desat) yang mengandungi "mu-oxo-bridged diiron-carboxylate enzyme" yang dikategorikan di dalam keluarga "ferritin-like protein". Pemblotan Soutern juga menunjukkan bahawa *Jb*SAD adalah gen "multiple-copy". Kajian pengekspresan juga telah dijalankan dengan mengekspreskan *Jb*SAD sebagai protein gabungan "His-tagged". Dengan rangsangan 1 mM IPTG dan inkubasi pada suhu berlainan, *Jb*SAD muncul sebagai protein bersaiz ≈ 60 kDa pada SDS-PAGE, dan ini berpadanan dengan saiz jangkaan protin iaitu 59.61 kDa. Identifikasi jalur protein protein gabungan His-tagged-SAD (≈ 60 kDA) dijalankan dengan menggunakan teknik pemblotan Western. Manakala analisis komposisi asid lemak perumah *E. coli* telah dilakukan dengan menggunakan teknik kromatografi gas. Didapati terdapat peningkatan peratusan asid oleik sehingga 15% akibat pengekspressan *Jb*SAD di dalam perumah *E. coli* ini menunjukkan *Jb*SAD yang diekspreskan adalah dalam keadaan aktif dan kofaktor yang hadir di dalam *E. coli* mampu memenuhi keperluan proses penyah tepuan oleh *Jb*SAD.

© COPYRIGHT UPM

ACKNOWLEDGEMENTS

Bismillahirrahmanirrahim, First and foremost, I thank Allah SWT for endowing me with health, patience, and knowledge to complete this work.

I would like to express my deep and sincere gratitude to my supervisor, Assoc. Prof. Dr. Parameswari Namasivayam, Her wide knowledge and her logical way of thinking have been of great value for me. I am also grateful to my other supervisory committee member, Assoc. Prof. Dr. Ho Chai Ling, for her constructive guidance, valuable advice and cooperation.

I owe my most sincere gratitude to Dr. Umi Salamah bt Ramli, who gave me the opportunity to work under your group and gave me untiring help and support during my difficult moments.

The special thank goes to my helpful friend, Dr. Teh Ooi Kock. The wide knowledge and support that he gave, truly help the progression and smoothness of the lab work progress. I want to thank my former colleagues from the Division of Biotechnology and Advanced Breeding for all their help, support, interest and valuable hints.

Last but not least, I would like to give my special thanks to my parents, Tn. Haji Sharian b. Ahmad, Pn. Hajjah Noraini bt Mohd Ariffin, my wife, Farah bt Ikram and also my big family for the encouragement and love toward my successful research

© COPYRIGHT UPM

I certify that a Thesis Examination Committee has met on 27th September 2012 to conduct the final examination of Muhamad Syafiq Bin Sharian on his thesis entitled "Cloning, expression and Characterization Of Stearoyl-ACP Desaturase (SAD) gene From *Jessenia Bataua*" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Masters in Science.
Members of the Thesis Examination Committee were s follows:

Sieo Chin Chin, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairperson)

Tan Wen Siang, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Adam Leow Thean Chor, PhD
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Clemente Michael Wong Vui Ling, PhD
Associate Professor
Timbalan Pengarah (Akademik & HEP)
Universiti Malaysia Sabah,
(External Examiner)

SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date : 19 December 2012

© COPYRIGHT UPM

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Parameswari Namasivayam, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Ho Chai Ling, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Umi Salamah bt Ramli, PhD
Senior Research Officer
Advanced Biotechnology and Breeding Division
Malaysian Palm Oil Board
(Member)

_____
BUJANG KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

© COPYRIGHT UPM

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

_____

MUHAMAD SYAFIQ BIN SHARIAN

Date: 27 July 2012

© COPYRIGHT UPM

TABLE OF CONTENTS

© COPYRIGHT UPM

© COPYRIGHT UPM

© COPYRIGHT UPM