



UNIVERSITI PUTRA MALAYSIA

***DEVELOPMENT OF SUBMERGENCE TOLERANT RICE VARIETY
THROUGH MARKER-ASSISTED BACKCROSS BREEDING
BETWEEN MR219 AND SWARNA-SUB1***

FAHIM AHMED

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By

FAHIM AHMED

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Philosophy**

December 2015

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DEDICATION

To my beloved parents...



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

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THROUGH MARKER-ASSISTED BACKCROSS BREEDING
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December 2015

Chairman : Professor Mohd Rafii Yusop, PhD
Faculty : Agriculture

Flash floods can result in yield losses of rice up to 100% depending upon different factors of submergence prone ecosystem. In such condition, development of submergence tolerant high yielding variety is an effective and economic way for the affected farmers to grow rice successfully. Marker-assisted selection (MAS) is an effective approach than the conventional breeding for rice varietal development. In this study, a popular high yielding but susceptible to submergence, MR219 rice variety was crossed with submergence tolerant variety, Swarna-Sub1 for development of variety tolerant to submergence through MAS. Specific objectives were to introgress submergence tolerant gene, *Sub1* from Swarna-Sub1 into high yielding MR219 through marker-assisted backcross breeding, to determine suitable polymorphic SSR markers for foreground and background selection, to analyze SSR markers associated with submergence in different backcross populations for recurrent parent genome (RPG) recovery, and to identify superior improved lines to develop a new submergence tolerant rice variety. Out of 381 SSR markers including *Sub1* gene linked markers, 86 were found polymorphic between the two parental varieties. These markers were used to identify the genotypic segregation ratio and RPG recovery in different backcross populations. Out of 13 foreground and recombinant primers for submergence tolerance in rice, 3 polymorphic primers, namely RM8300, RM219 and RM23805 were used to identify the target gene. These markers were used as foreground and recombinant markers in F₁, BC₁F₁, BC₂F₁ and BC₂F₂ population. From genotypic and phenotypic verification in the respective generations, the *Sub1* gene was successfully introgressed into the MR219. The results revealed that the RPG recovery ratio of the best lines ranged from 77.8 to 72.3% (average 74.4%) and 78.79 to 95.5 % (average 92.62%) in BC₁F₁ and BC₂F₁ populations, respectively. Meanwhile, in the selected lines of BC₂F₂ population, the RPG recovery ranged from 96.2 to 93.0% (average 94.47%), that was higher than the expected RPG recovery ratio (87.5%). Among the selected homozygous lines of BC₂F₂ population, the best plant with gene of interest, UPM3-BC₂F₂-34 was selected for the development of submergence tolerant variety. Phenotypic data were taken considering 11 traits. The UPGMA (The Un-weighted Pair Group Method with Arithmetic Means) algorithm and SAHN clustering were applied to determine genetic relationships among the genotypes. The PCA analysis of 30 lines

and MR219 were calculated by EIGEN and PROJ modules of NTSYS-pc and Minitab software (version 15). From yield performances evaluation of the 30 newly developed submergence tolerant lines of BC₂F₃ population, lines L7, L13, L24, L12, L26, L10, L30, L14, L27 and L9 comparatively had high yields and better morphological traits. From the 30 lines, more than 70% heritability values were found for plant height, days to maturity, tiller numbers per hill, panicles per hill, percentage of filled grains and days to 50% maturity. Heritability value of more than 80% was found for yield per hill, and more than 90% were for grain length, grain width and 1000-filled grain weight. In case of phenotypic coefficient of variation (PCV), the highest value was shown by number of tillers / hill (22.20 %) and followed by panicle/hill (17.56 %) and panicle length (12.84 %) traits. Genotypic coefficient of variation (GCV) shows variation of results as in this case, number of tillers /hill (19.45%) is the highest value followed by panicle/ hill (14.84%) and panicle length (11.05%). In case of genetic advance (GA) the highest value was shown by Number of tiller / hill (35.10) of which is close to the value of panicle/hill (25.83) and panicle length (19.59). Cluster analysis based on the quantitative traits grouped these 30 improved lines and MR219 into four clusters, and 20 of the improved lines were grouped together with the recurrent parent, MR219. From this study, the newly developed improved lines, L7, L13, L24, L12, L26, L10, L30, L14, L27 and L9 are recommended for large-scale field evaluation for the release of submergence tolerance and high yielding rice variety.

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

PEMBANGUNAN VARIETI PADI TOLERAN SUBMERGEN MELALUI PEMBIAKBAKAAN KACUKBALIK BANTUAN PENANDA DIANTARA MR219 DAN SWARNA-SUB1

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Banjir kilat boleh mengakibatkan kerugian hasil padi sehingga 100% bergantung kepada faktor yang berbeza terhadap ekosistem yang selalu berlaku banjir. Dalam persekitaran ini, pembangunan varieti toleran submergen serta hasil yang tinggi adalah merupakan kaedah yang berkesan dan ekonomi bagi pesawah yang terlibat untuk penanaman padi dengan jayanya. Pemilihan berbantuan penanda (MAS) merupakan pendekatan efektif berbanding dengan pembiakabakaan secara konvensional untuk pembangunan varieti padi. Dalam kajian ini, varieti padi popular yang berhasil tinggi tetapi rentan kepada submergen, MR219 dikacukkan dengan varieti padi toleran submergen, Swarna-Sub1 bagi menghasilkan varieti toleran submergen melalui kaedah MAS. Objektif khusus adalah untuk introgresi gen toleran submergen, *Sub1* dari varieti Swarna-Sub1 ke MR219 melalui pembiakabakaan kacukbalik bantuan penanda, untuk menentukan penanda SSR yang polimorfik bagi pemilihan pemulihan genom dari induk penerima dan penentuan gen yang berkait rapat dengan ciri submergen, untuk menganalisa penanda SSR yang berkait rapat dengan ciri submergen dalam populasi kacukbalik yang berlainan bagi pemulihan genom induk penerima (RPG), dan untuk mengenalpasti waris unggul maju bagi menghasilkan varieti padi toleran submergen. Dari 381 penanda SSR termasuk penanda berkait rapat dengan gen *Sub1*, didapati 86 penanda adalah polimorfik di antara kedua-dua varieti induk. Penanda ini telah digunakan untuk mengenalpasti nisbah segregasi genotip dan penentuan pemulihan RPG dalam populasi kacukbalik yang berbeza. Dari 13 penanda khusus dan rekombinan untuk toleran submergen padi, 3 penanda polimorfik, iaitu RM8300, RM219 dan RM23805 telah digunakan untuk mengenalpasti gen berkait rapat dengan ciri submergen. Penanda ini telah digunakan sebagai penanda untuk pemilihan toleran submergen dan rekombinan ke atas populasi F_1 , BC_1F_1 , BC_2F_1 dan BC_2F_2 . Melalui verifikasi genotip dan fenotip dalam setiap generasi yang dihasilkan, gen *Sub1* telah berjaya diintrogresikan ke dalam varieti MR219. Hasil kajian menunjukkan bahawa kadar pemulihan RPG bagi kalangan waris terbaik adalah di antara 77.8 hingga 72.3% (purata 74.4%) dan 78.79 hingga 95.5% (purata 92.62%) dalam populasi BC_1F_1 dan BC_2F_1 masing-masing. Manakala dalam kalangan waris terpilih populasi BC_2F_2 , pemulihan RPG adalah di antara 96.2 hingga 93.0% (purata 94.47%), iaitu lebih tinggi dari kadar pemulihan RPG yang dijangka (87.5%). Dari kalangan waris homozigus

terpilih dari populasi BC₂F₂, pokok terbaik, UPM3-BC₂F₂-34 yang mengandung gen yang dikehendaki telah dipilih untuk pembangunan varietas toleran submergen. The UPGMA algoritma dan SAHN kluster telah digunakan untuk menentukan hubungan genetik antara genotip. Analisis PCA 30 titisan dan MR219 telah dikira melalui modul EIGEN dan Modul PROJ NTSYS-pc dan perisian Minitab (versi 15). Dari penilaian prestasi hasil terhadap 30 titisan rintang submergen daripada populasi BC₂F₃ yang baru dibangunkan, titisan L7, L13, L24, L12, L26, L10, L30, L14, L27 dan L9 relatifnya mempunyai hasil yang tinggi dan ciri-ciri morfologi yang lebih baik. Dari 30 titisan, nilai heritabiliti adalah lebih daripada 70% untuk ciri ketinggian tumbuhan, tempoh matang, bilangan tangkai per rumpun, tangkai per rumpun, peratus bilangan bijirin dan tempoh untuk 50% matang. Nilai heritabiliti lebih daripada 80% telah didapati untuk hasil per rumpun, dan lebih daripada 90% adalah untuk panjang bijirin, lebar bijirin dan berat 1000 bijirin. Nilai pekali variasi fenotip (PCV) yang tertinggi adalah bilangan anak padi per rumpun (22.20%) dan diikuti oleh ciri tangkai per rumpun (17.56%) dan panjang tangkai (12.84%). Pekali variasi genotip (GCV) menunjukkan keputusan yang berbeza. Nilai GCV tertinggi adalah bilangan anak padi per rumpun (19.45%) dan diikuti oleh bilangan tangkai per rumpun (14.84%) dan panjang tangkai (11.05%). Berdasarkan kemajuan genetik (GA), nilai yang paling tinggi telah ditunjukkan oleh bilangan anak padi per rumpun (35.10), diikuti oleh ciri tangkai per rumpun (25.83) dan panjang tangkai (19.59). Analisa kluster berdasarkan ciri kuantitatif, 30 waris maju dan MR219 telah asing kepada empat kluster, dan didapati 20 dari waris maju tersebut adalah di dalam kelompok yang sama dengan induk penerima, MR219. Dari kajian ini, waris maju terpilih iaitu L7, L13, L24, L12, L26, L10, L30, L14, L27 dan L9 adalah disyorkan untuk dibuat penilaian berskala besar dalam rangka usaha menghasilkan varietas padi toleran submergen serta berhasil tinggi.

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TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	iii
ACKNOWLEDGEMENTS	v
APPROVAL	vi
DECLARATION	viii
LIST OF TABLES	xiii
LIST OF FIGURES	xv
LIST OF ABBREVIATIONS	xviii
CHAPTER	
1. INTRODUCTION	1
1.1. General Introduction	1
1.2. Problem Statement	1
1.3. Research objectives	3
2. LITERATURE REVIEW	4
2.1. Rice: Origin and distribution	4
2.1.1. Rice in Malaysia	5
2.2. Submergence tolerance of rice	5
2.2.1. Submergence	6
2.2.2. Mechanism of tolerance to submergence	6
2.2.3. Inheritance of submergence tolerance	10
2.3. Microsatellite Markers	10
2.3.1. Mapping of <i>Sub1</i> gene	15
2.4. Marker-assisted backcross breeding for submergence tolerance	16
2.4.1. Gene recovery by MABC	17
2.4.2. Foreground selection	18
2.4.3. Recombinant selection	18
2.4.4. Background selection	19
2.4.4.1. Mapping information needed for background recovery	20
2.5. Rice varieties developed by marker-assisted backcross breeding (MABC)	21
2.5.1. MABC for biotic stresses	21
2.5.2. MABC for abiotic stresses	22
2.5.3. MABC for grain quality traits	23
2.5.4. MABC for submergence tolerant	24
3. INTROGRESSION OF SUBMERGENCE TOLERANT <i>SUB1</i> GENE INTO HIGH YIELDING MALAYSIAN RICE VARIETY, MR219 USING MARKER- ASSISTED BACKCROSS BREEDING	27
3.1. Introduction	27
3.2. Materials and method	28
3.2.1. Breeding scheme and plant materials	28

3.2.2.	Molecular marker analysis	30
3.2.3.	Foreground and recombinant selection	30
3.2.4.	Background selection	31
3.2.5.	Allele scoring and data analysis	33
3.2.6.	Screening for submergence tolerance	33
3.3.	Results	33
3.3.1.	Foreground selection	34
3.3.2.	Recombinant selection	35
3.3.3.	Selection using gene-based markers	36
3.3.4.	Background Selection	37
3.3.5.	Introgression size and position of the <i>Sub1</i> QTL	41
3.3.6.	Submergence screening	41
3.3.7.	Phenotyping of newly developed introgression lines	42
3.4.	Discussion	44
3.5.	Conclusion	46
4.	PARENTAL GENOME RECOVERY OF BACKCROSS POPULATIONS DERIVED FROM A CROSS BETWEEN MR219 AND SWARNA-SUB1 VARIETY	47
4.1.	Introduction	48
4.2.	Materials and method	48
4.2.1.	Plant materials and crossing scheme	48
4.2.2.	DNA Extraction	48
4.2.3.	Molecular marker analysis	48
4.2.4.	Allele scoring and data analysis	48
4.2.5.	Foreground selection and recombinant selection	49
4.2.6.	Background selection	49
4.3.	Results	51
4.3.1.	Foreground selection	51
4.3.2.	Recombinant selection	51
4.3.3.	Background Selection	53
	4.3.3.1. BC ₁ F ₁ generation	53
	4.3.3.2. BC ₂ F ₁ generation	57
	4.3.3.3. BC ₂ F ₂ generation	61
4.4.	Discussion	64
4.5.	Conclusion	67
5.	PERFORMANCE OF YIELD AND YIELD CONTRIBUTING CHARACTERS OF BC₂F₃ POPULATION WITH THE ADDITION OF SUBMERGENCE TOLERANT <i>SUB1</i> GENE	68
5.1.	Introduction	69
5.2.	Materials and method	69
5.2.1.	Experimental location and soil	69
5.2.2.	Plant materials	69
5.2.3.	Experimental design and management practices	69
5.2.4.	Raising seedlings	69
5.2.5.	DNA Extraction	70
5.2.6.	Data collection	70
5.2.7.	Statistical analysis	72
	5.2.7.1. Genotypic variance	72

5.2.7.2.	Phenotypic variance	72
5.2.7.3.	Error variance	73
5.2.7.4.	Phenotypic and genotypic coefficient of variation	73
5.2.7.5.	Heritability Estimate	73
5.2.7.6.	Expected genetic advance	73
5.2.8.	Cluster and principal component analysis	74
5.3.	Result	74
5.3.1.	Verification of selected advance lines using linked SSR markers	74
5.3.2.	Yield and agro-morphological performance	79
5.3.3.	Phenotypic coefficient of variation (PCV), Genetic coefficient of variation (GCV) and the estimation of	79
5.3.4.	Cluster analysis	82
5.3.5.	Principal component analysis	83
5.4.	Discussion	85
5.5.	Conclusions	88
6.	GENERAL CONCLUSION AND RECOMMENDATIONS	89
6.1.	Conclusion	89
6.2.	Recommendations for future study	91
	REFERENCES	93
	APPENDICES	111
	BIODATA OF STUDENT	126
	LIST OF PUBLICATIONS	127

LIST OF TABLES

Table		Page
3.1	List of foreground and recombinant markers (highlighted markers are polymorphic)	32
3.2	Background recovery with <i>Sub1</i> in BC ₁ F ₁ population where the recipient (A), donor (B), or heterozygous (H)	39
3.3	Number of markers homozygous for the recipient (A), donor (B), or heterozygous (H) for BC ₁ F ₁ plants subjected to background selection using 66 markers	39
3.4	Number of markers homozygous for the recipient allele (A), for the donor allele (B) and heterozygous (H) for selected BC ₂ F ₁ plants	40
3.5	submergence screening for the tolerant lines (including MR219- <i>Sub1</i>), and tolerant and susceptible checks	42
3.6	Yield and yield contributing characters of the newly developed MR219- <i>Sub1</i> and recurrent parent MR219 under non-submerged condition	43
4.1	List of foreground and recombinant markers (highlighted markers are polymorphic)	50
4.2	Number of markers homozygous for the recipient (A), donor (B), or heterozygous (H) for BC ₁ F ₁ plants subjected to background selection using 66 markers	54
4.3	Background recovery of best four plants with foreground markers in BC ₁ F ₁ population	55
4.4	Number of markers homozygous for the recipient allele (A), for the donor allele (B) and heterozygous (H) for selected BC ₂ F ₁ plants	58
4.5	Background recovery of best four plant with foreground markers in BC ₂ F ₁ population	58
4.6	Background recovery of best four plants with foreground markers in BC ₂ F ₂ population	61
5.1	Different parameters used in this study	71
5.2	ANOVA outline	72

5.3	ANOVA Table for 11 morphological characters	77
5.4	Yield and yield contributing characteristics of 30 selected advance lines derived from UPM3-BC ₂ F ₂ .34	78
5.5	Estimation of genetic variables of 11 morphological characteristics of selected 30 lines derived from UPM3-BC ₂ F ₂ .34	81
5.6	Advance line with MR219 based on 11 yield contributing factors according to cluster	83
5.7	Eigen vectors and Eigen values of first four components of PCA	85



LIST OF FIGURES

Figure		Page
2.1	Graphical representation of chromosome 9 and <i>Sub1</i> QTL, (positioned at the tip of the chromosome) (Left) and details of <i>Sub1</i> QTL (Right) as described by Septingish et al. (2009)	15
3.1	Development of the submergence-tolerant MR219- <i>Sub1</i> with details of markers used for foreground (RM8300), recombinant (RM219, RM23805), and background selection. The numbers of plants selected in each generation are indicated in parentheses	29
3.2	Seed formation after the crossing between MR219× <i>Swarna-Sub1</i> at two weeks of pollination	30
3.3	Selection of heterozygous plant carrying submergence tolerant gene at BC ₂ F ₁ generation using tightly linked foreground marker for <i>Sub1</i> , RM8300 (A; MR219, B; <i>Swarna-Sub1</i> , H; Heterozygous, M; 50bp ladder)	34
3.4	Selection of homozygous plant carrying submergence tolerant gene at BC ₂ F ₂ generation using tightly linked foreground marker for <i>Sub1</i> , RM8300 (A; MR219, B; <i>Swarna-Sub1</i> , H; Heterozygous, M; 50bp ladder)	35
3.5	Selection of recombinant marker at proximal end of <i>Sub1</i> QTL using marker RM23805 at BC ₁ F ₁ generation (A; MR219, B; <i>Swarna-Sub1</i> , H; Heterozygous, M; 50bp ladder)	36
3.6	Selection of recombinant marker at distal end of <i>Sub1</i> QTL using marker RM219 at BC ₁ F ₁ generation (A; MR219, B; <i>Swarna-Sub1</i> , H; Heterozygous, M; 50bp ladder)	36
3.7	Confirmation of <i>Sub1</i> QTL in BC ₁ F ₁ generation using gene specific marker for submergence tolerance, G _n S ₂ after digestion (A; MR219, B; <i>Swarna-Sub1</i> , H; Heterozygous, M; 50bp ladder)	37
3.8	Physical map for the best plant of BC ₂ F ₂ population (UPM3-BC ₂ F ₂ -34) with background and foreground markers	38
3.9	Selection of homozygous plant for recurrent parental genome recovery at BC ₁ F ₁ generation using background marker RM517 (A; MR219, B; <i>Swarna-Sub1</i> , H; Heterozygous, M; 50bp ladder)	40
3.10	Size of Introgressed <i>Sub1</i> gene	41
3.11	Pictures showing screening test of newly developed lines on tank (left) and survived plants after desubmergence (right)	44

4.1	Selection of heterozygous plant carrying submergence tolerant gene at BC ₂ F ₁ generation using tightly linked foreground marker for <i>Sub1</i> , RM8300 (A; MR219, B; Swarna- <i>Sub1</i> , H; Heterozygous, M; 50bp ladder)	51
4.2	Selection of recombinant marker at proximal end of <i>Sub1</i> QTL using marker RM23805 at BC ₁ F ₁ generation (A; MR219, B; Swarna- <i>Sub1</i> , H; Heterozygous, M; 50bp ladder)	52
4.3	Selection of recombinant marker at distal end of <i>Sub1</i> QTL using marker RM219 at BC ₁ F ₁ generation (A; MR219, B; Swarna- <i>Sub1</i> , H; Heterozygous, M; 50bp ladder)	52
4.4	Frequency distribution of Parental genome recovery (%) in BC ₁ F ₁ population	53
4.5	Chromosome-wise recovery of four best plants of the population BC ₁ F ₁	55
4.6	Physical map for the best plant of BC ₁ F ₁ population (P12-2) with background and foreground markers	56
4.7	Frequency distribution of Parental genome recovery (%) in BC ₂ F ₁ population	57
4.8	Chromosome-wise recovery of four best plants of the population BC ₂ F ₁	59
4.9	Physical map for the best plant of BC ₂ F ₁ population (P12-2-49) with background and foreground markers	60
4.10	Chromosome-wise recovery of four best plants of the population BC ₂ F ₂	62
4.11	Physical map for the best plant of BC ₂ F ₂ population (UPM3-BC ₂ F ₂ -34) with background and foreground markers	63
5.1	Selection of heterozygous plant carrying submergence tolerant gene at BC ₂ F ₁ generation using tightly linked foreground marker for <i>Sub1</i> , RM8300 (A; MR219, B; Swarna- <i>Sub1</i> , H; Heterozygous, M; 50bp ladder)	75
5.2	Selection of homozygous plant carrying submergence tolerant gene at BC ₂ F ₂ generation using tightly linked foreground marker for <i>Sub1</i> , RM8300 (A; MR219, B; Swarna- <i>Sub1</i> , H; Heterozygous, M; 50bp ladder)	75
5.3	The dendrogram of advance lines with MR219 based on 11 yield and yield contributing characteristics	82

5.4	Three dimensional plots of PCA indicating the 30 advance lines with MR219 based on 11 morphological Characters	84
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LIST OF ABBREVIATIONS

ADH	Alcohol dehydrogenase
AF	Alcoholic fermentation
AFLP	Amplified fragment length polymorphism
ALP	Amplified length polymorphism
BAC	Bacterial artificial chromosomes
BB	Bacterial blight
BBS	Bangladesh Bureau of Statistics
BC	Backcross
bp	Base pair
BRRRI	Bangladesh rice research institute
CAPS	Cleaved amplified polymorphic sequence
cM	Centimorgan
CTAB	Cetyl trimethyl ammonium bromide
DAF	DNA amplification fingerprinting
DAT	Days after transplanting
df	Degree of Freedom
DH	Doubled haploid
DNA	Deoxyribo nucleic acid
dNTPs	Deoxynucleoside triphosphate set
DR	Double recombinant
EDTA	Ethylene diamine tetra acetate acid
EMV	Enhanced mega variety.
ERF	Ethylene response factor
EST	Expressed sequence tag
GA	Expected genetic advance
GCV	Genotypic coefficient of variance
GGT	Graphical genotypes
HI	harvest index
HYV	High yielding variety
Indel	Insertion-deletion
IRGSP	International rice genome sequence project
Kb	Kilo base (1000 bp)
LSD	Least significant difference
MAB	Marker-assisted breeding
MABC	Marker-assisted backcrossing
MAS	Marker-assisted selection
Mb	Mega base (1000 000 bp)
MCP	1-mehtyl cyclopropene
Na ₂ EDTA	Disodium ethylene diamine tetraacetate

NADH	Nicotinamide adenine dinucleotide
NIL	Near isogenic line
PCR	Polymerase chain reaction
PDC	Pyruvate decarboxylase
PIL	Precision introgression line
QTL	Quantitative trait locus
RFLP	Restriction fragment length polymorphism
RIL	Recombinant inbred line
RLR	Rainfed lowland rice
RM	Rice microsatellites
RPG	Recurrent (or recipient) parent genome
SDS	Sodium dodecyl sulphate
SES	Standard evaluation system
SNP	Single nucleotide polymorphism
SRD	Single recombinant at distal-end
SRP	Single recombinant at proximal-end
SSCP	Single strand conformation polymorphism
SSLP	Simple sequence length polymorphism
SSR	Simple sequence repeats
STS	Sequence tagged sites
TAE	Tris-acetate EDTA buffer
TB	Tris borate
TBE	Tris-borate EDTA buffer
TEMEL	Tetramethyl ethylene diamine
Tris	Tris(hydroxymethyl) aminomethane
χ^2	Chi-square
YAC	Yeast artificial chromosomes

CHAPTER 1

INTRODUCTION

1.1 General introduction

During the past 40 years, rice improvement efforts have been directed towards increasing grain yield, shortening growth duration, improving grain quality and incorporating disease and insect resistance. For another quantum jump in total rice production, we must explore the possibility of development of high yielding varieties with tolerance against abiotic stresses for unfavorable ecosystems. Among the abiotic stresses, submergence is one of the important factors in the flash flood prone rice growing area.

Rainfed lowland ecosystem almost covers 25% of rice cultivable area of the world (i.e. 38 million hectares). In South and Southeast Asia about 25 million hectares of rice area falls under rainfed lowland.

With the recent trend of change in the world climatic factors, particularly global warming, flora-fauna and agro ecosystems have been badly affected. Malaysia has become one of the potential victims of climate change and is already affected by the unfavorable changes of climate. The agro ecosystems of the country are facing various environmental stresses.

1.2 Problem statement

The most devastating natural disaster experienced in Malaysia is flood. Throughout Malaysia, including Sabah and Sarawak, there is total of 189 river basins with the main channels flowing directly to the south china sea and 85 of them are prone to recurrent flooding (89 of the river basins are in Peninsula Malaysia, 78 in Sabah and 22 in Sarawak). The estimated area vulnerable to flood disaster is approximately 29,800 km² or 9% of the total Malaysia area, and is affecting almost 4.82 million people which is around 22% of the total population of the country (DID, 2009). Recorded data showed 29 flood events from 1980 to 2010 in Malaysia (Ching et al., 2013).

Complete submergence due to frequent flooding can adversely affect plant growth and yield. Flash flood which results in the rapid rise of water levels with submergence causes severe damage to rice crop. Traditional varieties adapted to these submergence-prone environments are however low yielding due to their poor tillering ability, long droopy leaves, susceptibility to lodging and poor grain quality. Improved varieties are needed which can combine high yield attributes with submergence tolerance. Most rice cultivars cannot survive

if the plants are completely submerged for more than 7 days (Adkin et al., 1990).

Conventional breeding for the development of submergence tolerant rice cultivar has been going on since 1940. But the efforts have not become much effective because of the complex genetic inheritance of submergence tolerance trait and also due to the influence of environment (Mackill, 1986). The use of molecular marker-assisted selection (MAS) significantly overcame these hindrances. Molecular markers provide the ideal tool for improving the effectiveness of selection for desirable traits in new rice varieties. They offer great scope for improving the efficiency of conventional plant breeding by carrying out selection not directly on the trait of interest but the molecular markers linked to that trait.

If Malaysian popular variety, MR219 can be converted into a submergence tolerant type maintaining its all other characteristics unchanged, a significant portion of the rice supply could be met in the future from increased production in the submergence prone areas.

The present study was designed to develop the rice variety MR219 into *Sub1*-type by two backcrosses. It was thus necessary to find out the most effective and appropriate strategies for developing double recombinant type MR219-*Sub1* by two backcrosses in BC₂F₂ generation. A double recombinant type *Sub1* was obtained by 2 to 3 backcrosses previously by researchers (Neeraja et al., 2007; Iftekharuddaula et al. 2011). Another major target of the present marker-assisted backcrossing (MABC) scheme was to reduce introgression size in the converted mega variety by as small as possible.

Present study comprised foreground, recombinant and background selection for MABC. This approach actually comprised all the regular steps of MABC as was described by Neeraja et al. (2007) and Iftekharuddaula et al. (2011).

The *Sub1* lines required to be tested for their submergence tolerance and phenotypic adaptabilities. The present investigation was also aimed to compare the genetic background of the *Sub1* lines to the genome of recurrent parent with respect to grain yield and yield-contributing attributes.

1.3 Research objectives

General objective

To develop submergence tolerant with high yielding rice variety adapted to local environments

Specific objectives

- i. To introgress *Sub1* gene from Swarna-*Sub1* (submergence tolerant variety) into MR219 (a susceptible variety) through marker-assisted backcross breeding.
- ii. To determine suitable polymorphic SSR markers for background selection
- iii. To analyze SSR markers associated with submergence in backcross populations.
- iv. To select potential improved lines with best agronomical characteristics for the development of a new variety.

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