UNIVERSITI PUTRA MALAYSIA

OPTIMIZATION OF MALAYSIA RICE MR219 DERIVATIVES CELL SUSPENSION CULTURE AND RESPONSES OF STRESS-RELATED ENZYMES UNDER DROUGHT CONDITION

ER CHOON YEONG

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By

ER CHOON YEONG

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

October 2019

DEDICATED TO:

FATHER (ER CHAI HENG), MOTHER (TAN NG MOI),
BROTHERS (ER CHOON HAW and ER CHOON SIONG)

WHO ALWAYS HAVE FAITH IN ME

AND

THEIR SUPPORTS HAVE GUIDED ME TO GONE THROUGH
ALL THE OBSTACLES IN LIFE

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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Chairman : Noor Azmi Shaharuddin, PhD
Faculty : Biotechnology and Biomolecular Science

Rice is the staple food for a majority of the population around the world. Drought is one of the many factors that affect rice production yield. Researches have used various methods to develop suitable indicator in rice to defend the crop against drought environment. Among the biotechnology techniques, cell culture is commonly used to develop drought tolerant cell line. In Malaysia, drought has greatly affected rice production; as water is the essential element for rice and the heavy dependence of rice on irrigation will make rice as the crop most affected by water shortage crisis. Hence, it is a need to develop drought-tolerant rice in order to fulfil rice demand in Malaysia.The aim of this research is to investigate the biochemical responses in Malaysian rice by using plant cell culturing technique under water-limited condition. The cell suspension of Malaysian rice MR219 line 4 was established and it was cultured *in vitro* with drought agent (PEG6000) in order to prevent water up take by plant cell. After that, biochemical analyses were performed such as glyoxalase I (Gly I) enzyme assay, methylglyoxal, antioxidant enzymes activities – superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and lipid peroxidation of PEG6000 treated cultures were determined. The results showed that while PEG6000 concentration increased from 0%, 5%, 10%, 15% to 20%, the settle cell volume (SCV), cell viability and relative water content (RWC) were declined maximum to 115%, 34% and 24% respectively as a result of drought condition. When drought stress occurred, it eventually activated the plant cell defensive system by upregulating antioxidant enzymes activities (SOD, CAT and APX) to overcome oxidative injury under drought stress condition. It exhibited the highest relative activities for SOD (13.7708 U / mg proteins), CAT (0.1238 µmoL / mg protein / min) and APX (0.4852 µmoL / mg protein / min). Same trend has been applied to Gly I (4.4633 µmoL / mg protein / min). It implicated that the Gly I could be one of the potential drought stress indicator for rice. The output of this study would contribute tremendously to exploit potential drought-tolerant rice cultivar in the future.

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

PENGOPTIMUMAN KULTUR SEL AMPAIAN DERIVATIF PADI MALAYSIA MR219 DAN TINDAKBALAS ENZIM BERKAIT TEKANAN DI BAWAH KONDISI KEMARAU

Oleh

ER CHOON YEONG

Oktober 2019

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

Beras, makanan ruji untuk manusia di seluruh dunia. Kekeringan adalah salah satu faktor yang mempengaruhi pengeluaran beras. Penyelidikan menggunakan kaedah yang berbeza untuk membangunkan penunjuk yang sesuai dalam padi untuk mempertahankan persekitaran kemarau. Antara teknik bioteknologi, kultur sel biasanya digunakan untuk membangunkan sel tumbuhan yang tahan kemarau. Di Malaysia, kemarau banyak memberi kesan kepada pengeluaran beras kerana air adalah unsur penting untuk beras dan ketergantungan berat beras pada pengairan akan menjadikan beras sebagai tanaman paling terjejas oleh krisis air. Oleh itu, adalah keperluan untuk membangunkan beras yang tahan toleran untuk memenuhi keperluan beras di Malaysia.Tujuan kajian ini adalah untuk mengkaji tindak balas biokimia di beras Malaysia dengan menggunakan teknik kultur sel tumbuhan di bawah keadaan kemarau. Suspensi sel beras Malaysia MR219-4 telah ditubuhkan dan dibiakkan secara *in vitro* dengan agen kekeringan (PEG6000) untuk mengelakkan air diambil oleh sel tumbuhan. Selepas itu, ujian biokimia termasuk enzim glyoxalase I (Gly I), enzim, metilglyoxal, aktiviti enzim antioksidan - superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) dan peroksidasi lipid PEG 6000 yang telah dirawat. Keputusan menunjukkan bahawa, manakala kepekatan PEG6000 meningkat 0%, 5%, 10%, 15% hingga 20%, volume sel menetap (SCV), viabilitas sel dan kadar air relatif (RWC) masing-masing menurun maksimum menjadi 115%, 34% dan 24% akibat kondisi kemarau. Apabila tekanan kemarau berlaku, ia akhirnya mengaktifkan sistem pertahanan sel tumbuhan dengan mengawal selia aktiviti enzim antioksidan (SOD, CAT dan APX) untuk mengatasi kecederaan oksidatif dalam keadaan kemarau. Ini menunjukkan aktivitas relatif tertinggi untuk SOD (137708 U / mg protein), CAT (0,1238 µmoL / mg protein / min) dan APX (0,4852 µmoL / mg protein / min). Situasi yang sama telah digunakan untuk Gly I (4.4633 µmoL / mg protein / min). Ini membuktikan bahawa Gly I boleh menjadi salah satu penunjuk tekanan kemarau berpotensi

untuk beras. Hasil kajian ini akan memberi sumbangan besar untuk mengeksploitasi kultivar beras yang tahan kemarau di masa depan.

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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 Master of Science. The members of the Supervisory Committee were as follows:

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

Zetty Norhana Balia Yusof, PhD
Associate Professor
Faculty Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

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

_____

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 12 November 2020

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LIST OF ABBREVIATIONS

BAP	N6-benzylaminopurine
BSA	Bovine serum albumin
Dicamba	2-Methoxy-3,6-d ichlorobenzoic acid
DTT	DL-Dithiothreitol
EB	Evans blue
EDTA	Ethylenediamine-tetraacetic acid
IAA	lndol acetic acid
IBA	lndol butyric acid
IPA or 2iP	N6-(2-lsopentyl) adenine
H_20_2	Hydrogen peroxide
NAA	a-naphthalene acetic acid
NBT	Nitroblue-tetrazolium
PEG	Polyethylene glycol
Picloram	4-Amino-3, 5,6-trichloropicolinic acid
PMSF	Phenylmethanesulfonylflouride
2,4-D	2,4-Dichlorophenoxyacetic acid
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid
Kinetin	6-Furfurylaminopurine
Thidiazuron	1-Phenyl-3-(1, 2, 3-thiadiazol-5-yl) urea
Zeatin	4-Hydroxy-3-methyl-trans-2- - butenylaminopurine
g/L	Gram per litre
W/V	Weight per volume
v/v	Volume per volume
%	Percentage
glyl	Glyoxalase I
glyll	Glyoxalase ll
SCV	Settle cell volume
SDS	Sodium dodecyl sulfate
SLG	S-D lactoylglutathione
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
MG	Methylglyoxal
MDA	Malondialdehyde
GSH	Glutathione
SOD	Superoxide dismutase
APX	Ascorbate peroxidase
CAT	Catalase
GR	Glutathione reductase
H_20_2	Hydrogen peroxide
$\bullet 0_2^-$	Superoxide anion radicals
0_2^1	Singlet oxygen
•OH	Hydroxyl radical
ROIS	Reactive oxygen intermediates

CHAPTER 1

INTRODUCTION

Rice has been planted by mankind for at least 10,000 years in the world (Yang *et al*., 2015). Most of the countries produce rice, excluding Antarctica, and more than 122 countries are growing rice crop on earth. The co-ordination for rice growth on the planet starts from the equator to latitudes of fifty degrees on north (in China) and thirty five degrees to forty degrees and to elevations (in tropical regions) as high as 2,400 m above sea level (Kenmore, 2003). According to the paddy statistic of Malaysia (2012), the total area rice plantation is estimated to be 100 million hectare with yearly rice yield with average of 720 million metric tons. From the above data, rice occupied 29% in the total grain crops on planet (Xu and Shen, 2003). In the year 2010, rice became the major food resources for more than 50% of human population in terms of protein and calories uptake. In average, each person on Earth consume 62-190 kg of rice yearly (zhao *et al*., 2020). Besides, in order to solve food-shortage problems, rice production was increased during the Green Revolution of the 1960s and 1970s. This answered the food demands around the world year by year. Presently, Asians consume rice to provide 80% of energy source for themselves. Chukwu *et al*. (2019) mentioned in their research that it is expected that 3.5 billion people will rely on rice for their daily food intake in the year 2025. This explain why rice is very important for food security globally.

Rice is the main food resource in Asia (Micheal *et al*., 2013). Irrigated rice possesses very low water use efficiency and consumes 3,000–5,000 litres of water to produce 1 kg of rice, which is about 2–3 times more than the quantity required to produce 1 kg of other cereals, such as maize or wheat (Ethan *et al*., 2010). The available amount of water for irrigation is, however, increasingly getting scarce worldwide. In Asia, more than 80% of the fresh water resources are used for irrigation. Among of this, 90% of fresh water is used for rice crop (Ejlali *et al*., 2012). The uneven distribution of the rainfall resulted in limited availability of water in some parts of the country.

In Malaysia, more than 75% water usage is for irrigation in agriculture sector, which is the largest water withdrawal and it is mainly restricted to irrigated rice production (Moquammel Haque *et al*., 2018). Irrigated rice is usually grown in flooded condition during most of its growing period. However, water usage efficiency for rice is undesirable (Othman, 2009). Water is the essential element for rice and the heavy dependence of rice on irrigation will make rice as the crop most affected by the water crisis (Akinbile *et al*., 2011). All kinds of pollution, urbanisation, and abiotic stress such as drought caused water shortage in Malaysia and it affects rice production yield in Malaysia. Since rice is the main food resource in Malaysia, there is a need to ensure security to fullfil the needs of Malaysian and cope with the nation's increasing demand for rice.

At present, the tremendous development in the scientific field brings significant contribution to human beings. Biotechnology has become a very powerful tool to develop or improve crop variety in order to help farmers globally. It has become one of the efficient way to develop drought-tolerant rice to enhance rice production in order to meet the high food demand (Quan *et al.,* 2010). Plant tissue culture has become one of the important tool for plant production. Cell suspension cultures are some cramps of calli immersed in liquid culture medium in a closed system, usually in a flask and are agitated by rotary shaker machine (Fidemann *et al.,* 2018). In the cell system, the continuous agitation between cramps of callli in the liquid culture medium cause the calli to collide with each other to separate and become a uniform distribution of cell suspension. It helps the exchange between medium and gas in the flask. Because of this unique features, cell suspension culture is suitable to be used for investigating its stress responses under *in vitro* drought stress condition.

A large amount of plant injury that are affected by climate change is related to oxidative damage in the plant cell. In the last few years, reactive oxygen species (ROS) is widely known to be important as signalling molecules in abiotic stress responses (Foyer and Noctor, 2009). Besides, recent studies show that the efficiency of antioxidant system and glyoxalase system are correlated to minimise the abiotic stress and oxidative damage in plant cell. (Hasanuzzaman *et al*., 2017). Thus, the investigation of glyoxalase enzyme activity in the glyoxalase system might discover another potential stress indicator for abiotic stress for example drought. The glyoxalase possesses two-steps reaction by using glyoxalase I and glyoxalase II enzyme. For the first reaction, methylglyoxal (MG) (which is the cytotoxic substance, where induced from the ROS) was catalysed by glyoxalase I (glyI) to form S-D lactoylglutathione (SLG) and glutathione (GSH). The second reaction, glyoxalase II (gly II) hydrolyses the SLG to form D-lactate and the GSH is released (Mustafiz *et al*., 2010). According to the information above, we came up with the hypothesis that glyoxalase I enzyme is a potential drought biomarker for Malaysian rice.

According to the paddy statistics of Malaysia (2012), MR219 has occupied 61% hectare of paddy varieties for overall granary area. Therefore, researches co-operated to improve MR219 cultivar in order to benefit farmers and came out with new genotype, MR219-4, which is able to produce a high yield and survive under minimal water condition. This cultivar was selected in this study that was carried out according to the objectives below:

1. To optimization rice cell suspension culture.
2. To study the responses of stress-related enzymes in Malaysian rice under drought stress condition.

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BIODATA OF STUDENT

Er Choon Yeong was born in Johor Bahru, Johor on the 28th April 1986. He received his primary education at Sekolah rendah Jenis Kebangsaan Chien Chi started from year 1993 to year 1998. He continued his secondary education at Sekolah Menengah Kebangsaan Permas Jaya from 1999 to 2003. After obtaining his Sijil Pelajaran Malaysia (SPM), he began his diploma in Tunku Abdul Rahman University College (TARUC) from year 2004 to 2006. Later, he continued in the same university for pursuing his degree. In June 2008, he has successfully earned his Bachelor of Science major and biology and chemistry. He was given an opportunity to get his second bachelor degree in Liverpool John Moores University and third bachelor degree under Campbell University. Due to his inquisitive nature in plant sciences, a decision has been made to enrol himself as a Masters candidate under the supervision of Dr. Noor Azmi Shaharuddin, Prof. Dr. Maziah Mahmood and Dr. Zetty Norhana Balia Yusof. During his candidature, he was offered the Graduate Research Assistantship (GRA) as well as Ministry of Higher Education - MyBrain15 scholarship and an opportunity to work on a rice tissue culture project funded by the National LRGS Food Security (Rice) Grant. He was also given numerous opportunities to participate in several national conferences, symposiums, workshops and seminars in order to better equip himself with the current development of plant sciences.

LIST OF PUBLICATIONS

Conferences and symposiums attended

Poster Presenter

Choon Yeong, E., Noor Azmi, S, Maziah, M. and Zetty, N. B. Y. (2012). Establishment of *in vitro* cell suspension culture of Malaysian MR219-4 genotype (*Oryza sativa* L.). International Agriculture Congress. 4-6th September 2012. Marriott Putrajaya, Malaysia.

Choon Yeong, E., Noor Azmi, S, Maziah, M. and Zetty, N. B. Y. (2013). *In vitro* response of Malaysia rice cultivar (MR219-4) cell suspension culture to drought stress. The First National LRGS Food Security Rice Research Colloquium. 31st January 2013. Auditorium Jurutera, Faculty of Engineering, Universiti Putra Malaysia, UPM Serdang, Selangor.

Choon Yeong, E., Noor Azmi, S, Maziah, M. and Zetty, N. B. Y. (2014). Develooping a potential drought tolerant biomarker in rice (*Oryza sativa* L.) using cell suspension culture. International Conference on Advances in Plant Biochemistry and Biotechnology: Empowering Urban Agriculture. 9-10th December 2014. Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, UPM Serdang, Selangor.

Oral Presenter

1. Choon Yeong, Er., Noor Azmi, S., Maziah, M. and Zetty, N. B. Y. (2013). *In vitro* response of Malaysia rice cultivar (MR219-4) cell suspension culture to drought stress. LRGS workshop. 16-18th November 2013. Faculty of Agrotechnology and Food sciences, Universiti Malaysia Terengganu, Terengganu.