UNIVERSITI PUTRA MALAYSIA

GENETIC DIVERSITY OF MALAYSIAN AROMATIC RICE GERMPLASM REVEALED BY QUANTITATIVE TRAITS, MICROSATELLITE AND INTERSIMPLE SEQUENCE REPEAT MARKERS

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By

SABA JASIM MOHAMAD AL-JUMAILY

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

October-2015

DEDICATION

This thesis is dedicated to Allah, the almighty God, his prophet, his beloved companions, my parents and my beloved family.

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

GENETIC DIVERSITY OF MALAYSIAN AROMATIC RICE GERMPLASM REVELED BY QUANTITATIVE TRAITS, MICROSATELLITE (SSR) AND INTER SIMPLE SEQUENCE REPEAT (ISSR) MARKERS

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The study of genetic diversity among 50 aromatic rice accessions from Peninsular Malaysia, Sabah and Sarawak with 3 released varieties as a control was carried out through quantitative traits and molecular markers. The objectives of this research were (i) to evaluate the performance of different accessions of Malaysian aromatic rice, (ii) to determine the genetic diversity among the aromatic rice accessions, (iii) to quantify the genetic divergence of the aromatic rice accessions using microsatellite (SSR) and inter simple sequence repeat (ISSR) markers, and (iv) to identify superior accessions among the germplasm for future aromatic rice breeding program. Results from genetic diversity analysis based on 14 quantitative traits, showed that all the traits had significant variation among the accessions. The eight traits, namely plant height, 1000-grain weight, yield per hill, number of panicles, spikelet fertility, number of grains per hill, flag leaf length to width ratio and panicle length indicated high level of broad sense heritability and genetic advance.

These traits are regarded as important yield components for selection of superior rice genotypes. The broad sense heritability values for these traits were more than 91%, while genetic advance values of those traits ranged from 31.02 to 56.95%. Cluster analyses based on morphological traits grouped the 53 accessions into six clusters. The first four principal components based on the quantitative traits resulted into 71.3% of the total variation. Based on the quantitative analysis, Accessions Acc6288, Acc9993, Acc11816, Acc9936, Acc9971 and Acc10538 indicated among the highest

average values for some traits namely, number of tillers per hill, 1000-grain weight, grain yield per hill, spikelet fertility and number of grains per hill. Genetic diversity analysis of the 53 rice accessions using 32 SSR and 25 ISSR polymorphic markers clustered the accessions into 10 groups and 8 groups respectively. Based on Analysis of molecular variance (AMOVA), SSR markers detected a high polymorphism within population (89%) and low polymorphism among populations (11%). ISSR markers also revealed similar trend with a high polymorphism within population (87%) and low polymorphism among populations (13%). Gene diversity (h) among the 53 accessions ranged from 0.045 to 0.976 using SSR markers, and while from ISSR markers, it ranged from 0.129 to 0.849. Several superior accessions have been identified for the future aromatic rice breeding program. These include Accession Acc6288 (Peninsular Malaysia), Acc9936 and Acc9971 (Sabah), and Acc11816 and Acc10538 (Sarawak). The selected accessions can be subjected to further evaluation and subsequent crossing program for aromatic rice varietal development.

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

KEPELBAGAIAN GENETIK JANAPLASMA PADI BERAROMA BERDASARKAN CIRI KUANTITATIF, PENANDA MIKROSATELIT DAN ANTARA JUJUKAN BERULANG MUDAH

Oleh

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Kajian kepelbagaian genetik antara 50 aksesi padi beraroma dari Semenanjung Malaysia, Sabah dan Sarawak, dengan 3 varieti yang telah diistiharkan sebagai kawalan telah dijalankan melalui ciri kuantitatif dan penanda molekul. Objektif kajian ini adalah (i) untuk menilai prestasi aksesi berbeza padi beraroma Malaysia (ii) untuk menentukan kepelbagaian genetik aksesi padi beraroma tesebut (iii) untuk mengukur perbezaan genetik aksesi padi beraroma dengan penanda mikrosatelit (SSR) dan antara jujukan berulang mudah(ISSR), dan (iv) untuk mengenal pasti aksesi padi beraroma yang unggul untuk program pembiakbakaan dimasa hadapan. Keputusan analisa kepelbagaian genetik berdasarkan 14 ciri kuantitatif, menunjukkan bahawa semua ciri yang dinilai mempunyai perbezaan yang ketara diantara semua aksesi.

Lapan ciri iaitu ketinggian pokok, berat 1000-bijian, hasil setiap perdu, bilangan tangkai, bilangan tangkai bernas, bilangan bijian setiap perdu, nisbah panjang ke lebar daun pengasuh dan panjang tangkai menunjukkan paras tinggi bagi kedua-dua nilai iaitu keterwarisan luas dan kemajuan genetik. Ciri ini adalah merupakan komponen hasil yang penting untuk pemilihan genotip padi unggul. Nilai keterwarisan luas untuk ciri tersebut adalah lebih daripada 91%, sementara itu nilai kemajuan genetik pula adalah diantara 31.02 hingga 56.95%. Analisa kluster berdasarkan ciri morfologi membahagikan 53 aksesi tersebut kepada enam kluster. Empat pertama komponen principal berdasarkan ciri kuantitatif yang dikaji adalah 71.3% dari jumlah keseluruhan variasi. Berdasarkan analisa kuantitatif, Aksesi Acc6288, Acc9993,

Acc11816, Acc9936, Acc9971 dan Acc10538 memberikan nilai purata yang tinggi bagi sebahagian ciri, iaitu bilangan anak pokok, berat 1000-bijian, hasil bijian setiap perdu, kesuburan tangkai dan bilangan bijian setiap perdu. Analisa kepelbagaian genetik daripada 53 aksesi padi menggunakan 32 penanda SSR dan 25 penanda ISSR polimorfik telah membahagikan aksesi kepada 10 kluster dan 8 kluster masing-masing.

Berdasarkan analisa varians molekular (AMOVA), penanda SSR mendapati tahap polimorfisme yang tinggi dihkalangan aksesi dalam populasi (89%) dan polimofisma yang rendah dihantara populasi (11%). Penanda ISSR juga mendapati corak yang sama iaitu tahap polimorfisme yang tinggi dikalangan aksesi dalam populasi (87%) dan polimorfisme rendah diantara populasi (13%). Kepelbagaian gen (h) dikalangan 53 aksesi adalah diantara 0.045 hingga 0.976 mengunakan penanda SSR, manakala nilainya diantara 0.129 hingga 0.849 melalui penanda ISSR. Beberapa aksesi telah dikenalpasti untuk program pembiakbakaan padi aromatik selanjutnya. Aksesi tersebut adalah Acc6288 (Semenanjung Malaysia), Acc9936 dan Acc9971 (Sabah), dan Acc11816 dan Acc10538 (Sarawak). Aksesi terpilih ini perlu penilaian lanjutan dan seterusnya untuk program kacukan bagi pembangunan varieti padi beraromatik.

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

I certify that a Thesis Examination Committee has met on 7 October 2015 to conduct the final examination of Saba Jasim Mohamad on her thesis entitled "Genetic Diversity of Malaysia Aromatic Rice Germplasm Revealed by Quantitative Traits, Microsatellite and Intersimple Sequence Repeat Markers" 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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LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
AMOVA	Analysis of molecular variance
bp	Base pairs
h^2_B	Broad sense heritability
CTAB	Cetyltrimethylammonium bromide
CV	Coefficient Of Variation
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
MSE	Mean square of error
MSG	Mean square of genotype
h	Nei's (1973) gene diversity
n_a	Number of alleles
PPL	Percent of polymorphic Loci
PCV	Phenotypic coefficient of variation
PCR	Polymerase chain reaction
PC	Principal component
PCA	Principal component analysis
NTSYS	Numerical taxonomy multivariate analysis system
RAPD	Random amplified polymorphic DNA

RFLP	Restriction fragment length polymorphism
SSR	Simple sequence repeat
Std	Standard deviation
SE	Standard error
Taq	Thermos aquatics
TBE	Tris-borate/EDTA
TE	Tris EDTA buffer
TM	Melting temperature
UV	Ultraviolet
UPGMA	Unweight pair group method using arithmetic averages

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Rice (*Oryza sativa*L.) belonging to the family *Gramineae* is a staple food for over half of the world's population (FAO, 2004).Rice is the world's most important food crop for people compared to other cereals. Approximately three billion people of the world consumed rice as a basic food that provides between 50 to 80% of their daily calories. It not only supplies carbohydrate but also provides some essential food elements like protein, iron, calcium, thiamine, riboflavin, niacin and vitamin E to the human body (Akinbile et al., 2011). In Malaysia, approximately 72% rice is being granary areas (Teh, 2010). The aromatic rice is preferred over non-aromatic rice during special occasions and for export, and thus they command a higher market price. Major feature of these aromatic rice varieties is aroma which is being appreciated by many people and represents a high value added trait (Joseph et al., 2004). Three different things seem to have led to the growth in popularity of aromatic rice: globalization, health-consciousness and culinary changes (Hore, 2005). So, rice needs attention toward improvement in its cooking qualities as well as several biochemical and morphological characteristics (Golam et al., 2011).

The demand for aromatic rice is increasing day by day. Unfortunately, the aromatic rice production is been affected by some abiotic and biotic, susceptibility to pests and diseases, and strong shedding (Ahn et al., 1992). The agronomic value of a variety depends on many characteristics (Regmi et al., 2002). The most important characteristics are high yielding ability, resistance to diseases and pests, resistance to undesirable environmental factors and high quality of the products. Plant breeding launches with genetic diversity that is utilized as a source for improving new features or transforming undesirable varieties. Breeders can reveal genome structure and find new techniques for modification and developing crops by genetic diversity (Ahmadikhah, 2009). The first stage for effective technique in breeding programs is knowing the genetic diversity.

Using genetic diversity information can aid reasonable utilization of genetic resources among closely related crop varieties. Furthermore, the exploration of genetic diversity can assist breeders to observe germplasm and to predict potential genetic diversity is quite remarkable for evolving crops. Genetic variation analysis facilitates breeders in observation germplasm as well as in predict of potential genetic achievements (Chakravarthi and Naravaneni, 2006). The improvement of rice breeding plummeted progressively during the last ten years due to poor basis of the parent materials (Zhao et al., 2009). The research of rice genetic variety is essential for cultivars rating,

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

Among the PCR-based markers, for example the SSR markers, are proved very efficacious tools in the study of genetic diversity and organism relationships among all types of molecular markers, since they show higher level of polymorphism (Ishii et al., 2001; He et al., 2003). For marker-assisted selection as well as gene tagging, rice microsatellites (RM) had shown their utility (Chen et al., 1997;McCouch et al., 1997). The SSR markers can be effectively applied for developing unique DNA profiles of rice genotypes because of having high level of polymorphism and greater information. Moreover, these profiles might be valuable to clearly differentiate rice cultivars in order to get plant variety protection (Rahman et al., 2009).

Similarly, the ISSR markers also play important role in the determination of genetic diversity and organism relationships. The ISSR markers are useful not only in understanding the evolutionary relationships of Oryza but also in the fingerprinting of cultivated and wild species of germplasm. Moreover, these markers have high resolution power in fingerprinting and diversity analysis of rice observed by Joshi et al.(2000). Furthermore, using the ISSR markers it was found that higher diversity among rice species and variation exist between wild and cultivated rice as noticed by Girma(2007), in Ethiopiaamong the different types of molecular markers, PCR-based molecular markers such as microsatellites and inter-simple sequence repeat are valuable tools for studying genetic diversity and organism relationships, because they can show high levels of polymorphism (Ishii et al., 2001; He et al., 2003).

Problem statement

Low yield is a common phenomenon of aromatic rice and consequently rice breeders are trying to improve the agronomic characters to gain a better grain yield (Faruq et al., 2011). In addition, Malaysia is a tropical country, so it is highly potential area for cultivating aromatic rice. Unfortunately, there is no enough information published with regards to breeding, genetics, and morphological characteristics of aromatic rice. Obtaining comprehensive information on genetics and morphological characteristics as well as genetic diversity of aromatic rice in Malaysia is important for crop breeding programs.

Research objectives

The objectives of this research were:

i. To evaluate the performance of different accessions of Malaysian aromatic rice.
ii. To determine the genetic diversity of aromatic rice accessions with SSR and ISSR markers.
iii. To identify several superior accessions of aromatic rice for future breeding programs.

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