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MARKER-ASSISTED BACKCROSSING TO DEVELOP A FRAGRANT RICE VARIETY FROM CROSSING BETWEEN RICE VARIETIES MR269 AND BASMATI 370

WENDY LAU CHUI PHING

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By

WENDY LAU CHUI PHING

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Chairman: Professor Mohd Rafii bin Yusop, PhD
Faculty: Agriculture

Fragrant rice is highly demanded by Malaysian consumers. Malaysia has to import fragrant rice from fragrant rice-producing countries, which is a costly expenditure. Furthermore, traditional Basmati and Thai fragrant rice varieties are not suitable to be grown in Malaysia due to low yield and their fragrance expression is specific to their native area. In order to reduce the dependency on fragrant rice import, it is crucial to develop a fragrant rice variety that is able to satisfy local consumers' preferences and adapt to the local environment. Among the volatile compounds that contribute to fragrance in rice, 2-acetyl-1-pyrroline (2AP) plays an important role in conferring the distinct fragrances of Basmati and Jasmine rice. The accumulation of 2AP in fragrant rice is due to a mutation on chromosome 8 that results in a non-functional betaine aldehyde dehydrogenase 2 protein (BADH2). Marker-assisted backcross breeding (MABC) enables the introgression of the desired gene and accelerates the restoration of the recurrent parent genome; thus, MABC was applied in this study. MR269 variety is a high-yielding but non-fragrant rice variety and was used as the recurrent parent. Basmati 370 variety is famous for its fragrance and was used as the donor parent of the *BADH2* gene to introgress the *BADH2* gene into the MR269 genome. Three functional markers of the *BADH2* gene were found to be polymorphic between the two parents. These functional markers were used in foreground selection to identify the desired fragrance genotype. The functional markers were used in the F_1 generation and confirmed true F_1 plants that were backcrossed to MR269. Chi-square analyses in the BC_1F_1 and BC_2F_1 generations showed that the functional markers segregated in a 1:1 ratio in the single gene model. All of the functional markers showed similar banding patterns for all of the backcross generations in this study. In the BC_2F_1 generation, aroma phenotype was evaluated. Most of the selected BC_2F_1 plants were scored as having a faint or moderate fragrance. Of the 324 simple sequence repeats (SSR) markers surveyed, 70 were polymorphic between the two parents and were used in background screening to determine the recovery of the recurrent parent genome. The average recovery of the recurrent parent genome in selected plants was 69.0% in the

BC_1F_1 generation and increased to 83.6% in the BC_2F_1 generation. After two generations of backcrossing and one generation of self-pollination, the average recovery of the recurrent parent genome increased to 88.4% in selected BC_2F_2 plants with morphological phenotype similar to that of MR269. The 14 advanced fragrant rice lines that were selected from the BC_2F_2 generation had fragrance, and most of their morphological and agronomical characters were similar to those of MR269. These advanced fragrant rice lines can be further used in the development of a fragrant rice variety. In conclusion, this study demonstrated the successful introgression of the fragrance gene and restored the high-yielding characteristics of MR269 in advanced fragrant rice lines. It is anticipated that the new variety developed from the advanced fragrant rice lines will be utilized by local farmers and consumers in the near future.

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

KACUKAN BALIK BERBANTUAN PENANDA UNTUK MENGHASILKAN VARIETI PADI WANGI DARIPADA KACUKAN ANTARA VARIETI PADI MR269 DAN BASMATI 370

Oleh

WENDY LAU CHUI PHING

Januari 2017

Pengerusi: Professor Mohd Rafii bin Yusop, PhD
Fakulti: Pertanian

Beras wangi mempunyai permintaan yang sangat tinggi oleh pengguna Malaysia. Oleh itu, Malaysia perlu mengimport daripada negara pengeluar beras wangi, dan ianya merupakan satu perbelanjaan yang tinggi. Selain itu, varieti padi wangi tradisional Basmati and Thai adalah tidak sesuai untuk ditanam di Malaysia kerana hasilnya yang rendah and pengekspresan kewangiannya adalah khusus kepada kawasan asal mereka. Bagi mengurangkan kebergantungan kepada import beras wangi, ia adalah penting untuk membangunkan varieti padi wangi yang mampu memenuhi keperluan pengguna dan dengan keupayaan varieti beradaptasi dengan persekitaran setempat. Antara sebatian meruap yang menyumbang kepada sifat wangian dalam beras, ialah 2-asetil-1-pirolin (2AP) yang memainkan peranan penting dalam memberikan aroma yang unik kepada beras Basmati and Jasmin. Akumulasi 2AP dalam beras wangi adalah disebabkan oleh mutasi pada kromosom 8 yang mengakibatkan protein betain aldehid dehidrogenase 2 (BADH2) tidak berfungsi. Kacukan balik berbantuan penanda (MABC) membolehkan introgresi gen yang dikehendaki dan mempercepatkan pemulihan genom induk penerima; justeru itu, MABC diaplikasikan dalam kajian ini. Varieti MR269 merupakan varieti padi berhasil tinggi tetapi tidak wangi telah digunakan sebagai induk penerima. Varieti Basmati 370 yang terkenal dengan wangiannya telah digunakan sebagai induk penderma gen *BADH2* untuk diintrogresikan ke dalam genom MR269. Tiga penanda yang berfungsi untuk gen *BADH2* didapati polimorfik di antara kedua-dua induk. Penanda berfungsi tersebut telah digunakan dalam pemilihan untuk mengenalpasti genotip kewangian yang dikehendaki. Penanda berfungsi tersebut telah digunakan untuk verifikasi generasi F_1 dan pokok F_1 tersebut telah dikacukbalik dengan MR269. Analisis khi-kuasa dua ke atas generasi BC_1F_1 dan BC_2F_1 menunjukkan ketiga-tiga penanda berfungsi bersegregasi dengan nisbah 1:1 mengikut model gen tunggal. Semua penanda berfungsi memberikan jalur yang serupa untuk semua generasi kacukan balik dalam kajian ini. Dalam generasi BC_2F_1, penilaian fenotipik aroma telah dijalankan. Kebanyakan pokok BC_2F_1 yang terpilih mendapat skor rendah dan sederhana wangi.

Daripada 324 penanda mikrosatelit (SSR) yang disaring, 70 penanda adalah polimorfik antara kedua-dua induk dan telah digunakan dalam penentuan pemulihan genom induk penerima. Purata pemulihan genom induk penerima bagi pokok terpilih adalah 69.0% pada generasi BC_1F_1 dan meningkat kepada 83.6% pada generasi BC_2F_1. Selepas dua generasi kacukan balik dan satu generasi penyendirian, purata pemulihan genom induk penerima meningkat kepada 88.4% dalam pokok BC_2F_2 terpilih dengan mempunyai fenotip morfologi yang menyerupai MR269. Empat belas titisan padi maju berwangi yang dipilih daripada generasi BC_2F_2 mempunyai ciri kewangian dan kebanyakan ciri morfologi dan agronomi adalah serupa dengan MR269. Titisan padi maju berwangi yang telah dihasilkan boleh dimajukan seterusnya untuk pembangunan varieti padi wangi. Kesimpulannya, kajian ini telah menunjukkan kejayaan introgressi gen wangian dan memulihkan ciri hasil tinggi varieti MR269 dalam titisan padi maju berwangi. Adalah dijangkakan penggunaan varieti baharu padi dari titisan maju berwangi ini oleh petani dan pengguna tempatan pada masa hadapan.

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I certify that a Thesis Examination Committee has met on 12 January 2017 to conduct the final examination of Wendy Lau Chui Phing on her thesis entitled "Marker-Assisted Backcrossing to Develop a Fragrant Rice Variety from Crossing Between Rice Varieties MR269 and Basmati 370" 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 Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Halimi bin Mohd Saud, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Datin Siti Nor Akmar binti Abdullah, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Syahida binti Ahmad, PhD
Senior Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Bing Yang, PhD
Associate Professor
Iowa State University
United States
(External Examiner)

NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 22 March 2017

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)

Mohd Razi Ismail, PhD
Professor
Institute of Tropical Agriculture and Food Security
Universiti Putra Malaysia
(Member)

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

Mohammad Abdul Latif, PhD
Plant Pathology Division
Bangladesh Rice Research Institute
Bangladesh
(Member)

Asfaliza Ramli, PhD
Rice and Industrial Crop Research Centre
Malaysian Agriculture Research and Development Institute
Malaysia
(Member)

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LIST OF ABBREVIATIONS

2AP	2-acetyl-1-pyrroline
AC	Amylose content
AFLP	Amplified Fragment Length Polymorphisms
ARM	Aroma sensory test
ASA	Allele-specific amplification
ASV	Alkali spreading value
BAC	Bacterial artificial chromosome
BADH2	Betaine aldehyde dehydrogenase 2
BC_1F_1	First filial of first backcross generation
BC_2F_1	First filial of second backcross generation
BC_2F_2	Second filial of second backcross generation
bp	Base-pair
CKE	Cooked kernel elongation ratio
cM	Centi Morgan
CTAB	Cetyltrimethylammonium bromide
df	Degrees of freedom
DF	Days to flowering
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
DM	Days to maturity
EDTA	Ethylenediaminetetraacetic acid
EPP	Entry Point Project
ET	Effective tillers per plant
F_1	First filial
FGP	Filled grains per panicle

fgr	Fragrance locus
GC	Gel consistency
GC-MS	Gas chromatography-mass spectrometer
GL	Grain length
GT	Gelatinization temperature
GW	Grain width
GYP	Grain yield per plant
HRR	Head rice recovery
HSD	Tukey's honest significant difference
IRRI	International Rice Research Institute
KOH	Potassium hydroxide
MABC	Marker-assisted backcrossing
MAB	Marker-assisted breeding
MARDI	Malaysian Agricultural Research and Development Institute
MAS	Marker-assisted selection
$MgCl_2$	Magnesium chloride
MRL	Milled rice length
MLWR	Milled rice length-to-width ratio
MRR	Milling recovery
MRW	Milled rice width
NKEA	National Key Economic Areas
NaCl	Sodium chloride
NGS	Next-generation sequencing
NPT	New plant type
PCR	Polymerase chain reaction
PEMANDU	Performance Management & Delivery Unit
PH	Plant height

PL	Panicle length
PVP	Polyvinylpyrrolidone
QTL	Quantitative trait loci
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction fragment length polymorphism
RIL	Recombinant inbred line
rpm	Revolutions per minute
SNP	Single nucleotide polymorphism
SPME/GC-MS	Solid-phase microextraction/gas chromatography-mass spectrometer
SR	Seed-setting rate
SSR	Simple sequence repeats
TBE	Tris-borate -EDTA
TE	Tris-EDTA
TGW	1000-grain weight
TSP	Total spikelets per panicle
TT	Total tillers per plant
t/ha	Tonne per hectare
Tris-HCl	Tris-Hydrochloride

CHAPTER 1

INTRODUCTION

1.1 Background

Rice (*Oryza sativa* L.) is an important food crop, serving as staple food for nearly half of the world's population (International Rice Research Institute (IRRI), 2014). Rice is also a pivotal source of carbohydrates and accounts for the diet of people mostly from Asia, Latin America, Africa and the Middle East (Brar and Khush, 2002). Rice is also the staple food of Malaysia and important to the Malaysian way of life, culture and traditions. To cater to local consumption, farmers in Malaysia produce more than 2 million tonnes of rice every year (Department of Statistics Malaysia, 2014). However, Malaysia still has to import rice, such as fragrant rice, to meet the local demand.

Although rice is considered common in rice-consuming countries, rice is not a "one-size fits all" crop. Rice quality is subjective in that consumer preferences vary across regions and cultures. The Japanese prefer short-grain, sticky rice which is usually used in making *sushi*. In contrast, in India, Pakistan and the Middle East, Basmati rice is well-liked because of its fragrance and elongated and dry grains when cooked (Suwannaporn and Linnemann, 2008). Italians prefer *Arborio* rice, a medium-grain rice that gives the "al dente" texture to *risotto* (Suwannaporn and Linnemann, 2008).

Generally, rice is eaten as cooked whole grain with little or no seasoning and is served as main part of the meal, thereby making rice quality a determining factor of acceptance by consumers. Fragrance is an important quality trait, as fragrant rice is more appealing to consumers. Furthermore, there is a growing demand for better quality, rice such as Jasmine and Basmati, especially in Asia (Pingali *et al.*, 1997). The growing demand for better quality rice is due to higher household income, allowing consumers to afford to pay more for better quality rice (Unnevehr *et al.*, 1992).

The method for evaluating fragrance in rice has changed with advances in science and technology. Substantial effort by rice breeders has identified the major compound that contributes to fragrance in rice. The advent of molecular marker technology, along with researchers' substantial efforts in molecular mapping, has identified chromosome regions carrying the genes responsible for fragrance. The identification of the genes responsible for the fragrance compound has facilitated the development of molecular markers. Plant breeding has benefited from the advent of molecular marker technology. Currently, molecular markers are incorporated into breeding programmes, known as marker-assisted breeding (MAB). The availability of molecular markers and the MAB approach enable breeders to tailor desirable traits and develop varieties according to consumer demand. In this study, the fragrance gene was successfully introgressed into a Malaysian high-yielding rice variety, MR269, through marker-assisted backcrossing (MABC).

1.2 Significance of the study

The increasing demand for fragrant rice among local consumers justifies the need to develop a fragrant rice variety. The advanced lines of this study will redound to the benefit of society considering that local consumers look forward to fragrant rice. This study will be a significant endeavour in promoting the approach of MAB for rice breeding in Malaysia. Thus, the application of MAB may accelerate rice varietal improvement and development in Malaysia. This study may serve as a future reference for quality rice breeders, particularly for those that study Basmati rice or its derivatives. In addition, the advanced lines will add to the genetic diversity of the fragrant rice varieties in Malaysia. The advanced fragrant rice lines derived in this study can be used as breeding material to develop a fragrant rice variety for Malaysia to meet the local demand for fragrant rice.

1.3 Problem statement

Malaysia's rice self-sufficiency level can only meet up to approximately 70% of the demand; the remaining 30% consists of fragrant rice and other rice types and has to be imported from other countries (Department of Statistics Malaysia, 2015). Based on the rice imports in 2011, fragrant rice accounts for up to 63% of the total import of rice, with a value of approximately RM 1.1 billion (Department of Agriculture Peninsular Malaysia, 2012). Furthermore, it is unfeasible to grow the traditional fragrant rice variety, such as Basmati 370 and Khao Dawk Mali 105 in Malaysia because these traditional fragrant rice varieties has low yield and their fragrance expression is specific to certain geographical area (Pachauri *et al.*, 2010). To achieve 100% self-sufficiency in rice and to be independent from fragrant rice import, it is crucial to develop fragrant rice varieties that can meet local consumers' preferences and that are adapted to our local environment. Therefore, this study aimed to develop high-yielding fragrant rice variety through MABC from MR269×Basmati 370 for local cultivation and consumption.

1.4 Objectives

The main objective of this study was to develop high-yielding fragrant rice variety for local cultivation and consumption.

The specific objectives were:

i. To identify polymorphic DNA markers between MR269 and Basmati 370 for background selection and fragrance for foreground selection in MABC.
ii. To introgress the *BADH2* gene from Basmati 370 into a Malaysian high-yielding rice variety, MR269, using the MABC method.
iii. To quantify the recovery of the recurrent parent genome in backcross generations.
iv. To determine the morphological and agronomical characteristics and grain quality attributes in the advanced BC_2F_2 lines.

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