UNIVERSITI PUTRA MALAYSIA

GENETIC DIVERSITY OF UPLAND RICE REVEALED BY QUANTITATIVE TRAITS AND MICROSATELLITE POLYMORPHISMS

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ITA 2012 17

GENETIC DIVERSITY OF UPLAND RICE REVEALED BY QUANTITATIVE TRAITS AND MICROSATELLITE POLYMORPHISMS

BY:

MEHDI SOHRABI

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

May 2012

Special Dedication to My Beloved Parents

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Abstract of Thesis Presented to the Senate of Universiti Putra Malaysia in the Fulfilment of the Requirement for the Degree of Master of Science

GENETIC DIVERSITY OF UPLAND RICE REVEALED BY QUANTITATIVE TRAITS AND MICROSATELLITE POLYMORPHISMS

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May 2012

Chairman : Assoc. Prof. Mohd Rafii Yusop, PhD

Institute : Tropical Agriculture

In Malaysia, upland rice is cultivated mainly in Sabah and Sarawak, and a small area in Peninsular Malaysia. In this study, the genetic diversity was evaluated among fifty Malaysian upland rice accessions. The objectives of this research were (i) to study the genetic diversity of the upland rice population revealed by quantitative traits and microsatellite polymorphism, (ii) to determine genetic control and heritability of quantitative traits, and (iii) to identify several potential upland rice accessions for further breeding program.

In the first experiment the genetic diversity was evaluated based on 12 quantitative traits. All traits were significant or highly significant among all accessions. Four traits indicated both high level of broad sense heritability and genetic advance, namely flag leaf length to width ratio, spikelet fertility, grain yield, and days to flowering. All accessions were divided into six

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groups by morphological clustering and accessions 6040, 6041, 6048, 6068, 6070, and 6067 indicated higher average values for most of traits. This clustering was related to geographical divergence between Sabah state and Peninsular Malaysia. The twelve morphological traits provided around 77% variation among accessions.

Twenty three SSR markers were used for estimation of genetic relationships, which finally helped in characterization of the upland rice germplasm.Ten primers indicated polymorphism among 50 accessions of upland rice. The Jaccard similarity coefficient between accessions was clustered in 7 groups which correlated to regional diversity. Shannon's information index ranged from 0.5269 to 2.0050. Gene diversity (*h*) ranged from 0.3432 to 0.8273. Overall gene flow was 0.0011. Some accessions suggested for further breeding program according to their Jaccard's similarity. They are including accessions 07537, 07538, 03826, 07574, 07588, 07585, 07540, 07575, 07541, 07543, 07544, 07576, 07571, 07539, 03825, and 03830 from group six and also accessions 07531, 07534, and 07535 from group five.

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

KEPELBAGAIAN GENETIK PADI HUMA BERDASARKAN CIRI KUANTITATIF DAN POLIMORFISM PENANDA MIKROSATELIT

Oleh

MEHDI SOHRABI

Mei 2012

Pengerusi : Prof. Madya Mohd Rafii Yusop, PhD

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Di Malaysia, padi huma banyak ditanam di Sabah dan Sarawak, dan sebahagian kecilnya di Semenanjung Malaysia. Dalam kajian ini, kepelbagaian genetik dinilai dikalangan 50 aksesi padi huma. Objektif kajian ini adalah (i) untuk mengkaji kepelbagaian genetik populasi padi huma berdasarkan polimorfism penanda mikrosatelit dan ciri kuantitatif, (ii) untuk menentukan pengawalan genetik dan keterwarisan ciri kuantitatif, dan (iii) untuk mengenalpasti beberapa aksesi padi huma yang berpotensi untuk program pembiakbakaan yang seterusnya.

Dalam eksperimen yang pertama, kepelbagaian genetik telah dinilai berdasarkan 12 ciri kuantitatif. Berdasarkan keputusan tersebut, kesemua ciri adalah mempunyai perbezaan yang bererti atau sangat bererti di kalangan semua aksesi. Empat ciri menunjukkan nilai keterwarisan luas dan kemajuan genetik yang tinggi, iaitu kadar panjang kepada lebar daun,

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kesuburan spikelet, hasil bijian, dan tempoh masa untuk berbunga. Kesemua aksesi tersebut telah dibahagikan kepada enam kumpulan berdasarkan kluster ciri morfologi, dan aksesi 6040, 6041, 6048, 6068, 6070,dan 6067 memberikan nilai purata yang tinggi bagi kebanyakan ciri tersebut. Pengklusteran ini mempunyai kaitan dengan pencapahan kawasan geografi di antara negeri Sabah dan Semenanjung Malaysia. Dua belas ciri morfologi menunjukkan sebanyak 77% perbezaan di antara aksesi.

Dua puluh penanda SSR telah digunakan untuk menganggarkan perhubungan genetik, yang seterusnya membantu dalam mengkategorikan gemplasma padi huma tersebut. Sepuluh penanda telah menunjukkan polimorfism dikalangan 50 aksesi padi huma tersebut. Persaman pekali Jaccard di antara aksesi telah diklusterkan kepada tujuh kumpulan yang menunjukan perhubungan dengan kepelbagaian kawasan. Indeks informasi Shannon adalah di antara 0.5269 hingga 2.0050. Kepelbagaian gen (*h*) dari 0.3432 hingga 0.8273. Keseluruhan aliran gen adalah 0.0011. Sebahagian aksesi disaran untuk program pembiakbakaan selanjutnya berdasarkan persamaan Jaccard aksesi tersebut. Aksesi tersebut adalah 07537, 07538, 03826, 07574, 07588, 07585, 07540, 07575, 07541, 07543, 07544, 07576, 07571, 07539, 03825, dan 03830 dari kumpulan keenam, dan juga aksesi 07531, 07534 dan 07535 daripada kumpulan kelima.

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ACKNOWLEDGEMENT

I would like to express my sincerest appreciation to my research supervisor, Associate Professor Mohd Rafii Yusop, PhD for giving me the opportunity to work in his laboratory and for his advice and encouragement throughout this entire project. Without his support and confidence over the years this dissertation would not have been possible.

I wish to give my special thanks to my committee members Professor Mohamed Hanafi Musa, PhD and Associate Professor Datin Siti Nor Akmar Abdullah, PhD for their valuable suggestions, comments and help during the past few years.

I wish to express my profound gratitude to Dr. Md. Abdul Latif to provide excellent help during the project. I am very grateful to MahmmodReza Shabanimofrad and Alireza Biabanikhankahdani who have been always graciously willing to encourage me, treat and help me during the project with true friendship. Thanks to all my friends in the breeding lab.

I would like to express my deepest gratitude to my parents for their endless love and faith. Their unwavering support lifted my spirit and confidence.

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I certify that a thesis examination Committee has met on 18 May 2012 to conduct the final examination of Mehdi Sohrabi on his thesis entitled "Genetic diversity of upland rice revealed by quantitative traits and microsatellite polymorphism" 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 Master of Science.

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DECLARATION

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

MEHDI SOHRABI

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LIST OF ABBREVIATION

AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
bp	Base pairs
h^2_B	Broad sense heritability
cm	Centimetre
CTAB	Cetyltrimethylammonium bromide
CV	Coefficient of variation
r	Correlation coefficient
DNA	Deoxyribonucleic acid
dNTP	2'-Deoxynucleoside 5'- triphosphate
n_e	Effective number of alleles
EDTA	Ethylenediamine tetra-acetic acid
FAO	Food and Agriculture Organization of the United Nations
G_{ST}	Gene differentiation
Nm	Gene flow
GA	Genetic advance
GCV	Genotypic coefficient of variation
GV	Genotypic variance
ha	Hectare
MS_E	Mean square of error
MS_G	Mean square of genotype
µg	Microgram
µl	Microlitre
µM	Micromolar
h	Nei's (1973) gene diversity
n_a	Number of alleles
NTSYS	Numerical taxonomy multivariate analysis system
PPL	Percent of polymorphic Loci

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PCV	Phenotypic coefficient of variation
PCR	Polymerase chain reaction
PC	Principal component
PCA	Principal component analysis
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
I	Shannon's information index
s	Second
SSR	Simple sequence repeat
Std.	Standard deviation
SE	Standard error
Taq	*Thermus aquaticus*
TBE	Tris-borate/EDTA
TE	Tris EDTA buffer
TM	Melting temperature
ton	Tons
UV	Ultraviolet
UPGMA	Unweighted pair group method using arithmetic averages
v/v	Volume per volume

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Rice (*Oryza sativa*) is one of the most important food crops for human compared to other cereals. Around three billion people of the world consumed rice as a critical or basic food that provides 50 to 80% of their daily calories. Rice is cultivated on more than 150 million hectares, and annual world production is around 600 million tons (Delseny *et al.*, 2001; Guimarães, 2009; Tyagi *et al.*, 2004). It is one of the crops responsible for the so-called green revolution of the 1960s and 1970s (Guimarães, 2009). With the global population of the world rising, a plan for increasing the productively of this crop is needed (Ram *et al.*, 2007).

Asian planted rice was domesticated from its wild ancestor about 11,500 years ago. Rice can grow in different geographical conditions including in tropical and subtropical countries (Normile, 1997; Ram *et al.*, 2007). More than half of global rice is cultivated and consumed in Asia: China, India, and Indonesia show a high yield of this crop (Chakravarthi and Naravaneni, 2009; Hossain, 2007).

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There are different types of rice such as deepwater rice, irrigated rice, and rainfed lowland rice (Poehlman and Sleper, 1995). Upland rice comprises eleven percent of global rice production and is cultivated on around 14 million hectares. Upland rice has a small role in total production but it is a major food source in some tropical countries (Kondo *et al.*, 2003). Bangladesh, Indonesia, and Philippines are the areas that plant the most the upland rice, but the yield is so low (about 1 t/ha on average) and highly variable (Thanh *et al.*, 1999).

In Malaysia, two types of rice are cultivated: wet paddy in Peninsular Malaysia (503,184 ha) and upland rice in Sabah and Sarawak (165,888 ha). The average yield of wet paddy is around 3.3 t/ha; in good conditions, however, it can increase to around 10 t/ha. In contrast, the average yield of upland rice ranges from 0.46 to 1.1 t/ha. In 2005, the total national rice production was roughly 2.24 million metric tons. In Malaysia, upland rice is usually cultivated for home consumption by rural people living in Sabah and Sarawak (Hanafi *et al.*, 2009).

Plant breeding begins with genetic diversity, which is used as a source for developing new characteristics or transforming unfavorable varieties. Breeders can detect genome architecture and find new methods for modification and improvement of crops by genetic diversity (Ahmadikhah *et al.*, 2008).

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One step for successful in breeding programs is genetic diversity. In evolutionary biology, specification and quantification has been main target. Using genetic diversity information can assist in logical utilization of genetic resources within and among closely related crop varieties. The analysis of genetic diversity can help breeders to monitor germplasm and to predict potential genetic gains (Chakravarthi and Naravaneni, 2009). The awareness of genetic diversity is so important for improving crops and on the other hand, rice is the biggest ex situ germplasm collection in the world, so it is essential to study genetic diversity in the germplasm for developing genetic variation in rice breeding (Zhao *et al.*, 2009).

Genetic diversity is mainly measured based on the physiological and morphological differences of quantitative and economically important traits. However, this method has some disadvantages, such as time and labor costs. Moreover, this method cannot define the exact level of genetic diversity among germplasms, because the trait appears through interaction between genes and the environment (Schulman, 2007; Zeng *et al.*, 2004). Gene expression is affected by environment, so selection-based morphological traits are seductive (Asif *et al.*, 2005; Astarini *et al.*, 2004; Kumar *et al.*, 1998).

Molecular markers are powerful tools for analyzing genetic diversity within and among varieties. There are different molecular markers which are

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based on polymorphism of protein or DNA. Molecular markers can show differences between accessions at the DNA level and are direct and reliable for germplasm protection and management.

In rice, molecular markers have been used to identify accessions (Olufowote *et al.*, 1997; Virk *et al.*, 1995), to determine the genetic structure and pattern of diversity for cultivars of interest (Akagi *et al.*, 1997; Mackill, 1995; Yang *et al.*, 1994; Zhang *et al.*, 1992), to optimize the assembly of core collections (Schoen *et al.*, 1995), and to compute QTL (Kishima *et al.*, 2005).

Among the different types of molecular markers, PCR-based molecular markers such as microsatellites are valuable tools for studying genetic diversity and organism relationships, because they can show high levels of polymorphism (He *et al.*, 2003; Ishii *et al.*, 2001).

1.2 Statement of problem

Malaysia is a tropical country, so it is a good place for cultivating upland rice. Unfortunately, there is no information published with regards to breeding, genetics, and morphological characteristics of upland rice. Obtaining comprehensive information on genetics and morphological

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