MOLECULAR CHARACTERISATION OF MALAYSIAN RICE GERMPLASM FOR BACTERIAL BLIGHT

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To my beloved parents, my brother and my sister and my friends

Thanks for unconditional love and support throughout my whole life

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ABSTRACT

Bacteria blight caused by *Xanthomonas oryzae pv oryzae* is one of the most destructive bacterial diseases of rice among the biotic stress that occurring worldwide. This disease is a significant constraint to food security in Asia, which cause yield loss in rice. Therefore, there is an urgent need for controlling bacterial blight disease through resistance cultivars. However, the genetic potential of Malaysian rice cultivar has not yet been investigated. Hence, present study was conducted to screen the presence and absence of resistance genes in 38 of modern cultivated Malaysian varieties using thirteen Simple Sequences Repeats (SSR) markers and one Sequence Tagged Sites (STS) marker. Rice cultivar MRQ74 had maximum 10 resistance genes while MR81 had only one resistance gene. However, MR263 and MR84 did not exhibits any resistance gene. Among the Malaysian rice varieties, the highest resistance level was observed in RM317 locus while the lowest resistance level was found in RM21 locus. Amplified product specific to *xa13* is not detected. A dendrogram was constructed to classify 38 Malaysian rice varieties into seven major clusters at 0.0, 0.25 and 0.3 of similarity coefficient. MR84 and MR263 were formed in cluster 1 and cluster 2 alone. Both varieties were the least genetic related to other Malaysian cultivars because they do not possess any resistance gene. Cluster 5 was the largest group comprised of ten rice cultivars. Rice cultivars carrying multiple resistance genes was grouped in cluster 5. The result can be served as the source of parent donor gene for gene pyramiding through marker-assisted selection and select appropriate parent cultivars for hybridization programmes to develop cultivars possessing durable resistance against bacterial blight.

ABSTRAK

Penyakit hawar daun bakteria disebabkan oleh *Xanthomonas oryzae pv oryzae* merupakan salah satu penyakit bakteria utama menyebabkan keruntukan poducksi padi di seluruh dunia. Penyakit ini adalah halangan utama kepada keselamatan makanan di Asia yang menyebabkan keruntukan hasil padi. Oleh itu, tindakan boleh diambil bagi mengawal penyakit ini adalah melalui meningkatan resistensi tanaman padi. Walau bagaimanapun, potensi genetik kultivar padi Malaysia masih belum disiasat. Tujuan penyelidik ini adalah untuk mengenapasti perkembangan penyakit hawar daun bakteri pada varietas Malaysia dengan menggunakan tiga belas marka SSR dan satu marka STS. Hasil penelitian menunjukkan padi kultivar MRQ74 memiliki maksimum 10 resistant gen resistant gen manakala, MR81 hanya memiliki satu gen resistant. Tetapi, MR263 dan MR84 tidak memiliki resistant gen. Antara jenis padi Malaysia, tahap resistant yang paling tinggi adalah terdapat dalam RM317 locus manakala tahap resistant yang paling rendah adalah ditemui dalam RM21 locus. Amplikasi produk tidak dapat dikesan dalam *xa13*. Oleh itu, dendogram telah dibina untuk mengasifikasikan padi Malaysia kepada 7 kelompok utama pada 0.0, 0.25 dan 0.3 pekali persamaan. MR84 dan MR263 ditakluk dalam kelompok 1 dan kelompok 2 persendirian. Kedua-dua kultivar adalah genetic yang amat berbeza dengan lain-lain kultivar kerana mereka tidak mempunyai gen resistant. Kelompok 5 adalah kumpulan terbesar terdiri daripada sepuluh kultivar padi. Kelompok 5 adalah kumpulan yang mengandungi gen-gen resistans. Informasi ini boleh digunakan sebagai sumber gen resistans dalam program pemuliaan masa depan dan memberi manfaat kepada tanaman padi untuk memilih penderma gen yang sesuai untuk memindahkan gen penahanan penyakit hawar daun bakteri ke dalam cultivar beras melalui seleksi berbantuan penanda.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

%	-	Percentage
°C	-	Degree celsius
A	-	Adenine
A	-	Ampere
AFLP	-	Amplified fragment length polymorphism
BLB	-	Bacterial leaf blight
Bp	-	Base pair
C	-	Cytosine
cM	-	Centimorgan
DNA	-	Deoxyribonucleic acid
dNTPs	-	Deoxynucleotide triphosphates
EDTA	-	Ethylenediaminetetra acetic acid
et al	-	And others
G	-	Guanine
g	-	Gram
ISSRs	-	Inter-simple sequence repeats
kb	-	Kilo base pair
LRR	-	Leucine-rich repeat
Mb	-	Megabases
mg/mL	-	Milligram per millilitres
mL	-	Milliliter

mM	-	Millimolar
NaCl	-	Sodium chloride
NaOH	-	Sodium hydroxide
ng/µL	-	Nanogram per microliter
NBS	-	Nucleotide-binding site
NILs	-	Near isogenic lines
PAGE	-	Polyacrylamide gel electrophoresis
PCoA	-	Principal Coordinates Analysis
PCR	-	polymerase chain reaction
PIC	-	Polymorphism information content
RAPD	-	Random amplified polymorphic DNA
RFLP	-	Restriction fragment length polymorphism
rpm	-	Rotation per minute
SNP	-	Single nucleotide polymorphism
SSR	-	Simple sequence repeat
STS	-	Simple Tag sequence
T	-	Thymine
TAE	-	Tris-acetate-EDTA
TBE	-	Tris-Borate-EDTA
TE	-	Tris-EDTA
µg/mL	-	Microgram per milliliter
µL	-	Microliter
UPGMA	-	Unweighted Pair Group Method with Arithmetic Mean
UV	-	Ultraviolet
V	-	Voltage
Xoo	-	*Xanthomonas oryzae pv oryzae*

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of study

Rice is an important staple food for over half of the global population and occupies approximately one-fifth to the total cultivation lands covered by cereal (Prabakaran *et al.,* 2010). It ranked the third most important cereal crop after wheat and maize. It is also known as an ideal grass model among the cereal crops to study genetic potential for better crop improvement due to its relatively small genome size (430Mb), diploid genome (2n=24), wide range of genetic variation (McCouch *et al.,* 1988) and accessible whole genome sequences (Pervaiz *et al.,* 2010; Rabbani *et al.,* 2010). It provides over 90% of the basic diet for the Asian peoples. It is Malaysia's third largest agricultural crops after rubber and palm oil. However, biotic stress and abiotic stresses influences future rice yield. In Malaysia, the self-sufficiency level of rice has decreased from 78.6% in 1990 to 73.5% in 2013 (Harun *et al.,* 2015). Bacterial blight is one of the causes of low rice productivity in Malaysia. Recently, the yield loss of susceptible Malaysian rice caused by bacterial blight is about 60000 metric ton in Padang Besar, Perlis (Jonit *et al.,* 2016).

Bacteria blight caused by *Xanthomonas oryzae* is one of the most destructing bacterial diseases of rice among the biotic stress that occurring worldwide (Mew, 1987; Nino-Liu *et al.,* 2006). In certain areas of Asian, this disease can causes yield reduction up to 50% (Qi *et al.,* 2003) and even up to 80% (Srinivasan and Gnanamanickam, 2005). It is highly destructive to susceptible cultivators in tropical

and subtropical regions especially in south-eastern Asian (Nino-Liu *et al.*, 2006). It is also widely distributed throughout Asia, Australia, United States, Latin America and Africa (Sun *et al.*, 2003). Thus, bacterial blight disease is a significant constraint to food security in a country. Hence, there is an urgent need to manage this disease through identifying the rice varieties that are bacterial blight resistant. However, chemical control management strategy causes harm to environment and farmers. The pattern of antibiotics sensitivity is highly variable against the pathogen population. Therefore, this disease can be managed by developing the effective disease control strategies. Host plant resistance is proved to be one of the reliable and environment friendly strategies for bacterial blight management. Many rice cultivars with resistance gene to biotic stress are widely adopted by farmers with the application of molecular marker technologies (Miedaner and Korzun, 2012).

Conventional breeding method is not efficient for identification of resistance gene in rice cultivar based on phenotype characterisation due to epistasis of genes. One of resistance gene can replace the action of another resistance gene without improving the resistance phenotype in rice cultivars. The recessive resistance genes such as *xa5* and *xa13* are difficult to be identified using conventional breeding methods (Khan *et al.*, 2015). Therefore, resistance genes can be identified through the molecular analysis using host resistance cultivars. SSR markers can be used to select rice cultivars containing multiple resistance genes without actual pathogen inoculation. Thus, these markers can be utilised for profiling genotype of rice cultivars to select the suitable bacterial blight resistance parents for the future breeding program in Malaysia.

Molecular marker technologies are useful tools for the identification of desirable genes for biotic and abiotic resistances as well as analysis of genetic diversity, and it helps the plant breeder to release desirable plants with tolerance/resistance to these stresses. Among various molecular markers techniques, simple sequence repeat markers are codominant, hypervariable, abundant and well distributed throughout the rice genome (Temnykh *et al.*, 2001). Several studies reported the genes for resistance to these diseases and molecular markers which are tightly linked to genes through the fine mapping and cloning of resistance genes (Pha

and Lang, 2004; Jiang *et al.*, 2006; Korinsak *et al.*, 2009, Jayawardana *et al.*, 2015). Therefore, it is important to acquire the information for genetic potential of bacterial blight resistance genes in Malaysian rice cultivars.

1.2 Statements of problems

Bacterial blight disease is one of the main constraints causing yield loss in rice. As a consequence, bacterial blight disease would result in rice yield reduction ranged from 50 to 80 % (Ogawa and Khush, 1989; Pha and Lang, 2004). Crop yield loses caused by bacterial blight diseases is mainly due to insufficient information regarding the strategies management for bacterial blight of rice (Waheed *et al.*, 2009). For this matter, molecular markers linked to resistance gene can be incorporated into the marker-assisted breeding by allowing selection at early stage and reduce the number of breeding cycles in plant growth and development.

Host resistance is a reliable approach for disease management, which is more cost effective and environmentally-friendly. In general, a single resistance gene against some race-specific pathogen is usually incorporated into the breeding programs. However, this method is not durable for long term breeding programs because of resistance to *Xoo* pathogen gene for only a very short time (Suh *et al.*, 2009). Due to the evolution of pathogen, rice cultivars containing single resistance gene can be susceptible to the bacterial blight disease. It is important to have multiple resistant genes in rice cultivars against bacterial blight diseases for a sustainable rice production. Rice cultivars containing multiple resistance genes have been shown to deliver durable resistance against bacterial blight (Rajpurohit *et al.*, 2011). However, the genetic potential of Malaysian rice cultivars possessing resistance to this disease has not been explored yet. Therefore, identification of rice varieties possessing multiple genes for resistance to bacterial blight would be a prerequisite in this direction. Molecular screening of rice germplasm for these stresses, by using the microsatellite markers tightly linked to genes of interest, became a routine exercise in

rice breeding program. The present study aims at detecting resistance genes in Malaysian rice varieties by using the SSR markers for resistance to bacterial blight.

1.3 Objectives

The objectives of this study are:

1. To record the marker data to identify the presence or absence of respective genes resistance to bacterial blight in Malaysian rice cultivars.
2. To categorise Malaysia rice cultivars based on resistance against bacterial blight

1.4 Scope of Study

The present study was conducted to detect the bacterial blight resistance genes in Malaysian rice varieties using microsatellite markers. To achieve this objective, DNA were extracted from 38 Malaysian rice varieties using lkeda method and the appropriate DNA sample for each variety were selected based on quality and quantity of DNA. PCR-based molecular markers linked to resistance genes were selected based on the previously published journal before subjecting DNA samples to PCR analysis. For screening of bacterial blight resistance genes in Malaysian rice cultivars, specific DNA fragment carrying resistance genes were amplified using appropriate PCR-based markers in a thermal cycler. Clear and visible amplified fragments specific to resistance genes were scored as present (1) or absent (0) indicating the level of genetic potential in the Malaysian rice cultivars. Based on the genetic distance between the rice cultivars, a dendrogram using the UPGMA clustering methods in PAST software was constructed to investigate the genetic relationship among the Malaysian varieties.

1.5 Significance of study

This research would provide information to plant scientists about the genetic potential of Malaysian rice cultivars regarding the resistance of bacterial blight disease. The identification of resistance potential in Malaysian rice varieties, as a source of bacterial blight resistance genes, would be utilised to improve Malaysian rice cultivars through pyramiding approach by marker assistant selection. Furthermore, the resistant varieties/cultivars with multiple resistance genes would be utilized in rice breeding programs in Malaysia.

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