UNIVERSITI PUTRA MALAYSIA

IMICROARRAY ANALYSES OF GRAIN FILLING IN MALAYSIAN INDICA RICE VARIETIES (*Oryza sativa* L.) MR84 AND MR219

TEO CHIN JIT

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By

TEO CHIN JIT

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MICROARRAY ANALYSES OF GRAIN FILLING IN MALAYSIAN INDICA RICE VARIETIES (*Oryza sativa* L.) MR84 AND MR219

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TEO CHIN JIT

October 2008

Chairman : Associate Professor Ho Chai Ling, PhD

Faculty : Biotechnology and Biomolecular Sciences

Rice is a dominant staple food in Asia, including Malaysia. In this study, microarray analysis has been undertaken to identify the genes that were expressed at different stages of rice grain filling in MR219 and MR84 as it is a vital factor that affects the yield of rice directly. Two microarrays were used in this present study: cDNA microarray developed from 3840 PCR-amplified cDNAs from flag leaves and panicles; and commercial NSF 20 K rice oligonucleotide array. cDNA microarray analysis of panicles at 1, 5, 10 and 15 days after heading (DAH) of MR219 compared to heading revealed that a high proportion of storage proteins (glutelin and prolamin) were differentially expressed at 5, 10 and 15 DAH compared to heading in MR219. To compare the expressed genes in MR219 and MR84 at early grain filling period (5 and 10 DAH), microarray data generated from NSF 20K rice oligonucleotide array was analyzed using limmaGUI. Differentially expressed genes at 5-10 DAH from both MR219 and MR84 exhibited diversified functions, suggesting that rice grain filling is a complex biological process involving many different biochemical pathways. Genes encoding transcripts related to transcriptional regulation and signal

transduction were differentially expressed at 5 and 10 DAH. In MR84, transcriptional factor EREBP, ethylene receptor, ERF-like protein and 1-aminocyclopropane-1-carboxylate oxidase 1 were up-regulated at 10 DAH while other transcription factors such as RING finger, zinc finger, GRAS family transcription factor were up-regulated in MR219. The up-regulation of genes related to sugar signaling and sensing may correlate to the assimilate partitioning and transport which occurred during 5-10 DAH in both varieties. Changes in gene expression caused by 'varieties' and 'developmental stage' effects involved transcripts related to stress and defense response, signal transduction and assimilate transportation in MR84 only. The expression patterns of 2 out of 3 selected genes examined were consistent when analyzed with microarray and RT-PCR. Southern analyses showed that putative ethylene responsive transcriptional coactivator and putative ABI3 interacting protein 2 may exist in more than one copy in the genome of both MR84 and MR219 whereas putative BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor may be a single copy gene in both varieties. Real time RT-PCR analysis demonstrated that putative BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor and putative ethylene responsive transcriptional coactivator were differentially expressed at heading, fertilized and 9-12 DAH compared to booting in MR84 and MR219.

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

ANALISIS MIKROATUR BAGI PENGISIAN BIJI PADI INDICA (*Oryza sativa* L.) MALAYSIA VARIETI MR84 DAN MR219

Oleh

TEO CHIN JIT

Oktober 2008

Pengerusi : Professor Madya Ho Chai Ling, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Beras adalah makanan ruji utama di Asia, termasuk Malaysia. Dalam kajian ini, mikroatur telah dilaksanakan untuk mengenalpasti gen-gen yang diekspreskan pada peringkat pengisian biji padi berlainan di MR219 dan MR84 kerana ia adalah faktor penting yang mempengaruhi hasil padi secara langsung. Dua jenis mikroatur telah digunakan dalam kajian ini: mikroatur cDNA yang dibina daripada 3840 cDNA hasil amplifikasi PCR daripada daun bendera dan malai; dan NSF 20K mikroatur oligonukleotida komersial. Mikroatur cDNA bagi malai MR219 pada peringkat 1, 5, 10 dan 15 hari selepas keluar malai (DAH) berbanding dengan peringkat keluar malai menunjukkan bahawa sebahagian besar gen-gen yang terekspres pada peringkat 5, 10 dan 15 DAH di MR219 adalah protein penyimpanan (glutelin dan prolamin). Untuk membandingkan gen-gen yang terekspres di MR219 dan MR84 pada peringkat awal pengisian biji padi (5 dan 10 DAH), data mikroatur oligonukleotida komersial telah dianalisis dengan menggunakan ‘limmaGUI’. Gen-gen yang diekspreskan secara berbeza antara MR219 and MR84 pada peringkat 5-10 DAH mempamerkan kepelbagaian dalam fungsi, mencadangkan bahawa pengisian biji padi adalah satu proses yang kompleks dan melibatkan banyak tapak jalan

biokimia. Gen mengekodkan transkrip yang berkaitan dengan pengawalan transkripsi dan transduksi isyarat diekspreskan pada 5 dan 10 DAH. Di MR84, pengekspresan faktor transkripsi EREBP, 'ethylene receptor', protein 'ERF-like' dan '1-aminocyclopropane-1-carboxylate oxidase 1' dipertingkatkan pada 10 DAH manakala ekspresi faktor transkripsi lain misalnya 'RING finger', zinc finger', factor transkripsi family 'GRAS' diatur naik di MR219. Peningkatan pengekspresan gen-gen yang terlibat dengan pengesanan dan pengisyaratan gula mungkin berkait rapat dengan pembahagian dan pengangkutan produk fotosintesis yang berlaku semasa 5-10 DAH dalam kedua-dua varieti. Perubahan pengekspresan gen yang disebabkan oleh faktor 'varieti' dan 'peringkat perkembangan' melibatkan transkrip yang berkaitan dengan respon terhadap tekanan dan pertahanan, transduksi isyarat serta pengangkutan produk fotosintesis hanya didapati dalam MR84. Corak pengekspresan bagi dua daripada tiga gen yang terpilih didapati konsisten bila dianalisis dengan mikroatur dan RT-PCR. Analisis Southern menunjukkan bahawa koaktivator transkripsi respon etilena putatif (putative ethylene responsive transcriptional coactivator) dan protein ABI3 berinteraksi 2 putatif (putative ABI3 interacting protein 2) mungkin mempunyai lebih daripada satu salinan dalam genom MR219 dan MR84, manakala putatif '*BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor*' mungkin hanya mempunyai satu salinan dalam kedua-dua varieti. Analisis Tindakbalas Rantai Polimerase Masa Nyata menunjukkan bahawa putatif 'BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor' dan koaktivator transkripsi respon etilena putatif diekspreskan secara berbeza pada peringkat keluar malai, persenyawaan dan 9-12 DAH berbanding dengan peringkat bunting dalam MR84 dan MR219.

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I certify that an Examination Committee has met on 28 October 2008 to conduct the final examination of Teo Chin Jit on her Master of Science thesis entitled "Microarray analyses of grain filling in Malaysian indica rice varieties (*Oryza sativa* L.) MR84 and MR219" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree.

Members of the Examination Committee were as follows:

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

Norihan Mohd Saleh, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Faridah Bte Qamaruzzaman, PhD
Lecturer
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Roohaida Othman, PhD
Associate Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
Malaysia
(External Examiner)

HASANAH MOHD. GHAZALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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:

Ho Chai Ling, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Parameswari Namasivayam, PhD
Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

HASANAH MOHD. GHAZALI, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 12 February 2009

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

_____

TEO CHIN JIT

Date:

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LIST OF ABBREVIATIONS

aRNA	Amplified RNA
bp	basepairs
BSA	bovine serum albumin
cDNA	Complementary DNA
Cy 5	Cyanine 5
Cy3	Cyanine 3
DAH	Days after heading
ºC	Degree Celsius
dATP	2'-deoxy-adenosine-5'-triphosphate
dCTP	2'-deoxy-cytidine-5'-triphosphate
dGTP	2'-deoxy-guanosine-5'-triphosphate
dTTP	2'-deoxy-thymidine-5'-triphosphate
DEPC	Diethyl pyrocarbonate
DNA	Deoxyribonucleic acid
DNase	Deoxynuclease
dNTPs	Deoxynucleotides
DTT	Dithiothreitol
DMSO	Dimethylsulphonyl oxide
EDTA	Ethylenediaminetetraacetic acid
g	Gravitational acceleration
g	Gram
HCl	Hydrochloric acid
LiCl	Lithium chloride
LIMMA	Linear Model for Microarray Analysis

µg	Microgram
µL	Microliter
nM	nanomolar
HEPES	N-2-hydroxyethylpiperazine-N'-2ethanesulfonic acid
%	Percentage
PVP	Polyvinylpyrrolidone
PCR	Polymerase chain reaction
QTL	Quantitative trait loci
RNA	Ribonucleic acid
RNase A	Ribonuclease A
NaOAc	Sodium acetate
SDS	Sodium dodecyl sulphate
NaOH	Sodium hydroxide
NaCl	Sodium chloride
$NaBH_4$	Sodium borohydride
TAE	Tris acetate EDTA
TE	Tris-EDTA
Ct	Threshold cycle
U	Unit
v/v	Volume per volume

CHAPTER 1

Introduction

Rice (*Oryza sativa* L.) is the most important cereal crop species that feeds more than two thirds of the world population. It was estimated that in year 2015, Asia will need to produce 38 million more tons of rice than it produced in 2005 (IRRI, 2008) to meet the demand of growing population. Many studies on cultivation and breeding have been conducted to increase rice production. However, the performance of hybrid rice is often inconsistent and the process is time-consuming. More extensive studies should be carried out to accelerate the introduction and adoption of higher yielding rice varieties addressing future food shortage issues.

The occurrence of empty grains or poor grain filling in rice has led to decreased grain yield. However, much of the available information on rice yield is not devoted to studies on grain filling. Grain filling is a multigenic agronomic trait that directly affects the yield of rice. It involves numerous genes at various levels and coordination of gene expression from many important biochemical pathways. During grain filling, nutrient accumulation is governed by the coordination of two major processes: photosynthesis which produces photoassimilates; and partitioning which determines how much of photoassimilates is translocated into the grains. Biochemical pathways of individual storage components are known but factors that determine relative ratios of each are poorly understood.

Therefore, a functional genomics approach is necessary to understand the key events

which occur during grain filling and to elucidate how the expression of these genes is coordinated during grain development. Rapid progress in rice genetics and genome studies have enabled the examination of the expression of genes involved in rice grain filling to be done. It is hoped that the issues of poor grain filling can be improved and thus, maximize the production potential of rice.

In this study, microarray technology was used to study differential gene expression during grain development. It is a powerful tool that allows simultaneous monitoring of the expression levels of thousands of genes. cDNA fragments (probes) isolated from different cDNA libraries or- designed oligomers that match parts of the sequence of known or predicted mRNAs from fully-sequenced rice genome are fixed to glass slides for hybridization to labeled cDNA targets prepared from different RNA samples of cells and tissues. Information on the genome-wide regulatory network of a biological process can be obtained through the study of differential expression pattern.

This study is focused on the gene expression at different stages of grain development in two Malaysian rice varieties (MR84 and MR219). The objectives of this study are:

a) To identify genes that are involved at different stages of development in rice grain filling through microarray analysis.
b) To compare the expressed genes in MR219 and MR84 at early grain filling (5 and 10 DAH) stage.
c) To verify the gene expression profiles of selected genes involved in rice grain filling using real time PCR.
d) To characterize phytohormone-related transcripts using Southern hybridization and

real time PCR.

CHAPTER 2

Literature Review

2.1 *Oryza sativa* L.

Rice belongs to the family of Gramineae. *Oryza sativa*, or the Asian rice, is believed to have evolved from an annual progenitor in a broad belt extending from the Gangetic plain below the foothills of Himalayas, across upper Myanmar, Northern Thailand, to North Vietnam and South China. Domestication could have occurred independently and concurrently at many sites within this area. Man took annual wild types, subjected them to the selection pressure of cultivation, harvesting and sowing, which gave rise to the *O. sativa* cultivars in Asia (Barker et al., 1985).

There are two cultivated and 21 wild species of *Oryza*. *Oryza sativa* is grown all over the world, whereas *O. glaberrima*, the African rice, is grown at a limited scale in West Africa (Purseglove, 1972). These two species of rice has a diploid chromosomal number of 24 (2n= 24). Numerous cultivars were established due to human selection and environmental factors that influence the evolution of rice types. Today, it is estimated that about 120,000 rice varieties exist in the world and a number of classifications were developed (Khush, 1997). Based on their genetic similarities, *O. sativa* are classified into six groups. The widely known *indica* and *japonica* correspond to group I and group VI respectively (Khush, 1997). The so-called *javanica* rice belonging to group VI a tropical ecotype of *japonica* (Glaszmann et al., 1986). Besides, rice is also being divided

according to their cultural types - irrigated, rainfed lowland, rainfed upland and flood prone area (Barker et al., 1985).

2.1.1 The growth phases and developmental stages in rice

Rice is a monocarpic and semi-aquatic plant with a main stem and many tillers. Plant height can range from 0.6 to 6 m depending on its variety. The reproductive tillers bear ramified panicles between 20 to 30 cm wide and each panicle have 50 to 300 spikelets (floret) which form the grains. Generally, the growth of rice plant is divided into three phases:

1. Vegetative (germination to panicle initiation)
2. Reproductive (panicle initiation to flowering)
3. Ripening (flowering to mature grain)

The reproductive phase and ripening phase take about 65 days in the tropics. The differences in growth duration of rice are mostly determined by changes in the duration of the vegetative phase (Wang and Li, 2005).

Vegetative phase is characterized by active tillering, gradual increase in plant height, and leaf emergence at regular intervals marks the beginning of vegetative phase. It encompasses stages from germination, emergence, seedling, tillering to stem elongation (IRRI, 2002). The reproductive phase is characterized by culm elongation (which increases plant height), decline in tiller number, and emergence of flag leaf, booting, heading and flowering. The initiation of panicle primordium at the tip of the growing shoot usually starts at about 25 to 30 days before heading. The young panicle increases

in size and its upward extension inside the flag leaf sheath causing the leaf sheath to bulge. The bulging of the flag leaf sheath is called booting. Heading is marked by the emergence of the panicle tip from the flag leaf sheath. The panicle continues to emerge until it partially or completely protrudes from the sheath.

Flowering / anthesis begins when anthers protrude from the spikelet and then fertilization takes place. At flowering, the florets open, the anthers protrude from the flower glumes because of stamen elongation, and the pollens are shed when pollination occurs. The florets then close. Flowering occurs with heading or a day after heading. Generally, florets open in the morning and take about 7 days for all spikelets in a panicle to open. The tillers of the rice plant have been separated at the start of the flowering and grouped into bearing and nonbearing tillers (Yoshida, 1981; IRRI, 2002).

Leaf senescence, increases in grain size and weight and changes in grain color marks ripening phase. This last growth stage is subdivided into milky, dough, yellow ripe and matures (Yoshida, 1981; Wong and Li, 2005). During milky grain stage, the grain is filled with milky materials, which can be squeezed out by pressing the grain between the fingers. The panicle looks green and starts to bend. Senescence at the base of the tillers is progressing. The flag leaves and the two lower leaves are green. During dough grain stage, the milky portion of the grain first turns into soft dough and later into hard dough. The grains in the panicle begin to change from green to yellow. Senescence of tillers and leaves is noticeable. As the panicles turn yellow, the last two remaining leaves of each tiller begin to dry at the tips. In mature grain stage, the individual grain is mature, fully developed, hard and yellow (IRRI, 2002).

2.1.2 The importance of rice

2.1.2.1 Global food supply

Rice is the principal food of nearly half of the world's population. It accounts for 50 to 80% of their daily calorie intake and is an important source of vitamins (thiamine, riboflavin and niacin) and minerals (phosphorus, iron and potassium). Besides, it contains eight essential amino acids for human body (Purseglove et al., 1972). More than 90% of rice is grown in developing countries where food supply is a problem. With the establishment of International Rice Research Institute (IRRI) in 1960, many efforts have been put in to increase rice production over the past 40 to 50 years. The enhancement of rice yielding from 257 million tons to 596 million tons in 1999 (Peng et al., 1999) was attributed to the large scale adoption of improved rice varieties (IR8, the first variety with improved plant type has doubled the yield potential of tropical rice) and technology developed by IRRI and other national rice improvement programs, based on Mendelian genetics and conventional breeding techniques (Matsushima, 1980).

However, in Asia, Africa and Latin America where rice is of top priority, the population is expected to increase 1.5 fold by 2025 (Sasaki et al., 2002). In Asia-Pacific region, on average, the production of rice have to increase from 524 tons in 1995 to 700 tons in 2025, an enhancement of about 25%, to meet the demand of the growing population (Peng et al., 1999).

Urbanization has limited arable land and irrigation for rice farming. In addition, poor