

MUTAGENESIS AND BIOTECHNOLOGICAL STUDIES FOR ABIOTIC STRESS TOLERANCE IN RICE

By

MOHAMED ALI MOHAMED ALI

B. Sc. Agric. Sc. (Genetics), Zagazig University, 2004

A thesis submitted in partial fulfillment

of

The Requirements for the Degree of

**MASTER OF SCIENCE
in
Agriculture Sciences
(Genetics)**

**Department of Genetics
Faculty of Agriculture
Ain Shams University**

2016

Approval Sheet

MUTAGENESIS AND BIOTECHNOLOGICAL STUDIES FOR ABIOTIC STRESS TOLERANCE IN RICE

By

MOHAMED ALI MOHAMED ALI

B. Sc. Agric. Sc. (Genetics), Zagazig University, 2004

This thesis for M.Sc. degree has been approved by:

Dr. Hassan Mohamed Zaki Allam

Prof. Emeritus of Genetics, Faculty of Agric., Minia University

Dr. Eman Mahmoud Fahmy

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

Dr. Alia Ahmed Mohamed El-Seoudy

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

Date of Examination: 4 / 6 /2016

MUTAGENESIS AND BIOTECHNOLOGICAL STUDIES FOR ABIOTIC STRESS TOLERANCE IN RICE

By

MOHAMED ALI MOHAMED ALI

B. Sc. Agric. Sc. (Genetics), Zagazig University, 2004

Under the supervision of:

Dr. Alia Ahmed Mohamed El-Seoudy

Prof. Emeritus of Genetics, Department of Genetics, Faculty of
Agric., Ain Shams University (Principal supervisor)

Dr. Lamyaa Mostafa Kamal Sayed

Lecturer of Genetics, Department of Genetics, Faculty of Agric., Ain
Shams University

Dr. Abd EL-Shafy Ibrahim Ragab

Prof. Emeritus of Genetics and Plant Breeding, Nuclear Research
Center, Egyptian Atomic Energy Authority

ABSTRACT

Mohamed Ali Mohamed Ali. Mutagensis and biotechnological studies for abiotic stress tolerance in rice. Unpublished M.Sc. Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2016.

Water stress is one of the major threats for sustainable rice productivity. Combining drought resistance with yield potential is the most promising challenge for the rice breeders. The present study was conducted using three local rice cultivars (*Oryza sativa* L.); Sakha 101, Sakha103 and Giza 178, in addition to IET 1444 indica rice cultivar to explore their response against three water stress intervals. Grains of the cultivars were treated with different doses of gamma rays (200, 250 and 300 Gy) to create genetic variation to obtain drought tolerant and earlier genotypes *via* selection in the field and *in vitro* in M₂ and M₃ generations. Significant differences were observed between genotypes and their parents in all generations for most studied traits under both well watered and stress conditions. The most tolerant genotypes were identified due to their performances in the field. Callus initiated from mature embryos of all tested genotypes, then maintained on MS medium containing different concentrations (5, 10 and 15%) of Polyethylene glycol (PEG). Callus growth traits and number of regenerated plants decreased with increasing PEG concentration. Forty six SSRs primers were used to assess the 10 elite genotypes and their four original parents. The total number of amplified bands and polymorphic bands were (189 bands), representing 100% polymorphism. Thirty nine unique markers identified the drought tolerant genotypes and may be responsible for tolerance to drought.

Key words: Rice, Gamma radiation, Mutations, Drought tolerance, Early maturing, Mature embryo, Callus induction, Plant regeneration, PEG, SSRs.

ACKNOWLEDGMENT

Thanks for Allah, the greatest and almighty for his uncountable and infinite graces guided me to the Islam and taught me things that I didn't know.

Deep thanks are extended to **Dr. Alia El-seoudy**, Professor of Genetics, Genetics Dept., Ain Shams University for her kind supervision, suggesting the problem, the facilities she offered to me during the preparation of this work, her valuable advices and for reviewing the manuscript.

I wish to express my sincere appreciation and deep gratitude to **Dr. Lamyaa Mostafa Kamal**, Assistant Prof in Genetics, Ain Shams University for her kind supervision, energetic guidance, conclusive instructions throughout the course of this investigation and reviewing the manuscript.

My deep thanks are offered to **Dr. Abdel-shafy Ibrahim Ragab**, Professor of Genetics and Plant Breeding, Plant Research Dept., Nuclear Research Center, Atomic Energy Authority, for his kind supervision, suggesting the problem, the facilities he offered to me during the preparation of this work and for writing the manuscript.

I would like to extend my deepest and greatest thanks to **Prof. Dr. Mohamed A. M. Kassem** Professor of Plant Breeding, Plant Research Dept., Nuclear Research Center, Atomic Energy Authority for his supervision, fruitful help, kind offer to finish this work. I will always cherish his generous help.

I would like to extend my deepest and greatest thanks to **Prof Dr. Ali El-Gohary**, Sakha Research and Training Center, for helping me and his kind offer to finish this work. I will always cherish his generous help.

My deepest thanks and sincere appreciation to my best friends **Dr. Mohammed Ayaad, Mr. Ibrahim Osamy, Dr. Khaled Elazab, Mr.**

Mohammed Basyouny and MS Wafaa Adly for helping me in every part of my work.

I dedicate this work to whom my heartfelt thanks; to the soul of my lovely **mother** for her encouragement and valuable advices. As well as to **my father, my sisters** and my **brother** for their continuous encouragement and praying for me at all time.

Also, I dedicate this investigation to the soul of my cousin **Walaa** for her encouragement and moral support.

CONTENTS

	Page
LIST OF TABLES	
LIST OF FIGURES	
LIST OF ABBREVIATIONS	
1. INTRODUCTION	1
2. REVIEW OF LITERATURE	4
2.1. Evolve drought-related traits <i>via</i> gamma radiation	4
2.2. Drought tolerance in rice	9
2.2.1. Screening of drought tolerance in field.....	9
2.2.2. Screening of drought tolerance <i>in vitro</i> through tissue culture.....	16
2.2.2.1. <i>In vitro</i> regeneration from mature embryos.....	16
2.2.2.2. <i>In vitro</i> selection for drought tolerance.....	23
2.3. Molecular markers of rice <i>via</i> SSR-PCR technique.....	27
3. MATERIALS AND METHODS	33
3.1. Materials.....	33
3.2. Methods	34
3.2.1. Testing radio sensitivity of the rice cultivars.....	34
3.2.2. Gamma rays source	34
3.2.3. Field experiments	34
3.2.3.1. M ₁ generation	34
3.2.3.2. M ₂ generation	35
3.2.3.3. M ₃ generation	36
3.2.3.4. Data Recorded in the field experiments.....	37
3.2.4. Data Statistical Analysis.....	37
3.2.5. Tissue culture experiments	37
3.2.5.1. Mature embryo culture	37
3.2.5.2. <i>In vitro</i> selection for drought tolerance.....	38

3.2.5.3. Plant regeneration.....	39
3.2.6. Molecular genetic analysis.....	40
3.2.6.1. DNA extraction procedure.....	40
3.2.6.2. DNA Extraction.....	41
3.2.6.3. Determination of DNA concentration	42
3.2.6.4. Simple sequence repeats (SSRs).....	42
3.2.6.4.1. SSRs protocol.....	42
3.2.6.4.2. PCR reaction mix.....	46
3.2.6.4.3. PCR amplification.....	46
3.2.6.4.4. Separation of the amplification products.....	46
3.2.6.4.5. Genetic similarities based on SSRs bands.....	48
3.2.6.4.6. Cluster analysis for the rice genotypes based on SSR.....	48
4. RESULTS AND DISCUSION.....	49
4.1. Rice mutations breeding experiments.....	49
4.1.1. Testing radiosensitivity of the studied rice cultivars	49
4.1.2. Field experiments (Effect of water stress, Irradiation and Genotype).....	52
4.1.2.1. M ₂ bulks and their parents.....	53
4.1.2.1.1. Analysis of variance of M ₂ bulks and their parents...	53
4.1.2.1.2. Mean performance.....	54
4.1.2.1.2.1. Number of days to maturity.....	55
4.1.2.1.2.2. Plant height (cm).....	58
4.1.2.1.2.3. Panicle length (cm).....	60
4.1.2.1.2.4. Panicles (effective tillers) number/ plant.....	62
4.1.2.1.2.5. Grains number per panicle	65
4.1.2.1.2.6. Panicle weight (g).....	68
4.1.2.1.2.7. Hundred-grain weight (g).....	70
4.1.2.1.2.8. Grain yield per plant (g).....	72
4.1.3. M ₃ elite genotypes and their parents.....	75

4.1.3.1. Analysis of variance of M ₃ genotypes.....	75
4.1.3.2. Mean performance.....	76
4.1.3.2.1. Number of days to maturity.....	77
4.1.3.2.2. Plant height (cm).....	80
4.1.3.2.3. Panicle length (cm).....	82
4.1.3.2.4. Panicles (productive tillers) number/plant.....	84
4.1.3.2.5. Grains number per panicle.....	87
4.1.3.2.6. Panicle weight (g).....	89
4.1.3.2.7. 100-grain weight (g).....	91
4.1.3.2.8. Grain yield/plant g.....	93
4.2. Tissue culture experiments.....	97
4.2.1. Mature embryo culture experiment.....	97
4.2.1.1. Callus induction.....	97
4.2.2. In vitro selection for drought tolerance.....	99
4.2.2.1. Callus growth under PEG.....	99
4.2.2.1.1. Effect of PEG concentration.....	101
4.2.2.1.2. Irradiation effect.....	101
4.2.2.1.3. Role of genotype.....	104
4.2.2.2. Plant regeneration.....	107
4.2.2.2.1. Effect of PEG.....	108
4.2.2.2.2. Irradiation effect.....	108
4.2.2.2.3. Role of genotypes.....	110
4.3. Molecular genetic analysis using SSRs-PCR technique	110
4.3.1. SSRs analysis of the 10 elite rice genotypes and their parents.....	111
4.3.2. Molecular markers of elite rice genotypes via SSRs- PCR technique.....	116
4.3.2.1. SSRs molecular markers for early maturing	120
4.3.2.2. SSRs molecular markers for drought tolerance	122

4.3.3. Genotypes identification by unique DNA markers	135
4.3.4. The genetic relationships among the 14 rice genotypes using the 46 SSRs primers.....	137
4.3.5. Cluster analysis using SSR-PCR technique.....	139
4.4. Conclusion	142
5. SUMMARY	145
6. REFERENCES	152
ARABIC SUMMARY	

LIST OF TABLES

No.	Title	Page
1.	The origin and pedigree of the used rice cultivars	33
2.	Physical analysis of the soil at the two locations of this study.....	35
3.	Primers name, sequences and chromosome number (Chr. no.) of SSRs primers used for detection of drought tolerance and early heading (www.gramene.org).....	43
4.	Analysis of variance for seedling height of four rice cultivars as affected by different gamma ray doses.....	49
5.	Effect of different gamma ray doses on means of seedling height (cm) of the four rice cultivars and percentages of change as compared to control	50
6.	Analysis of variance for studied traits of four rice cultivars as affected by different gamma ray doses in the M ₂ generation evaluated under non-stress and water stress conditions (2013 season).....	54
7.	Effect of different gamma ray doses on means of number of days to maturity of the four rice cultivars under water stress (S ₁ and S ₂) and non-stress (NS) conditions and percentages of change as compared to NS conditions.....	57
8.	Effect of different gamma ray doses on means of plant height of the four rice cultivars under water stress (S ₁ and S ₂) and non-stress (NS) conditions and percentages of change as compared to NS conditions.....	59
9.	Effect of different gamma ray doses on means of panicle length of the four rice cultivars under water stress (S ₁ and S ₂) and non-stress (NS) conditions and percentages of change as compared to NS conditions.....	61

10.	Effect of different gamma ray doses on means number of panicles per plant of the four rice cultivars under water stress (S_1 and S_2) and non-stress (NS) conditions and percentages of change as compared to NS conditions.....	64
11.	Effect of different gamma ray doses on means number of filled grains per panicle of the four rice cultivars under water stress (S_1 and S_2) and non-stress (NS) conditions and percentages of change as compared to NS conditions.....	67
12.	Effect of different gamma ray doses on means of panicle weight (g) of the four rice cultivars under water stress (S_1 and S_2) and non-stress (NS) conditions and percentages of change as compared to NS conditions.....	69
13.	Effect of different gamma ray doses on means of 100-grain weight per plant (g) trait of the four rice cultivars under water stress (S_1 and S_2) and non-stress (NS) conditions and percentages of change as compared to NS conditions	71
14.	Effect of different gamma ray doses on means of grain yield per plant (g) of the four rice cultivars under water stress (S_1 and S_2) and non-stress (NS) conditions and percentages of change as compared to NS conditions.....	73
15.	Analysis of variance for studied traits of the 19 elite rice genotypes and their parents in the M_3 generation evaluated under non-stress and water stress conditions (2014 season)...	76
16.	Mean of numbers of days to maturity of the 19 elite rice genotypes and their parents under water stress (S_1 , S_2) and well watering (NS) conditions and percentages of change as compared to NS conditions.....	78
17.	Means of plant height (cm) of the 19 elite rice genotypes and their parents under water stress (S_1 , S_2) and well watering (NS) conditions and percentages of change as compared to NS conditions.....	81
18.	Means of panicle length (cm) of the 19 elite rice genotypes and their parents under water stress (S_1 , S_2) and well watering (NS) conditions and percentages of change as compared to NS conditions.....	83

19.	Mean number of panicles per plant of the 19 elite rice genotypes and their parents under water stress (S_1 , S_2) and well watering (NS) conditions and percentages of reduction as compared to NS conditions.....	85
20.	Mean number of filled grains/panicle of the 19 elite rice genotypes and their parents under water stress (S_1 , S_2) and well watering (NS) conditions and percentages of change as compared to NS conditions.....	88
21.	Means of panicle weight (g) of the 19 elite rice genotypes and their parents under water stress (S_1 , S_2) and well watering (NS) conditions and percentages of change as compared to NS conditions.....	90
22.	Mean 100 grain weight per panicle (g) trait of the 19 elite rice genotypes and their parents under water stress (S_1 , S_2) and well watering (NS) conditions and percentages of change as compared to NS conditions.....	92
23.	Means of grain yield/plant (gm) of the 19 elite rice genotypes and their parents under water stress (S_1 , S_2) and well watering (NS) conditions and percentages of change as compared to NS conditions.....	94
24.	Callus induction response of the four rice cultivars and their irradiated treatments.....	99
25.	Analysis of variance of CFW, CGR and number of regenerated plants/300mg callus of sixteen rice genotypes grown on MS medium supplemented with four concentrations of PEG.....	100
26.	Effect of different concentrations of PEG on mean CFW (g) of irradiated rice genotypes and their parents maintained on MS medium supplemented with 2 mg/l 2, 4-D.....	102
27.	Mean CGR g/day as affected by genotypes, gamma rays doses and different concentrations of PEG supplemented to the MS maintenance medium.....	103
28.	Mean number of regenerated plants/300mg of CFW of irradiated rice genotypes and their parents as affected by different concentrations of PEG on MS medium supplemented with 2 mg/l BAP and 0.5 NAA.....	109

VIII

29.	Total number of bands, polymorphic bands and percentage of polymorphism revealed by 46 SSR primer pairs for the 14 rice genotypes, there is no monomorphic bands	112
30.	Number of amplified DNA bands revealed using 46 SSR primer pairs for the 14 rice genotypes.....	115
31.	Positive and negative detected SSR markers in 10 selected rice genotypes as compared with their four parents, molecular size (bp) and total number of markers identifying each genotype	117
32.	The scoring data of SSRs bands for the selected rice genotypes using five primers related to early maturing.....	121
33.	The scoring data of SSRs bands for the selected rice genotypes using 41 SSR primers related to drought tolerance.....	125
34.	Unique positive and negative SSR markers related to drought tolerance and their molecular sizes generated for the 14 rice genotypes.....	137
35.	The genetic similarity matrices among the 14 rice genotypes via the 46 SSRs primers.....	139

LIST OF FIGURES

No.	Title	Page
1	Molecular weight of DNA marker (100 bp DNA ladder).....	47
2	Seedling height of the four rice cultivars at 14 days from sowing following irradiation of their seeds with 10 doses of gamma rays.....	52
3	Mut.1 earlier 25 days than its parent SK101-control	56
4	The genotype Sk101-200 GY (115 days) at the left earlier 25 days than its parent Sk101 (140 days) at the right	79
5	Differences in plant height of rice mutant lines developed by gamma rays mutagenesis.....	82
6	Panicle length of Mut.5 (long panicle) and their parent Gz178.....	84
7	High tillering genotypes SK103-250Gy ₂ and Gz178-250Gy ₂ compared with their parents SK103 and Gz178, respectively.....	86
8	Performance of genotype Mut.2 at lower compared with their parent Gz178 cultivar at upper in two different stages of plant growth duration of rice under normal and water stress conditions	96
9	Callus induction and plant regeneration in Gz178-250 Gy on MS medium supplemented with 2mg/l 2, 4-D. (A) Grain culture, (B) Callus induction from mature embryo, (C) Subcultured callus, (D) Shoot induction from embryogenic callus, (E) root formation, (F) fully regenerated shoots, (G) transplanting into pots and development of regenerated plant during adaptation.....	98
10	In vitro screening of rice callus of Gz178 cultivar and its derived genotype for drought stress using different concentrations of PEG (6000). Gz178 control; (A) 0%, (B)	105