UNIVERSITI PUTRA MALAYSIA

DETERMINATION OF GENETIC RELATEDNESS AMONG SELECTED RICE CULTIVARS USING MICROSATELLITE MARKERS FOR CULTIVARS IMPROVEMENT THROUGH MARKER ASSISTED BREEDING

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FBSB 2009 33

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Degree of Master of Science
Universiti Putra Malaysia

2009

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By

Ali Etemad

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

November 2009

This Thesis is Dedicated to

You

MY Wife,

MY Mother,

And also to

My kind father which was my best teacher and friend also, I have a memory of him in my heart during my lifetime which was like a treasure, but unfortunately he passed away during my Master study and I miss him so much, God bless him

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Sciences

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Chairman: Maziah Mahmood, PhD

Faculty: Biotechnology and Biomolecular Sciences

Rice is grown in diverse environmental conditions. In this study, genetic variation among thirteen Iranian and thirteen Malaysian rice cultivars was determined using Microsatellite markers. Microsatellites are polymerase chain reaction (PCR) based and deoxyribonucleic acid (DNA) markers which are abundant, co-dominant and widely used in various organisms. This study consisted of two parts, the first part was DNA extraction, which consisted of comparing between four different DNA extraction methods, namely the Dellaporta and CTAB as conventional methods also, Promega and Axyprep as commercial protocols kits. Comparison was also made on the effect of different leaf age as well as leaf position on different quality and yield of DNA obtained. The results of the study showed significant difference ($P<0.05$) between different extraction methods in relation to optical density $OD_{260/280\ nm}$ and DNA yield from each method. The Dellaporta method ($OD_{260/280}=2\pm0.07_{nm}$ and DNA yield 2073±196 ng) gave the best results. The positions of different leafs (from top to bottom leaf number 4 to 1)

and the ages of leafs (2, 4, 6 and 8 weeks) were also monitored for optimum DNA extraction. The results of the Duncan test showed that there was no significant difference ($P>0.05$) between leaf positions for 2 to 4 weeks old leaf. However, the age of leaves in young and fresh stages of tissue showed significant difference ($P<0.05$) in ratio of $OD_{260/280}$ 2±0.03 and DNA yield (1373±70 ng). The results (based on method of extraction, leaf age and position) were used for subsequent DNA extraction of the 26 rice cultivars. The second part consisted of molecular work using twenty one microsatellite primer pairs which were selected from the Gene Bank. The estimation of genetic diversity among two rice groups (Iranian and Malaysian cultivars) were done with the assistance of two softwares UVIdoc (ver.98) and POPGENE (ver.1.31). A total of 21 loci (75 alleles) were observed, of which 20 loci (95.24 %) were polymorphic, except RM338. Microsatellite loci RM1 and RM271 showed the highest polymorphism (between 94 to 136 bp in size). The Polymorphism Information Content (PIC) value was (0.578±0.170). The dendogram constructed based on genetic distance values (UPGMA) grouped the cultivars into five clusters. All of the Iranian rice cultivars were placed in cluster I and III while Malaysian rice cultivars were in clusters IV and V. However cluster II consisted of both Iranian and Malaysian rice cultivars. The results of genetic diversity among selected cultivars in this study can be used for screening of the high grain quality rice accession for backcrossing and breeding programs.

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

Persaman Genetik di Antara Kultivar Padi Terpilih Dikesan Mengganakan penanda Mikrosatelit Untuk Penambahbaikan Kultivar Secara Pembiakbakaan Berpandukan Penanda

Oleh

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Padi ditanam pelbagai jenis keadaan perselitaran. Dalam kajian ini, variasi genetik di antara tiga belas kultivar padi Iran and tiga belas kultivar padi Malaysia (berjumlah dua puluh enam) dikesan menggunakan penanda mikrosatelit. Mikrosatelit adalah berasaskan tindakbalas rantai polimerase (PCR) dan penanda asid deoksi ribonukleik (DNA) yang mana ianya mudah diperolehi, ko-dominan, dan digunakan secara meluas dalam pelbagai organisma. Kajian ini mengandungi dua bahagian. Bahagian pertama ialah membezakan pengekstrakan DNA di antara empat jenis pergekstrakan DNA iaitu Dellaporta dan CTAB sebacai kaedab konvensional serta kaedah penggunaan kit Promega dan Axyprep. Perbandingan turut dilakukan bagi kesan umur daun, kedudukan daun ke atas kualiti dan hasil DNA yang diperolehi. Keputusan menunjukkan perbezaan yang ketara ($P<0.05$) di antara kaedah pergekstrakan yang berbeza di mana OD $260/280_{nm}$ dan kepekatan diukur untuk DNA setiap kaedah. Kaedah Dellaporta (OD $260/280_{nm}$ 2 ± 0.07 $_{nm}$ dan kepekatan 2073 ± 196 ng) menunjukkan hasil yang terbaik.

Kedudukan daun yang berbeza (dari atas ke bawah, daun dinomborkan 4 kepada 1) dan umur daun (2, 4, 6 and 8 minggu) juga dipantau untuk pengekstrakan DNA yang optimum. Keputusan ujian Duncan menunjukkan tidak ada perbezaan yang ketara ($P>0.05$) di antara kedudukan daun yang berumur antara 2 dan 4 minggu menunjukkan perbezaan yarg ketara ($P<0.05$) bagi nisbah OD $260/280_{nm}$ 2±0.03 dan kepekatan DNA (1372±70 ng). Keputusan dari kajian pergekstrakan DNA (berasaslian kaedah pengekstrakan, umur dan kedudukan daun) digunakan untuk pengekstrakan DNA bagi dua puluh enam kultivar padi. Bahagian kedua, ialah bahagian molekul yang menggunalian dua puluh satu pasaugan primer mikrosatelit yang dipilih daripada *Gene Bank*. Anggaran diversiti genetik antara dua kumpulan padi (kultivar Iran dan Malaysia) dianalisis dengan mengunakan dua perisian iaitu UVIdoc (ver. 98) dan POPGENE (ver. 1.31). Sejumlah 21 lokus (75 alel) telah dikesan, di mana 20 lokus (95.24%) davipadanya aolalah polimorfik, kecuali lokus RM 338. Lokus mikrosatelit (lokus RM1 dan RM271) menunjukkan polimorfisma yang tertinggi (bersaiz 94 hingga 136 bp). Purata nilai PIC dalam analisis ini ialah 0.578± 0.170. Dendogram berdasarkan nilai jarak genetik (UPGMA) mengumpulkan kultivar ini ke dalam lima kluster yang berlainan. Seuwa kultivar padi Iran digolongkan dalam kluster I dan III, manakala kultivar padi Malaysia dalam kultivar IV dan V. Walau bagaimanapum, kluster II menganolungi gabungan kedua- dua kultivar padi Iran dan Malaysia. Keputusan kepelbagaian genetik di antara kultivar terpilih dalam kajian ini boleh digunakan untuk pemilihan assesi padi yang berkualiti tinggi bagi program pembiakan silang dan pembiakbakaan.

ACKNOWLEDGEMENTS

Beauty and Admire to Allah (SWT), the Omnipotent, Omniscient and Omnipresent, for opening doors of opportunity to me throughout my life and for giving me the strength and health to achieve what I have so far.

First and foremost, my deepest gratitude to my mother and father (God bless them) who advised and supported me emotionally and financially in my pursuit for higher education and academic excellence and expressed understanding and consideration towards me. Words cannot express my gratitude for their love, support, and patience that have sustained me during my life and study. What can I say, except thank you and I shall never forget your kind ness and sacrifice.

My heart felt thanks and appreciation goes to my understanding wife, Marsa and her family, whom I am thankful to their patience and understanding throughout the course of my study.

I would like to express my greatest gratitude to my respected supervisor, Prof. Dr. Maziah Mahmood, chairman of my supervisory committee, for her advice and invaluable guidance towards the period of the study.

I would like to express my deepest thanks and gratitude to my co-supervisor Assoc. Prof. Dr. Siti Khalijah Daud, for her suggestions, guidance and encouragement throughout this study.

Also I would also like to extend my thanks to the Department Head and all of the staff members, the Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences and Department of Biology, Faculty of Science followed by the staff of School of Graduate studies of University Putra Malaysia for helping me in one way or another during the course of my study at UPM.

I would like to acknowledge Mr. Reza Ostadsaraee in Rasht Gilan Iran Rice Research Institute and Mr. Abduollah Mohd Zain in Malaysian Agriculture Research and Development Institute MARDI for their kind cooperation and help in collection, support and delivery the rice seeds as the experimental materials samples for this study.

Special thanks to my friends, Iranians, Malaysians, and those from other places, in particular to Arash Javanmard, Majid Masoumian, Arash Asadzade, Fariborz Momayezi, Mostafa Norozi, Yousef Emami poor, Fateme Mahdavi, Ayyob Akbari, Hassan Moini, Kourosh jome Khaledi, Tan Suat Hian, Mohd Hakiman Awang Mansor, Chong Haw Eong, Vijendren Krishnan and others for their help.

I certify that a Thesis Examination Committee has met on 23 November 2009 to conduct the final examination of Ali Etemad on his Thesis entitled ‘‘Determination of Genetic Relatedness among Selected Rice Cultivars using Microsatellite Markers for Cultivar Improvement Through Marker-Assisted Breeding’’ in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Sciences.

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

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Date: 11 February 2010

DECLARATION

I declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

(Signature)

Ali Etemad

Date:

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LIST OF ABREVIATIONS

µl	microliter
µM	micromole
^{0}C	Centigrade Celsius
1X	one time
AFLP	Amplified Fragment Length Polymerase
bp	Base Pair
CRD	Completely Random Design
CSB	Clone Sequence based
CTAB	Cetyl Trimethyl Ammonium Bromide
DDI H_2O	Distilled De-Ionized Water
DNA	Desoxy Nucleic Acid
dNTP	dinucleotide triphosphate
DC	Direct Current
EDTA	ethylene diamine tera acetic Acid
EtBr	ethidium bromide
F	Fixation Index
FAO	Food and Agriculture Organization
GDP	Gross Domestic Products
GLM	General Linear Method
H_0	observed Heterozygosity
H_e	Heterozygosity
HWE	Hardy-Weinberg Equilibrium
IRRI	International Rice Research Institute
MARDI	Malaysian Agriculture Research and Development Institute
MAS	Marker Assisted Selection
$MgCl_2$	Magnesium Chloride
ml	milliliter
mM	millimole
Na	Observed Number of Alleles
Ne	Effective Number of Alleles
ng	nanogram
OD	Optical Density
PCR	Polymerase Chain Reaction
PIC	Polymorphism Information Content
QTL	Quantitative Trait Loci
RAHM	Random Amplified Hybridization Microsatellite
RAMPO	Random Amplified Microsatellite Polymorphism
RAPD	Random Amplification of Polymorphic DNA,
RFLP	Restriction Fragment Length Polymorphism

SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats
STR	Short Tandem Repeats
TBE	Tris Borate Ethylene Diamine Tetra Acetic Acid
TE	Tris Ethylene Diamine Tera Acetic Acid
Tm	Melting Temperature
U/µL	Unite Per Microlitter
UN	United Nations
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
UPM	Universiti Putra Malaysia
UV	Ultraviolet
V	Volt
Ver	Version

CHAPTER 1

INTRODUCTION

1.1 Background

Developing countries are facing the challenge to quickly increase the productivity of agriculture sources to feed their growing populations. Rice is one of the most important and strategic crops in the world. Approximately, 50% to 80% of the world people receive their calories from rice (Khush, 2005).

Rice is the most important crop directly consumed by humans. With around 689 million tons produced annually on 149 million hectares of area in 2008, most of the rice (90%) is grown in Asia (IRRI, 2008). Also, rice needs specific climate to have optimum growth. Sometimes the plant has challenges during its growth period to survive through the environmental limitations such as water availably. Rice production increased by 130% between 1966 and 2000, while the population of low income countries increased by an average of 90% over the same period (Khush, 2005). The world's population is predicted to reach approximately eight billion by 2030, (UN, 2007) and therefore, there is a need to further increase rice production by 40% in the next 25 years (Khush, 2005). The studying of genetic diversity between selected Iranian and Malaysian rice cultivars, on which no research was carried out so far, might help to access the high potential and

tolerant cultivars. The majority of selected rice cultivars have acceptable phenotypic characteristics which were collected from Malaysian Agriculture Research and Department Institute (MARDI) and Rasht, Gilan Rice Research Institute in Iran.

1.2 Rice Consumption in Iran and Malaysia

In Iran, the rice yield from 1961 till 2006 has increased from 2.14 (t/ha) to 5.81 (t/ha) with the rice production of 709,000 (ton) and reaches 3,300,000 (ton) per year (IRRI, 2008). The populations, however, increase approximately three times in the same period of time resulting in high consumption of rice in Iran. Similar trends were observed in the rice consumption in Malaysia from 1961 to 2006 wile the rice yields increased from 2.11 (t/ha) to 3.36 (t/ha) and the production also increased from 1,152,000 (ton) to 2,277,000 (ton) per year over 40 years. The Malaysian population also increased as well and the import of rice increased from 423,000 (ton) in 1961 to 700,000 (ton) in 2006 (IRRI, 2008).

1.3 Iranian and Malaysian Rice

1.3.1 Iranian Rice

Iran is a Middle Eastern country bordering the Gulf of Oman, the Persian Gulf and the Caspian Sea, between Iraq and Pakistan, comprising 1.6 million km^2 of mainly deserts and fringing, arid mountainous areas (Appendix 3). There are also coastal places where crops such as rice are grown. Ten percent of the land is arable. One third of the