



**UNIVERSITI PUTRA MALAYSIA**

**SCREENING OF MALAYSIAN RICE ACCESSIONS AND MARKER-  
ASSISTED SELECTION OF A CROSS BETWEEN MR263 × SWARNA-  
SUB1 VARIETIES FOR SUBMERGENCE TOLERANCE**

**WELLAND COSMAS MOJULAT**

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By

**WELLAND COSMAS MOJULAT**

**Thesis Submitted to the School of Graduate Studies, Universiti  
Putra Malaysia, in Fulfilment of the Requirements for the Degree of  
Master of Science**

**November 2015**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

**SCREENING OF MALAYSIAN RICE ACCESSIONS AND MARKER-ASSISTED SELECTION OF A CROSS BETWEEN MR263 × SWARNA-SUB1 VARIETIES FOR SUBMERGENCE TOLERANCE**

By

**WELLAND COSMAS MOJULAT**

**November 2015**

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

Submergence is considered as one of the principal cause of crop failure worldwide causing major constraint in world rice production as well as in Malaysia. Several rice fields at Kelantan, Terengganu, and Pahang in Malaysia have been affected by flash flood and resulted in rice yield loss. *Sub1*, a gene which is responsible for submergence tolerance and has been utilized to developed several rice submergence tolerance varieties namely, Swarna-Sub1, BR11-Sub1, IR64-Sub1 and Samba Mahsuri-Sub1.

The discovery of *Sub1* gene is a breakthrough which can be introgressed into local popular variety to produce high yielding variety with submergence tolerance in Malaysia. Thus, improving crops capacity to withstand such stress is therefore a fundamental importance. In this study, 12 accessions were identified with high survival against submergence, namely, Mayang, Pagalan, Siong pelanduk 6, Labou, Bangkok (H), Babang, Renjan bembang, Bedor, Semilai, Tatalunalis, Langsat and Merjat. This outcome shows that Malaysia has its own submergence tolerance genetic source yet extensive research in the future is essential to test stability of these accessions to be used as alternative source for submergence tolerance.

The study also shown that the Malaysian commercial rice varieties namely, MR219, MR220 and MR263 were severe susceptible to submergence. These results provide the real purpose to develop submergence tolerance variety for stability in crop production with an increased economic value in nowadays extreme changing environments. First step towards the development of submergence tolerance variety starts with the breeding program by crossing MR263 and Swarna-Sub1.

Marker-assisted selection (MAS) was carried out through the utilization of simple sequence repeats (SSR) considering it is rapid and reliable for pre-selection tools. F<sub>1</sub> generations were confirmed by polymorphic linked marker, RM8300 and RM219. Out of 180 SSR markers, 30 markers were found polymorphic between two parents. Association of molecular markers and submergence tolerance were determined using Chi-square revealed that the MR263 × Swarna-Sub1 F<sub>2</sub> lines carried *Sub1* tolerance gene associated with SSR markers, RM413, RM1233, RM8225, RM5961, RM219 and RM8300. These markers showed a good fit to the expected marker segregation ratio (1:2:1) in a Mendelian single gene model (DF=1.0, p≤0.05).

Eleven homozygous lines with linked *Sub1* gene marker were selected for future development of submergence tolerant varieties. We thus selected a subset of 11 fitness compromised tolerance lines out of 256 lines linked *Sub1* gene from the F<sub>2</sub> generation and screened them for submergence tolerance for phenotypic performance. The selected 11 lines from previous molecular experiment were verified by evaluating its submergence survival and yielding performance for selection of the best lines. The 11 lines were confirmed phenotypically submergence tolerance. Through yield performance evaluation, all accessions were grouped into three clusters. Finally, from this study the best four accessions, namely L05, L08, L09 and L11 were identified for the further development of high yielding submergence tolerant rice variety.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Master Sains

**SARINGAN AKSESI PADI MALAYSIA DAN PEMEMILIHAN BANTUAN  
PENANDA KEATAS KACUKAN DIANTARA VARIETI MR263 x SWARNA-  
SUB1 UNTUK TOLERAN SUBMERGEN**

Oleh

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Submergen adalah merupakan salah satu penyebab utama kemerosotan tanaman di seluruh dunia yang mengakibatkan kekangan utama dalam pengeluaran beras dunia dan juga di Malaysia. Beberapa sawah padi di Kelantan, Terengganu dan Pahang di Malaysia telah terjejas oleh banjir kilat dan menyebabkan pengurangan hasil padi. Gen *Sub1* yang mengawal toleransi submergen telah digunakan untuk menghasilkan beberapa varieti padi toleran submergen seperti Swarna-*Sub1*, BR11-*Sub1*, IR64-*Sub1* dan Samba Mahsuri-*Sub1*.

Penemuan gen *Sub1* merupakan satu kejayaan yang mana gen ini boleh diintrogresikan ke dalam padi tempatan yang popular untuk menghasilkan varieti padi yang berhasil tinggi serta submergen toleran di Malaysia. Oleh itu, kajian ini merupakan kepentingan asas bagi meningkatkan keupayaan tanaman padi untuk menahan masalah submergen. Dalam kajian ini, 12 aksesori telah dikenalpasti mempunyai daya tahan yang tinggi terhadap submergen, iaitu, Mayang, Pagalan, Siong Pelanduk 6, Labou, Bangkok (H), Babang, bembang Renjan, Bedor, Semilai, Tatalunalis, Langsung dan Merjat. Keputusan ini menunjukkan bahawa Malaysia mempunyai sumber genetik padi toleran submergen. Walau bagaimanapun, seterusnya kestabilan aksesori tersebut perlu diuji di masa hadapan untuk digunakan sebagai sumber alternatif bagi toleransi submergen.

Kajian ini juga menunjukkan bahawa padi komersial Malaysia MR219, MR220 dan MR263 adalah rentan terhadap submergen. Hasil kajian jelas menunjukkan bahawa pembangunan padi yang toleran submergen adalah amat perlu untuk kestabilan dalam pengeluaran tanaman dengan nilai ekonomi yang meningkat sesuai dengan perubahan persekitaran yang melampau. Langkah pertama ke

arah pembangunan pelbagai toleran submergen bermula dengan program pembiakbakaan dengan mengacukkan varieti MR263 dan Swarna-Sub1.

Program pemilihan bantuan penanda (MAS) telah dijalankan melalui penanda jujukan berulang mudah (SSR) didapati lebih pantas dan boleh dipercayai sebagai alat untuk pra-pemilihan. Generasi F<sub>1</sub> telah disahkan dengan penanda polimorfik yang berkait rapat dengan submergen, RM8300 dan RM219. Daripada 180 penanda SSR, 30 penanda ditemui polimorfik antara kedua-dua induk tersebut. Hubungan antara penanda molekul dan toleransi submergen dijalankan dengan menggunakan Chi-kuasa dua menunjukkan bahawa titisan F<sub>2</sub> MR263 × Swarna-Sub1 mengadungi gen *Sub1* yang boleh dikenalpasti dengan penanda SSR, RM413, RM1233, RM8225, RM5961, RM219 dan RM8300. Penanda ini menunjukkan padanan yang baik kepada kadar jangkauan nisbah segregasi genotip Mendel (1:2:1) (df= 1.0, p≤0.05).

Sebelas titisan tulen homozigot yang berkait rapat dengan penanda gen *Sub1* telah dipilih untuk pembangunan padi toleran submergen di masa hadapan. Oleh itu, 11 titisan tersebut telah dipilih berdasarkan padanan penanda dari 256 titisan yang dikaitkan gen *Sub1* dari populasi F<sub>2</sub>. Sebelas titisan terpilih dari penyelidikan molekul sebelumnya telah diverifikasi terhadap kebolehan hidup terhadap submergen serta prestasi hasil bagi pemilihan titisan terbaik. Semua sebelas titisan tulen disahkan mempunyai ketahanan fenotipik submergen. Melalui penilaian ciri hasil, semua aksesori tersebut telah dikelompokkan kepada tiga kluster. Dari hasil kajian ini, empat titisan terbaik L05, L08, L09 dan L11 telah dipilih untuk pembangunan seterusnya varieti padi berhasil tinggi serta toleran submergen.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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## LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
CRD	Completely randomized design
CTAB	Cetyl trimethylammonium bromide
CV	Coefficient of variation
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
DF	Degree of variation
dGTP	Deoxyguanosine triphosphate
DNA	Deoxyribonucleic acid
dTTP	Thymidine triphosphate
EDTA	Ethylenediaminetetraacetic acid
EMBOSS	European molecular biology open software suite
F <sub>1</sub>	First generation
F <sub>2</sub>	Second generation
HSD	Tukey's honest significance test
IRRI	International rice research institute
KCl	Potassium chloride
MAB	Marker-assisted selection
MAS	Marker-assisted breeding
P <sub>1</sub>	First parent
P <sub>2</sub>	Second parent
PCA	Principal component analysis
QTL	Quantitative trait loci
R <sup>2</sup>	Coefficient of determination
RCBD	Randomized complete block design
RFLP	Restriction fragment length polymorphism
RNAse	Ribonuclease
SCAR	Sequence characterized amplified region
SNP	Single-nucleotide polymorphism
SSR	Microsatellite
TE	Tris EDTA buffer
Tris-Cl	Tris hydrochloride
V	Volt
r	Correlation coefficient

## CHAPTER 1

### INTRODUCTION

Rice, *Oryza sativa* L. ( $2n = 24$ ) belongs to the family *Poaceae* and subfamily *Oryzoideae* is the staple food for more than half of the world's population. With a compact genome, the cultivated rice represents a model for other cereals as well as other monocot plants (Shimamoto and Kyojuka, 2002). Rainfed lowlands constitute highly fragile ecosystems, always prone to flash-floods submergence. Among biotic and abiotic stresses affecting rice production, submergence has been identified as the major constraint which causes total yield loss in rice productivity (Sarkar *et al.*, 2006). It has been estimated that about 4 million tons of rice being lost every year because of submergence (CNN, 2009).

The severity of flooding and the scale of damage are alarmingly increasing over the years. Moreover, under changing climatic scenarios, crops will be exposed more frequently to episodes of drought, high temperature and flood. Even though rice is being cultivated under flooded and irrigated condition, most of the rice varieties are susceptible to flooding if the plants are submerged under water for more than seven days (Adkins *et al.*, 1990). Hence, developing submergence tolerant rice varieties will be useful in reducing yield loss in the affected areas. Submergence tolerance is a metabolic adaptation in response to an anaerobiosis that enables cells to maintain their integrity to survive in hypoxia without any major damage. High starch levels prior to submergence favoured tolerance by utilizing non-structural carbohydrate to supply the required energy for growth and maintenance metabolism (Jackson and Ram, 2003).

As with other major abiotic stresses, breeding and selecting highly submergence tolerant rice varieties have not yet met with notable commercial success till some years ago. Germplasm survey revealed the existence of limited amount of genetic variation for submergence tolerance. Through efforts by IRRI, Philippines, the identification of "FR13A" a flood tolerant rice line; showed submergence tolerance of up to 14 days and the exploitation of this genetic material in various breeding programs and mapping studies led to the understanding of genetic and molecular basis of improved submergence tolerance in rice genotypes. Submergence tolerance in FR13A is controlled by a putative ethylene responsive factor (Xu *et al.*, 2006) located in the *Sub1* quantitative trait loci (QTL) on chromosome 9 (Xu and Mackill, 1996).

Introgression of *Sub1* gene into a high yielding submergence susceptible Indian variety "Swarna" was successfully carried out through marker-assisted selection (MAS). The improved Swarna called "Swarna-*Sub1*" showed

improved level of tolerance to submergence than the original variety and it possessed all the other desirable characteristics of the Swarna (Neeraja *et al.*, 2007). This report clearly showed the possibility of improving submergence tolerance in rice through MAS of *Sub1* gene. MAS approach on desirable gene of interest through foreground and background selection improves efficiency of the breeding program.

## **1.2 Problem statement**

The occurrence of flood becomes more frequent in the past years in Malaysia. This issue has been affecting Malaysian rice industry which recorded high losses in yield, resulting in farmers loss of income. One of the solutions is to improve the current commercial varieties by incorporating with submergence tolerance gene to withstand damage from submergence. Therefore, with the current increasing flood problems in Malaysia, the need to developed tolerant rice variety is a necessity.

## **1.3 Research Objectives**

Based on these facts, the present study was undertaken with the following objectives:

1. To evaluate submergence tolerance on Malaysian rice accessions.
2. To analyse SSR markers associated with submergence tolerant gene up to F<sub>2</sub> generation derived from crossing between MR263 × Swarna-*Sub1*.
3. To identify promising advanced submergence tolerant lines from the MR263 × Swarna-*Sub1* cross.

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## PUBLICATION

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