



UNIVERSITI PUTRA MALAYSIA

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

SEYEDEHSAREH SEYEDI

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UNIVERSITI PUTRA MALAYSIA
BERILMU BERBAKTI

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GENE FROM ROSELLE (*Hibiscus sabdariffa* L.)**

By

SEYEDEHSAREH SEYEDI

**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

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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 respectively. 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.

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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:

Tan Soon Guan, PhD

Professor

Faculty of Biotechnology and Biomolecular of Science

Universiti Putra Malaysia

(Chairman)

Christina Yong, PhD

Senior Lecturer

Faculty of Science

Universiti Putra Malaysia

(Member)

Parameswari A/P Namasivayam, PhD

Associate Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Member)

BUJANG BIN KIM HUAT, PhD

Professor and Dean

School of Graduate Studies

Universiti Putra Malaysia

Date

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