UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF ALLELOPATHIC RICE GENOTYPE WITH HIGH GRAIN ZINC CONTENT THROUGH MARKER-ASSISTED BACKCROSS

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By

MUHAMMAD SIRAJUL MAZID

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

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DEDICATION

THIS THESIS IS SPECIALLY DEDICATED

TO

MY BELOVED PARENTS, BROTHERS, FAMILY AND THE PEOPLE WHO SUFFER DUE TO ZINC DEFICIENCY PROBLEMS

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

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February 2018

Chairman : Professor Abdul Shukor Juraimi, PhD
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Rice cultivation is hindered by biotic and abiotic stresses. Among the biotic stresses, weeds like barnyard grass can cause significant reduction of rice yield. The efficacious approach to control barnyard grass is by using allelopathic rice genotype integration management with minimum chemical control method. Zinc (Zn) deficiency is one of the critical problems of global malnutrition for humans. The problem affected approximately half of the world's population. The genetic biofortification together with agronomic biofortification of staple food crop is considered the most effective approach to prevent Zn deficiency. Marker-assisted selection (MAS) can potentially hasten breeding programs for varietal development. Marker-assisted backcrossing (MABC) is one of the most-useful ways of transferring specific gene(s) or QTL(s) into a popular variety. In this study, high grain Zn QTLs were transferred from an *indica* rice donor BRRI dhan62, using MABC method, into an allelopathic elite *indica* rice variety, MR73 to develop one superior genotype which has both allelopathic trait along with high grain Zn. Twenty eight microsatellite markers linked to the Zn QTLs or grain Zn content were selected and used in this study to determine potential association with Zn QTLs. These polymorphic foreground markers were used for confirmation of Zn QTLs or presence of grain Zn content gene in the F_1 population. Subsequently, only three microsatellite markers, RM152, RM3331 and RM3909 that indicated Zn QTLs located on chromosomes 8, 12 and 9 respectively, were used on BC_1F_1, BC_2F_1 and BC_2F_2 populations. Out of 405 microsatellite markers, 75 were found to have polymorphism between parental varieties and were used for recurrent parent genome recovery (RPG) analysis in each generation. Chi-square analyses of foreground markers showed an expected segregation ratio of 1:1 in BC_1F_1 and BC_2F_1 generations indicating inheritance in simple Mendelian fashion. This result confirmed that grain Zn content trait in BRRI dhan62 is governed by single dominant gene. The genotypic segregation of BC_2F_2 population applying RM152, RM3331 and RM3909 exhibited 1:2:1 ratio. Results confirmed that single dominant gene control high grain Zn in BRRI

dhan62 located on chromosomes 8, 12 and 9 are linked to RM152, RM3331 and RM3909 markers respectively. The RPG analysis of the improved genotypes indicated recurrent parent genome recovery ranging from 70.20-91.40% in BC_1F_1 generation, 80.50-93.00% in BC_2F_1 and 93.10-95.40% in BC_2F_2. The average RPG in BC_2F_2 selected improved genotypes were 93.86%, explaining the very close similarity at phenotypic level with the recipient parental variety MR73. Ten homozygous dominant Zn QTLs plants which are phenotypically closely similar for agro-morphological traits to MR73 were selected as improved high grain Zn breeding genotypes. The agronomic traits showed no significant difference between MR73 parent and Zn QTLs containing MR73 improved genotypes except the trait days to 50% flowering. Moreover, average Zn content in brown rice of improved genotypes was 40 mg kg^{-1} which was higher than the recurrent parent MR73 (27.99 mg kg^{-1}). Furthermore, the ability of newly improved rice genotypes to suppress the growth of barnyard grass which is expressed through average percent inhibition was 39.98%. The improved rice genotypes including these research findings could be used as valuable source for further high grain Zn rice breeding programmes in future.

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

PEMBANGUNAN GENOTIP PADI ALELOPATIK DENGAN KANDUNGAN ZINK BIJIAN YANG TINGGI MELALUI KACUK BALIK BANTUAN PENANDA

Oleh

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Penanaman padi menghadapi kekangan oleh tekanan biotik dan abiotik. Di antara tekanan biotik, rumpai seperti rumput sambau boleh mengakibatkan pengurangan hasil padi yang ketara. Pendekatan yang berkesan untuk mengawal rumput sambau ini adalah melalui penggunaan genotip padi alelopatik serta pengurusan bersepadu dengan penggunaan kaedah pengawalan kimia yang minima. Kekurangan unsur zink (Zn) adalah salah satu masalah malnutrisi yang kritikal di peringkat global. Masalah ini melibatkan kira-kira separuh penduduk dunia. Peningkatan nutrien tanaman makanan ruji secara genetik dan agronomik dianggap sebagai cara yang paling berkesan untuk menangani masalah kekurangan unsur Zn. Pemilihan dengan bantuan penanda (MAS) berpotensi untuk mempercepatkan program pembiakbakaan dalam pembangunan sesuatu varieti. Kacuk balik dengan bantuan penanda (MABC) adalah salah satu kaedah yang paling berjaya untuk memindahkan gen tertentu atau QTL ke dalam sesuatu varieti popular. Dalam kajian ini, QTLs untuk unsur Zn bijian yang tinggi telah dipindahkan dari padi *indica* induk BRRI dhan62 kepada satu varieti elit padi *indica* beralelopatik, MR73 dengan kaedah MABC, untuk menghasilkan satu genotip baharu yang mempunyai ciri unsur Zn bijian yang tinggi berserta beralelopatik. Dua puluh lapan penanda mikrosatelit yang berkaitan dengan QTL Zn atau kandungan Zn bijian dipilih dan digunakan dalam kajian ini untuk mengenalpasti kaitannya dengan QTL-Zn. Penanda polimorfik berkait rapat dengan Zn ini digunakan untuk pengesahan QTL-Zn atau kewujudan gen kandungan Zn bijian dalam populasi F_1. Selepas itu, hanya tiga penanda mikrosatelit iaitu RM152, RM3331 dan RM3909 yang menunjukkan QTL Zn yang masing-masing terletak di kromosom 8, 12 dan 9 telah digunakan ke atas populasi BC_1F_1, BC_2F_1 dan BC_2F_2. Dari 405 penanda mikrosatelit, 75 penanda telah didapati memberikan polimorfik di antara varieti induk dan ianya telah digunakan untuk analisis pemulihan genom induk penerima (RPG) untuk setiap generasi kacuk balik. Analisa khi-kuasadua ke atas penanda berkait rapat dengan Zn

telah menunjukkan nisbah segregasi 1:1 seperti nisbah Mendelian dijangka iaitu mengikut pewarisan gen tunggal. Keputusan ini menunjukkan ciri kandungan zink bijian varieti BRRI dhan62 adalah dikawal oleh gen dominan tunggal. Nisbah segregasi genotip dalam populasi BC_2F_2 yang dikesan oleh penanda RM152, RM3331 and RM3909 memberikan nisbah 1:2:1. Keputusan ini juga mengesahkan bahawa gen tunggal dominan mengawal kandungan zink bijian dalam varieti BRRI dhan62 yang terletak di kromosom 8, 12 dan 9 yang berkaitan masing-masing kepada penanda-penanda RM152, RM3331 dan RM3909. Analisis RPG ke atas hasil kacukan menunjukkan pemulihan genom induk penerima adalah dari 70.20 - 91.40% dalam generasi BC_1F_1, 80.50-93.00% dalam BC_2F_1 dan 93.10-95.30% dalam BC_2F_2. Purata RPG untuk genotip terpilih BC_2F_2 adalah 93.85%, dan ianya adalah hampir menyamai fenotip dengan varieti induk penerima, MR73. Sepuluh pokok dominan homozigot QTL zink yang fenotip agro-moforlogi adalah hampir sama dengan MR73 telah dipilih sebagai genotip maju padi yang tinggi kandungan bijian Zn. Analisis ciri-ciri agronomik menunjukkan tiada perbezaan antara induk MR73 dan genotip maju terpilih MR73 yang mengandungi QTL Zn yang tinggi kecuali bilangan hari 50% berbunga. Tambahan pula, purata kandungan Zn dalam beras perang genotip maju terpilih adalah 40 mg kg^{-1}, iaitu lebih tinggi daripada varieti induk penerima MR73 (27.99 mg kg^{-1}). Seterusnya, keupayaan genotip maju terpilih padi baharu ini untuk mengawal pertumbuhan rumput sambau yang ditunjukkan melalui purata peratus perencatan adalah 39.98%. Genotip maju padi ini serta dapatan kajian ini adalah sumber yang bernilai yang boleh digunakan dalam program peningkatan kandungan Zn bijian seterusnya dalam program pembiakbakaan di masa hadapan.

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LIST OF ABBREVIATIONS

bp	Base pair
CTAB	Cetyltrimethylammonium bromide
χ^2	Chi-square
df	Degree of Freedom
DNA	Deoxyribo Nucleic Acid
EDTA	Ethylene Diamine Tetra Acetate Acid
GA	Expected Genetic Advance
GCV	Genotypic Coefficient of Varience
MABC	Marker Assisted Backcrossing
MAS	Marker Assisted Selection
MABB	marker-assisted backcross breeding
ABL	Advance breeding line
MS	Mean Square
MSE	Mean Square of Error
MSG	Mean Square of Genotypes
PCA	Principal component analysis
PCR	Polymerase Chain Reaction
PCV	Phenotypic Coefficient of Varience
PVP	Polyvinyl Pyrrolidone
QTL	Quantitative Trait Loci
SSR	Simple Sequence Repeat
RAPD	Randomly Polymorphic DNA
RCBD	Randomized Complete Block Design
RH	Relative Humidity
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
TE	Tris/ EDTA
UV	Ultra Violet
ml	Milliliter

mm	Millimeter
Min	Minute
M	Molar
n	Nano
PCR	Polymerase chain reaction
%	Percentage
RPG	Recurrent parent genome
µ	Micro
RNA	Ribo Nucleic Acid
ºC	Degree celsius
cm	Centimeter
cM	Centi Morgan
g	Gram
rpm	Rounds per minute
s	Second
V	Volt
v/v	Volume/volume
β-BE	B-mercaptoethanol
ddH2O	Double distilled water
mg	Milligram
kg	Killogram
ha	Hectare
GA	Genetic Advance
ZIP	Zrt-,Irt-like protein
SNP	Single-nucleotide polymorphism

CHAPTER 1

INTRODUCTION

1.1 General introduction

More than last five decades, availability and growth of calorically enriched staple crops production were the main targets of agricultural research in developing countries. Rice development programmes have been conducted towards enhancing yield, resistance to biotic factors, tolerance to abiotic stress, and curtailing growth period for the last four decades. Amount of micronutrients present in the rice grain is one of the vital characteristics. We should investigate the prospect of development of high yielding rice varieties with high micronutrients content in order to combat micronutrients deficiency. Among the micronutrients deficiency, zinc (Zn) scarcity is one of the important micronutrients deficiency problem for humans.

More than half of the global humans consume rice as a staple food. Rice grain innately contain lower amount of micronutrients like Zn. Insufficiency of Zn is a significant issue not only for the health of world population but also nutrition. One third of the global people are under threat owing to poor nutritional eating of Zn (Myers et al., 2014). Most of the people who are under risk widely dependent on cereals; particularly rice (*Oryza sativa* L.). Malaysian people also consume rice as their staple food. Therefore, people of Malaysia could be potential victims of Zn deficiency in near future.

Weed causes significant problems including yield loss in rice crop. Although many controlling measures are adopted involving popular chemical control but it significantly affect environment (Kabir et al., 2010), human health and is responsible for the emergence of herbicide resistant weed variety (Heap, 2014). Therefore, development of weed resistant rice variety or use of rice allelopathy has been suggested. In this perspective, advantageous/desired trait development in the background of allelopathic rice variety could be one of the logical approaches for weed control combined with other controlling methods.

1.2 Problem statement

Deficiency of Zn in diet is considered a significant public health and dietary problem in the current world. In the globe, half of the soils are fundamentally poor in terms of Zn. Inadequate of Zn in soils is considered as extensive nutritional crisis all over the rice producing nations. Scarcity of Zn in soil is generally related to high fixation of Zn, poor phyto-availability (Rehman et al., 2012) and cultivation of high yielding rice varieties which eliminate huge amount of soil Zn at each reaps (De Steur et al., 2014).

Zinc deficiency in humans can lead to many unexpected consequences such as dwarfism, hypogonadism, diarrhoea, pneumonia and many more (Prasad et al., 1961; Gibson et al., 2008). Among the different approaches, Zn biofortification is highly suggested to combat Zn deficiency problem.

Many efforts have been taken more than last one decade to biofortify food crops particularly for Zn. Consequently, information is available with regards to genetic, molecular and physiological basis of high Zn accumulation, influence of environmental features and agronomic practices on Zn uptake, translocation and loading into rice grain. Generally, biofortification efforts have been carried out through agronomic, transgenic and breeding approach. Agronomic biofortification is a remedy for short time and legal systems of many countries do not support products from transgenic approach. Therefore, genetic Zn biofortification through breeding is highly recommended. Rice variety should be developed in such a way so that it can include high yielding characteristics with high Zn content in grain.

Conventional breeding for the development of high Zn rice cultivar has been going for the last few years in different countries. But the efforts became less effective due to complex genetic basis of grain Zn and environmental effects (Swamy et al., 2016). Moreover, it took long time through this method and the developed line had moderate yield potential as well as modest increase in Zn concentration. However, selection of correct breeding methods, crossing programmes, selection of individual plant and processes of crop field evaluation play crucial role for fruitful development of high Zn rice as suggested by Swamy et al. (2016). Conventional breeding method is a lingering process in which normally six to eight backcrosses required to recover complete recurrent parent genome. Moreover the method includes arduous evaluation of field performance which is some time unreliable due to genotype × environment (G×E) interactions and expensive, plant breeders require wide experience etc. The application of molecular marker-aided selection (MAS) extensively removes these impediments. Molecular markers allow the perfect tool for developing the efficacy of selection for expected traits in new rice lines. These molecular markers provide vast scope for increasing the efficacy of conventional breeding by executing selection not straightforwardly on the desirable feature but the molecular markers related to that trait. Furthermore, application of molecular marker decreases the time required for the recovery of recurrent parent genome compared to conventional method. Thus, molecular marker-aided backcrossing (MAB) facilitates to select the desired plant and to reduce the overall time requirement for completing the breeding procedure.

If high Zn trait could be introgressed into Malaysian modern allelopathic rice variety MR73 keeping its other traits unaltered, a significant amount of Zn enriched rice could be produced and simultaneously application of herbicide could be decreased for the betterment of environment.

The current study was designed to improve allelopathic MR73 rice variety into high Zn content rice through marker-aided backcrossing (MABC). The study consisted of foreground and background selection for MABC.

The improved lines required to be tested for their Zn content in grain and phenotypic adaptabilities. Moreover, grain yield and yield-contributing characteristics were also compared.

1.3 Objective of the study

1. To analyse microsatellite (SSR) markers linked to high grain Zn QTLs in MR73 and BRRI dhan62 crosses.

2. To analyse the recipient parent genome reappearance in marker-aided backcross breeding programmes.

3. To pyramid high grain Zn QTLs in MR73-rice variety through marker-aided backcross breeding.

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