

GENETIC STUDIES ON BIODIVERSITY AND EVOLUTION IN RICE

By

MAHMOUD FAZAA ABDEL-MAGIED MOHAMED OMAR

B.Sc. Agric. Sc. (Genetics), Tanta Univ. 2003

M. Sc. Agric. Sc. (Genetics), Kafr El-Sheikh Univ. 2010

A thesis submitted in partial fulfillment

Of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Agricultural Sciences

(Genetics)

Department of Genetics

Faculty of Agriculture

Ain Shams University

2017

Approval Sheet

**GENETIC STUDIES ON BIODIVERSITY
AND EVOLUTION IN RICE**

By

MAHMOUD FAZAA ABDEL-MAGIED MOHAMED OMAR

B.Sc. Agric. Sc. (Genetics), Tanta Univ. 2003

M. Sc. Agric. Sc. (Genetics), Kafr El-Sheikh Univ. 2010

This thesis for Ph.D. degree has been approved by:

Dr. Said Abd El-Salam Dora

Prof. Emeritus of Genetics, Faculty of Agriculture, Kafr El-Sheikh
University

Dr. Alia Ahmad ElSeoudy

Prof. Emeritus of Genetics, Faculty of Agriculture, Ain Shams University

Dr. Fathy Mohamed Abdel-Tawab

Prof. Emeritus of Genetics, Faculty of Agriculture, Ain Shams University

Dr. Eman Mahmoud Fahmy

Prof. Emeritus of Genetics, Faculty of Agriculture, Ain Shams University

Date of examination: / / 2017

GENETIC STUDIES ON BIODIVERSITY AND EVOLUTION IN RICE

By

MAHMOUD FAZAA ABDEL-MAGIED MOHAMED OMAR

B.Sc. Agric. Sc. (Genetics), Tanta Univ. 2003

M. Sc. Agric. Sc. (Genetics), Kafr El-Sheikh Univ. 2010

Under the supervision of:

Dr. Eman Mahmoud Fahmy

Prof. Emeritus of Genetics, Department of Genetics, Faculty of
Agriculture, Ain Shams University (Principal Supervisor).

Dr. Fatthy Mohamed Abdel-Tawab

Prof. Emeritus of Genetics, Department of Genetics, Faculty of
Agriculture, Ain Shams University

Dr. Abd El-Salam Ebaid Draz

Head of Research of Rice, Rice Research and Training Center, Field
Crops Research Institute, Agricultural Research Center

ABSTRACT

Mahmoud fazaa Abdel-Magied Mohamed Omar: Genetic Studies on Biodiversity and Evolution in Rice. Unpublished PhD. Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2017.

A set of two hundred and fifty-eight rice accessions divided into five groups were evaluated under normal and stress conditions. The study was divided into two parts:

- 1) Assessment of genetic biodiversity, which include phenotypic evaluation, genetic parameters, correlation coefficient estimation and morphological clustering for all the studied accessions.
- 2) Estimation of the genetic structure and divergence by utilizing marker assisted selection (MAS), SSRs to tag favorable QTLs, molecular profiling for establishing phylogenic tree to detect the origin/evolution among all the tested accessions.

Eight primers (RM 315, RM 318, RM 166, RM 302, RM 201, RM 234, RM 526 and RM 144) revealed different levels of polymorphism to tag the related traits of interest as tolerant to abiotic stress, resistant to biotic stress and yield-related traits. Two primers (RM 190 and RM 278) were monomorphic. The percentage of the polymorphism was nearly 80 %. The size of detected fragments ranged from 105–325 bp. A total of 186 bands were scored from the amplification products with the ten SSR primers. Genetic diversity analyses were conducted on the basis of the scores with 176 unique bands. Phylogenic tree for the fifteen selected rice accessions from each group were established according to the molecular data and based on ten SSRs. A marked genetic diversity was observed in the five groups and the origin genotypes from the five groups were (Sakha 101, IR 03N137, IR 83142-12, IR 87856-10-AJY-1-B, HHZ 12-Y4-

DT1-Y2 and IR 1552), respectively which showed higher levels of diversity and hence can be used as donors for the effective conservation, utilization and providing favorable genes and valuable germplasm in rice breeding.

Key words: Rice, Genotypes, Biodiversity, Evolution, SSRs.

ACKNOWLEDGMENT

First, all thanks for God the Lord of the world,

I would like to express my deep gratitude and appreciation to my dear supervisor **Prof. Dr. Fatthy M. Abdel-Tawab**, Professor Emeritus of Genetics, Genetics Department, Faculty of Agriculture, Ain Shams University for his kind supervision, scientific advices, encouragement and reviewing this thesis. .

My deepest gratitude and sincere appreciation to **Prof. Dr. Eman Mahmoud Fahmy**, Professor Emeritus of Genetics, Genetics Department, Faculty of Agriculture, Ain Shams University for her kind supervision, effective help, encouragement and reviewing the manuscript.

Deep thanks to **Prof. Dr. Abd El-Salam Ebaid Draz**, Head of Research, Rice Research and Training Center, Field Crops Research Institute, Agricultural Research Center for his kind supervision and support with all facilities of RRTC and valuable advices.

My appreciation and gratitude to **Prof. Dr. El-Sayed H. Hassanien**, Professor Emeritus of Genetics, Genetics Department, Faculty of Agriculture, Ain Shams University, for his advices and support.

Thanks are also due to all the staff members of **Genetics Department, Faculty of Agriculture, Ain Shams University**, and many thanks are due to my colleges and **to all RRTC staff members**.

A great gratitude to my mother **Nabah El-Sayed** who backed me all these years around to achieve my goals and dreams in life only with the help of her prayers and warm feelings. Also, my deep thanks to all my family; sisters and brothers and the beauty of their children, Zeinab Fazaa (Ossama, Salma, Mostafa, Shahd, Hala), Rehab Fazaa (Asmaa, Aisha, Abd Elrhman, Annas, Salma, Ahmed), Mohamed Fazaa (Haneen, Omar, Eyad), Ahmed Fazaa (Yassmeen, Mohamed, Mahmoud, Khadijah) and Asmaa Fazaa (Mohamed, Moemen, Malk) And I would like to express

my grand gratitude to my father **Fazaa Omar**, for his wisdom and friendship, God bliss his soul.

Grand gratitude to **Charles Darwin** and special thanks to my friends (Hytham Mesbah, Reda Ali, Mahmoud Mohamed, A.Gaber and Emad Elmoghazy) who urged me to be what I dreamed of and get what I deserve.

Thanks for everyone who gave me a hand and stood up for me to accomplish this study.

CONTENTS

	Page
I. INTRODUCTION	1
II. REVIEW OF LITERATURE	5
2.1. Importance of genetic biodiversity in rice	5
2.2. Assessment of genetic biodiversity and evolution among rice genotypes	6
2.3. Genetic parameters	9
2.4. Correlation coefficient	14
2.5. Marker assisted selection in rice breeding	15
III. MATERIAL AND METHODS	20
3.1. Plant materials	20
3.1.1. Genotypes	20
3.2. Methods	31
3.2.1. Phenotypic studies	31
3.3. Statistical analysis	35
3.3.1. Analysis of variance	35
3.3.2. Estimation of variance components	35
3.3.3. Estimation of Phenotypic correlation	37
3.4. Molecular analysis	37
3.4.1. DNA isolation and quantification	37
3.4.2. SSRs protocol	39
3.4.3. Electrophoresis, staining and analysis	41
IV. RESULTS AND DISCUSSION	42
4.1.1. Mean squares, mean performances and analysis of variance	42
4.1.2. Genetic parameters	74
4.1.3. Correlation coefficients	86
4.1.4. Morphological clustering	94
4.2. Estimation of the genetic structure and divergence	101
4.2.1. Molecular profiling for the selected rice accessions	101

4.2.2. Genetic polymorphism and phylogenic relationships for the selected rice accessions	109
V. SUMMARY	115
VI. REFERENCES	121
الملخص العربي	

LIST OF TABLES

	Page
Table (1): Entry, Pedigree and country of origin of 258 studied genotypes	21
Table (2): Source of variation, degrees of freedom and the expected mean squares for each genotype	35
Table (3): Forward and reverse sequences of ten SSR primers	40
Table (4): PCR amplification of DNA fragments	41
Table (5): Mean performance for group I	44
Table (6): Analysis of variance for group I.	45
Table (7): Mean performance for subgroup II. A. (ex-situ)	46
Table (8): Mean performance for subgroup II. A. (ex-situ)	49
Table (9): Mean performance for subgroup II. B. (in-situ) ur normal conditions	51
Table (10): Analysis of variance for subgroup II.B.(in-situ) under normal conditions	53
Table (11): Mean performance for subgroup II. B. (in-situ) under drought conditions	54
Table (12): Analysis of variance for agronomic and yield related traits in subgroup II. B. under drought conditions	56
Table (13): Mean performance for group III. under normal conditions	57
Table (14): Analysis of variance for group III. under normal conditions	59
Table (15): Mean performance for group III. under saline stress conditions	60
Table (16): Analysis of variance for group III. under saline stress conditions	61
Table (17): Mean performance of agronomic traits in group IV.	63
Table (18): Mean performance for yield related traits in group IV.	66
Table (19): Analysis of variance for agronomic traits in group IV.	69

Table (20): Analysis of variance for yield related traits in group IV.	69
Table (21): Mean performance of agronomic and yield related traits in group V.	70
Table (22): Analysis of variance for group V.	73
Table (23): Range, general mean, phenotypic and genotypic variation, phenotypic and genotypic coefficient of variation, heritability in the broad sense and genetic advance for group I.	75
Table (24): Range, general mean, phenotypic and genotypic variation, phenotypic and genotypic coefficient of variation, heritability in the broad sense and genetic advance subgroup II. A. (ex-situ)	76
Table (25): Range, general mean, phenotypic and genotypic variation, phenotypic and genotypic coefficient of variation, heritability in the broad sense and genetic advance for subgroup II.B.(in-situ) under normal conditions	78
Table (26): Table (26): Range, general mean, phenotypic and genotypic variation, phenotypic and genotypic coefficient of variation, heritability in the broad sense and genetic advance for subgroup II. B. (in-situ) under drought conditions	79
Table (27): Range, general mean, phenotypic and genotypic variation, phenotypic and genotypic coefficient of variation, heritability in the broad sense and genetic advance for group III. under normal conditions	81
Table (28): Range, general mean, phenotypic and genotypic variation, phenotypic and genotypic coefficient of variation, heritability in the broad sense and genetic advance for group III. under saline stress conditions	82

Table (29): Range, general mean, phenotypic and genotypic variation, phenotypic and genotypic coefficient of variation, heritability in the broad sense and genetic advance for group IV.	83
Table (30): Range, general mean, phenotypic and genotypic variation, phenotypic and genotypic coefficient of variation, heritability in the broad sense and genetic advance for group V.	85
Table (31): Correlation coefficients for group I.	87
Table (32): Correlation coefficients in subgroup II.A. ex-situ	88
Table (33): Correlation coefficients for subgroup II.B. in-situ under normal conditions	89
Table (34): Correlation coefficients for subgroup II.B. in-situ under drought conditions	90
Table (35): Correlation coefficients for agronomic and yield related traits for group III. under normal conditions	91
Table (36): Correlation coefficients for group III. under saline conditions	91
Table (37): Correlation coefficients for group IV.	92
Table (38): Correlation coefficients for group V.	93
Table (39): The selected rice accessions from all the studied groups	102

LIST OF FIGURES

Figure (1): The morphological, agronomical and yielding clustering for group I.	94
Figure (2): The morphological, agronomical and yielding clustering for subgroup II.A.	96
Figure (3): The morphological, agronomical and yielding clustering for subgroup II.B.	97
Figure (4): The morphological, agronomical and yielding clustering for group III.	98
Figure (5): The morphological, agronomical and yielding clustering for group IV.	99
Figure (6): The morphological, agronomical and yielding clustering for group V.	100
Figure (7): SSR profile of the 15 selected accessions from group I. with RM 190.	105
Figure (9): SSR profile of the 15 selected accessions from group II.A. with RM 318.	106
Figure: (10) SSR profile of the 15 selected accessions from group II.B. with RM 166.	107
Figure: (11) SSR profile of the 15 selected accessions from group II.B. with RM 302.	107
Figure: (12) SSR profile of the 15 selected accessions from group III. with RM 201.	107
Figure: (13) SSR profile of the 15 selected accessions from group III. with RM 234.	108
Figure: (14) SSR profile of the 15 selected accessions from group VI. with RM 278.	108
Figure: (15) SSR profile of the 15 selected accessions from group VI. with RM 526.	108
Figure: (16) SSR profile of the 15 selected accessions from group V. with RM 144.	109

Figure (17): the phylogenic relationships among the selected 112
accessions based on SSR markers.

INTRODUCTION

Genetic biodiversity and evolution in rice have the potential of revealing new insights about genetic pathways, and creating new opportunities to meet both anticipated and unforeseen challenges in rice breeding strategies. Genetic biodiversity is considered as the foundation of the genetic improvement in rice and finding out knowledge of multiple facets about rice genetic diversity from phenotype to molecule which is essential for the effective conservation, utilization and providing favorable genes and valuable germplasm in rice breeding (IRRI, 2006).

The accessible genetic biodiversity whether natural or induced is a prerequisite for understanding the biological and evolutionary process of a particular set of rice genotypes represented in a given geographical region or during the long-term domestication and cultivation under various ecological niches (IRRI, 2010).

Genetic biodiversity is showing the genetic changes that result in specific characteristics and which might make breeding new varieties more straightforward. On the other hand, the limitations of continuous application of traditional breeding methods in rice has resulted in the utilization of the uncharacterized genetic diversity. So, characterization of genetic biodiversity and discovery of favorable genes to be integrated into new varieties are vital to achieve advanced breeding materials.

Among all crops, rice is exceptionally well endowed with genetic resources and advanced genetic tools that make it a model of genomic system and genetic biodiversity. Genetic erosion of several crops has already occurred leading to the world's dependence for food on just a few species. Rice, maize, and wheat – account for 69% of the calories and 56% of the proteins that people derive from plants. Rice, *Oryza sativa* L. (2n=24) is the third largest cereal crop of the world.