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GENETIC AND MOLECULAR ANALYSES FOR ORYZA SATIVA L. CV. MRQ74 FRAGRANCE TRAIT THROUGH QUANTITATIVE TRAIT LOCI MAPPING USING GENE-BASED AND MICROSATELLITE MARKERS

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ITA 2015 5

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By

FARAHNAZ SADAT GOLESTAN HASHEMI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

May 2015

DEDICATION

To my love, Ruzbeh
and
my dear parents, Sedigheh and Kamal

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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May 2015

Chairman: Professor Mohd Rafii BinYusop, PhD
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Rice fragrance is a complex trait and one of the aim of rice breeding programs is to improve this quality and to further explore its genetic basis. Grain 2AP (2-acetyl-1-pyrroline) as the primary reason for the distinctive aroma is the most attractive characteristic of high-quality rice. MRQ74, a popular Malaysian aromatic variety, has considerably higher prices than non-aromatic varieties. Thus, breeding this profitable trait has become a priority for Malaysian rice breeding programs. In spite of many studies on aroma genetics, ambiguities about its genetic basis remain. Identifying quantitative trait locus (QTL) based on markers that are associated with candidate genes controlling a trait of interest, can increase the power of QTL detection. This study aimed to locate QTLs that influence natural variations in rice scent using microsatellites and candidate gene-based sequence polymorphisms. For this purpose, an F_2 mapping population, including 189 individual plants, was developed through the cross 'MRQ74', an elite Malaysian scented cultivar, with 'MR84', a Malaysian non-scented cultivar. Qualitative (sensory test) and quantitative (GC-MS) approaches were applied to obtain a phenotype data framework. Totally, 512 pairs of microsatellite primers and six gene-based markers that cover entire rice genome were combined for a comparative polymorphic analysis among the two parents and two randomly selected F_1 progenies with 108 markers proving polymorphic for evaluating segregation ratios among the F_2 progenies. The observed segregations of each locus were then checked against the expected Mendelian ratio in the segregating population. Results revealed that 88 markers fitted well with 1:2:1 ratio for an F_2 population. However, segregation distortion was also found for 20 marker loci that were distributed across 9 chromosomes, except chromosomes 2, 8 and 11. Among these 20 markers, 11 (55%) and 9 (45%) were sloped toward MR84 and MRQ74, respectively. Two QTLs were identified on chromosomes 4 and 8. These QTLs explained from 3.2% to 39.3% of the total fragrance phenotypic variance. In addition, we could resolve linkage group 8 by adding six gene-based primers in the interval harboring the most robust QTL. Hence, we could locate a putative *fgr* allele in the QTL on chromosome 8 in the interval RM223-SCU015RM (1.63 cM).Developing fragrant rice through marker assisted/aided selection (MAS) is an economical and profitable approach enriching an elite genetic background with a pleasant aroma worldwide. PCR-based DNA markers that distinguish alleles of the main genes of rice fragrance have been synthesized to develop rice scent biofortification by MAS. The present study also examined the aroma biofortification potential of co-dominant markers in the F_2 progenies to determine the most influential diagnostic markers for odour biofortification. The SSR as well as functional DNA markers RM5633 (on chromosome 4), RM515, RM223, L06, NKSbad2, FMbadh2-E7, BADEX7-5, Aro7, and SCU015RM (on chromosome 8) were highly associated with 2AP content across the population. The alleles detected via these markers considered for approximately 12.1, 27.05, 27.05, 27.05, 25.42, 25.42, 20.53, 20.43, and 20.18% of the total phenotypic variation, respectively. F_2 plants harboring the favorable alleles of these effective markers produced higher levels of fragrance. Such rice plants can be used as donor parents to speed up the development of fragrance biofortified tropical rice varieties adapted to growing conditions and consumer preferences, thus contributing to rice global market. Moreover, the identified QTLs represent an important step towards clarification of the rice flavor genetic control mechanism. This identification will accelerate the progress of the use of molecular markers for gene isolation, gene-based cloning, and marker-assisted selection breeding programs aimed at improving rice cultivars.

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

ANALISIS GENETIK DAN MOLEKUL PADI, *ORYZA SATIVA* L. CV. MRQ74 UNTUK CIRI AROMA MELALUI PEMETAAN CIRI LOKUS KUANTITATIF MENGGUNAKAN PENANDA BERASASKAN GEN DAN MIKROSATELIT

Oleh

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Aroma adalah sifat kompleks tanaman padi dan salah satu matlamat pembiakbakaan padi adalah untuk meningkatkan kualiti tersebut dan seterusnya mendalami asas genetik ciri ini. Aroma bijirin 2-acetyl-1-Pirolina (2AP) adalah komponen kimia utama beras aromatik, merupakan ciri utama beras yang berkualiti tinggi. MRQ74, varieti aromatik Malaysia yang popular, harga lebih tinggi di pasaran daripada padi biasa. Oleh itu, pembiakbakaan ciri ini telah menjadi keutamaan program pembiakbakaan padi Malaysia. Walaupun banyak kajian mengenai genetik aroma, kesamaran asas genetiknya masih ada. Mengenal pasti lokus ciri kuantitatif (QTL) berdasarkan penanda molekul utama, khususnya gen yang mengawal sifat yang dikehendaki, dapat meningkatkan keupayaan mengesan QTL. Kajian ini bertujuan untuk mencari QTL yang mempengaruhi variasi semulajadi aroma beras menggunakan mikrosatelit dan polimorfisme berasaskan jujukan gen calon. Untuk tujuan ini, sejumlah 189 individu populasi pemetaan F_2, dibangunkan daripada kacukan 'MRQ74', kultivar wangi elit Malaysia, dengan 'MR84', kultivar bukan wangi Malaysia. Ujian, kualitatif (deria) dan kuantitatif (GC-MS) telah digunakan untuk mendapatkan data fenotip. Sejumlah 512 pasang primer mikrosatelit dan enam penanda berdasarkan gen yang meliputi seluruh genom padi digabungkan untuk menjalankan analisis polimorfik perbandingan di antara kedua induk dan progeni F_1 yang dipilih secara rawak dengan 108 terbukti polimorfik untuk menilai nisbah segregasi di antara progeni F_2. Segregasi yang dicerap pada setiap lokus kemudian disemak dengan nisbah Mendel yang dijangka dalam populasi tersebut. Hasil kajian menunjukkan bahawa 88 penanda mengikut dengan baik nisbah 1:2:1 untuk populasi F_2. Walau bagaimanapun, segregasi juga telah dicerap pada 20 lokus penanda yang meliputi di 9 kromosom, kecuali kromosom 2, 8 dan 11. Di antaranya 20 penanda, 11 (55%) dan 9 (45%) cerun bergerak kearah MR84 dan MRQ74. Dua QTL telah dikenal pasti pada kromosom 4 dan 8. QTL dijelaskan dari 3.2% hingga 39.3% jumlah varians fenotip padi wangi. Di samping itu, kita boleh mengatasi kumpulan silang 8 dengan menambah enam primer berdasarkan gen-dalam

selangan QTL yang paling kuat. Oleh itu, kita boleh mencari alel *fgr* putatif QTL yang terdapat pada kromosom 8 selang diantara RM223-SCU015RM (1.63 cM). Pembiakbakaan beras wangi melalui bantuan pemilihan penanda (MAS) merupakan satu pendekatan yang ekonomi dan menguntungkan dengan memperkaya latar belakang genetik daripada padi aroma elit seluruh dunia. Penanda DNA PCR yang membezakan alel gen utama aroma padi disintesis untuk membangunkan beras wangi biofortifikasi melalui MAS. Kajian ini juga meneliti kemungkinan biofortifikasi aroma penanda dominan bersama dalam progeni F_2 untuk mendiagnostik penanda yang paling berpengaruh untuk aroma biofortifikasi. SSR dan juga penanda berfungsi DNA RM5633 (pada kromosom 4), RM515, RM223, L06, NKSbad2, FMbadh2-E7, BADEX7-5, Aro7 dan SCU015 RM (pada kromosom 8) sangat berkaitan dengan kandungan 2AP keseluruhan populasi. Alel dikesan melalui penanda tersebut adalah dianggar 12.1,27.05, 27.05, 27.05, 25.42, 25.42, 20.53, 20.43, dan 20.18% daripada jumlah variasi fenotip. Tumbuhan F_2 yg mengandungi alel yang diingini daripada penanda berkesan menghasilkan tahap wangian yang lebih kuat. Tanaman padi tersebut dapat digunakan dengan induk penderma untuk mempercepat pembiakbakaan wangian biofortifikasi varieti padi tropika disesuaikan dengan keadaan tumbuh dan keutamaan pengguna, seterusnya menyumbang kepada pasaran global padi. Selain itu, QTL yang dikenal pasti merupakan langkah penting ke arah pengiktirafan mekanisma kawalan genetik aroma nasi. Selain itu, pengenalan ini mungkin akan mempercepat kemajuan penggunaan penanda molekul untuk pengasingan gen, berdasarkan gen-klon dan program pemilihan bantuan penanda bertujuan untuk pembiakbakaan kultivar padi.

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I certify that a Thesis Examination Committee has met on 11 May 2015 to conduct the final examination of Farahnaz Sadat Golestan Hashemi on her thesis entitled "GENETIC AND MOLECULAR ANALYSES FOR *ORYZA SATIVA* L. CV. MRQ74 FRAGRANCE TRAIT THROUGH QUANTITATIVE TRAIT LOCI MAPPING USING GENE-BASED AND MICROSATELLITE MARKERS" in accordance with the Universities and Universiti Collages 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 Doctor of Philosophy.

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LIST OF ABBREVIATIONS

AB-ald	γ-aminobutyraldehyde
ADC	Arginine decarboxylase
AE	Additive effect
ALA	5-aminolevulinic acid
ASA	Allele Specific Amplification
AT	Annealing Temperature
BADH2	Betaine aldehyde dehydrogenase
Bp	base pair
CE	Capillary Electrophoresis
carboxen/DVB/PDMS	divinylben-zene/carboxen/polydimethylsiloxane stableflex
cM	centimorgan
χ^2	Chi-square
CIM	Composite Interval Mapping
CTAB	Cetyltrimethyl ammonium bromide
˚C	Degree Centigrade
DAO	Dominance effect
DE	Diamine oxidase
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotides
DOA	Department of Agriculture
EDTA	Ethylenediaminetetraacetic acid
ES	Expected Size
ESP	External sense primer

EAP	External anti-sense primer
EST	Expressed Sequence Tag
EHK	Extended Haley-Knott regression
EM	Expectation-maximization algorithm
FM	Functional marker
GAD	Glutamic acid decarboxylase
G	Gram
ga	Gametophytic selection gene
GABA	4-aminobutyric acid
GC-MS	Gas chromatography-mass spectrometry
h	hour
HCL	Hydrochloric acid
HK	Haley-Knott regression
INSP	Internal Non-fragrant Sense Primer
IRRI	International Rice Research Institute
IFAP	Internal Fragrant Anti-sense Primer
IS	Internal Standard
KDML 105	Khao Dawk Mali 105
RD15	Kor Kho 15
kb	Kilo base-pair
L	Liter
LOD	log of odds
MARDI	Malaysian Agriculture Research and Development Institute
MABC	Marker assisted backcrossing
MAS	Marker assisted/aided selection

MITE	Miniature Interspersed Transposable Element
MI	Multiple Imputation
µl	Microliter
µM	Micromolar
M	Molar
Mbp	Mega base pair
Mg	Milligram
mg g^{-1}	Miligram per gram
mL	Milliliter
Mg	Microgram
µL	Microliter
ng	Nano gram
NIST	National Institute of Standards and Technology
NLM	Nearest left marker
NRM	Nearest right marker
NILs	Near-isogenic lines
ODC	Ornithine decarboxylase
OAT	Ornithine aminotransferase
P5C	Δ^1-pyrroline 5-carboxylate
P5CS	Δ^1-pyrolline-5-carboxylic acid synthetase
ppm	parts per million
ppb	parts per billion
PCR	Polymerase Chain Reaction
PVP	Polyvinyl pyrrolidone
KOH	Potassium hydroxide

QTLs	Quantitative Trait Loci
RI	Retention Indices
RIL	Recombinant Inbred Line
rpm	rounds per minutes
RFLP	Restriction Fragment Length Polymorphism
RAPD	Random Amplified Polymorphic DNA
RNase A	Ribonuclease A
S	Sterility gene
SD	Segregation Distortion
SDL	Segregation Distortion Loci
SDR	Segregation Distorted Region
SSR	Simple Sequence Repeat
NaCl	Sodium chloride
SPME	Solid Phase Micro Extraction
SNPs	Single Nucleotide Polymorphisms
STSs	Sequence-Tagged Sites
TAE	Tris-acetate-EDTA
TE	Tris-EDTA
TMP	2,4,6-trimethylpyridine
2AP	2-acetyl-1-pyrroline
2AT	2-acetyl-2-thiazoline
UPM	Universiti Putra Malaysia
UV	Ultra Violet
v/v	volume/volume

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Rice (*Oryza sativa* L.) is consumed as the main food by more than half of the world population (Brar and Khush, 2002; Sabouri *et al.,* 2012), while about 95% of its production is in Asia (Yang *et al.*, 2008). Rice produces around one fourth of the calories consumed worldwide (Subudhi *et al.,* 2006).

The average yield of Malaysian rice varieties has fluctuated around 3.5 to 4.0 tons per hectare (t/ha) over the last ten years. Hence, self-sufficient level has been stabilized from 73.6% to 79.3% for the past 10 years. The total importation is about 30% which is comprised of normal rice, fragrant rice, basmati rice, glutinous rice, and other specialty rice types. Efforts have been made to improve yield from introducing new varieties to adopting revised agronomic package and the use of excessive amount of additional growth enhancer. These efforts finally could increase rice yield (Ramli, 2014).

The inherent quality of rice kernel is highly important since most consumers cook and consume it as a grain, whereas the proportion of rice becoming flour or flakes is negligible (Huang *et al.,* 1998). These quality features are identified by the physico-chemical properties of the rice kernel which in turn are genetically governed by some expression modulation in the growth environment (Amarawathi *et al.,* 2008).

Fragrant rice cultivars are small and special group of rice. Rice kernels emitting a pleasant scent are in high demand by consumers (Shi *et al.*, 2008). In the global market, aromatic rice is greatly popular (Singh *et al.*, 2000b) due to its natural chemical components giving it a unique aroma when cooked. Additionally, the retail price of scented rice is considerably higher than common rice types (Qiu and Zhang, 2003; Shi *et al.*, 2008). In Malaysia, the importation of fragrant rice was 481.619 metric tons in 2010. This amount is equivalent to RM 840 Million (DOA, 2012).

One hundred fourteen different volatile compounds in charge of flavor and aroma of rice have been identified (Yajima*et al.,* 1979). 2-acetyl-1-pyrroline (2AP), as one of these compounds, is a potent fragrance component, with a lower odor threshold giving both basmati and jasmine distinct fragrances (Buttery *et al.,* 1983; Shi *et al.,* 2008). 2AP can be found in all parts of the rice plant except the roots (Lorieux*et al.,* 1996). It can also be found in non-aromatic rice cultivars with concentrations up to 100 times lower (Grosch and Schieberle, 1997; Jain *et al.,* 2006).

Furthermore, aromatic species have high nutritional values and rich amino acid profiles (Sun *et al.,* 2008). For instance, Basmati 370 rice has higher amount of methionine, lysine, phenylalanine, and leucine content compared to non-fragrant species (Yin, 2012). Thailand, Pakistan and India are the pioneers in the trade of fragrant rice. India and Pakistan are the suppliers of Basmati, while Jasmine rice comes from Thailand (Yin, 2012). Jasmine rice is produced from varieties Khao Dowk Mali 105 (KDML 105) and Kor Kho 15 (RD15) (Pitiphunpong *et al.,* 2011). KDML 105, Bahra (Afganistan), Siamati (Thailand), Sadri (Iran), Texami, Della, and Kasmati (USA) are other important aromatic varieties worldwide (Singh *et al,* 2000b). Singh *et al.,* (2000b) reported that popular fragrant rice varieties in the world market were long grained, but a majority of the Indian indigenous fragrant rice cultivars was small and medium-grained. A large number of land races of these varieties were placed in the Himalayan Tarai area of the state of Uttar Pradesh and Bihar of India. Thus, this area is probably the origin of fragrant rice.

For the breeders, it is cumbersome to select the quality through conventional approaches because of shortage of discrete phenotypic classes in the progeny and having difficult methods for quality assay. Accurate knowledge regarding the genes that control high yielding superior quality in basmati rice varieties is required for breeding of these attributes. The majority of these grain quality traits are governed by quantitative trait locus (QTL) as derived from continuous phenotypic variation in the segregating progeny of inter-varietal crosses. Evaluation of rice kernel quality becomes more complex by the triploid nature of the endosperm and the impact of the environment on the expression of such characteristics (He *et al.,* 1999). However, several studies investigated rice quality genetics using molecular markers (Ahn *et al.,* 1992, 1993; Redona and Mackill, 1998; Aluko *et al.,* 2004; Wanchana *et al.,* 2005, Wan *et al.,* 2006; Chen *et al.,* 2006).

1.2 Significance of Study

Aromatic rice has increasingly become in demand, and aromatic varieties command a higher price than non-aromatic ones. Therefore, developing new improved local cultivars is necessary. Despite the universal importance of fragrant rice, no information is available on molecular and genetic base of scent characteristics in local scented rice in Malaysia. Furthermore, its genetic analysis has not yet been carried out. Thus, rice breeders are encouraged to identify the genetic determinants of aroma (Cordeiro *et al.,* 2002) because the exploitation of local aromatic genotypes can profit the national rice-breeding program. Hence, development of Malaysian rice germplasm with fragrance is a main goal for plant breeding, and effective techniques should be developed. Discovery of QTLs that control aroma in local genotypes can assist rice breeders to target specific loci on different chromosomes and generate fragrant cultivars. QTL discovery should also be employed as maker-assisted selection (MAS) technologies for an accurate and efficient selection, resulting in the rapid development of new crop cultivars with improved agronomic features. Thus, developing fragrant rice varieties by finding appropriate microsatellite loci and identifying QTLs using SSR and gene-based primers for fragrance are the most economical and practical methods. The results of the present study is the first SSR-based QTL map of aroma trait in fragrant rice in Malaysia.

1.3 Problem Statement

Although aroma is one of the most important grain quality traits in rice, the origin and evolution of the *BADH2* gene that underlies this trait remains ambiguous (Kovach *et al.*, 2009). There are also conflicting studies on the number of genes, nature of inheritance, and the location of aroma genes (Sakthivel *et al.*, 2009b). In Malaysia, insufficient knowledge on the molecular and genetic information for varieties which have been used extensively in breeding program is the major constraint to rice breeding. Furthermore, Malaysian fragrant rice cultivars have less 2AP in comparison with jasmine and basmati varieties. Thus, almost 100% of scented rice has been imported to Malaysia from other countries since aromatic rice is highly becoming popular in the market (MARDI, 2007). Consequently, in Malaysia there is a wide scope for research on fragrant rice, and Malaysian rice breeders should develop high quality rice varieties to meet the market demand and reduce Malaysia's expenses in importing rice.

1.4 Research Objectives

The objectives of the present study were as follows:

1. To identify polymorphism and segregation pattern of SSR and gene-based markers for aroma trait in an F_2 population of the crosses between MR84, a non-aromatic cultivar, and MRQ74, an elite Malaysian aromatic cultivar.
2. To determine inheritance of fragrance in the F_2 population through qualitative (leaf sensory test) and quantitative (gas chromatography-mass spectrometry) approaches.
3. To map QTLs for fragrance characteristics in the scented rice MRQ74 using microsatellite and gene-specific markers.
4. To identify tightly linked markers with aroma QTLs for marker-assisted selection.

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