UNIVERSITI PUTRA MALAYSIA

MORPHOLOGICAL, NUTRITIONAL AND MOLECULAR CHARACTERIZATION OF SELECTED GENOTYPES FOR UPLAND RICE IMPROVEMENT THROUGH MARKER-ASSISTED BACKCROSS BREEDING

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By

JANNATUL FERDOUS

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

April 2012

DEDICATIONS

This Thesis is Special Dedicated to

My Parents, My beloved husband and son Fahim

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

MORPHOLOGICAL, NUTRITIONAL, AND MOLECULAR CHARACTERIZATION OF SELECTED GENOTYPES FOR UPLAND RICE IMPROVEMENT THROUGH MARKER-ASSISTED BACKCROSS BREEDING

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Chairman : **Professor Mohamed Hanafi Musa, PhD, *AMIC***

Institute : **Tropical Agriculture**

Upland rice genotypes are low yielder but possess special traits. Marker assisted backcross breeding is a convenient technique for improvement of upland genotype in terms of yield and quality, where desirable trait is selected in early backcross generation. The aims of this study were to introduce high amylose content and high vitamin E alleles in BC1F1 generation as well as select the plant with desirable alleles through marker assisted selection (MAS) using SSR markers. Thirty nine rice genotypes were evaluated for parent selection based on morphology, nutritional composition, and genetic diversity. Three upland genotypes: Bukit Garam582, Bukit Garam1449, and Karingam were selected and back crossed with two high yielding varieties (BR16 and MR219). Identification of desirable plants for amylose content and vitamin E in BC_1F_1 generation was performed by MAS. The significant variation was observed among the genotypes for yield contributing characters, chlorophyll content, amylose content, glycemic index (GI), and antioxidant activity. The rice genotypes were grouped into 3 clusters, where Pulut Hitam Pahang (PHP), Lansan, and Karibang fell into 3 different solitary positions by morphological clustering. The first four principle components explained 76.5% variation for all

morphological characters and showed positive correlation between tiller number, panicle number, filled grain percentage and grains per panicle. The highest apparent amylose content (27.1%) and the lowest GI (71.1) were observed in BR16. The highest GI (99.2) was found in Bukit Hitam (waxy rice) which contained low amylose (1.5%). The highest antioxidant activity (89.8%) was found in Karingam and the lowest (50.8%) was in BR16. The highest Fe, Zn, and Mg were observed in white and high yielding genotypes. The highest Mn and Cu were detected in local upland black genotype Bukit Hitam (54.4 µg/g) and red genotype Padi Hijau Manis (23.2 µg/g) genotypes, respectively. The genetic diversity was assessed using 25 SSR markers. Cluster analysis showed differentiation of rice genotypes into 4 major groups and several sub-groups. Cross and reciprocal cross in 10 combination between selected five genetically diverse parents were performed. The F_1 plants were confirmed using Wx, RM190, RM3187, and RM3827 markers. The RM190 and Wx markers were used to select waxy allele for high amylose content. The RM3187 and RM3827 were used to select desirable allele of HGGT and HPT, respectively, which are responsible for tocopherols and tocotrienols biosynthesis. The following markers were amplified: for high amylose content (RM190 and Wx at 107 bp fragment); desirable allele of qTOC-6-2 (tocophelol) (RM3187 at 148 bp) and qT3-6-1 (tocotrienol) (RM3827 at 181 bp). A total of 28 plants were selected in BC_1F_1 generation, which contained all desirable alleles for high amylose content, tocopherol and tocotrienol. On the basis of morphological characters, plant number 9 of BR16/2 × Karingam (BC_1F_1) was selected for high yield potential. This plant produced the highest grain weight per plant (10.7 g) among 28 selected plants with 77.2% filled grain and grain color of this plant was white which can be used for the development of good quality and high yield potential upland variety.

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

PENCIRIAN MORFOLOGI, NUTRISI, DAN MOLEKULAR UNTUK PENAMBAHBAIKAN GENOTIP PADI BUKIT MELALUI PEMBIAK-BAKAAN KACUKAN BALIK

Oleh

JANNATUL FERDOUS

April 2012

Pengerusi : **Prof Mohamed Hanafi Musa, PhD,** ***AMIC***

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Genotip padi bukit mempunyai hasil pengeluaran yang rendah tetapi menunjukkan trait-trait yang istimewa. Pembiakbakaan kacuk balik dibantu penanda terpilih merupakan teknik yang berguna dalam penambahbaikan genotip padi bukit dari aspek hasil dan kualiti, dimana trait-trait yang dikehendaki akan dipilih dalam proses awal penghasilan generasi kacuk balik. Tujuan kajian ini adalah untuk memasukkan kandungan alel-alel amilosa dan Vitamin E yang tinggi dalam generasi BC_1F_1 serta memilih pokok dengan alel yang dikehendaki melalui marker assisted selection (MAS) dengan menggunakan penanda molekul jujukan ringkas terulang, SSR. Sebanyak 39 genotip padi telah dinilai untuk pemilihan pokok induk berdasarkan morfologi, kandungan nutrisi dan kepelbagaian genetik. Sebanyak tiga genotip padi bukit iaitu Bukit Garam582, Bukit Garam1449 dan Karingam telah dipilih dan dikacukan balik dengan dua varieti yang mempunyai hasil pengeluaran yang tinggi (BR16 dan MR219). Kaedah MAS digunakan untuk mengenalpasti pokok yang dikehendaki dengan kandungan amilosa dan Vitamin E dalam generasi BC_1F_1. Ciri-ciri variasi yang penting telah diperhatikan antara genotip yang menyumbang kepada hasil, kandungan klorofil, kandungan amilosa, indeks glycemic, dan aktiviti

antioksida. Kepelbagaian 39 genotip padi telah dianalisis dengan meneliti 14 ciri-ciri morfologi. Tiga puluh enam genotip padi telah dikumpulkan kepada 3 kluster dan genotip Pulut Hitam Pahang(PHP), Lannsan, dan Karibang telah dikumpulkan kepada 3 kedudukan yang berbeza melalui analisis kluster morfologi. Empat komponen pertama dalam analisis komponen prinsipal (PCA) menerangkan variasi untuk semua ciri-ciri morfologi dengan kadar 76.5% dan menunjukkan kolerasi positif di antara bilangan anak, bilangan tangkai padi, peratusan biji terisi, dan bilangan biji per tangkai padi. Kandungan amilosa yang tinggi (27.1%), dan indeks glycemic yang rendah (71.1%) telah didapati pada BR16. Nilai indeks glycemic yang tertinggi (99.2%) pada Bukit Hitam (beras pulut) dan juga mempunyai kandungan amilosa yang rendah (1.5%). Indeks glycemic dan kandungan amilosa menunjukkan kolerasi negatif. Kandungan antioksida yang tinggi adalah pada Karingam (89.8%) dan paling rendah pada BR16 (50.8%). Kandungan Fe, Zn, dan Mn yang tinggi didapati pada genotip putih dan genotip yang tinggi dalam hasil pengeluaran. Kandungan Mn dan Cu yang tinggi telah dikesan pada genotip hitam tempatan (54.4 µg/g) dan genotip merah padi hijau manis (23.2 µg/g). Kepelbagaian genetik untuk 39 genotip telah dinilai dengan 25 penanda molekul SSR. Analisis kluster menunjukkan perbezaan genotip padi kepada 4 kumpulan utama dan beberapa subkumpulan. Sepuluh kombinasi kacukan telah dibuat daripada kacukan silang tunggal dan kacukan silang berganda diantara 5 induk yang mempunyai kepelbagaian genetik. Pokok F_1 telah dibuktikan menggunakan dengan penanda Wx, RM190, RM3187, dan RM3827. Penanda Wx dan RM190 digunakan untuk memilih alel berlilin untuk kandungan amilosa yang tinggi. Penanda RM3187 dan RM 3827 telah digunakan untuk memilih alel HGGT dan HPT yang berperanan untuk biosintesis tocoferol dan tocotrienol. Penanda berikut diamplifikasi: untuk

kandungan amilosa yang tinggi (RM190 dan Wx pada fragmen bersaiz 107 bp); tinggi aktiviti alel qTOC-6-2 (tocoferol) (RM3187 pada 148 bp) dan qT3-6-1 (tocotrienol) (RM3827 pada 181 bp). Sebanyak 28 tumbuhan telah dipilih daripada generasi BC_1F_1 yang mempunyai semua kandungan alel-alel yang dikehendaki untuk kandungan amilosa, tocoferol, dan tocotrienol yang tinggi. Berdasarkan kepada ciri-ciri morfologi, pokok padi nombor 9 daripada BR16/2 × Karingam (BC_1F_1) telah dipilih untuk potensi tinggi hasil pengeluaran. Pokok ini memberikan berat biji per pokok yang tertinggi (10.7 g) daripada 28 pokok yang terpilih dengan 77.2% biji terisi dan warna biji pada tumbuhan ini adalah putih dan boleh digunakan untuk penambahbaikan varieti yang mempunyai kualiti yang baik dan hasil pengeluaran yang tinggi.

ACKNOWLEDGEMENT

Praise to Almighty Allah for His blessings, which enable me to complete this thesis. It is a great pleasure to express my profound sense of gratitude and ever indebtedness to my Chairman of the Supervisory Committee Professor Mohamed Hanafi Musa, for his untiring constant guidance, generous help and encouragement throughout the period of the research work. I also express my deepest sense of gratitude and indebtedness to the members of the Supervisory Committee, Associate Professor Dr. Mohd Rafii Yusop and Associate Professor Dr. Sharifah Kharidah Syed Muhammad for their constructive criticism and valuable suggestions during the study period.

I am thankful to the Organization for Women in Science for the Developing world (OWSD) for awarding me fellowship and grateful to the Bangladesh Rice Research Institute (BRRI) for allowing leave on deputation for completing the Ph D program. I am also grateful to UPM for providing research fund and Ministry of Higher Education (MOHE) for a partial research grant through top-down LRGS research grant (No. 9154900). Furthermore, I like to thank Dr. Sharifah Kharidah for giving permission to use lab facilities in BERNAS.

I wish to pay my sincere graduate to all members of the Institute of Tropical Agriculture, Universiti Putra Malaysia for their direct and indirect help during the study. I am especially grateful to Mrs. Norhasimah for her cordial cooperation and Nora, Hazi and Zainudin for their active help in the laboratory. I am grateful to Dr. Mahfuza for encouraging me to continue my study. I am also thankful to my friends and lab mate in the MTDC central lab, ITA and BERNAS, Shahanaz, Hasna, Laila,

Samanthi, Shamima, Azura, Yanti, Zaleha, Ahmed, Chee Yong, Hanif, Faiz, Fabien, Dr. Abdul Hakim, Parvez and Rita for their friendship, help and support.

I am grateful to my ever-respected mother who bears divine affection, brother, brother-in-law, sister, sister-in-law and other relatives who continuously encouraged and prayed for my success. I am especially thankful to my mother-in-law and sister-in-law Rekha, who had provided moral support and looked after my child in my absence. Special gratitude must go to my son Md. Fahim Mushfiq for his great sacrifice.

Finally, I express my sincere gratitude and appreciation to my husband Dr. Md. Monirul Islam for his encouragement, sacrifice and patience during the entire period of my study.

I certify that an Examination Committee met on 23 April 2012 to conduct the final examination of Jannatul Ferdous on her Doctor of Philosophy thesis entitled "Morphological, nutritional, and molecular characterization of selected genotypes for upland rice improvement through marker-assisted backcross breeding" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for equations and citations, which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

JANNATUL FERDOUS

Date: 23 April 2012

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