



**UNIVERSITI PUTRA MALAYSIA**

***GENETIC DIVERSITY ANALYSIS OF COLOURED UPLAND RICE  
GERMPLASM USING SSR MARKERS, QUANTITATIVE TRAITS, AND  
NUTRITIONAL QUALITY***

**FAIZ AHMAD**

**ITA 2014 7**



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NUTRITIONAL QUALITY**

By

**FAIZ AHMAD**

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

**April 2014**

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## **DEDICATION**

**THIS THESIS IS SPECIALLY DEDICATED**

**TO**

**MY PARENTS, MY BELOVED FAMILY AND FRIENDS**



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

**GENETIC DIVERSITY ANALYSIS OF COLOURED UPLAND RICE  
GERMPLASM REVEALED BY SSR MARKER, QUANTITATIVE TRAITS  
AND NUTRITIONAL QUALITY**

By

**FAIZ AHMAD**

**April 2014**

**Chairman: Professor Mohamed Hanafi Musa, PhD**

**Institute: Tropical Agriculture**

Rice (*Oryza sativa* L.) is a staple food for many people around the world. It contains high carbohydrate, which is as a source of energy. Currently, people are more concern about healthy food intake. Coloured rice is one of the alternatives for healthy diet because it has high nutritional value. In addition, coloured rice have antioxidants compound, such as polyphenols, carotenoids, and tocochromanols. These antioxidants have significant important health effect in human body. In this study, 42 selected coloured upland rice accessions were obtained from International Rice Research Institute (IRRI). There were collected from difference places around the world. To reveal the genetic diversity among the accessions, 25 short sequence repeated (SSR) markers were used in this study. Out of 25 SSR markers used in this study, only 21 were polymorphic. The polymorphic information content (PIC) value ranged from 0 (RM 338, RM 431, RM 118, RM 133) to 0.760 (RM 455). Mean of expected heterozygosity ( $H_e$ ) was 0.470 and Shannon's information index ranged from 0.380 to 1.660. From SSR markers, clustering analysis using Jaccard Similarity Coefficient showed that all the accessions were clustered into 7 groups. The seeds among all accessions were planted in the glasshouse for multiplication, morphological and assessments of agronomical characteristic using randomized complete block design (RCBD) with 3 replications for each accession. The following parameters were collected: number of tiller per plant, number of panicle per plant, plant height, length flag leaf, panicle length, percentage of filled grain, percentage of unfilled grain, harvest index, yield per plant, 100 grains weight, days to flowering, days to maturity, length

breath ratio of kernel, kernel length, chlorophyll content (SPAD reading) at 40 and 60 days. All data were analyzed by using SAS 9.2 software. Agro-morphological characters showed significant variation among all accessions. The heritability from 25 agro-morphological characters and nutrient content (macro & micro) in rice straw ranged from 56.52 to 99.31%. The nutrient contents (macro & micro) in rice grain varied greatly among accessions. The antioxidant activities for all rice accessions determined by 1,1- Diphenyl-2-picrylhydazyl (DPPH) radical scavenging effect method ranges from 31.85 to 98.45%. Based on the content of vitamin E (tocochromanols), the tocotrienol content was higher than that of tocopherol for all accessions selected. The selection of better rice accessions for future breeding program could be obtained using information from agro-morphological data, and nutritional status of the rice accessions.



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

**ANALISIS KEPELBAGAIAN GENETIK DALAM JENAPLASMA PADI BUKIT BERWARNA MENGGUNAKAN PENANDA SSR, TRAIT KUANTITATIF DAN KUALITI NUTRISI**

Oleh

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**April 2014**

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**Institut: Pertanian Tropika**

Padi (*Oryza sativa* L.) adalah makanan ruji pada kebanyakan orang di seluruh dunia. Ia mempunyai kandungan karbohidrat yang tinggi, iaitu salah satu daripada sumber tenaga. Belakangan ini, masyarakat lebih prihatin terhadap pengambilan makanan berkhasiat. Padi berwarna adalah salah satu alternative untuk diet berkhasiat kerana mempunyai kandungan nutrisi yang tinggi. Di samping itu, padi berwarna mempunyai komponen antioksidan, seperti “polyphenols”, “carotenoids”, dan “tocochromanols”. Komponen antioksidan ini mempunyai kepentingan yang signifikan kepada kesihatan tubuh manusia. Dalam kajian ini, sebanyak 42 aksesori padi bukit berwarna diperolehi dari Institut Penyelidikan Padi Antarabangsa (IRRI). Ia terdiri daripada koleksi pelbagai tempat dari seluruh dunia. Untuk mendedahkan kepelbagaian genetik antara aksesori, 25 penanda SSR telah digunakan dalam kajian ini. Daripada 25 penanda SSR digunakan dalam kajian ini, hanya 21 yang polimorfik. Min He adalah 0.470 dan indeks informasi Shannons adalah antara julat daripada 0.380 sehingga 1.660. Daripada penanda SSR, analisis kluster menggunakan “Jaccard Similarity Coefficient” menunjukkan kesemua aksesori telah diklusterkan sebanyak 7 kumpulan. Biji benih antara aksesori telah ditanam di rumah kaca untuk multiplikasi, ciri-ciri penilaian morfologi dan agronomi menggunakan model RCBD dengan 3 replikasi bagi setiap aksesori. Parameter tersebut telah dikumpulkan: bilangan batang per pokok, bilangan tangkai per pokok, tinggi pokok, panjang daun bendera, panjang tangkai, buah terisi per tangkai, buah tak terisi per tangkai, indeks tuaian, hasil per pokok, 100 berat buah, hari untuk berbunga, hari

untuk matang, nisbah panjang lebar biji, panjang biji, kandungan klorofil (bacaan spad) pada 40 dan 60 hari. Kesemua data ini telah dianalisis menggunakan perisian SAS 9.2. Ciri-ciri morfologi dan agronomi menunjukkan signifikasi pada variasi antara semua aksesori. Keterwarisan daripada 25 ciri-ciri agro-morfologi dan kandungan nutrisi (makro&mikro) dalam jerami padi antara julat dari 56.52 sehingga 99.31%. Kandungan nutrisi (makro&mikro) dalam biji padi amat berbeza antara aksesori. Aktiviti antioksidan untuk kesemua aksesori padi ditentukan oleh kaedah radikal memerangkap 1,1- Diphenyl-2-picrylhydrazyl (DPPH) antara julat dari 31.85 sehingga 98.45%. Berdasarkan kandungan vitamin E (tocochromanols), kandungan “tocotrienol” adalah tinggi daripada “tocopherol” bagi semua aksesori. Pemilihan untuk aksesori beras yang lebih baik untuk program pembiakbakaan boleh dibuat menggunakan maklumat daripada agro-morfologi dan status nutrisi aksesori.

## ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious, the Most merciful, I would like to praise to the Allah Almighty for His blessings which enable me to complete my thesis. I would like to express my appreciation to my supervisor Professor Dr. Mohamed Hanafi Musa for his guidance, good advice, and idea, from beginning to the end of this research. Without his supports and encouragement, my research study may not finished on the time.

I would like to give special thanks to the members of the Advisory Committee, Professor Dr. MohdRafiiYusop and Professor Datin Dr. SitiNorAkmar Abdullah for their good advice and suggestion for my research study.

I am thankful to Universiti Putra Malaysia for awarding me scholarship and research facilities for my master study. Additionally, I want to acknowledge for the Long-term Research Grant Scheme (LRGS: 55525001/Food Security) for the financial support to conduct this research.

I wish to give thanks to all the staff of Institute of Tropical Agriculture (ITA), UPM especially to Mrs. Norhasimah, Mr. Zainuddin, Mr. Zahardin, and Mrs. NorRafidah for their help during my research and study period. Furthermore, I would like to thank Associate Professor Dr. SharifahKharidha Syed Muhamad and DrAzmilHaizam

Ahmad Tarmizi for giving permission to use the lab facilities in UPM-Bernas and Malaysia Palm Oil Board (MPOB) laboratories, respectively.

I am thankful to my friends in ITA laboratory especially to Dr. Jannatul, Dr. Nusaibah, Ms. Mayzaitul, Ms. Akmal, Mrs. Shuhada, Mr. Hanif, Ms. Yee Min, Mr. Shahrudin, Mr. Mahboud, Mrs. Hasmah and to all individual who helped me directly or indirectly during the tenure of my research.

Special appreciation goes to my beloved parents, Mr. Ahmad Bin MohamadSom, and Mrs. Soa'atBintiJohari for their blessings and encouragements to me to finish the studies. Special gratitude also to my siblings who also gave me moral support during my study.



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:

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## LIST OF ABBREVIATIONS

%	-	Percentage
$\alpha$	-	Alpha
$\beta$	-	Beta
$\gamma$	-	Gama
$\delta$	-	Delta
$\sigma^2_g$	-	Genotypic variance
$\sigma^2_p$	-	Phenotypic variance
$^{\circ}\text{C}$	-	Degree celsius
ANOVA	-	Analysis of variance
B	-	Boron
bp	-	Base pair
Ca	-	Calcium
cm	-	Centimeter
CTAB	-	Cetyltrimethyl ammonium bromide
Cu	-	Copper
DNA	-	Deoxyribose nucleic acid
dNTPs	-	Deoxyribonucleotide triphosphate
EDTA	-	Ethylene diaminetetracetate
Fe	-	Iron
GA	-	Genetic advance
g	-	Gram
GCV	-	Genotypic coefficient variance
H <sub>2</sub> SO <sub>4</sub>	-	Sulphuric acid
H <sub>2</sub> O <sub>2</sub>	-	Hydrogen peroxide
h <sup>2</sup> B	-	Heritability
He	-	Expected heterozygosity
IRRI	-	International Rice Research Institute
K	-	Potassium
L	-	Liter
M	-	Molar
Mg	-	Magnesium
mg	-	Milligram
mm	-	Milimeter
mM	-	Millimolar
min	-	Minute
mL	-	Milliliter
Mn	-	Manganese
Mo	-	Molybdenum
N	-	Nitrogen
NaCl	-	Sodium Chloride

P	-	Phosphorus
PCR	-	Polymerase chain reaction
PIC	-	Polymorphic information content
PVP	-	Polyvinylpolypyrrolidone
rpm	-	Rotation per minute
SSR	-	Short sequence repeated
TBE	-	Tris-borate-EDTA
UV	-	Ultraviolet
µg	-	Microgram
µL	-	Microliter
V	-	Volt
v/v	-	Volume per volume
w/v	-	Weight per volume
Zn	-	Zinc

## CHAPTER 1

### INTRODUCTION

Rice is the staple food for most Asian countries including Malaysia, Thailand and Philippines. Currently, the level of rice sufficiency in Malaysia is at 72% and the yield at about 3.8 t/ha/season (Oryza, 2012). The government had spent millions of ringgit to increase domestic rice production and to cut the rice import. This is due to the food crisis in 2008 where countries, such as Thailand, Vietnam, and Philippines have reduced their rice export. This problem caused the shortage of rice during that period. In 2011, the Malaysian Government had spent about RM 26.6 million under the Economic Transformation Plan (ETP) to introduce the fragrant rice in non granary area (ETP, 2011). About RM 300 million were spent by the government in order to increase the domestic rice production within the period of 8 to 9 years (Oryza, 2012). This investment is very important action to reduce the importation of rice, to increase the farmers income, and as a food security programme.

The world population is increasing and has reached 7 billion in 2011. The population will reach around 8 billion by 2025 (Rosenberg, 2012). About half of the populations depend on rice as a main food source especially people from the Asian countries. The reduction in productive rice area is the major problem facing us today. Inconvenient divergence will occur, if the sufficiency to feed people is less than the demand. Many alternative ways have been done to increase rice production, such as developing high yielding varieties resistant to diseases and pests, introducing sustainable management practice to farmers, and introducing non granary area for planting rice.

Upland rice can grow in non-flooded soil without the need for irrigation system. It has special characteristics, such as fragrance, colour and shape (Hanafi et al., 2009). Usually, upland rice is planted on the hills and the yield is very low compared to irrigated rice. In Malaysia, the cultivation of upland rice has been performed mainly by farmers in upland area in Peninsular, Sabah and Sarawak. The worldwide rice production and planted area is about 4 and 13%, respectively (Gupta and O'toole, 1986). The main problem of the upland rice cultivation is to low number of tillers, lodging, and imbalanced supply of nutrients. More research is required to increase the yield of upland rice as an alternative to irrigated rice, since the scarcity of granary areas.

Health-conscious people is increasing in this modern society. They prefer to choose healthy and nutritious food in their daily meal. The composition of the mineral elements in food is decreasing during 1979-1995 period (Ya-Wen et al., 2004). The increasing case of chronic disease nowadays are also related to nutrient deficiency in food. Thus, coloured upland rice is one of the alternatives for healthy food, since it contains high antioxidant that has significant effect on human health. This pigmented rice accession have potential use for breeding programme that is beneficial for various nutraceutical and functional food development. Thus, assessments of the nutrient in these accessions is important for better understanding and knowledge of coloured rice germplasms.

There are little information and research about the coloured upland rice accessions. The documentation of the accession must be done in order for further breeding programme. Agro-morphological data are the important characteristic needed to select the good quality accessions. Each accession has a unique varietal characteristic, such as plant height, panicle length, and time of flowering. While, molecular markers is another tool used for selection of the accession for breeding programe. These include Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphism (SNP). The most widely used is SSR since it has high polymorphism and high reproducibility. Molecular marker is more powerful compared to morphology characteristic since it is not influenced by the environment.

Documentation of the accessions must be done for better understanding and reveal the special characteristic of the accessions. Hence, the nutritional value of the accession must be assessed to improve the the quality of upland rice This study was conducted to determine the genetic diversity and nutritional qualities of 42 coloured upland rice germplasm of rice (*Oryza sativa* L.) obtained from the International Rice Research Institute, Philippines. The specific objectives were:

- i) To assess genetic diversity using SSR markers and calculate genetic similarities among accessions.
- ii) To determine agro-morphological characteristics, heritability, and genetic advance.
- iii) To determine the mineral content, antioxidant activities, and Vitamin E content.

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