UNIVERSITI PUTRA MALAYSIA

ISOLATION AND CHARACTERIZATION OF CELLULOSE SYNTHASE GENE FROM ROSELLE (Hibiscus sabdariffa L.)

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FBSB 2014 7

ISOLATION AND CHARACTERIZATION OF CELLULOSE SYNTHASE GENE FROM ROSELLE (*Hibiscus sabdariffa* L.)

By

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

June 2014

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

ISOLATION AND CHARACTERIZATION OF CELLULOSE SYNTHASE GENE FROM ROSELLE (*Hibiscus sabdariffa* L.)

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June 2014

Chairman: Professor. Tan Soon Guan, PhD

Faculty: Biotechnology and Biomolecular Sciences

Hibiscus sabdariffa (Roselle) has the potential to be an alternative fibre source for cellulose as a strengthening material in polymer composites. A literature survey has shown scant information regarding utilization of this fiber as reinforcing material. Although roselle was hypothesized to be a potential natural fibre source, there is no study focusing on the fundamental genetics underlying the cellulose biosynthesis machinery in roselle. Isolation of CesA gene is important to understand the biosynthesis pathway in roselle. Therefore, the first cellulose synthase gene (*HsCesA1*) of *H. sabdariffa* was isolated and characterized. Full-length *HsCesA1* cDNA of 3528 bp was isolated using RACE PCR, and the start and stop codons, poly A signal, poly A tail, 3´ UTR and 5´ UTR were identified using in silico analyses. The full-length *HsCesA1* gene with a total length of 5489 bp, which consisted of 12 exons, 11-introns and a promoter region of 737 bp was also isolated using PCR walkin and genome walking repectively. Several important and conserved characteristics were predicted in the HsCesA1 deduced amino acid sequence such as Cellulose-synt, Glycosyltransferase family A (GT-A), bcsA, Zn-finger domain, RING/U-box and domains A and B. These predicted characteristics provided evidence that the isolated gene is possibly a *CesA* and it belongs to the processive class in the glycosyltransferase family A. Semi-quantitative RT-PCR expression study was carried out on both leaf and stem tissues, it was found that *HsCesA1* was expressed in both tissue types and it had similar levels of expression in both young tissues. Phylogenetic and Blast analyses also supported that *HsCesA1* might play roles in both primary and secondary cell wall depositions. However, further investigations must be carried out to confirm the function of this *HsCesA1*. We have isolated the first cellulose synthase gene, full-length *HsCesA1* cDNA with total length of 3528 bp (accession no: KJ608192) and full length *HsCesA1* gene with total length of 5489 bp (accession no: KJ661223). This study generated some genetic information about the structure and putative function of the cellulose synthase gene in the genome of *H. sabdariffa*. In the other words, this study provides information on the primary structure of the *HsCesA1* gene, which is fundamental for working towards understanding the function of the gene in the roselle plant in the future.

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

PENGASINGAN DAN PENCIRIAN GEN SELULOSA SINTASE DARIPADA ROSELLE (*Hibiscus sabdariffa* L.)

Oleh

SEYEDEHSAREH SEYEDI

Jun 2014

Pengerusi: Profesor. Tan Soon Guan, PhD

Fakulti: Bioteknologi dan Sains Biomolekul

Hibiscus sabdariffa (Roselle) mempunyai potensi untuk menjadi sumber serat alternatif untuk selulosa sebagai bahan pengukuhan komposit polimer. Tinjauan literatur telah menunjukkan sedikit maklumat mengenai penggunaan serat ini sebagai bahan memperkukuh. Walaupun roselle dihipotesis sebagai sumber serat semula jadi yang berpotensi, tidak ada kajian keatas asas genetik yang mengawal jentera selulosa biosintesis dalam roselle. Pengasingan Cesa gen adalah penting untuk memahami laluan biosintesis dalam roselle. Dengan ini, selulose sintase *H. sabdariffa* yang pertama (*HsCesA1*) telah dipencilkan. Jujukan lengkap cDNA *HsCesA1* 3528 bp (accession no: KJ608192) telah dipencilkan menggunakan RACE PCR, dan codon permulaan dan pemerhentian, isyarat poli A, ekor Poli A, 3' UTR dan 5' UTR telah dikenalpasti menggunakan analisis *in-silico*. Jujukan lengkap gen *HsCesA1* dengan panjang keseluruhan 5489 bp (accession no: KJ661223), terdiri daripada 12 ekson, 11 intron dan rantau pengawalatur sepanjang 737 bp juga berjaya dipencilkan. Beberapa ciri-ciri penting telah diramalkan dalam jujukan amino acid HsCesA1 seperti selulosa-synt, famili Glycosyltransferase A (GT-A), BCSA, domain Zn-jari, RING / U keselamatan, domain A dan B. Ini membuktikan bahawa gen terpencil mungkin adalah satu gen *CesA* dan ia tergolong dalam kelas prosesif dalam famili glikosiltransferas A. Kajian pengekspresan semi-kuantitatif RT-PCR dijalankan keatas tisu daun dan batang, dan *HsCesA1* mempunyai tahap yang sama bersuara dalam kedua-dua tisu muda. Filogenetik dan analisis Blast juga menyokong bahawa *HsCesA1* mungkin memainkan peranan dalam deposisi sel dinding primer dan sekunder. Walau bagaimanapun, kajian lanjut perlu dilakukan untuk mengesahkan fungsi *HsCesA1*. Kajian ini telah menjana maklumat genetic mengenai struktur dan fungsi putatif gen selulosa sintase di dalam genom *H. sabdariffa*. Dalam kata lain, kajian ini menyediakan maklumat mengenai struktur primer gen HsCesA1, yang mana merupakan asas ke arah memahami fungsi gen dalam tumbuhan roselle pada masa akan datang.

ACKNOWLEDGEMENTS

First and foremost, my gratitude goes to my truly kind and devoted supervisor Professor Dr. Soon-Guan Tan. I especially want to extend my heartfelt thanks and deepest gratitude to my dear co-supervisor Dr. Christina Yong who was incredibly efficient, supportive and excellent in guiding me through this novel experience, I also felt having a kind and deeply considerate friend beside me. My grateful appreciation is also due to my co-supervisor Prof. Madya Dr. Parameswari A/P Namasivayam. This project is funded by Research University Grant Scheme initiative 5 (9199675).

My profound and warmest thanks and love to my mother for her love, patience, and encouragement and to my father for his constant support, love and prayers throughout my study. I am forever indebted to my parents. My heartfelt thanks are extended to my sister, Hamideh, for her care, endless emotional support, love and assistance throughout my course of study. I wish to express my sincere appreciation to my close friends Sonia Nikzad, Parastoo Khajeian and Nahid Kalhor for their support and kind wishes. Last but certainly not least, I wish to express my sincere appreciation to all those who not mentioned here that helped me to ensure the completion of my research.

APPROVAL

I certify that a Thesis Examination Committee has met on 9 June 2014 to conduct the final examination of Seyedehsareh Seyedi on her thesis entitled "Isolation and Characterization of Cellulose Synthase Gene from Roselle (*Hibiscus sabdariffa* L.)" 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 Master of Science.

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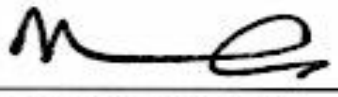

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Declaration by graduate student

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