UNIVERSITI PUTRA MALAYSIA

MORPHO-PHYSIOLOGY AND YIELD PERFORMANCE, AND LEA GENES EXPRESSION FOR DROUGHT TOLERANCE OF ADVANCED MUTANT RICE LINES

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By

ZARIFTH SHAFIKA KAMARUDIN

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

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DEDICATION

This thesis is dedicated to my parents (Kamarudin Abu Samah, Norainan Ibrahim), husband (Umair Abdul Halim), grandparents (Ibrahim Mokhtar, Mahedah Abdul Karim), brother (Muhammad Haikal), sisters (Zarifth Shahira, Zarifth Shamira) and parents-in-law (Abdul Halim Othman, Dr. Nor Hayati Alwi) for their boundless love, understanding, encouragement, support and sacrifice throughout my study. And my loving daughter, Nur Iman Umayrah binti Umair.

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

MORPHO-PHYSIOLOGY AND YIELD PERFORMANCE, AND *LEA* GENES EXPRESSION FOR DROUGHT TOLERANCE OF ADVANCED MUTANT RICE LINES

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Increasing genetic variability with mutagenic agents has been broadly employed in plant breeding because this method alters one or more desirable traits for an effective breeding programme. Based on this background, induced mutation was adopted to improve the popular Malaysian rice variety, MR219 for drought tolerance. The main objective of this study was to select the high-yielding drought-tolerant advanced mutant rice lines for commercial cultivation in Malaysia. The performance of selected mutant rice and check varieties were evaluated under different drought stress treatments for morpho-physiological and biochemical characteristics, yield and yield components in glasshouse and field conditions. Glasshouse study showed that mutants MR219-4 and MR219-9 had significant higher yields compared to the check varieties when the plants were imposed with moderate and severe drought stresses at active tillering, booting and 50% flowering stages. Analysis of the drought tolerance indices indicated that the ability of drought tolerance for the MR219-4 and MR219-9 were different in response to each drought stress intensity. Based on stress tolerance level, stress tolerance index, stress susceptibility index and drought tolerance efficiency, MR219-4 was identified as drought-tolerant genotype with high yield under both drought conditions, moderate and severe, meanwhile MR219-9 was high-yielding drought-tolerant genotype only under moderate stress condition. Further experiment was carried out to evaluate the yield performances of these two advanced mutant rice lines under field drought stress condition at reproductive stage over two planting seasons. Study of drought stress under field condition showed MR219-4 was the best performance followed by MR219-9 under drought stress among all the rice genotypes for all the traits studied. These advanced mutant lines were selected as drought-tolerant genotype with high yield. *LEA* genes expression analysis was carried out on the selected rice genotypes to identify genes controlling the drought tolerance characteristic. The *OsLEA1, OsLEA2, OsLEA3, OsLEA4* and *OsLEA5* genes were found to be up-regulated with more than four-fold increase when exposed to drought stress treatment. The pattern of *LEA* expression in the MR219-4 and MR219-9 were highly similar with the drought-tolerant check variety

(Aeron1) and therefore, MR219-4 and MR219-9 were confirmed as drought-tolerant mutant rice lines. These advanced mutant lines are highly recommended for large scale field evaluation in development of high-yielding drought-tolerant rice varieties.

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

PRESTASI MORFO-FISIOLOGI DAN HASIL SERTA PENGEKSPRESAN GEN-GEN *LEA* UNTUK TOLERANSI KEMARAU KE ATAS TITISAN-TITISAN MAJU PADI MUTAN

Oleh

ZARIFTH SHAFIKA KAMARUDIN

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Peningkatan kepelbagaian genetik melalui agen mutagenik telah digunakan secara meluas dalam pembiakbakaan tumbuhan kerana kaedah ini dapat mengubah satu atau lebih ciri yang diingini bagi suatu program pembiakbakaan yang berkesan. Berdasarkan latar belakang ini, mutasi induksi telah digunakan untuk memperbaiki toleransi terhadap kemarau bagi varieti padi popular di Malaysia, MR219. Objektif utama kajian ini adalah untuk memilih titisan maju padi mutan berhasil tinggi yang toleransi kemarau untuk penanaman secara komersial di Malaysia. Prestasi padi mutan terpilih dan varieti kawalan telah dinilai dalam keadaan kemarau yang berbeza untuk ciri-ciri morfo-fisiologikal dan biokimia, hasil dan komponen hasil di rumah kaca dan ladang. Kajian rumah kaca menunjukkan mutan MR219-4 dan MR219-9 mengeluarkan hasil yang lebih tinggi secara signifikan berbanding varieti kawalan apabila pokok diberikan rawatan kemarau yang sederhana dan melampau pada peringkat pengeluaran aktif anak pokok, bunting dan 50% pokok berbunga. Analisa indeks-indeks toleransi kemarau menunjukkan keupayaan toleransi kemarau bagi MR219-4 dan MR219-9 adalah bertindakbalas secara berbeza pada setiap tahap intensiti kemarau. Berdasarkan tahap toleransi tekanan, indeks toleransi tekanan, indeks rentan tekanan dan kecekapan toleransi tekanan, MR219-4 telah dikenalpasti sebagai genotip yang toleran kemarau serta berhasil tinggi dalam kedua-dua keadaan tekanan kemarau, sederhana dan melampau, manakala MR219-9 adalah genotip berhasil tinggi serta toleran kemarau hanya dalam keadaan tekanan kemarau yang sederhana. Eksperimen seterusnya telah dijalankan untuk menilai prestasi hasil dua titisan maju padi mutan di ladang dalam keadaan kemarau pada peringkat reproduktif untuk dua musim penanaman. Dalam kajian ini, MR219-4 menunjukkan prestasi terbaik diikuti dengan MR219-9 dalam keadaan kemarau di kalangan semua genotip padi untuk semua ciri yang dikaji. Titisan-titisan maju padi mutan ini telah dipilih sebagai genotip toleran kemarau berhasil tinggi. Analisa pengekspresan gen *LEA* telah dijalankan ke atas genotip padi terpilih bagi mengenalpasti gen-gen yang mengawal ciri toleransi kemarau. Gen *OsLEA1, OsLEA2, OsLEA3, OsLEA4* dan *OsLEA5* didapati menunjukkan pengekspresan secara menaik sebanyak

empat kali ganda apabila didedahkan kepada rawatan tekanan kemarau. Corak pengekspresan *LEA* dalam MR219-4 dan MR219-9 adalah mempunyai persamaan yang tinggi dengan varieti kawalan toleran kemarau (Aeron1) dan dengan demikian, MR219-4 dan MR219-9 telah disahkan sebagai genotip padi toleran kemarau. Titisan-titisan maju mutan ini adalah sangat disyorkan untuk penilaian ladang berskala besar dalam pembangunan varieti padi berhasil tinggi toleran kemarau.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

AA	Ascorbic Acid
ABA	Abscisic Acid
ANOVA	Analysis of Variance
APX	Ascorbate Peroxidase
bp	Base pair
h_B^2	Broad-sense heritability
ºC	Degree centigrade
CAT	Catalase
cDNA	Complementary Deoxyribonucleic Acid
cm	Centimeter
CO_2	Carbon Dioxide
DAS	Days After Sowing
DAT	Days After Transplanting
DEPC	Diethylpyrocarbonate
DMRT	Duncan's Multiple Range Test
DNA	Deoxyribonucleic Acid
DTE	Drought Tolerance Efficiency
EC	Enzyme Nomenclature
EDTA	Ethylenediaminetetraacetic Acid
et al.	et alia
€	Extinction Coefficient
F_1	First filial generation
F_m	Maximal fluorescence level from dark-adapted leaves
F_v/F_m	Maximum quantum yield of PSII
F_o	Minimal fluorescence level from dark-adapted leaves
F_v	Mean values of variable fluorescence level from dark-adapted leaves
g	Gram
GCV	Genotypic Coefficient
Gy	Gray
h	Hour
ha	Hectare
H_2O_2	Hydrogen Peroxide
IUP	Intrinsically Unstructured Protein
K	Selection differential
KCl	Potassium chloride
kDa	Kilodalton
kg	Kilogram
kPa	Kilopascal
L	Litre
LDH	Lactate Dehydrogenase
LEA	Late Embryogenesis Abundant
M_1	First mutation generation
M	Molar
MARDI	Malaysian Agricultural Research and Development Institute
MDH	Malate Dehydrogenase
min	Minute

ml	Millilitre
MNA	Malaysian Nuclear Agency
mol	Mole
MPI	Mean Productivity Index
mRNA	Messenger Ribonucleic Acid
MRP	Mean Relative Performance
µ	Micro
mg	Milligram
MPa	Megapascal
NCBI	National Center of Biotechnology Information
nm	Newton meter
O_2	Oxygen
%	Percentage
PCR	Polymerase Chain Reaction
PCV	Phenotypic Coefficient
POX	Peroxidase
PS	Photosystem
P5C	Pyrroline-5-carboxylatem
P5CS	P5C reductase
qRT-PCR	Quantitative Real Time PCR
RCBD	Randomized Complete Block Design
REI	Relative Yield Index
RNA	Ribonucleic Acid
rpm	Revolutions per minute
ROS	Reactive Oxygen Species
Sec	Seconds
sp	Species
SAS	Statistical Analysis System
SE	Standard Error
SES	Standard Evaluation System
SI	Stress Intensity
NaCl	Sodium chloride
SOD	Superoxide Dismutase
SSI	Stress Susceptibility Index
SSL	Self-sufficiency Level
STI	Stress Tolerance Index
TOL	Stress Tolerance Level
σ^2	Variance

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

The current status of climate change has led to a strong El Niño weather pattern that causes higher temperature, prolonged dry seasons and severe drought, and thereby affects granary areas of Malaysia. Such environmental conditions are the major factors that limit the production of crop at a worldwide level. Approximately 40% of the world's population is exaggregated by drought (GIEWS, 2017). In Malaysia, about 114,324 hectares of land are affected by drought (Tiara *et al.*, 2015).

Rice (*Oryza sativa* L.) is widely cultivated mostly as a staple food crop. In Malaysia, the total area of growing rice is about 490,500 hectares, which is prominent to the production volume of rice, totalling approximately 1.77 million metric tonnes. However, rice yield, in contrast, is very low in the dry soil. It is essential to identify the changes in physiological and biochemical attributes in plants under drought stress to upsurge the yield of rice.

1.2 Problem Statement

Increasing of drought severity and lack of high-yielding varieties suitable for cultivation under drought condition leads to a sharp decline in rice yields. The declining of water supply and severe depletion of watersheds affects the rice production. Apart from the water shortage, the El Niño phenomenon in Malaysia's rice granary areas for example between the June 2015 and March 2016 has disrupted agricultural activities that leads to the decline of rice production. This had resulted in a decline of total rice production at 1.8 million tons for 2015/16 which showed about 20% decrease from 2014/15 (Tiara *et al.*, 2015).

Stresses due to severe drought can be detrimental at all stages of plant development. Understanding how the plants respond to the stresses is a challenging area of research. Climate change especially severe drought condition is projected to negatively impact future agricultural production worldwide. Membranes and proteins are the most affected cell components during stress condition. Changes in these components can alter several processes such as uptake of water and ions, translocation of solutes, photosynthesis and respiration, and produce inactivation of enzymes, accumulation of unprocessed peptides, and proteolysis. As a result, metabolic damage is produced and plant growth is reduced (Chen *et al.*, 1982).

Therefore, understanding the effects and mechanisms of drought tolerance on rice are important issues for the improvement of rice quality in Malaysia. As domestic requirement is not fulfilled with native production, Malaysia depends on imported rice from main rice exporters such as Thailand and Vietnam (Tiara *et al.*, 2015). Under this

context, to feed the increasing human population along with decreasing cultivable land, more innovative research and technological advancement is essential to increase rice production. Therefore, it is necessary to identify genotypes having high yield potentials as well as tolerance against drought condition to be suitable for lowland cultivation.

Presently, the commercial rice farmers are quite aware about the importance of new varieties having high yield potential with uniformity in maturity, tolerance to abiotic stresses, and better quality as compared to the standard varieties. The commercial production of mutant rice has been found to be successful. The experience on the possibility of exploiting the genetic modification in rice has shown considerable promise. Under this context, it is important to understand the effects and mechanisms of drought tolerance and the expression of *LEA* genes on newly developed mutant rice lines as this might be underlying selection criteria for a drought tolerance rice breeding programme.

1.3 Objectives of the Study

The study was conducted to select high-yielding drought-tolerant mutant rice lines for commercial cultivation in Malaysia with the following specific objectives:

1. To evaluate the performance of yield and biochemical characteristics of advanced mutant rice lines under drought conditions.
2. To quantify genetic variance and heritability values among the selected rice genotypes.
3. To identify drought-tolerant genotypes of advanced mutant rice lines.
4. To identify *LEA* protein genes and its expression among advanced mutant rice lines against drought stress.

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In her personal life, Mrs. Zarifth is happily married with Mr. Umair bin Abdul Halim and blessed with a daughter Nur Iman Umayrah binti Umair. She can be contacted through zarifth_shafika@yahoo.com; zarifthshafika@gmail.com.

LIST OF PUBLICATIONS

Zarifth Shafika Kamarudin, Mohd Rafii Yusop, Mahmud Tengku Muda Mohamed, Mohd Razi Ismail and Abdul Rahim Harun (2018). Growth performance and antioxidant enzyme activities of advanced mutant rice genotypes under drought stress condition. *Agronomy*, 8 (279): 1-15. **Q2**

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