UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION AND CHARACTERIZATION OF DROUGHT-INDUCED GENES IN MALAYSIA RICE CULTIVARS, MR220 AND MR211 USING RNA-SEQUENCING PLATFORM

TAJUL ARIFFIEN BIN OTHMAN

FBSB 2019 33

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By

TAJUL ARIFFIEN BIN OTHMAN

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

June 2019

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

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Chair : Assoc. Prof. Noor Azmi Shaharuddin, PhD
Faculty : Biotechnology and Biomolecular Sciences

Drought is one of the abiotic stresses on plants, causing significant detrimental impacts, especially to lowland rice (*Oryza sativa L.*) ecosystems. In order to obtain new insights on osmotic stress in rice, a comparative study using a Next-Generation Sequencing platform was conducted to elucidate osmotic-responsive genes from two local Malaysian rice cultivars, namely the commercially available drought-tolerant MR220 and the drought-sensitive MR211. In the study, 21-day-old seedlings of MR220 and MR211 were exposed to 6% PEG 6000 for 24 hours, which produced osmotic stress that mimicked the drought condition. The samples were collected and total RNA were extracted. Two transcriptomic libraries were constructed from both rice cultivars using the Illumina HiSeq 2000 platform. A total of 77,964,138 and 92,699,454 raw sequence reads were generated from these libraries. From the expressed genes from both libraries, around 44, 902 genes have been found overlapping each other. Then, 8,095 and 2,081 gen have been found uniquely in MR220 and MR211, respectively. Based on the gene annotation of O. sativa, a total of 106 genes were identified as differentially and significantly expressed in drought-tolerant and drought-susceptible cultivars, and a total of 29 genes were categorized as unknown genes. From the 106 differentially expressed genes (DEGs), 14 genes were up-regulated, while another 92 were down-regulated in MR220. Gene Ontology (GO) and KEGG analysis were conducted to obtain the functional and biological role of the differentially expressed genes. Six drought related genes were selected for validating the RNA-Seq analysis using semi-quantitative PCR. The validation result showed four out of six DEGs followed the RNA-Seq analysis. Then, two genes were selected form six semi-quantitative analysis, *HP* and *Thia* genes were validated using real-time qRT-PCR. The result showed same expression with semi-quantitative PCR. Overall, this study gives further insight on rice defense mechanisms during osmotic stress at early stage.

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

MENGENAL PASTI DAN MENGKAJI CIRI-CIRI GEN YANG TERLIBAT DENGAN KEMARAU PADA PADI MALAYSIA, MR220 AND MR211 DENGAN MENGUNAKAN PLATFORM PENJUJUKAN RNA

Oleh

TAJUL ARIFFIEN OTHMAN

Jun 2019

Pengerusi : Prof. Madya Noor Azmi Shaharuddin, PhD
Fakulti : Bioteknologi dan Sains Biomolekul

Kemarau merupakan salah satu juzuk abiotic terhadap tumbuh-tumbuhan, yang memberi kesan yang sangat buruk terutamanya ekosistem padi (*Oryza sativa L*.) tanah rendah. Untuk mendapat gambaran sebenar kesan kemarau terhadap padi, kajian perbandingan mengunakan platform Penjujukan Generasi Seterusnya telah dijalankan dalam mencari gen yang terlibat kepada kemarau, daripada dua jenis padi tempatan, padi komersal tahan kemarau, MR220 dan padi sensitif terhadap kemarau, MR211. Dalam kajian ini, anak padi MR220 dan MR211 yang berusia 21 hari dikenakan 6% daripada PEG 6000 selama 24 jam, menghasilkan tekanan osmotik yang meniru keadaan kemarau. Sampel padi dikumpul dan total RNA akan diekstrak dari sampel pokok. Dua perpustakaan transkriptomik telah dibina dari kedua-dua kultivar padi dengan mengunakan HiSeq Platform 2000. Sebanyak 77, 964,138 dan 92,699,454 'sequence read' telah dijana dari data tersebut. Daripada gen yang terzahir daripada kedua-dua perpustakaan, sebanyak 44,902 gen telah dijumpai terzahir. Sementara itu, sebanyak 8,095 dan 2,081 gen yang unik dijumpai dalam MR220 dan MR211. Berdasarkan anotasi gen O. Sativa, sebanyak 106 gen telah dikenal pasti sebagai yang sangat ketara dalam kultivar tahan kemarau dan tidak tahan kemarau, dan sejumlah 29 gen adalah gen baru yang tidak dikenal pasti. Daripada 106 gen yang dinyatakan sebagai (DEG), 14 gen telah menunjukkan peningkatan dan 92 gen menunjukkan penurunan dalam MR220. Gen Ontologi (GO) dan analisis KEGG telah dijalankan untuk memperolehi fungsi dan proses biologi untuk setiap gen tersebut. Enam gen telah dipilih untuk mengesahkan analisis RNA-Seq dengan mengunakan RT-PCR separa-kuantitatif. Keputusan pengesahan menunjukkan daripada empat gen menyerupai analisis RNA-Seq tersebut. Kemudian, dua gen dipilih, *HP* dan *Thia* gen dalam mengesahkan keputusan RNA-Seq, dengan mengunakan qRT-PCR kuantitatif. Hasil kajian ini menunjukkan penzahiran yang sama dengan PCR separa kuantitatif. Secara keseluruhan, kajian ini memberikan satu pandangan berkenaan dengan mekanisma pertahanan pokok padi semasa tekanan osmosis pada peringkat awal.

ACKNOWLEDGEMENTS

In the of Allah, Most Gracious, Most Merciful, I have completed this thesis

I would like to express my appreciation to Universiti Putra Malaysia (UPM) for giving me a place to pursue my master study in Plant Biotechnology Program and finally accomplish my study. I also would like to thank to the Dean of Faculty of Biotechnology and Biomolecular Science, Prof. Dr. Arbakariya Ariff, for his support and help towards postgraduate student affairs.

Special appreciation goes to my supervisor, Assoc. Prof. Dr. Noor Azmi Shaharuddin, for his supervision and constant support, fantastic idea and guidance to increase the value of my project as well as taught me the great value of how to be a good scientist. In addition, heartfelt appreciation conveys to all my co-supervisors, Dr. Norliza Binti Abu Bakar, Puan Rabiatual Adawiyah Binti Zainal Abidin, Allahyarhamah Prof. Dr. Maziah Mahmood and Dr. Nor Baity Binti Saidi who have contributed to the success of this research.

Also thanks goes to Assoc. Prof. Dr. Awang Yahya as his guidance and contribution on field experiments. Special thanks for laboratory assistants of Faculty of Biotechnology and Biomolecular Sciences for giving me help, support, and guidance during my lab work.

Sincere thanks also to all my friends and lab mates for their kindness and moral support during my study. Finally yet importantly, my deepest gratitude goes to my beloved parents, (Othman bin Mamat and Alimah binti Amirusin), my siblings and my wife, Sharifah Nur Nusrah binti Syed Yassin for their endless love, prayers and encouragement. To those who indirectly contributed in this research, your kindness means a lot to me.

Thank you very much.

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:

Noor Azmi bin Shaharuddin, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Nor Baity binti Saidi, PhD
Senior Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Maziah binti Mahmood, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

_____
ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 17 October 2019

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LIST OF ABBREVIATIONS

bp	BasePair
cDNA	Complementary Deoxyribonucleotide acid
°C	Celsius
DEGs	Differential Expressed Genes
DNA	Deoxyribonucleic Acid
ds	Double Stranded
FPKM	Fragment Per Kilobase of transcript per Million mapped reads
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
MARDI	Malaysia Agricultural Research and Development Institute
min	Minute
µg	Microgram
mM	MiliMolar
µL	Microlitre
NGS	Next Generation Sequencing
NCBI	National Centre for Biotechnology Information
nt	nucleotide
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
qPCR	Quantitative polymerse chain reaction
RIN	RNA integrity number
RNA	Ribonuclease acid
RNA-Seq	RNA Sequencing

Rpm	revolutions per minute
RT	Reverse-transcription
RT-PCR	Reverse-transcription polymerase chain reaction
ROS	Reactive Oxygen Species
RPKM	Read Per Kilobase of transcript per Million mapped reads
s	Second
Ss	Single Stranded
UPM	Universiti Putra Malaysia
UK	United Kingdom
US	United State Of America

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Rice, (*Oryza sativa L.*) is one of the most important crops in the world as over half of the world's populations consume rice as their staple food (Hadiarto and Tran, 2011). In 2017, over 167.25 million hectares were planted with rice all over the world with 87.02% of the rice harvested from Asia. Total production yield from all over the world is around 769.65 million tonnes, with 89.98% of the rice production came from Asia (FAO, 2018). In Malaysia, rice is grown on 689,268 hectares of land which produced 2.9 million tons of paddy grain annually (FAO, 2018). In the midst of meeting with the increasing world demand, rice production is still facing limitations, mostly due to abiotic and biotic stresses, as well as drought stress and the latter is a major issue as reported in numerous scientific reports (Najmuddin, *et al.,* 2018; Todaka *et al.,* 2015; Mostajeran and Rahimi-Eichi, 2009).

Drought is one of the environmental factors that affect the rice production. It is severely influencing many regions in the world especially in lowland rice ecosystems (Zhang *et al.,* 2018; Shukla *et al.,* 2012; Passioura, 2007). Statistical analysis showed that the land affected by drought have increased more than twice in percentage from 1970s to early 2000 (Isendahl and Schmidt, 2006). The situation becomes more serious due to global climate change in agricultural areas and increasing world population (Lesk *et al.,* 2016; Hongbo *et al.,* 2005).

Rice is a crop that is susceptible to drought due to its shallow root system, rapid stomatal closure and leaf senescence in mild water stress (Obidiegwu *et al.,* 2015; Hirasawa, 1999). The drought stress in different stage of rice growth gives different effects. At vegetative stage, the height of rice plant is significantly reduced and lower grain yield is produced (Sarvestani *et al.,* 2008). Therefore, it is highly important to study the molecular mechanism of drought tolerance for future production of drought tolerant rice.

Rice genome has been fully sequenced and generated and 2859 unique genes were discovered and need to be characterized (Sasaki, 2005). Due to the lack of information about their functional characterization, validation needs to be carried out. RNA-Seq is a high-throughput sequencing-based method, which has become an important technique used in transcriptome study. Much information can be obtained and studied from the RNA-Seq data. This includes identification of transcription sites and new splicing variants, monitoring of allele expression, cataloguing species transcripts, quantifying expression level of each transcript in different conditions and quantification of exon expression and splicing site (Nagalakshmi *et al.,* 2010; Tsuchihara *et al.,* 2009; Wang

et al., 2009). As the RNA-Seq is one of the most used methods in most transcriptome study, this technology can be used to identify drought related tolerant genes.

MR220 has been identified as Malaysian drought tolerant commercial rice cultivar (Zulkarnain *et al.,* 2013) while MR211 has been identified as susceptible cultivar to drought stress (Abdul Rahim *et al.,* 2012). Moreover, MR220 gives high yield (Zain *et al.,* 2014) and has been planted widely in Malaysia for the past few years. Hence, a study was designed to characterize and profile the expression of drought-induced genes in both these Malaysia cultivars, MR220 and MR211 *via* high-throughput sequencing technology. These results can reveal pathways, alternative splicing sites for genes and help to identify novel genes that could play major roles during plant tolerance to drought stress.

1.2 Research Objectives

Therefore, the objectives of this study were:

1. To induce drought treatment to Malaysian rice cultivars, MR220 and MR211 using PEG 6000 and generate transcriptome libraries of drought-induced MR220 and MR211 *via* RNA-Seq technology
2. To identify Differential Expressed Genes (DEGs) from the transcriptome libraries
3. To validate the expression of the DEGs using reverse transcription-polymerase Chain Reaction (RT-PCR) and real-time quantitative reverse-transcription Polymerase Chain Reaction (qRT-PCR)

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