UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF WEED COMPETITIVE RICE VARIETY UNDER WATER DEFICIT CONDITIONS THROUGH MARKER-ASSISTED BACKCROSS BREEDING

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By

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Weed is one of the most troublesome pests in rice cultivation especially in water deficit areas, where farmers are commonly used herbicides to control it. Hence, it is important to develop rice variety that has capability to withstand water deficit and higher weed interference conditions, using an environmentally friendly method, such as marker-assisted backcross breeding. Eight rice varieties *viz.* AERON1, MR185, MR211, MR219, MR232, MR253, MR263 and MRQ74 were evaluated for their performances in water and weed stress conditions. From this study, two varieties (AERON1 and MRQ74) with the best performances were selected as donor and recurrent parental plants, respectively, for molecular breeding study. In molecular study, two foreground and 57 background markers were found as suitable to be used along the breeding study. The foreground markers were RM242 and RM263, which are linked markers to root length genes for drought tolerance. MRQ74 and AERON1 were crossed and backcrossed to generate F_1, BC_1F_1 and BC_2F_1 populations, respectively. Rice lines of BC_2F_1 population were self-pollinated to produce BC_2F_2 population. Chi-square (χ^2) analysis for BC_1F_1 and BC_2F_1 populations showed good fit to the expected segregation ratio (1:1) for a single dominant gene model (d.f.=1, P>0.05) in Mendelian law for both foreground markers. The Chi-square (χ^2) values for RM242 and RM263 were χ^2=3.1 and χ^2=0.03, and χ^2=1.01 and χ^2=0.22, in BC_1F_1 and BC_2F_1 populations, respectively. In each BC_1F_1 and BC_2F_1 populations respectively, 18 and 25 rice lines were revealed as to carry both foreground markers. The highest RPG percentage for BC_1F_1 and BC_2F_1 populations were found in line (L)

50-39 with 71.4% and L45-6-24 with 90.8%, respectively. In BC_2F_2 population, the Chi-square (χ^2) values for RM242 and RM263 were χ^2=2.81 and χ^2=2.97, respectively, and showed good fit to the expected marker segregation ratio (1:2:1) for a single dominant gene model (d.f.=2, P>0.05) in Mendelian law. The highest RPG percentage was discovered in L45-6-24-23 with 93.0%. BC_2F_2 population had also been evaluated for its agro-morphological traits and tolerance to water deficit conditions by using several drought indices, rank and standard deviation of rank method. Through BC_2F_2 population, 12 rice lines *viz.* L45-27-3-7, L50-39-8-5, L50-39-8-8, L45-27-10-18, L45-27-10-28, L45-27-8-13, L45-27-8-28, L45-27-8-34, L50-39-7-15, L45-6-24-10, L45-6-24-20 and L45-6-24-23 were found to carry both foreground markers, whereas L2 (L50-39-8) was discovered as the most tolerant line to water deficit conditions in this study. The usage of molecular markers in this study is responsible to accelerate the development of new rice lines that can withstand water deficit conditions and possessed good weed competitive ability.

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

PEMBANGUNAN VARIETI PADI YANG BERDAYA SAING DENGAN RUMPAI PADA KEADAAN KURANG AIR MELALUI PEMBIAKBAKAAN BERSILANG DENGAN PENANDA BANTUAN

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Rumpai adalah salah satu perosak penanaman padi terutamanya di kawasan yang kurang air, dimana petani menggunakan herbisid untuk mengawal rumpai. Oleh itu, adalah penting untuk membangunkan varieti yang berkebolehan untuk hidup pada keadaan kurang air dan tinggi kehadiran rumpai, menggunakan kaedah mesra alam seperti pembiakbakaan silang dengan penanda bantuan. Lapan varieti padi iaitu AERON1, MR185, MR211, MR219, MR232, MR253, MR263 dan MRQ74 dinilai keupayaannya pada keadaan stres air dan rumpai. Dalam kajian ini, dua varieti (AERON1 dan MRQ74) dengan keupayaan terbaik dipilih sebagai pokok induk penderma dan penerima, masing-masing, untuk kajian pembiakbakaan molekular. Dua penanda utama dan 57 penanda latarbelakang ditemui sesuai untuk digunakan sepanjang kajian pembiakbakaan ini. Dua penanda yang dipilih adalah RM242 dan RM263, yang merupakan penanda yang berkait dengan gen kepanjangan akar untuk kerintangan terhadap kemarau. MRQ74 dan AERON1 masing-masing dikacukkan dan dikacukbalikkan untuk menghasilkan populasi-populasi F_1, BC_1F_1 dan BC_2F_1. Titisan-titisan padi dari populasi BC_2F_1 dikacuksendirikan untuk menghasilkan populasi BC_2F_2. Analisis Chi-kuasa dua (χ^2) menunjukkan padanan yang baik kepada nisbah pemisahan dijangka (1:1) bagi model gen tunggal dominan (d.f.=1, P>0.05) dalam peraturan Mendel untuk dua penanda utama. Nilai-nilai Chi-kuasa dua (χ^2) untuk RM242 dan RM263 adalah χ^2=3.1 dan χ^2=0.03, dan χ^2=1.01 dan χ^2=0.22, masing-masing bagi populasi BC_1F_1 dan BC_2F_1. Populasi BC_1F_1 dan BC_2F_1 masing-

masing menunjukkan bahawa 18 dan 25 titisan padi membawa kedua-dua penanda utama. Peratusan RPG tertinggi bagi populasi BC_1F_1 dan BC_2F_1 masing-masing adalah dari L50-39 dengan 71.4% dan L45-6-24 dengan 90.8%. Dalam populasi BC_2F_2, nilai Chi-kuasa dua (χ^2) untuk RM242 dan RM263 masing-masing adalah χ^2=2.81 dan χ^2=2.97, dan menunjukkan padanan yang baik kepada nisbah pemisahan dijangka (1:2:1) bagi model gen tunggal dominan (d.f.=2, P>0.05) dalam peraturan Mendel. Peratusan RPG tertinggi adalah dari L45-6-24-23 dengan 93.0%. Titisan padi populasi BC_2F_2 juga dinilai dari segi ciri-ciri agro-morfologikal dan kerintangan terhadap keadaan stres air menggunakan kaedah indeks kemarau, kedudukan dan pembahagian standard kedudukan. Melalui populasi BC_2F_2, 12 titisan padi iaitu L45-27-3-7, L50-39-8-5, L50-39-8-8, L45-27-10-18, L45-27-10-28, L45-27-8-13, L45-27-8-28, L45-27-8-34, L50-39-7-15, L45-6-24-10, L45-6-24-20 dan L45-6-24-23 ditemui membawa kedua-dua penanda utama, manakala L2 (L50-39-8) ditemui sebagai titisan paling rintang terhadap keadaan kurang air dalam kajian ini. Kesimpulannya, penggunaan penanda molekular dapat mempercepatkan pembangunantitisan padi baru yang dapat bertahan pada keadaan kurang air dan berdaya saing dengan rumpai.

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analysis of Variance
AWD	Alternate Wetting and Drying
bp	base pair
BIL	Backcross Inbred Lines
CEC	Cation Exchange Capacity
cM	centi Morgan
CTAB	Centyltrimethylammonium bromide
χ^2	Chi-square
DAS	Day after sowing
d.f.	Degree of freedom
DI	Drought Resistance Index
DMRT	Duncan Multiple Range Test
DNA	Deoxyribonucleic acid
dSm	decisemens per meter
EDTA	Ethyenediaminetetraacetic acid
gmp	Geometric Mean Productivity
IMW	Integrated Weed Managament
kPa	kiloPascal
M	molar
MABC	Marker-assisted backcross breeding
MAS	Marker-assisted selection
Mb	megabytes
meq	millequivalents
mM	micromolar
MOP	Muriate of potash
MPI	Mean Productivity Index
MRP	Mean Relative Performance

NPK	Natrium/Potassium/Kalium
PCR	Polymerase Chain Reaction
ppm	parts per million
QTL	Quantitative Trait Loci
RCBD	Randomized Complete Block Design
RPG	Recurrent Parent Genome
SDR	Standard deviation of rank
SRI	System of Rice Intensification
SSC	Saturated Soil Culture
SSR	Simple Sequence Repeat
SSI	Stress Susceptibility Index
SSSI	Schneider’s Stress Susceptibility Index
STI	Stress Tolerance Index
RAPD	Randomly Amplified Polymorphic DNA
RCC	Relative Chlorophyll Content
RDI	Relative Drought Index
REI	Relative Efficiency Index
RFLP	Restriction Fragment Length Polymorphism
rpm	rounds per minute
SNP	Single Nucleotide Polymorphism
SPAD	Silicon Photon Activated Diode
TBE	Tris/Boric acid/EDTA
TOL	Stress Tolerance
TE	Tris/EDTA
TSP	Triple Super Phosphate
WAS	Week after sowing
YSI	Yield Stability Index
µl	microliter

CHAPTER 1

INTRODUCTION

Weeds are the major constraint in rice cultivation followed by nitrogen deficiency and pests and diseases (Anwar *et al.*, 2010). Generally, weeds are competing with rice for sunlight, water, soil and nutrients (Davies *et al.*, 2008). In water deficit conditions, more C_4 weeds are found, as they are able in adapting hot and dryland conditions due to having higher water use efficiency compared to C_3 plants (Abouziena *et al.*, 2015). Therefore, weed control in water deficit condition is more important and challenging as rice is most susceptible and severely affected to dry condition during booting and flowering stages that tend to cause yield loss (Valencia, 2015). Meanwhile, in typical rice cultivation with easiness of water access, weed had also become a major problem and lowered rice yield production. This is due to shifting of cultivation method from transplanting to direct-seeded method as a way to reduce labor cost and production risks (Karim *et al.*, 2004). In this situation, weeds emerge at the same time with rice seedlings. To overcome weed problem, farmers tend to use harzadous herbicides that can cause health problem, pollute the environment, damage the plant itself, as well as encourage the emergence of herbicides resistant weeds (Maclean *et al.*, 2002). Hence, a method known as Integrated Weed Management (IWM) was developed to overcome this problem. This method implements preventive, cultural, mechanical and chemical methods throughout rice cultivation period (Owombo *et al.*, 2014), as single method seldom meet the demanded outcomes (Webster and Levy, 2009).

Rice is one of the important cereals in the world as one-third of the world population consumes it. Rice use approximately one-forth to one-third of world's freshwater annual supply and cause a waste of this limited and costly resource (Africare, 2010). It demands water as much as three times than wheat and maize and typically grown in puddle-irrigated soil condition (Swamy and Kumar, 2012). However, water is scarce nowadays. China and India, the world's leader of rice production are severely affected by freshwater resource scarcity (Priyanka *et al.*, 2012). Uncertainty in climate and increase of human population all over the world are among contributing factors to water scarcity condition (Sariam and Anuar, 2010). Hence, to overcome the problem, scientists had come up with aerobic rice varieties that were believed to withstand water deficit condition efficiently. Aerobic rice is a combination of traits of upland and lowland rice varieties with low water usage and is responsive to input (Priyanka *et al.*, 2012). Despite of its ability, these varieties are facing serious weed problem since weeds are abundantly grow in inadequate water availability.

To manage water and weed stress problems, marker-assisted backcross breeding had been utilized to develop new rice varieties that can tolerate those stress conditions. Marker-assisted backcross breeding has given a huge positive impact in rice breeding program (Yi *et al.*, 2009). Markers are used in backcross breeding to control the target gene through foreground selection and genetic background through background selection, which aims to reduce donor parent genome through recurrent backcrosses (Hospital, 2001). Markers-assisted selection helps to choose the best plants during cultivation, time, space and cost saving, and raise no ethical issuses as in transgenic plants (Bernier *et al.*, 2008; Francia *et al.*, 2005; Hasan *et al.*, 2015a). Meanwhile, breeding for drought tolerance varieties is quite a challenging effort due to complexity of genetics and uncertainty in time and intensity of drought period (Tuberosa and Salvi, 2005). Thus, with the discovery of many QTLs related to drought, new routes to study the contributor traits of drought tolerance are now enlarge (Saadalla, 2008). For water stress, root traits play important role in resisting drought condition in rainfed lowland irrigation (Manickavelu *et al.*, 2006). Deep-rooting variety is more resistant to drought as it has abilities to extract water from deeper soil and produce higher yield than shallow-rooting variety (Saadalla, 2008). Meanwhile, rice plant's traits, such as plant height, tillers number, early growth vigor and allelopaty ability (Anwar, 2012) should be wisely manipulated to overcome weed occurrence in rice field.

Perhaps, resistance towards both water and weed stress conditions is an ideal type of rice plant in competing those stress simultaneously in rice field. Hence, to develop a new rice variety that can withstand water and weed stress conditions, these objectives need to be achieved:

i. To identify weed competitive traits in water deficit condition among selected rice varieties,
ii. To select suitable parental plants of rice for molecular breeding study,
iii. To improve selected high-yielding rice variety by enhancing the ability to compete with weeds and water deficit environments through molecular breeding,
iv. To evaluate the new improved rice lines towards different water and weed stress conditions.

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