**UNIVERSITI PUTRA MALAYSIA**

# *GENETIC ANALYSIS OF GRAIN QUALITY TRAITS AND MARKER ASSISTED SELECTION FOR FRAGRANCE TRAIT IN SELECTED MALAYSIAN RICE (Oryza sativa L.) VARIETIES*

**ASFALIZA RAMLI**

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**DOCTOR OF PHILOSOPHY**
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By

**ASFALIZA RAMLI**

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

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**GENETIC ANALYSIS OF GRAIN QUALITY TRAITS AND MARKER-ASSISTED SELECTION FOR FRAGRANCE TRAIT IN SELECTED MALAYSIAN RICE (*Oryza sativa* L.) VARIETIES**

By

**ASFALIZA RAMLI**

**May 2014**

**Chairman: Professor Mohd Rafii bin Yusop, PhD**

**Faculty: Agriculture**

Rice has reached a yield plateau and is susceptible to a major disease outbreak, so genetic information of the breeding material is important for developing a high quality variety. As a result of breeding that has focused on yields, grain quality has declined. This study is an evaluation of the combining ability, heritability, and correlation of grain quality traits for selected Malaysian rice varieties and the actions of the genes involved in the inheritance of these traits. It also introduces marker-assisted selection for the fragrance trait in a selected $F_2$ population. The mean squares values for general combining ability (GCA) were significant for grain length (GL), grain width (GW), milled grain length (MGL), milled grain width (MGW), length to width ratio (LW), milled rice recovery (MRR), head rice recovery (HRR), amylose (AMYL) and gel consistency (GC), which indicated the importance of additive gene effects to the inheritance of these traits. Rice varieties MR 84 and MR 267 were the best combiners for most of the traits. Q 85 was a good combiner for GC, GL and HRR. The results of the specific combining ability (SCA) effect promoted 7 out of 21 total combinations to the next generation cycle ($F_2$) for further phenotypic selection: MR 84 X MRQ 74, MR 84 X MRQ 76, MR 263 X Q84, MR 263 X MRQ 74, MR 267 X MRQ 74 and MRQ 76 X Q 84. Seven combinations showed large and significant REC effects: MRQ 76 X MR 84, MRQ 76 X MR 263, and Q 84 X MR 263 MRQ 76 X MRQ 74, Q 84 X MRQ 74, Q 85 X MRQ 74 and Q 84 X MRQ 76. The genetic parameters of the grain quality traits showed higher additive variance compared to the dominance variance. The broad sense heritability for GL was moderate, while it was comparatively higher in GW, MGL, MGW, LW AND HRR. The narrow sense heritability of the grain quality traits was high for GW, MGL, MGW and LW and moderate for AMYL, GC and GL. Positive correlations were observed between 10 pairs of grain quality traits: AMYL and HRR, GL and MGL, GL and LW, GW and MGW, MGL and LW, MGL and MRR, MGL and HRR, LW and MRR, LW and HRR and MRR and HRR. Generation mean analysis revealed the importance of additive gene action in GL, MGL and MRR for a population of high and low amylose parents. However, the populations of intermediate and high amylose parents and intermediate and low

amylose parents shared similar dominant gene actions for most of the physical grain quality traits. Large differences between the parents in the traits resulted in simple heritability. Heritability in the $F_2$ ranged from low to high in the population of high and low amylose parents despite the non-heritability of some traits in the populations of intermediate and high amylose parents and intermediate and low amylose parents. Marker-assisted selection (MAS) was introduced for fragrance detection, and allelic specific amplification (ASA) successfully differentiated the fragrant plants from the non-fragrant. Chi-squared analysis revealed that phenotyping and genotyping of the fragrance trait was not significant for a segregation ratio of 3:1 and significant for the segregation ratio of 1:2:1. Out of a total of 35 rice microsatellite markers (RMs) used, 10 were identified as polymorphic between the evaluated parents. The markers were RM 25, RM 44, RM 72, RM 80, RM 152, RM 210, RM 281, RM 330, RM 342 and RM 342A. However, none of these markers fit the 1:2:1 segregation ratio. The best breeding approach for MAS is at the seedling stage before transplant as opposed to conventional methods in which it is normally carried out at the mature stage. Marker assisted selection (MAS) is introduced for fragrance detection. Allelic specific amplification (ASA) had successfully differentiated the fragrance plant from the non-fragrant. Chi-square analysis revealed that phenotyping and genotyping of fragrance trait was non-significant for segregation ratio of 3:1 and significant for the segregation ratio of 1:2:1 respectively. Out of a total of 35 rice microsatellite marker (RM) used, 10 out of 35 markers were identified as polymorphic between the evaluated parents. The markers were RM 25, RM 44, RM 72, RM 80, RM 152, RM 210, RM 281, RM 330, RM 342 and RM 342A. However, none of these markers fit to 1:2:1 segregation ratio. The best breeding approach for MAS is at the seedling stage before transplanting as compared to the conventional methods which was normally carried out at maturity stage.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**ANALISA GENETIK BAGI CIRI KUALITI BIJI DAN PEMILIHAN BERBANTUAN PENANDA CIRI WANGI PADA VARIETI TERPILIH PADI MALAYSIA (*Oryza sativa* L.)**

Oleh

**ASFALIZA RAMLI**

**Mei 2014**

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Padi telah mencecah hasil yang statik dan mudah rentan kepada wabak penyakit dan perosak utama. Dengan itu, maklumat genetik bagi bahan baikbaka adalah penting untuk membangunkan varieti padi berkualiti tinggi. Hasil daripada objektif pembaikbakaan yang memfokus kepada ciri hasil, kualiti biji padi semakin berkurangan. Kajian ini meliputi penilaian keupayaan bergabung, kebolehwarisan dan hubungan ciri kualiti biji bagi varieti terpilih padi Malaysia, tindakan gen yang terlibat dalam kebolehwarisan ciri-ciri ini dan pemilihan berbantuan penanda untuk ciri wangi bagi populasi $F_2$ terpilih. Min kuasa dua bagi keupayaan bergabung (GCA) adalah bererti bagi panjang biji (GL), Lebar biji (GW), panjang beras (MGL), lebar beras (MGW), nisbah perpanjangan beras (LW), pulangan mengilang (MRR), pulangan beras kepala (HRR), amilos (AMYL) dan kekonsistenan gel (GC).dan menunjukkan kepentingan gen aditif dalam kebolehwarisannya. Varieti padi MR 84 dan MR 267 menunjukkan keupayaan bergabung terbaik bagi hampir kesemua ciri. Q 85 menunjukkan prestasi keupayaan bergabung tertinggi bagi ciri GC, GL, dan HRR. Hasil keupayaan bergabung spesifik (SCA) telah memajukan tujuh kombinasi ke generasi musim hadapan ($F_2$) untuk pemilihan fenotip iaitu MR 84 X MRQ 74, MR 84 X MRQ 76, MR 263 X Q84, MR 263 X MRQ 74, MR 267 X MRQ 74 dan MRQ 76 X Q 84 berbanding keseluruhan 21 kombinasi kacukan. Tujuh kombinasi menunjukkan keupayaan bergabung bersilang yang bererti iaitu MRQ 76 X MR 84 MRQ 76 X MR 263, Q 84 X MR 263, MRQ 76 X MRQ 74, Q 84 X MRQ 74, Q 85 X MRQ 74 dan Q 84 X MRQ 76. Parameter genetik bagi ciri kualiti biji menunjukkan varians aditif lebih tinggi berbanding varians dominan. kebolehwarisan bagi ciri GL adalah sederhana manakala GW, MGL, MGW, LW dan HRR adalah lebih tinggi. Kkebolehwarisan sempit adalah tinggi bagi GW, MGL dan LW dan sederhana bagi AMYL, GC dan GL. Korelasi positif diperolehi bagi 10 pasangan ciri kualiti biji padi iaitu AMYL dan HRR, GL dan MGL, GL dan LW, GW dan MGW, MGL dan LW, MGL dan MRR, MGL dan HRR, LW dan MRR, LW dan HRR dan MRR dan HRR. Analisa generasi min menunjukkan kepentingan tindakbalas gen aditif bagi ciri GL, MGL dan MRR pada populasi yang terhasil dari induk amilos tinggi dan rendah. Walaubagaimana pun, populasi yang terhasil dari induk amilos sederhana dan tinggi dan dari induk amilos sederhana dan rendah sama-sama menunjukkan tindakbalas gen dominan bagi

hampir kesemua ciri kualiti fizikal. Di dapati bahawa perbezaan yang besar bagi ciri kualiti padi di antara kedua-dua induk yang terlibat menghasilkan kebolehwarisan yang mudah. Kebolehwarisan pada $F_2$ bagi populasi yang terhasil dari induk amilos tinggi dan rendah adalah dalam sela rendah ke tinggi biarpun ianya menunjukkan ketidakbolehwarisan bagi sesetengah ciri kualiti biji padi bagi induk amilos sederhana dan rendah, Kaedah pemilihan berbantuan penanda molekul bagi penentuan ciri wangi telah diperkenalkan. Amplifikasi spesifik alel berjaya membezakan pokok yang wangi dari yang tidak wangi. Analisa chi-square mendapati fenotip dan genotip ciri wangi adalah tidak bererti bagi nisbah segregasi 3:1 tetapi bererti bagi nisbah segregasi 1:2:1. 10 penanda dari sejumlah 35 penanda molekul mikrosatelit (RM) telah dikenalpasti menunjukkan ciri polimorfik bagi kedua-dua induk yang diuji. Penanda molekul tersebut ialah RM 25, RM 44, RM 72, RM 80, RM 152, RM 210, RM 281, RM 330, RM 342 dan RM 342A. Namun begitu, tiada penanda molekul RM memberikan perbezaan tidak bererti bagi nisbah segregasi 1:2:1. Kaedah pembaikbakaan terbaik bagi pemilihan berbantuan penanda molekul ialah diperingkat anak pokok sebelum ianya di tanam berbanding kaedah konvensional yang dijalankan pada peringkat matang

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I certify that a Thesis Examination Committee has met on 23rd May 2014 to conduct the final examination of Asfaliza bt Ramli on her thesis entitle" Genetic analysis of grain quality traits and marker assisted selection for fragrance trait in selected Malaysian rice (*Oryza sativa* L.) varieties" in accordance with the Universities and University College Act1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15th March 1998. The committee recommends that the student be awarded the Doctor of Philosophy.

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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 degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows;

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| | |
|---|---|
| [d] | Additive gene effect |
| [h] | Dominance gene effect |
| [i] | Additive x additive gene effect |
| [j] | Additive x dominance gene effect |
| [l] | Dominance x dominance gene effect |
| [m] | Mean |
| µ | Overall mean |
| 2AP | 2-Acetyl-1-pyrolline |
| AFLP | Amplified fragment length polymorphism |
| AMYL | Amylose |
| ANOVA | Analysis of variance |
| B.C | Before Christ |
| $BC_1$ | Backcross 1 |
| $BC_2$ | Backcross 2 |
| BIP | Bi-parental |
| bp | Base pair |
| CTAB | Cetyltrimethylammonium bromide |
| DNA | Deoxy ribonucleic acid |
| DOA | Department of Agriculture |
| EDTA | Ethylenediamine tetra acetic acid |
| $F_1$ | First filial |
| $F_6$ | Sixth generation |
| $F_7$ | Seventh generation |
| FAO | Food Agriculture Organisation |
| FGR | Fragrance gene |
| GC | Gel consistency |
| GCA | General combining ability |
| $g_i$ | GCA effects of parents i |
| $g_j$ | GCA effects of parents j |
| GL | Grain length |
| GW | Grain width |
| $h^2_B$ | Broad sense heritability |
| $h^2_N$ | Narrow sense heritability |
| HRR | Head rice recovery |
| IADA | Integrated Agriculture Development Authority |
| IRRI | International Rice Research Institute |
| KADA | Kemubu Agriculture Development Authority |
| KETARA | Kawasan Pembangunan Pertanian Bersepadu Terengganu Utara |
| Kg/ha | Kilogram per hectare |
| KOH | Potassium hydroxide |
| LW | Length to width ratio |
| MADA | Muda Agriculture Development Authority |
| MAS | Marker assisted selection |
| MGL | Milled grain length |
| MGW | Milled grain width |
| MRR | Milled rice recovery |
| Mt | Metric tonne |

| | |
|---|---|
| NC1 | North Carolina design 1 |
| NC11 | North Carolina design 2 |
| NC111 | North Carolina design 3 |
| NMAT | Non maternal |
| NPK | Nitrogen phosphorus |
| PBLS | Projek Barat Laut Selangor |
| PCR | Polymerase chain reaction |
| RAPD | Random amplified |
| REC | Reciprocal |
| $r_{ij}$ | REC effects |
| RM | Rice microsatellite marker |
| RNase | Ribonuclease |
| rpm | Rotation per minute |
| SCA | Specific combining ability |
| $s_{ij}$ | SCA effects of the cross between parents i and j |
| SNP | Single nucleotide polymorphism |
| SSR | Simple sequence repeat |
| t/ha | Tonne per hectare |
| TE | Tris-EDTA |
| UPOV | International Union of the Protection of New Varieties |
| $V_G$ | Variance genotypic |
| $V_P$ | Variance phenotypic |
| $\varepsilon_{ij}$ | Error term |
| µl | Microlitre |
| $\sigma^2_A$ | Additive variance |
| $\sigma^2_D$ | Dominance variance |
| $\sigma^2_e$ | Error variance |
| $\sigma^2_g$ | Genotype variance |
| $\sigma^2_{gca}$ | General combining ability variance |
| $\sigma^2_p$ | Phenotypic variance |
| $\sigma^2_r$ | Reciprocal variance |
| $\sigma^2_s$ | Specific combing ability variance |
| $\sigma^2_{sca}$ | Specific combing ability variance |

# CHAPTER 1

# INTRODUCTION

Rice (*Oryza sp*.) belongs to the grass family (Gramineae). It is the second largest cereal crop and is a staple food of nearly half of the world's population (FAO, 2008). There are many rice species, but only *Oryza sativa* and *Oryza glaberrima*, which originated in Southeast Asia and the Niger basin in Africa, respectively, are cultivated worldwide. *O. sativa* is cultivated extensively due to its better adaptation to local growing conditions and its better yield (Grist, 1953). Asian cultivated rice has evolved into three distinct ecogeographic varieties, namely indica, japonica and javanica, and it is grown in three cultivation types, namely upland, lowland and deepwater.

The average yield of Malaysian rice varieties over the last 10 years has fluctuated by approximately 3.5 to 4.0 tonnes per hectare, and the level required for self-sufficiency had been stabilised between 73.6 and 79.3% for the previous 10 years (MARDITECH, 2004). The total importation is approximately 30%, comprised of normal rice, fragrant rice, basmathi rice, glutinous rice and other specialty rice types. Efforts have been made to improve yield, from introducing new varieties to adopting revised agronomic packages and the use of excessive amounts of additional growth enhancer, which have resulted in negligible increases in rice yields.

The increase in education level and income among Malaysians and the growing demand for higher-quality rice in terms of taste, appearance and other sensory qualities have created an avenue for the development of specialty rice varietals. These types of rice are priced higher than the normal white rice, and the demand for specialty rice such as jasmine fragrant rice, basmathi rice and glutinous rice has increased the importation of those varieties. In 2008, climate change, severe attacks of brown planthopper and poor harvest in most rice-exporting countries began a worldwide rice crisis (FAO, 2011). The price for normal rice, as well as high-quality rice including basmathi and fragrant rice, soared, and the most affected group was the rice retailers in exporting countries. The supply of fragrant rice became limited, and exporting countries such as India either discontinued the supply or increased the price by a few times. The dependency on these types of rice should be slowly decreased and replacing the imported rice with locally developed specialty or fragrant rice varieties is timely.

The current rice industry outlook has tailored varietal development towards varieties with high yield and high input. Therefore, breeders always prefer crossing between modern rice varieties or among advanced lines. Crossing widely separated varieties such as traditional varieties with modern varieties or indica type with japonica type has always been avoided. However, these types of crosses carry high risks over the long term, especially in the case of an outbreak of a major pest or disease, which would spread throughout all rice granary areas.

In addition, breeding objectives focused on yield rather than quality later can jeopardise the quality of marketable rice in terms of the grain appearance and the quality of the cooked rice. In addition, insufficient knowledge of the genetic backgrounds of varieties that have been used extensively in breeding programmes and the use of a narrow genetic base, such as varieties with similar phenotypes, often results in less inter-population segregation (Acquaah, 2007). These two factors have become the major constraints on rice breeding.

This study was conducted to gain information on the combining ability among Malaysian Agricultural Reseach and Developement Institute (MARDI) rice varieties by means of a diallel mating design. The general combining ability (GCA), specific combining ability (SCA) and reciprocal (REC) of grain quality traits were estimated following the diallel analysis by Griffing (1956), which also estimated the heritability and correlation of the grain quality traits. The gene effects of the grain quality traits were determined based on individual scaling tests for adequacy of the additive-dominance model and on generation mean analysis using either a three-parameter or a six-parameter model (Mather, 1949; Mather and Jink, 1971; Singh and Chaudary, 1977). Because rice fragrance is perfectly determined by molecular markers, genotyping of the fragrance was performed using the markers developed by Bradbury *et al*. (2005) for the segregating populations of a selected cross. Fragrance genotyping was also performed by evaluating the rice microsatellite marker (RM) that had been previously mapped onto chromosome 8. Therefore, the main objective of this study is to produce rice populations with improvements in grain-quality traits, with the following specific objectives:

a) To determine the combining abilities of grain quality traits using a diallel mating design for selected aromatic and non-aromatic rice varieties,
b) To estimate the heritability and correlation of the grain-quality traits in the $F_1$ progenies and subsequent generations,
c) To estimate the additive, dominance and epistasis effects of grain-quality traits in selected populations derived from different amylose and fragrance parents, and
d) To evaluate the effectiveness of allele-specific amplification markers and selected rice microsatellite markers for fragrance genotyping.

The overall research flow is presented in Appendix 1.

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