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

FP 2014 33
GENETIC ANALYSIS OF GRAIN QUALITY TRAITS AND MARKER ASSISTED SELECTION FOR FRAGRANCE TRAIT IN SELECTED MALAYSIAN RICE (*Oryza sativa* L.) VARIETIES

ASFALIZA RAMLI

DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA

2014
GENETIC ANALYSIS OF GRAIN QUALITY TRAITS AND MARKER ASSISTED SELECTION FOR FRAGRANCE TRAIT IN SELECTED MALAYSIAN RICE (Oryza sativa L.) VARIETIES

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

May 2014
COPYRIGHT

All material contained within the thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise state. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

Copyright © Universiti Putra Malaysia
Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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 F2 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 (F2) 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 F2 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 transplanting 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

Pengerusi: Profesor Mohd Rafii bin Yusop, PhD

Fakulti: Pertanian

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 keupayaan, kebolehwarisan dan hubungan ciri kualiti biji bagi varieti terpililih padi Malaysia, tindakan gen yang terlibat dalam kebolehwarisan ciri-ciri ini dan pemilihan berbantuan penanda untuk ciri wangi bagi populasi F\textsubscript{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\textsubscript{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 acuan. 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 varias aditif lebih tinggi berbanding varias dominan. Kebolehwarisan bagi ciri GL adalah sederhana manakala GW, MGL, MGW, LW dan HRR adalah lebih tinggi. Kekbolehwarisan 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, 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. Walau bagaimana 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

inya 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

dua-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
ACKNOWLEDGEMENTS

All praises and thanks due to Allah Almighty for His Mercy and Grace. Indeed Allah knows what lies in the heart of man.

I would like to express my sincere thanks to Professor Dr. Mohd Rafii Yusop for his dedicated guidance, efforts, invaluable advice and expertise during the accomplishment of this research work. I am indebted to my supervisory committee members, Professor Dr. Ghizan Saleh, Dr. Adam Puteh and Dato’ Dr. Othman Omar for their constructive comments and encouragements throughout the period of this study.

My sincere gratitude to the Director General, Deputy Director General, Director of Human Resource and Director of Rice and Industrial Crops Research Centre, personnel in the Human Resource Department, of Malaysian Agricultural Research and Development Institute (MARDI) for the financial and administrative support. I greatly appreciated all the help and facilities provided during my study at MARDI Station Seberang Perai.

My deepest gratitude goes to Dr Abdul Latif for the invaluable comments, my colleagues, Heri Yanto Mustafa, Nur Sufiah Sebaweh, Nurul Azida Azhar, Rasidah Jasman, Elixson Sunian Sulaiman and Shahida Hashim for giving me ‘the fast lane’ in the field and laboratory. My deepest gratitude goes to my husband Zolkiflee Amin, my beloved sister Asfazura, family members and friends for their prayers and moral support especially during the writing part of my thesis.
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 Act 1971 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.

Members of the Thesis Examination Committee were as follows:

Mohd Razi bin Ismail, PhD  
Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

Datin Siti Nor Akmar binti Abdullah, PhD  
Professor  
Institute of Tropical Agriculture  
Universiti Putra Malaysia  
(Internal examiner)

Maheran binti Abd Aziz, PhD  
Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Internal examiner)

Iftikhar H. Khalil, PhD  
Professor  
Plant Breeding and Genetics Department  
Agriculture University, Peshawar, Pakistan  
(External examiner)

NORITAH OMAR, PhD  
Associate Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 23 June 2014
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;

**Mohd Rafii Yusop, PhD**  
Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Ghizan Saleh, PhD**  
Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

**Adam Puteh, PhD**  
Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

**Dato’ Othman Omar, PhD**  
Fellow Scientist  
Rice and Industrial Crop Research Centre  
Malaysian Agriculture Research and Development Institute  
(Member)

---

**BUJANG BIN KIM HUAT, PhD**  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:
Declaration by graduate student

I hereby confirm that:

- this thesis is my original work
- quotations, illustrations and citations have been dully referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property fro the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before the thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software

Signature: __________________________ Date: ______________________

Name and Matric No: ASFALIZA BINTI RAMLI - GS 24263
Declaration by Members of Supervisory Committee

This is to confirm that:
- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: ___________________________  Signature: ___________________________
Name of Chairman of Supervisory Committee: Mohd Rafii Yusop, PhD
Name of Member of Supervisory Committee: Ghizan Saleh, PhD

Signature: ___________________________  Signature: ___________________________
Name of Member of Supervisory Committee: Adam Puteh, PhD
Name of Member of Supervisory Committee: Dato’ Othman Omar, PhD
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>ABSTRACT</strong></td>
<td>ii</td>
</tr>
<tr>
<td></td>
<td><strong>ABSTRAK</strong></td>
<td>iv</td>
</tr>
<tr>
<td></td>
<td><strong>ACKNOWLEDGEMENT</strong></td>
<td>vi</td>
</tr>
<tr>
<td></td>
<td><strong>APPROVAL</strong></td>
<td>vii</td>
</tr>
<tr>
<td></td>
<td><strong>DECLARATION</strong></td>
<td>ix</td>
</tr>
<tr>
<td></td>
<td><strong>LIST OF TABLES</strong></td>
<td>xv</td>
</tr>
<tr>
<td></td>
<td><strong>LIST OF FIGURES</strong></td>
<td>xviii</td>
</tr>
<tr>
<td></td>
<td><strong>LIST OF ABBREVIATIONS</strong></td>
<td>xix</td>
</tr>
<tr>
<td></td>
<td><strong>CHAPTER</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1. <strong>INTRODUCTION</strong></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2. <strong>LITERATURE REVIEW</strong></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2.1 Origin and domestication</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2.2 Malaysia rice industry</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2.3 Rice morphology</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2.4 The growth stages of rice</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>2.5 Varietal development in Malaysia</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>2.6 Breeding for specialty rice</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2.7 Rice grain quality</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2.7.1 Physical characteristics of rice quality</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2.7.1.1 Grain length and width</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2.7.1.2 Milled grain length and width</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2.7.1.3 Grain shape</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>2.7.1.4 Milled rice recovery</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>2.7.1.5 Head rice recovery</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>2.7.2 Chemical characteristics of rice quality</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>2.7.2.1 Gel consistency</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>2.7.2.2 Fragrance</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>2.7.2.3 Amylose</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>2.8 Breeding technique</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>2.8.1 Diallel mating design</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>2.9 Heritability</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>2.10 Gene actions in heritability of grain quality traits</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>2.11 Marker-assisted selection for fragrance trait in rice</td>
<td>22</td>
</tr>
</tbody>
</table>
# COMBINING ABILITY, HERITABILITY ESTIMATION AND CORRELATION OF GRAIN QUALITY TRAITS USING A DIALLEL MATING DESIGN

## 3.1 Introduction

## 3.2 Materials and Methods

### 3.2.1 Breeding materials

### 3.2.2 Development of the F<sub>1</sub> following a diallel mating design

#### 3.2.2.1 Emasculation of the female parent

#### 3.2.2.2 Preparation of pollen parent and pollination

#### 3.2.2.3 Experimental design and crop maintenance

### 3.3 Data collection

#### 3.3.1 Grain length

#### 3.3.2 Grain width

#### 3.3.3 Milled grain length

#### 3.3.4 Milled grain width

#### 3.3.5 Length to width ratio

#### 3.3.6 Milling recovery

#### 3.3.7 Head rice recovery

#### 3.3.8 Amylose

#### 3.3.9 Gel consistency

### 3.4 Statistical analysis

#### 3.4.1 Diallel analysis

#### 3.4.2 Heritability

#### 3.4.3 Correlation of grain quality traits and yield

### 3.5 Results and discussion

#### 3.5.1 Combining ability analysis

#### 3.5.2 General combining ability

#### 3.5.3 Specific combining ability

#### 3.5.4 Reciprocal effects

#### 3.5.5 Estimation of genetic parameters on grain quality traits

#### 3.5.6 Correlation of grain quality traits

### 3.6 Conclusion
4 GENERATION MEANS ANALYSIS AND RELATIONSHIP AMONG GRAIN QUALITY TRAITS IN SELECTED RICE POPULATIONS DERIVED FROM DIFFERENT AMYLOSE CHARACTERISTICS

4.1 Introduction 60
4.2 Materials and methods 61
  4.2.1 Crossing programme and population development 61
    4.2.1.1 Backcrossed population development 61
  4.2.2 Experimental design and crop maintenance 63
4.3 Data collection and statistical analysis 63
  4.3.1 Scaling test 63
  4.3.2 Components of generation means 64
    4.3.2.1 Three-parameter model (additive dominance model) 65
    4.3.2.2 Six-parameter model 66
  4.3.3 Estimations of heritability and correlation 67
4.4 Result and discussion 67
  4.4.1 Population derived from high amylose and low amylose parents 67
    4.4.1.1 Average performance of the generations 69
    4.4.1.2 Estimation of scaling test 71
    4.4.1.3 Estimation of additive and dominance gene effect (three-parameter model) 73
    4.4.1.4 Estimation of epistasis 75
  4.4.2 Population derived from intermediate and high amylose parents 77
    4.4.2.1 Average performance of the generations 77
    4.4.2.2 Estimation of scaling test 79
    4.4.2.3 Estimation of additive and dominance gene effect (three-parameter model) 81
    4.4.2.4 Estimations of epistasis 82
  4.4.3 Population derived from intermediate amylose and low amylose parents 84
    4.4.3.1 Average performance of the generations 84
    4.4.3.2 Estimation of scaling test 87
    4.4.3.3 Estimation of additive and dominance gene effect (three-parameter model) 88
    4.4.3.4 Estimations of epistasis 89
4.5 Estimation of heritability in the F2 population 91
4.6 Relationship among the grain quality traits
   4.6.1 Population derived from high and low amyllose parents
   4.6.2 Population derived from intermediate and high amyllose parents
   4.6.3 Population derived from intermediate and low amyllose parents

4.7 Conclusion

5 MARKER-ASSISTED SELECTION FOR FRAGRANCE CHARACTERISTICS IN A SELECTED F2 POPULATION

5.1 Introduction
5.2 Materials and methods
   5.2.1 Population development
   5.2.2 Experimental design and crop management
   5.2.3 Aroma detection by boiling method
   5.2.4 Aroma detection by MAS
      5.2.4.1 DNA extraction
      5.2.4.2 PCR assay and profile
      5.2.4.3 PCR using SSR markers
      5.2.4.4 Agarose gel electrophoresis
   5.2.5 Statistical analysis
5.3 Results and discussion
   5.3.1 Aroma detection by boiling method
   5.3.2 Aroma detection by Allelic Specific Amplification (ASA)
   5.3.3 MAS using rice microsatellite (RM) marker
   5.3.4 Aroma detection using the identified RM marker
   5.3.5 Breeding approach for MAS
5.4 Conclusion

6 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

REFERENCES
APPENDICES
BIODATA OF STUDENT
LIST OF PUBLICATION
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Rice varieties released by MARDI from 1972 to 2011</td>
<td>7</td>
</tr>
<tr>
<td>2.2</td>
<td>Classification of grain properties as stated in IRRI’s standard evaluation system for rice</td>
<td>11</td>
</tr>
<tr>
<td>2.3</td>
<td>Classification of milled grain size based on length</td>
<td>12</td>
</tr>
<tr>
<td>2.4</td>
<td>Classification of rice grain shape</td>
<td>12</td>
</tr>
<tr>
<td>2.5</td>
<td>Classification of gel consistency</td>
<td>13</td>
</tr>
<tr>
<td>2.6</td>
<td>Classification based on amylose content</td>
<td>14</td>
</tr>
<tr>
<td>3.1</td>
<td>Grain physical characteristics and chemical properties of the parental strains used for the diallel mating design</td>
<td>25</td>
</tr>
<tr>
<td>3.2</td>
<td>Schematic of the diallel crosses</td>
<td>26</td>
</tr>
<tr>
<td>3.3</td>
<td>Outline of the ANOVA for grain quality traits derives from a 7x7 diallel</td>
<td>30</td>
</tr>
<tr>
<td>3.4</td>
<td>Outline of the ANOVA table for diallel analysis in Griffing’s method 1 for the grain quality traits</td>
<td>31</td>
</tr>
<tr>
<td>3.5</td>
<td>The mean, standard deviation and maximum and minimum values of grain quality traits in the (F_1) progenies</td>
<td>32</td>
</tr>
<tr>
<td>3.6</td>
<td>Average performance of (F_1) progenies from the crossed and reciprocal population</td>
<td>34</td>
</tr>
<tr>
<td>3.7</td>
<td>ANOVA for grain quality traits derived from a 7x7 diallel</td>
<td>36</td>
</tr>
<tr>
<td>3.8</td>
<td>Mean squares values of general combining ability, specific combining ability and reciprocal, maternal and non maternal effects on grain quality traits from a 7x7 diallel cross</td>
<td>38</td>
</tr>
<tr>
<td>3.9</td>
<td>GCA effects of grain quality traits of the selected parents</td>
<td>41</td>
</tr>
<tr>
<td>3.10</td>
<td>SCA effects of grain quality traits for crosses of the selected parents</td>
<td>44</td>
</tr>
<tr>
<td>Section</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>3.11</td>
<td>REC effects of grain quality traits for the reciprocal crosses of the selected parents</td>
<td>48</td>
</tr>
<tr>
<td>3.12</td>
<td>Genetics parameters of grain quality traits from a 7x7 diallel cross</td>
<td>52</td>
</tr>
<tr>
<td>3.13</td>
<td>Pearson correlation coefficients among grain quality traits and yield</td>
<td>56</td>
</tr>
<tr>
<td>4.1</td>
<td>ANOVA for grain quality traits from six generations of the selected population</td>
<td>68</td>
</tr>
<tr>
<td>4.2</td>
<td>The means, standard errors and coefficients of variation for grain quality traits for population derived from high amylose and low amylose parents</td>
<td>70</td>
</tr>
<tr>
<td>4.3</td>
<td>The scaling test of grain quality traits for a population derived from high and low amylose parents</td>
<td>72</td>
</tr>
<tr>
<td>4.4</td>
<td>Estimates of gene effects of the grain quality traits for populations derived from high and low amylose parents</td>
<td>74</td>
</tr>
<tr>
<td>4.5</td>
<td>The means, standard errors and coefficients of variation of grain quality traits for population derived from intermediate and high amylose parents</td>
<td>78</td>
</tr>
<tr>
<td>4.6</td>
<td>The scaling test of grain quality traits for the population derived from intermediate and high amylose parents</td>
<td>80</td>
</tr>
<tr>
<td>4.7</td>
<td>Estimates of gene effects of the grain quality traits for populations derived from intermediate and high amylose parents</td>
<td>83</td>
</tr>
<tr>
<td>4.8</td>
<td>The means, standard errors and coefficients of variation of grain quality traits for the population derived from intermediate and low amylose parents</td>
<td>86</td>
</tr>
<tr>
<td>4.9</td>
<td>The scaling test of grain quality traits for population derived from intermediate and low amylose parents</td>
<td>88</td>
</tr>
<tr>
<td>4.10</td>
<td>Estimates of gene effects of the grain quality traits for population derived from intermediate and low amylose parents</td>
<td>90</td>
</tr>
<tr>
<td>4.11</td>
<td>Heritability of grain quality traits in the F$_2$ populations</td>
<td>92</td>
</tr>
</tbody>
</table>
4.12 Pearson correlation coefficients among grain quality traits for the population derived from high and low amylose parents

4.13 Pearson correlation coefficients among grain quality traits for the population derived from intermediate and high amylose parents

4.14 Pearson correlation coefficients among grain quality traits for the population derived from intermediate and low amylose parents

5.1 The sequence of ASA primers for MAS analysis of fragrance

5.2 PCR assay for fragrance detection

5.3 The sequence of selected rice microsatellite markers for fragrance detection

5.4 Phenotyping of the fragrance trait in the F$_2$ population as determined using the boiling method

5.5 Genotyping of the fragrance trait in the F$_2$ population as determined by ASA

5.6 Phenotyping of the fragrance trait in the F$_2$ population as determined by the identified markers and based on a 3:1 segregation ratio

5.7 Genotyping of fragrance trait in the F$_2$ population as determined by the identified markers based on the segregation ratio 1:2:1
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>The schematic diagram for the F$_2$ population derived from high amylose (MR 84) X low amylose (MRQ 76) parents and the backcrosses using MR 84 and MRQ 76 as the recurrent parent</td>
<td>62</td>
</tr>
<tr>
<td>5.1</td>
<td>Phenotyping of the fragrance trait in the F$_2$ population using ASA. L: 100 bp DNA ladder, P1: MR 84 (non fragrant), P2: MRQ 74 (fragrant), 1-22: individual plants of the F$_2$ population from a cross between MR 84 and MRQ 74</td>
<td>112</td>
</tr>
<tr>
<td>5.2</td>
<td>Phenotyping of the fragrance trait in the F$_2$ population using RM 80. L: 100 bp DNA ladder, P1: MR 84 (non fragrant), P2: MRQ 74 (fragrant), 1-22: individual plants of the F$_2$ population from a cross between MR 84 and MRQ 74</td>
<td>114</td>
</tr>
<tr>
<td>5.3</td>
<td>Phenotyping of the fragrance trait in the F$_2$ population using RM 210. L: 100 bp DNA ladder, P1: MR 84 (non fragrant), P2: MRQ 74 (fragrant), 1-22: individual plants of the F$_2$ population from a cross between MR 84 and MRQ 74</td>
<td>115</td>
</tr>
<tr>
<td>5.4</td>
<td>Phenotyping of the fragrance trait in the F$_2$ population using RM 330. L: 100 bp DNA ladder, P1: MR 84 (non fragrant), P2: MRQ 74 (fragrant), 1-22: individual plants of the F$_2$ population from a cross between MR 84 and MRQ 74</td>
<td>116</td>
</tr>
<tr>
<td>5.5</td>
<td>Phenotyping of the fragrance trait in the F$_2$ population using RM 152. L: 100 bp DNA ladder, P1: MR 84 (non fragrant), P2: MRQ 74 (fragrant), 1-22: individual plants of the F$_2$ population from a cross between MR 84 and MRQ 74</td>
<td>117</td>
</tr>
<tr>
<td>5.6</td>
<td>Phenotyping of the fragrance trait in the F$_2$ population using RM 281. L: 100 bp DNA ladder, P1: MR 84 (non fragrant), P2: MRQ 74 (fragrant), 1-22: individual plants of the F$_2$ population from a cross between MR 84 and MRQ 74</td>
<td>117</td>
</tr>
</tbody>
</table>
# LIST OF ABBREVIATIONS

[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  
\( \mu \) 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\(_B^2\) Broad sense heritability  
h\(_N^2\) 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
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC1</td>
<td>North Carolina design 1</td>
</tr>
<tr>
<td>NC11</td>
<td>North Carolina design 2</td>
</tr>
<tr>
<td>NC111</td>
<td>North Carolina design 3</td>
</tr>
<tr>
<td>NMAT</td>
<td>Non maternal</td>
</tr>
<tr>
<td>NPK</td>
<td>Nitrogen phosphorus</td>
</tr>
<tr>
<td>PBLS</td>
<td>Projek Barat Laut Selangor</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>RAPD</td>
<td>Random amplified</td>
</tr>
<tr>
<td>REC</td>
<td>Reciprocal</td>
</tr>
<tr>
<td>r_{ij}</td>
<td>REC effects</td>
</tr>
<tr>
<td>RM</td>
<td>Rice microsatellite marker</td>
</tr>
<tr>
<td>RNase</td>
<td>Ribonuclease</td>
</tr>
<tr>
<td>rpm</td>
<td>Rotation per minute</td>
</tr>
<tr>
<td>SCA</td>
<td>Specific combining ability</td>
</tr>
<tr>
<td>s_{ij}</td>
<td>SCA effects of the cross between parents i and j</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>SSR</td>
<td>Simple sequence repeat</td>
</tr>
<tr>
<td>t/ha</td>
<td>Tonne per hectare</td>
</tr>
<tr>
<td>TE</td>
<td>Tris-EDTA</td>
</tr>
<tr>
<td>UPOV</td>
<td>International Union of the Protection of New Varieties</td>
</tr>
<tr>
<td>V_G</td>
<td>Variance genotypic</td>
</tr>
<tr>
<td>V_p</td>
<td>Variance phenotypic</td>
</tr>
<tr>
<td>\varepsilon_{ij}</td>
<td>Error term</td>
</tr>
<tr>
<td>\mu_l</td>
<td>Microlitre</td>
</tr>
<tr>
<td>\sigma^2_A</td>
<td>Additive variance</td>
</tr>
<tr>
<td>\sigma^2_D</td>
<td>Dominance variance</td>
</tr>
<tr>
<td>\sigma^2_e</td>
<td>Error variance</td>
</tr>
<tr>
<td>\sigma^2_g</td>
<td>Genotype variance</td>
</tr>
<tr>
<td>\sigma^2_{gca}</td>
<td>General combining ability variance</td>
</tr>
<tr>
<td>\sigma^2_p</td>
<td>Phenotypic variance</td>
</tr>
<tr>
<td>\sigma^2_r</td>
<td>Reciprocal variance</td>
</tr>
<tr>
<td>\sigma^2_s</td>
<td>Specific combining ability variance</td>
</tr>
<tr>
<td>\sigma^2_{sca}</td>
<td>Specific combining ability variance</td>
</tr>
</tbody>
</table>
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 Research 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 F1 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.


Juliano, B. O. and Villareal. 1993. *Grain quality evaluation of world rices.* International Rice Research Institute, Los Banos, Philippines


Khush, G. S., Paulo, C. M. and Delacruz, N.M. 1979. *Rice grain quality evaluation and improvement at IRRI.* International Rice Research Institute, Los Banos, Philippines


Malaysian Agricultural Research and Development Institute MARDI. 2009. MARDI three decades of achievements in research and development. MARDI, Serdang, Selangor


Nguyen, T. L. and Bui, C. B. 2002. Identification and fine mapping of SSR marked linked to fgr gene of rice. Omonrice 10:14-20


Poapongsakorn, N. 2013. Rice in Thailand: Production, consumption, export and policy. Paper presented at Workshop on South-East Asian rice production. 18-22\textsuperscript{nd} March 2013, Bangkok, Thailand


Sao, A. 2002. Studies on combining ability and heterosis in F1 rice hybrids using cytoplasmic male sterile lines. Master Thesis. IGAU, Raipur, India


Subramanyan, D., Murty, V. V. S., Rao, A. V. 1986. Heritability of yield and other traits and inter-relationships among traits in *F*₂ to *F*₅ generations of three rice crosses. *Indian J. Genet.* 46(2):390-393


