UNIVERSITI PUTRA MALAYSIA

ISOLATION AND IN SILICO ANALYSIS OF PHOSPHOENOLPYRUVATE CARBOXYLASE GENE AND EFFECTS OF INDOLEACETIC ACID AND KINETIN ON SUCROSE PRODUCTION OF SUGARCANE (SACCHARUM OFFICINARUM, L., 1753)

NOOR FARIS HASSAN ALWASH

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By

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Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, In fulfilment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of the Universiti Putra Malaysia in fulfillment of the Requirements for the Degree of Master of Science

ISOLATION AND IN SILICO ANALYSIS OF PHOSPHOENOLPYRUVATE CARBOXYLASE GENE AND EFFECTS OF INDOLEACETIC ACID AND KINETIN ON SUCROSE PRODUCTION OF SUGARCANE (*SACCHARUM OFFICINARUM, L.*, 1753)

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Chair : Associate Prof. Siti Khalijah Daud, PhD

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Sugar is the most important food constituent in almost all countries around the world. The sugarcane plant is the most important source of sugar production in which 80% of the sugar production worldwide is derived from. This study aims to isolate, identify and characterize the phosphoenolpyruvate carboxylase gene (PEPcase) and finding out the effects of indoleacetic acid and kinetin hormones on glucose productivity of sugarcane. Moreover, various bioinformatics tools have been applied to further characterize the PEPcase protein. Three sugarcane cultivars, Tebu Hijau-GG (green stem, green leaf), Tebu Gula-RG (red stem, green leaf) and Tebu Gagak-RR (red stem, red leaf) were used to isolate the PEPcase gene GG-PEPcase, RG-PEPcase and RR-PEPcase respectively. Conserved regions have been detected such as protein active sites, and the phylogenetic tree has been constructed. Furthermore, the 3D protein structure (with predicting the ligand binding sites) has been modeled for these isolated PEPcase genes. In this study, tissue culture technique was used to propagate the plant and the effect of plant growth regulators on sugar content was determined. Twenty culture media have been prepared from different compositions and concentrations of plant hormones to propagate the three sugarcane cultivars and used them to find out the fastest and the healthiest growing plant. The best growing plant was the one that was tissue cultured in media containing (5mg/l) Indole-3-acetic acid (IAA) and (2mg/l) Kinetin (Kin). The juvenile plants were harvested and used to measure the sugar content. The concentrations of sugar were found 10% higher in GG, 24% in RR and 43% in RG than the original plant. Significant increase in sugar production has been detected in all of the three cultivars, and the highest increase has been found in the RG sugarcane cultivars. This may occur because of differences among these cultivars at the genetic level

and protein level. This study also showed that RG cultivars are more responsive to the hormone treatments than the other cultivars.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi Keperluan untuk Ijazah Master Sains

PENGASINGAN DAN SILICO ANALISIS PHOSPHOENOLPYRUVATE CARBOXYLASE GENE DAN KESAN ASID INDOLE-3-ASETIK DAN KINETIN ON SUKROSA PENGELUARAN TEBU (SACCHARUM OFFICINARUM, L., 1753)

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Gula adalah konstituen makanan yang paling penting di hampir semua negara di seluruh dunia. Sumber yang paling penting dalam pengeluaran gula ialah tebu di mana 80% daripada pengeluaran gula di seluruh dunia berasal dari tebu. Kajian ini bertujuan untuk mengasingkan, mengenal pasti dan mencirikan gen carboxylase phosphoenolpyruvate (PEPcase) dan mengetahui kesan asid indoleacetic dan hormon kinetin produktiviti glukosa tebu. Selain itu, pelbagai alat bioinformatik telah digunakan untuk terus mencirikan protein PEPcase itu. Tiga kultivar tebu, Tebu Hijau-GG (hijau batang, daun hijau), Tebu Gula-RG (batang merah, daun hijau) dan Tebu Gagak-RR (merah batang, daun merah) digunakan untuk memencilkan gen PEPcase GG-PEPcase, RG-PEPcase dan RR-PEPcase masing-masing. Pelbagai perision bioinformatik telah digunakan untuk menciriken, menganalisis dan membezakan PEPcase di kalangan ketiga-tiga kultivar tebu dan dibandingkan dengan Saccharum officinarum-PEPcase yang diperolehi dari pangkalan data dalam talian. Urutan protein PEPcase telah diramalkan. Selain itu, kawasan-kawasan yang dipelihara telah dikesan serta seperti tapak aktif protein, dan pokok filogenetik telah dibina. Tambahan pula, struktur protein 3D telah dimodelkan untuk gen PEPcase terpencil, dan menggunakan protein meramalkan struktur 3D untuk meramalkan laman mengikat ligan. Dalam kajian ini, teknik kultur tisu telah digunakan untuk propagasi tumbuhan dan kesan pengawal selia pertumbuhan tumbuhan pada kandungan gula juga ditentukan. Dua puluh media budaya telah disediakan daripada komposisi yang berbeza dan kepekatan hormon tumbuhan untuk menyebarkan tiga kultivar tebu dan digunakan mereka untuk mengetahui yang paling cepat dan tumbuhan yang sihat yang semakin meningkat. Kilang berkembang terbaik adalah salah satu yang merupakan tisu yang diternak dalam media yang mengandungi (5mg / l) asid Indole-3-asetik (IAA) dan (2 mg/l) Kinetin (Kin). Tumbuhan juvana telah dituai dan digunakan untuk mengukur kandungan gula. Kepekatan yang lebih tinggi gula daripada tumbuhan asli yang ditemui 10% dalam GG, 24% RR dan 43% RG. Terdapat banyak sebab untuk ini berlaku. Pada masa yang sama, peningkatan dalam kandungan gula dalam RG adalah lebih daripada GG dan RR, yang mungkin berlaku kerana perbezaan antara kultivar ini di peringkat genetik dan tahap protein.

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