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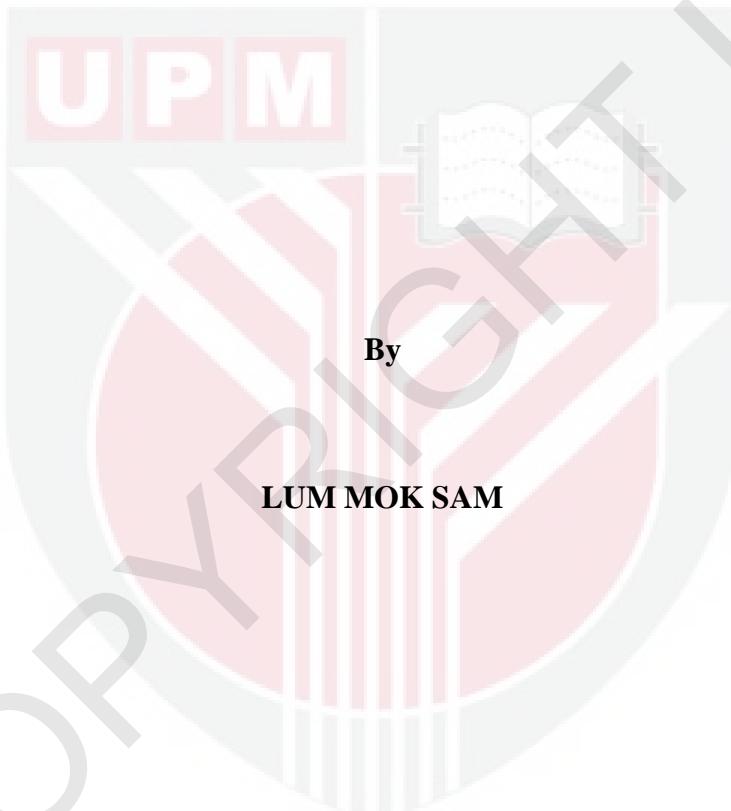
***ISOLATION AND GENE EXPRESSION PROFILING OF
DROUGHTRESPONSIVE
GENES IN UPLAND RICE (ORYZA SATIVA L.)***

LUM MOK SAM

IPTSM 2016 6



**ISOLATION AND GENE EXPRESSION PROFILING OF DROUGHT-
RESPONSIVE GENES IN UPLAND RICE (*ORYZA SATIVA* L.)**



**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

November 2016

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment
of the requirements for the degree of Doctor of Philosophy

**ISOLATION AND GENE EXPRESSION PROFILING OF DROUGHT-
RESPONSIVE GENES IN UPLAND RICE (*ORYZA SATIVA L.*)**

By

LUM MOK SAM

November 2016

Chairman : Professor Mohamed Hanafi Musa, PhD
Institute : Tropical Agriculture

Abiotic stresses negatively affect stable crop production yield world-wide. The yield of rice production is affected by drought or water deficit. Water shortage and uneven rainfall distribution limit the use of available water for rice production. The irrigation costs increase yearly in water limited area. Therefore, developing rice varieties with high water use efficient is needed currently. Upland rice performs better and drought tolerant under water limited environment although the yield is low. Therefore, the upland rice can serve as genetic resources for genetic manipulation of susceptible crops. The objectives of the study were: (i) to screen for drought-tolerant upland rice varieties, (ii) to isolate and identify the expressed drought-responsive genes in drought-tolerant upland rice under water limitation, and (iii) to examine the differential expression of transcripts under drought stress and normal conditions. Investigation was performed to evaluate 38 upland rice varieties with five drought levels at germination and early seedling growth stage using PEG 6000 as drought inducer. Drought tolerant variety, IR5533-15-1-1 (S24) had better germination indices, root length, the highest enzymatic antioxidant activity and non-enzymatic antioxidant level (proline) compared to sensitive varieties. Pot experiment was conducted on IR5533-15-1-1 (S24) variety that were (i) watered regularly (un-treated and control plants) and (ii) treated with PEG 6000 solution (-8 bar) for five days at reproductive stage. Then, isolated mRNA from leaves (control and treated plants) was used to generate subtracted cDNA library. The expressed sequence tags (ESTs) were analyzed. Reverse transcriptase PCR and real-time PCR were conducted to analyze the expression profiles of drought-responsive genes from upland rice. Two EST sequences identified showed 87% and 97% similarities to that of glutathione S-transferase (GST) and dehydrin (DHN) from rice, respectively. The expression levels of GST and DHN transcripts in PEG-treated plants were higher than those of untreated plants. The expression of GST was up-regulated and reached a peak at 3 days after treatment (DAT) in leaves (2.5 folds increase) and roots (2 folds increase). On the other hand, the levels of DHN expression showed higher expression in leaves and roots at 4 DAT (2.1 folds increase) and 3 DAT (2.7 folds increase), respectively. The up-regulation of GST and DHN transcripts indicate upland rice defense response

was activated during drought stress. This study has successfully identified the differentially expressed drought-responsive genes in upland rice under drought stress.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PENGASINGAN DAN PROFIL EKSPRESI GEN KEMARAU-RESPONSIF DALAM PADI HUMA

Oleh

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Tekanan abiotik memberikan kesan negatif terhadap kestabilan hasil pengeluaran tanaman di seluruh dunia. Hasil pengeluaran padi terjejas akibat daripada kemarau atau kekurangan air. Kekurangan air dan taburan hujan yang tidak sekata menghadkan penggunaan air dalam pengeluaran beras. Kos pengairan yang meningkat setiap tahun di kawasan air terhad. Oleh itu, pembangunan varieti padi yang berkecakapan penggunaan air yang tinggi diperlukan. Kecekapan penggunaan air padi huma adalah lebih baik dan tahan kemarau di persekitaran air yang terhad walaupun hasilnya adalah rendah. Oleh itu, padi huma boleh dijadikan sumber genetik untuk tujuan manipulasi genetik tanaman. Objektif kajian ini ialah (i) untuk menyaring padi huma yang tahan kemarau, (ii) untuk mengasingkan dan mengenalpasti gen kemarau responsif dalam padi huma yang tahan kemarau di bawah keadaan air terhad, dan (iii) untuk memeriksa ekspresi pembezaan transkrip di bawah tekanan kemarau dan keadaan biasa. Siasatan dilakukan untuk menilai 38 varieti padi huma terhadap lima tahap kemarau pada peringkat percambahan dan pertumbuhan awal menggunakan PEG 6000 sebagai kemarau simulator. Varieti tahan kemarau, IR5533-15-1-1 (S24) yang mempunyai indeks percambahan yang lebih baik, akar yang panjang, aktiviti antioksidan enzim dan antioksidan bukan enzim (proline) yang tertinggi berbanding dengan varieti sensitif. Kajian berpasu telah dijalankan ke atas varieti IR5533-15-1-1 (S24) dengan (i) penyiraman kerap (tumbuhan kawalan) dan (ii) rawatan larutan PEG 6000 (-8 bar) selama lima hari pada peringkat pembiakan. mRNA yang terasing telah digunakan untuk menjana perpustakaan cDNA tertolak. Tag urutan ekspresi (EST) telah dianalisis. Tindak balas berantai polimerase *reverse transcriptase* dan *real-time* dijalankan untuk menganalisis profil ekspresi gen kemarau responsif dari varieti IR5533-15-1-1 (S24). Dua urutan EST dikenalpasti menunjukkan 87% dan 97% persamaan dengan transkrip glutathione S-transferase (GST) dan dehydrin (DHN) masing-masing daripada padi. Tahap ekspresi GST dan DHN dalam tumbuhan yang dirawat dengan PEG adalah lebih tinggi daripada tumbuhan yang tidak dirawat (kawalan). Ekspresi GST telah dipertingkatkan dan mencapai puncak pada 3 hari selepas rawatan dalam

daun (meningkat sebanyak 2.5 kali ganda) dan akar (meningkat sebanyak 2 kali ganda). Sebaliknya, tahap ekspresi DHN menunjukkan ekspresi yang lebih tinggi dalam daun dan akar masing-masing pada 4 hari selepas rawatan (meningkat sebanyak 2.1 kali ganda) dan 3 hari selepas rawatan (meningkat sebanyak 2.7 kali ganda). Peningkatan ekspresi transkrip GST dan DHN menunjukkan tindak balas pertahanan padi huma telah diaktifkan semasa tekanan kemarau. Kajian ini telah berjaya mengenalpasti gen kemarau responsif yang ekspresi pembezaan dalam padi huma di bawah tekanan kemarau.

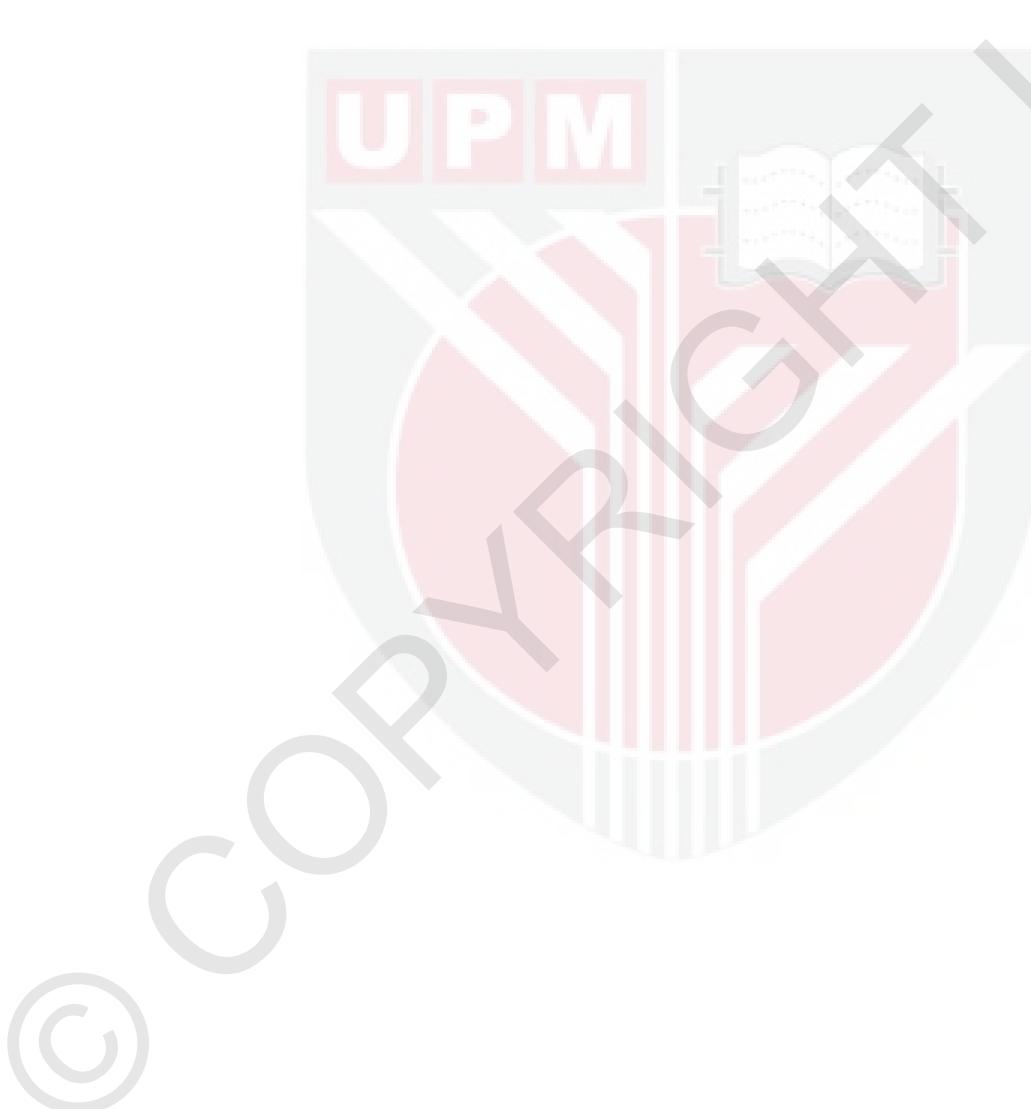


ACKNOWLEDGEMENTS

Firstly, I would like to thank my supervisors Prof. Dr. Mohamed Hanafi Musa, Prof Datin Dr. Siti Noh Akmar Abdullah and Prof. Dr. Mohd. Rafii Yusop, who were always available to provide guidance and delivered outstanding supervision throughout the project.

I would also like to thank Dr. Kwan Yee Min and Dr. Mahbod Sahebi for their excellent guidance.

This was a LRGS funded project (LRGS No. 5525001) so I would like to thank them for the financial support. I would also like to thank the rest of my lab mates for their assistance throughout the project.



I certify that a Thesis Examination Committee has met on 25 November 2016 to conduct the final examination of Lum Mok Sam on his thesis entitled "Isolation and Gene Expression Profiling of Drought-Responsive Genes in Upland Rice (*Oryza sativa* 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 Doctor of Philosophy.

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LIST OF ABBREVIATIONS

\geq	more than or equal
$^{\circ}\text{C}$	degree Celsius
ABA	abscisic acid
ABRE	ABA-responsive element
ANOVA	analysis of variance
bHLH	basic helix- loop- helix
BLAST	Basic Local Alignment Search Tool
bp	base pair
bZIP	basic-domain leucine zipper
CAT	catalase
CBL	calcineurin B-like
cDNA	complementary deoxyribonucleic acid
CRD	completely randomized design
CRT	C-repeat
DAT	days after treatment
DHN	dehydrin
DNA	deoxyribonucleic acid
dNTP	deoxyribose nucleoside triphosphate
DRE	dehydration-responsive element
EDTA	ethylenediaminetetraacetic acid
ERF	ethylene responsible element binding factor
EST	expressed sequence tag
G%	germination percentage
GB	glycine betaine
GI	germination index
GST	glutathione S-transferase
ha	hectare
hr	hour
IPTG	isopropyl β -D-1-thiogalactopyranoside
IRRI	International Rice Research Institute
ISTA	International Seed Testing Association

kb	kilo bases
LB	Luria-Bertani
LEA	late embryogenesis abundant
LSD	least significant difference
MAPK	mitogen-activated protein kinase
MGT	mean germination time
min	minute
mRNA	messenger RNA
NBT	nitrobluetetrazolium
NCBI	National Center for Biotechnology Information
nm	nanometre
OA	osmolyte accumulation
OD	optical density
P5CR	L-I-pyrroline-5-carboxylate reductase
PA	polyamines
PEG	polyethylene glycol
POD	Peroxidase
PS	photosystem
qPCR	real-time polymerase chain reaction
RNA	ribonucleic acid
ROS	reactive oxygen species
rpm	resolutions per minute
rRNA	ribosomal RNA
RT-PCR	reverse transcription polymerase chain reaction
RWC	relative water content
SA-PMPs	MagneSphere® Paramagnetic Particles
SE	standard error
SOD	superoxide dismutase
SSH	suppression subtractive hybridization
SSL	self-sufficiency level
t	tons
t/ha	tons per hectare
TAE	Tris-Acetic acid-EDTA

v/v

volume/volume

w/v

weight/volume

X-Gal

5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside



CHAPTER 1

INTRODUCTION

1.1 Introduction

Food is basic ingredients for sustainable living. Food should be adequately produced from agricultural products and food production is an important agenda in the agricultural sector. Food security is an important issue in recent years as a result of the impact of climate change uncertainty. The uncertainty of climate change is a major challenge for sustainable crop production. It is expected that extreme climate change will increase in the future and further affect the production of sufficient food from crops. Plant species must have a mechanism of adaptation to face climate change to ensure sustainable agricultural production (IPCC, 2014).

World statistics show an increase in world population which will reach 9 billion in 2050 (FAO, 2013). This will put pressure on the agricultural sector in producing enough food to feed the human population. Agricultural sector needs to increase the amount of food by 50% compared with the current situation, particularly in rice production. With the food production rate now, this will not be able to meet the food demand by 2050 (Khush, 2003).

Most Asian countries, including Malaysia, are dependent on rice as a staple food in their daily lives. Rice cultivation is the most important agenda in government efforts to ensure national security and stability in providing sufficient food for the local people. Malaysia is a country which does not produce enough rice for its people. It imports about 27% of its rice from other exporting countries, such as India, Vietnam, Pakistan and Thailand to meet local needs (Rosnani, 2015). Although the yield potential of rice is 10 tons (t), on average, the national yield for rice is only 4.2 t ha^{-1} (DOA Malaysia, 2013). National Food Policy in Malaysia (NPM9) focuses on self-sufficiency level (SSL) of rice production of 90% to be achieved by 2025; however, the SSL of rice production recorded in 2013 was only 73.5% (Rosnani, 2015). Reduction of this gap can be achieved through better management of crops.

There are many challenges in rice production, especially the threats of various environmental stresses (water scarcity, flood, salinity, and heat), other than the fertilizer crisis. Drought and high temperature affect the grain yield in the growth and flowering phases in rice life cycle. Flowering phase is very sensitive and susceptible to the effects of environmental stresses (Pandey and Shukla, 2015). Moreover, in Peninsular Malaysia, cultivation of rice is affected by drought, heat and extreme flood in the life cycle of rice. The climate change in certain areas in Malaysia will reduce grain yield significantly in the future (Munns and Tester, 2008).

Rice cultivation requires a lot of water and the water shortage is the major challenge that must be overcome especially in Malaysia. Effects of climate change and the poor irrigation system in Malaysia are expected to affect rice yields. The uncertainty of

drought and flood faced by local farmers cause damage to the rice crop and further reduce the yields.

There are two methods that can be used by Malaysia government to increase the rice yields, namely (1) the opening of more land for rice cultivation and equipped with better irrigation system, and (2) the production of rice variety that is resistant to drought. Globally, food safety issue can be minimized by producing a new variety of crop that can provide high yields when grown in the harsh environment because the lands suitable for planting are getting lesser. Opening wider lands is not practical and sustainable especially in developed industrial countries.

Upland rice contributes to 12% of global rice production and is major food in some tropical countries. Although the yield is low, upland rice shows better performance and drought tolerance under the water-limited environment (Hanafi *et al.*, 2009). Therefore, the upland rice can serve as genetic resources for genetic manipulation of susceptible crops in drought stress condition.

Understanding of the mechanism of plant responses to stresses gives insight for developing of self-defence crop varieties. It is important for us to exploit the response of rice plants to environmental stress (water deficit), as water resources are limited increasingly under influence of climate change. This project emphasized on the understanding of physiological, biochemical and molecular responses of upland rice growth and development under drought stress. Hence, the aims of this study were to investigate the molecular responses of upland rice to the drought stress and to isolate, identify and characterize the expressed drought-responsive genes in drought-tolerance upland rice.

1.2 Objectives of the study

The objectives were:

1. To screen for drought-tolerant upland rice varieties,
2. To isolate and identify the expressed drought-responsive genes in drought-tolerant upland rice variety under water stress condition, and
3. To examine the differential expression of transcripts under drought stress and normal conditions.

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