



**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**

**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  $\Delta^9$  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

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 *JbSAD* 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 *JbSAD* cDNA clone is  $\approx 1540$  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 *JbSAD* is  $\approx 45.04$  kDa and 5.91, respectively. The deduced amino acid sequence of the *JbSAD* shares approximately 81% sequence identity to SAD from the other plants, with the highest homology to that of oil palm ( $\approx 96\%$ ). *JbSAD* 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 *JbSAD* is a multiple-copy genes. Functional studies were also performed by expressing the *JbSAD* as a His-tagged cDNA fusion protein in *E. coli*. Upon induction by 1 mM IPTG and incubation at a different temperatures, *JbSAD* migrated as a  $\approx 60$  kDa protein on SDS-PAGE, in agreement with the predicted of 59.61 kDa molecular mass. Identification of protein band corresponds to His-tagged-*JbSAD* protein fusion ( $\approx 60$  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

(by  $\approx 15\%$ ) by overexpressing *JbSAD* in *E. coli*. The data suggested that *JbSAD* cDNA expressed in *E. coli* was in its active form.



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

**PENGLONAN, 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 permintaan 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

SAD *J. bataua* (*JbSAD*) telah berjaya dipencilkan dari tisu mesocarpa *J. bataua* menggunakan teknik RT-PCR dan RACE. Saiz klon tersebut adalah  $\approx 1540$  bp, dengan 1182 bp bingkai bacaan terbuka, 30 asid amino isyarat peptida dan 363 asid amino peptida matang. Berat molekul protein tersebut adalah lebih kurang 45.04 kDa dan titik isoelektrik jangkannya adalah 5.91. Jujukan asid amino *JbSAD* 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. *JbSAD* 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 *JbSAD* adalah gen “multiple-copy”. Kajian pengekspresan juga telah dijalankan dengan mengekspreskan *JbSAD* sebagai protein gabungan “His-tagged”. Dengan rangsangan 1 mM IPTG dan inkubasi pada suhu berlainan, *JbSAD* muncul sebagai protein bersaiz  $\approx 60$  kDa pada SDS-PAGE, dan ini berpadanan dengan saiz jangkakan protin iaitu 59.61 kDa. Identifikasi jalur protein protein gabungan His-tagged-SAD ( $\approx 60$  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 *JbSAD* di dalam perumah *E. coli* ini menunjukkan *JbSAD* yang diekspreskan adalah dalam keadaan aktif dan kofaktor yang hadir di dalam *E. coli* mampu memenuhi keperluan proses penyah tepuan oleh *JbSAD*.

## 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



I certify that a Thesis Examination Committee has met on 27<sup>th</sup> 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

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:

## 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



## TABLE OF CONTENTS

|   | <b>Page</b> |
|---|-------------|
| <b>ABSTRACT</b>   | ii          |
| <b>ABSTRAK</b>  | v           |
| <b>ACKNOWLEDGEMENTS</b>   | vii         |
| <b>APPROVAL</b>   | viii        |
| <b>DECLARATION</b>  | x           |
| <b>LIST OF TABLE</b>  | xiv         |
| <b>LIST OF FIGURE</b>   | xvi         |
| <b>LIST OF ABBREVIATIONS</b>  | xix         |
| <b>CHAPTER</b>  |             |
| <b>1 INTRODUCTION</b>   | <b>1</b>    |
| <b>2 LITERITURE REVIEW</b>  | <b>5</b>    |
| 2.1 Fatty Acid Biosynthesis in Plants   | 5           |
| 2.1.1 Introduction  | 5           |
| 2.1.2 Acyl Chain Elongation   | 6           |
| 2.1.3 Desaturation, Termination and Release of Fatty Acids  | 8           |
| 2.1.4 Triglycerides Formation   | 9           |
| 2.2 Fatty Acid Desaturase Enzymes   | 12          |
| 2.2.1 Introduction  | 12          |
| 2.2.2 Classes of Fatty Acid Desaturases   | 12          |
| 2.2.3 Soluble Plant $\Delta^9$ SAD  | 14          |
| 2.2.4 The Structures and Coding Properties of $\Delta^9$ SAD                                      | 16          |
| 2.2.5 Plant $\Delta^9$ SAD Enzyme and Their Structures  | 17          |
| 2.2.6 $\Delta^9$ SAD Mechanism and Regiospecificities of Desaturases                              | 22          |
| 2.2.7 Genetic Engineering of Plants for $\Delta^9$ SAD  | 24          |
| 2.3 <i>Jessenia bataua</i>  | 26          |
| 2.3.1 Introduction  | 26          |
| 2.3.2. Principal use: Beverage production and mesocarp oil  | 29          |
| 2.3.3 Secondary uses: Food, Construction Materials, Medicines, Toys, Weapons, Medicinal and Fibre | 31          |
| 2.3.4 Chemical-nutritive and industrial analysis  | 32          |
| <b>3 MATERIALS AND METHODS</b>  | <b>34</b>   |
| 3.1 Materials   | 34          |
| 3.1.1 Plant Materials   | 34          |
| 3.1.2 Bacteria and Growth Media   | 34          |
| 3.1.3 Oligonucleotides  | 35          |

|          |  |            |
|----------|--|------------|
| 3.1.4    | Antibody   | 35         |
| 3.1.5    | Chemicals  | 35         |
| 3.2      | Total RNA Extraction   | 36         |
| 3.3      | Total RNA Analysis   | 37         |
| 3.4      | Rapid Amplification of cDNA Ends (RACE) – PCR  | 38         |
| 3.5      | Amplification of the Full Length SAD cDNA from <i>J. Bataua</i>  | 40         |
| 3.6      | <i>In silico</i> Analysis of DNA and Protein Sequences   | 41         |
| 3.7      | Southern Blot Analysis   | 42         |
| 3.7.1    | Genomic DNA Extraction   | 42         |
| 3.7.2    | Southern Blot analysis   | 44         |
| 3.7.3    | Probe Labelling  | 44         |
| 3.7.4    | DNA Hybridization  | 45         |
| 3.8      | Expression of <i>J.bataua</i> SAD in <i>E. coli</i>  | 46         |
| 3.8.1    | Amplification of <i>JbSAD</i> Open Reading Frame   | 46         |
| 3.8.2    | Cloning of <i>JbSAD</i> into pET-32 Expression Vector  | 49         |
| 3.8.3    | Determining the Orientation of DNA Insert  | 50         |
| 3.9      | Characterization of Fusion Protein   | 51         |
| 3.9.1    | Recombinant Protein Extraction   | 51         |
| 3.9.2    | SDS-PAGE Analysis  | 52         |
| 3.9.3    | Western blotting   | 53         |
| 3.10     | Fatty Acid Analysis  | 55         |
| 3.10.1   | Fatty Acid Extraction  | 55         |
| 3.10.2   | Fatty Acid Methyl Esther (FAME) Analysis   | 56         |
| 3.10.3   | Statistical Analysis   | 57         |
| <b>4</b> | <b>RESULTS AND DISCUSSION</b>  | <b>58</b>  |
| 4.1      | Isolation and Molecular Characterization of $\Delta^9$ Stearoyl-ACP Desaturase ( <i>JbSAD</i> ) cDNA from <i>Jessenia bataua</i> | 58         |
| 4.2      | Total RNA Extraction   | 58         |
| 4.3      | Rapid Amplification cDNA Ends-PCR (RACE-PCR)   | 62         |
| 4.4      | Direct Amplification of Stearoyl-ACP Desaturase ( <i>JbSAD</i> ) Full Length cDNA  | 63         |
| 4.5      | Southern Blot Analysis   | 76         |
| 4.6      | Cloning of <i>J. bataua</i> Stearoyl-ACP Desaturase ( <i>JbSAD</i> ) into pET-32 Expression Vector                               | 81         |
| 4.7      | Orientation Analysis of <i>JbSAD</i> Fusion DNA Insert   | 84         |
| 4.8      | Production of <i>JbSAD</i> Fusion Protein  | 88         |
| 4.9      | Expression Studies of <i>JbSAD</i> in <i>E. coli</i>   | 94         |
| <b>5</b> | <b>CONCLUSION</b>  | <b>101</b> |
|          | <b>REFERENCES</b>  | <b>103</b> |

|  |     |
|--|-----|
| <b>APPENDICES</b>  | 124 |
| <b>A</b> Alignment of various GADPH gene in order the design the forward and the reverse primers   | 125 |
| <b>B</b> Map of pCR <sup>TM</sup> 2.1-TOPO cloning vector  | 127 |
| <b>C</b> Map of pBluescript II SK (+/-) cloning vector   | 128 |
| <b>D</b> Map of plasmid pET-32a-c(+)   | 129 |
| <b>E</b> Sequence alignment analysis of the 3' end and 5' end RACE products  | 130 |
| <b>F</b> Sequence alignment analysis of the <i>EcoR1-JbSAD-TSP-NotI</i> with the full-length sequence information of SAD from <i>J. Bataua</i> .   | 132 |
| <b>G</b> Sequence alignment analysis of the <i>EcoR1-JbSAD- NotI</i> with the full-length sequence information of SAD from <i>J. Bataua</i> .  | 133 |
| <b>H</b> Percentage of the targeted fatty acid (C16:1, C16:0, C18:0, C18:1) of the <i>JbSAD</i> cDNA transformed <i>E. coli</i> strain Origami <sup>TM</sup> (DE3) and control <i>E. coli</i> strain Origami <sup>TM</sup> (DE3)(untransformed).           | 134 |
| <b>I</b> Percentage of the targeted fatty acid (C16:1, C16:0, C18:0, C18:1) of the <i>JbSAD</i> cDNA transformed <i>E. coli</i> strain Rosetta-gami <sup>TM</sup> (DE3) and control <i>E. coli</i> strain Rosetta-gami <sup>TM</sup> (DE3)(untransformed). | 135 |
| <b>BIODATA OF STUDENT</b>  | 136 |
| <b>LIST OF PUBLICATION</b>   | 137 |