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

FS 2014 15

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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

April 2014

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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April 2014

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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Pengerusi : Prof. madya Siti Khalijah Daud, PhD

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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.

Peningkatan yang ketara dalam kandungan gula yang telah dikesan dalam semua tiga kultivar, dan peningkatan tertinggi telah ditemui di kultivar tebu RG .

ACKNOWLEDGEMENTS

First of all I would like to thank Allah (s.w.t) from my heart. He always helped me in everything. He was behind everything beautiful in my life.

This study could not be completed without the great support of my beloved supervisor and co-supervisors. I am greatly indebted to them. My special appreciation goes to my supervisor Associate Prof. Siti Khalijah Daud, for allowing me to work independently and providing me valuable comments to improve my work. I appreciate her belief in my work and supporting my study financially. She is such a good hearted professor that I will never forget. I would like to thank my co-supervisor Prof. Maziah Mahmood from the Faculty of Biotechnology and Biomolecular Sciences who provided me the opportunity to work in her lab and using her chemicals and instruments. She never hesitated to share with me her valuable experience in the field of plant tissue culture techniques. I will never forget my co-supervisor Dr. Christina Yong Seok Yien who was with me from the very first beginning of my journey in this study. She has generously allowed me to use her lab, kits and instruments, and sharing with me her valuable knowledge in the field of genetics. My special thanks go to Prof. Ahmad Ismail, the Head of the Biology Department. He helped me enormously in the bureaucratic processes during my study and provided me the privilege to access all the facilities in the department. I would like to thank all the Biology Department's staff for helping me in my research.

I would like also to thank my great family: my mother, my brother, my sister and my grand mom for their moral support and encouraging me to finish my research. And I would like to super thank my wonderful husband for all of his support in every single details in my study. May Allah bless all of them.

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

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CHAPTER 1

INTRODUCTION

Sugarcane is one of the most important crops from which 80% of the sugar is derived. Beside its valuable source for sugar, it has several other industrial uses which are unique to this important plant. It is used as an important source for products, like fibers which is used for various industrial applications and ethanol which is used as fuel for vehicles. This plant is adapted to tropical and subtropical areas. Sugarcane is a member of the grass family, Poaceae, classified under the Andropogoneae tribe; with maize and sorghum, as the closest relatives (Plomion et al., 2011).

There is great demand for sugar across the world because of its importance to a very wide range of food industries. However, sugar production nowadays does not meet the increased demand. The main product of sugarcane is sucrose which accumulates in the stalk internodes. The average yield of cane is about 60 tons per hectare per year. As approximately 12% of a cane stalk is sugar, one hectare can produce about 9 tons of sugar per year (James, 2004)

C_4 plant has better adaptation than C_3 plant to water stress, dry climate, high daytime temperatures, and nitrogen or CO_2 limitation in tropical and warm area. This is because of the presence of phosphoenolpyruvate carboxylase (PEPcase) in C4 plants. Sugarcane is an example of C4 plants, same as maize and sorghum. The unique leaf structure of C_4 plants that formed from surrounding the mesophyll cell around the bundle sheath cells, also helped a lot to enhance the photosynthesis process and make it more efficient.

The naming of C_3 and C_4 plants is because the first product of the CO_2 fixation process during photosynthesis was 3-phosphoglycerate in C_3 plant and it was oxaloacetate in C_4 plants, respectively.

PEP carboxylase (PEPcase) is an enzyme in the carboxy-lyases family which is responsible to make the four-carbon compound, oxaloacetate. The first step of photosynthesis process starts when the CO_2 molecules are absorbed from the environment and fixed in the chloroplast of the mesophyll cell as HCO_3, then converts to four carbon atom molecules called oxaloacetate. The presence of PEPcase helps the uptake of much more CO_2 molecules, which are stored in the mesophyll cell (Sage & Monson, 1998).

The oxaloacetate will be converted to malate by the presence of NADP malate dehydrogenase, which occurs in the chloroplast of the bundle sheath cell. The function of this molecule is to act as a transient store of the fixed CO_2 and transferred to the bundle sheath cell where the malate compound is converted into two compounds, pyruvate with the presence of NADP malic enzyme and CO_2, The pyruvate is exported to chloroplast in the mesophyll cell while the CO_2 will be released to enter the Calvin's cycle where the sugar and starch will be synthesized.

PEPcase is activated during night time in CAM plants whereas it is activated during the day in C_4 plants. In both plants, the PEPcase enzyme is inhibited by malate and activated by glucose-6-phosphate (Paul, 2012; Zhao, et al., 2012).

Many techniques have been developed to increase sugar productivity from sugarcane. Among the techniques, tissue culture technique is commonly used to enhance the growth of the plant and the production of secondary metabolites by adding different mixture and composition of plant hormones (George et al., 2008).

Plant hormone synthesized naturally in higher plants, which is needed for plant growth and development. Auxin and cytokinin are two of the most important classes of the plant hormones. The function of auxin is to induce cell division, callus formation and cell elongation; whereas cytokinin has an important role in shoot induction and cell division, as well as in stimulating growth, cell development and bud formation. Although many studies have proven that plant growth regulators such as Gibberellin have effects on sugar biosynthesis, there are no studies on the effect of IAA and Kin on sugar content in sugarcane.

The effect of plant growth regulators may reach to the gene level. It is thought that cytokinins have an effect on mitosis, which are cell cycle promoters, in which the DNA replication is affected by auxins, which are also known as cell cycle inducers. Advanced replication of DNA in cultured cells can cause chromosome rearrangement. At the same time, the cells do not enter into cell division without cytokinin, thus, it is thought that cytokinins may cause a change in the DNA sequences during mitosis (George et al., 2008; Zhang et al., 2005).

There are studies on Gibberellin (GA_3) that proved this hormone can increase the level of both starch and sucrose when applied to soybean leaves by increasing the activity of sucrose-phosphate synthase (SPS). The factors that affect sucrose synthesis can also affect starch synthesis. Spraying sugarcane with GA_3 can increase the growth rate, plant height and sucrose content (Gayler & Glasziou, 1968; Lu et al., 2010).

When some changes like infection, mutation at the gene level occur or the mechanism of production changes from one generation to another, then some changes may occur in the gene sequence, which will lead to the structural and functional changes in individuals (Cadotte et al., 2012; Pevsner, 2009).

1.1 Problem Statement

There is a big industrial interest to increase sugar production from sugarcane. Even a small increase in the efficiency of producing sugar has significant economic impact for the large commercial market. This study is attempted to enhance the productivity of sugar from sugarcane, and to understand the PEPcase background.

1.2 Objectives

The objectives of this study were:

1. To isolate PEPcase gene from three sugarcane cultivars GG, RR, and RG.

2. To analyze the PEPcase gene isolated from the three cultivars, using bioinformatic tools.

3. To determine the best medium to propagate the three cultivars through tissue culture technique, and to investigate the effects of IAA and Kin on sugar content in these three sugarcane cultivars.

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