

دراسات وراثية جزيئية على تنشيط الإجهاد البيئي للعناصر الوراثية المتنقلة الرجعية في حقيقيات النواة

رسالة مقدمة من

مروة محمود شحاتة أحمد

بكالوريوس علوم زراعية (وراثة)، كلية الزراعة، جامعة عين شمس، 2007

ماجستير علوم زراعية (وراثة)، كلية الزراعة، جامعة عين شمس، 2014

**كجزء من متطلبات الحصول على
درجة دكتور الفلسفة في العلوم الزراعية
(وراثة)**

قسم الوراثة
كلية الزراعة
جامعة عين شمس

2020

صفحة الموافقة على الرسالة

دراسات وراثية جزيئية على تنشيط الإجهاد البيئي للعناصر الوراثية المتنقلة الرجعية في حقيقيات النواة

رسالة مقدمة من

مروة محمود شحاتة أحمد

بكالوريوس علوم زراعية (وراثية)، كلية الزراعة، جامعة عين شمس، 2007

ماجستير علوم زراعية (وراثية)، كلية الزراعة، جامعة عين شمس، 2014

**كجزء من متطلبات الحصول على
درجة دكتور الفلسفة في العلوم الزراعية
(وراثية)**

وقد تمت مناقشة الرسالة والموافقة عليها:

اللجنة

د. أحمد عبد السلام محمود

أستاذ الوراثة المتفرغ، كلية الزراعة، جامعة الزقازيق

د. فتحي محمد عبد التواب

أستاذ الوراثة المتفرغ، كلية الزراعة، جامعة عين شمس

د. إيمان محمود فهمي

أستاذ الوراثة المتفرغ، كلية الزراعة، جامعة عين شمس

تاريخ المناقشة 2020/ 2 / 2

جامعة عين شمس
كلية الزراعة

رسالة دكتوراه

اسم الطالبة : مروة محمود شحاتة أحمد
عنوان الرسالة : دراسات وراثية جزيئية على تنشيط الإجهاد البيئي
للعناصر الوراثية المتنقلة الرجعية في حقيقيات النواة
اسم الدرجة : دكتور الفلسفة في العلوم الزراعية (وراثية)

لجنة الإشراف

د. إيمان محمود فهمي
أستاذ الوراثة المتفرغ، قسم الوراثة، كلية الزراعة، جامعة عين شمس (المشرف الرئيسي)

د. لمياء مصطفى كمال سيد
أستاذ الوراثة المساعد، قسم الوراثة، كلية الزراعة، جامعة عين شمس

تاريخ التسجيل 2015/4/15

الدراسات العليا

أجيزت الرسالة بتاريخ

2020 / /

ختم الإجازة

موافقة مجلس الجامعة

2020 / /

موافقة مجلس الكلية

2020 / /

MOLECULAR GENETIC STUDIES ON ENVIRONMENTAL STRESS ACTIVATION OF RETROTRANSPOSONS IN EUKARYOTES

By

MARWA MAHMOUD SHEHATA AHMED

B.Sc. Agric. Sc. (Genetics), Fac. Agric., Ain Shams University, 2007

M. Sc. Agric. Sc. (Genetics), Fac. Agric., Ain Shams University, 2014

**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**

2020

Approval Sheet
**MOLECULAR GENETIC STUDIES ON ENVIRONMENTAL
STRESS ACTIVATION OF RETROTRANSPOSONS
IN EUKARYOTES**

By

MARWA MAHMOUD SHEHATA AHMED

B.Sc. Agric. Sc. (Genetics), Fac. Agric., Ain Shams University, 2007

M. Sc. Agric. Sc. (Genetics), Fac. Agric., Ain Shams University, 2014

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

Dr. Ahmed Abdel-Salam Mahmoud

Prof. Emeritus of Genetics, Faculty of Agriculture, Zagazig
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: 2 / 2 / 2020

MOLECULAR GENETIC STUDIES ON ENVIRONMENTAL STRESS ACTIVATION OF RETROTRANSPOSONS IN EUKARYOTES

By

MARWA MAHMOUD SHEHATA AHMED

B.Sc. Agric. Sc. (Genetics), Fac. Agric., Ain Shams University, 2007

M. Sc. Agric. Sc. (Genetics), Fac. Agric., Ain Shams University, 2014

Under the supervision of:

Dr. Eman Mahmoud Fahmy

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

Dr. Lamyaa Mostafa Kamal Sayed

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

ABSTRACT

Marwa Mahmoud Shehata Ahmed: Molecular Genetic Studies on Environmental Stress Activation of Retrotransposons in Eukaryotes. Unpublished Ph.D. Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2020.

Retrotransposons comprise the major part of eukaryotic genomes. They have the ability to replicate themselves through RNA intermediate via reverse transcription process. During normal development, these elements become quiescent, but they are stimulated by stresses. The availability of PCR-based techniques to detect the variation in retrotransposition rate due to salinity was tested. IRAP and SCoT markers were applied in two salinity-tolerant eukaryotic genomes: Yeast (*Saccharomyces cerevisiae* L.) and Barley (*Hordeum vulgare* L.). Semi-quantitative analysis was applied with only the two barley cultivars.

The DNA of the yeast strain EMCC-49 and two barley cultivars Giza-123 and Giza-2000 were extracted. Five IRAP primers with two combinations and nine SCoT primers were applied. The yeast strain was grown in the YPG media with 0.5 M, 1 M, 1.5 M NaCl and the control. The barley cultivars were irrigated with 0.25 M, 0.6 M NaCl or just distilled water as the control.

This research aimed to study the effect of salinity stress on the activation of retrotransposition. IRAP technique developed three markers in the yeast under the different levels of salinity. ScM1 IRAP primer showed a band with molecular size of 456 bp in the yeast under 0.5 and 1.5 M only. Another band with molecular size (MS) of 409 bp appeared under the control only. The third IRAP marker was shown by the ScM2 primer with MS of 1952 bp under the 0.5 M treatment. While, two IRAP markers appeared in barley due to salinity stress. The 5'LTR IRAP primer showed an 886 bp band in the barley cultivar Giza-2000 under the control condition only. Sukkula IRAP

primer displayed the second IRAP marker in the cultivar Giza-2000 of barley with MS of 330 bp under the 0.6 M only.

SCoT markers showed 17 markers response to salinity stress in yeast with MS ranged from 1911 to 271 bp with SCoT-31 and SCoT-26 primers, respectively. SCoT-26 primer gave the highest number of markers per SCoT primer (five different markers). In barley, 18 SCoT markers were detected under salinity stress. They MS were between 1762 (SCoT-26) and 281 bp (SCoT-7). SCoT-32 primer showed five markers in barley under salinity. The results showed that the high levels of salinity led to new retrotransposition.

In semi-quantitative analysis the banding patterns obtained with actin primer as housekeeping gene showed the same pattern in the control and all treatments in Giza 123 and Giza 2000 with no difference in the band intensity. With specific primer of *TY1B* gene (*reverse transcriptase* gene), in Giza 123 had low intensity in 0.25 M NaCl (T1) compared with the control. Giza 2000 showed that the intensity of band was more in 0.6 M NaCl.

This study confirmed that PCR techniques; like IRAP and SCoT can exhibit the activation of retrotransposition due to salinity stress. Good positive results were obtained and we recommend the using of these techniques for different molecular purposes due to their advantage; easy, fast, cheap and effectiveness.

Keywords: Retrotransposon, salinity, IRAP, SCoT techniques and semi-quantitative

ACKNOWLEDGMENT

Thanks for **my God Allah, the great and almighty** on his uncountable and infinite graces, guided me to the Islam and learned me things that I didn't know.

I wish to express my sincere appreciation and deep gratitude to **Prof Dr. Eman Mahmoud Fahmy**, Prof. of Genetics, Genetics Dept., Ain Shams University for her kind supervision; suggesting the scientific problem, fruitful help, energetic guidance, conclusive instructions throughout the course of this investigation and in reviewing the manuscript.

Great and deep thanks is offered to **Prof Dr. Fatma Mohammad Ibrahim Badawy**, Prof. of Genetics, Genetics Dept., Fac. of Agric., Ain Shams Univ. for her kind supervision before her death and her valuable advices.

Sincere appreciation is due to **Dr. Lamyaa Mostafa-Kamal Sayed**; Associate Prof. of Genetics, Genetics Dept., Fac. of Agric., Ain Shams Univ. for her supervision, useful suggestions and for her facilities she offered to me during this work.

Great thanks are offered to **Prof. Dr. Fatthy M. Abdel-Tawab**, Prof. of Genetics, Genetics Dept., Fac. of Agric., Ain Shams Univ. For facilities he offered to me during this work. Great thanks are offered to **Prof. Dr. Ashraf bakry**, Prof. of Genetics, Genetics Dept., Fac. of Agric., Ain Shams Univ. for helping me in provide facilities during this study. Deep thanks are offered to **Prof. Dr. Khaled Abdel-Aziz**, Prof. of Genetics, Genetics Dept., Fac. of Agric., Ain Shams Univ. for his facilities he offered to me during this work.

Great thanks to **Dr. Asmaa abo-shady**; Associate Prof. of Genetics, Genetics Dept., Fac. of Agric., Ain Shams Univ. for her facilities she offered to me during this work.

Great and deep thanks to **Dr. Nouh E. Ahmed**, Assistant Prof. of Genetics, Genetics Dept., Fac. of Agric., Ain Shams Univ. for helping me in benefit discussions, helping me during this study and teaching me a lot of things. Deep thanks to **Dr. Mona Mohamed Moghazee** Assistant Prof. of Genetics, Genetics Dept., Fac. of Agric., Ain Shams Univ. for helping me in providing facilities during this study. Great and deep thanks to **my freind, Shaimaa Ahmed Ali** Assistant lecturer of Genetics, Genetics Dept., Fac. of Agric., Ain Shams Univ. for helping me in a lot of things during this work and encouragement. A lot of thanks for **Nour Elhoda Hany** Assistant lecturer. of Genetics, Genetics Dept., Fac. of Agric., Ain Shams Univ. For helping me in this study.

Finally, many thanks to my colleagues; the **staff members of Molecular and Biochemical Genetics Lab.**, Genetics Department, Faculty of Agriculture, Ain Shams University for their great help and encouragement.

I am indebted as a gift to **my parents and my family** for their continuous encouragement, helping me and praying for me.

CONTENTS

Title	Page
List of Tables	III
List of Figures	V
List of Abbreviations	VII
1. Introduction	1
2. Review of Literature	5
2.1. Transposable elements (TEs)	5
2.2. Retrotransposons	6
2.2.1. Relationship between genome size and LTR retrotransposons	7
2.2.2. Retrotransposons types	8
2.2.3. Promoters in active retrotransposons..	10
2.2.4. Silencing mechanisms controlling of TEs	10
2.3. Environmental stresses and retrotransposons activation	12
2.3.1 Proposed model of LTR retrotrasposon activation by stress	13
2.3.2. Active cereals retrotransposons	15
2.3.3. LTR-retrotransposons in Yeast	16
2.4. Retrotransposon-based marker systems	17
2.4.1. Inter-Retrotransposons Amplified Polymorphisms "IRAPs"	17
2.5. Definition, advantage and uses of start codon targeted markers "SCoT"	20
2.6. Semi-quantitative analysis of DNA	23
3. Materials and Methods	27
3.1. Materials	27
3.1.1. Yeast strain and barley cultivars	27
3.2. Methods	28
3.2.1. Growing the yeast-strain cells	28
3.2.2. DNA extraction	28
3.2.2.1. DNA extraction from yeast strain	28
3.2.2.2. Salinity treatments and DNA extraction from barley	30

II

cultivars	
3.2.3. PCR-based molecular genetic techniques	31
3.2.3.1. Inter retrotransposon amplified polymorphism- polymerase chain reaction (IRAP-PCR) technique	31
3.2.3.2. Start Codon Targeted Polymorphism (SCoT)	33
3.2.3.3. Semi-quantitative analysis.	35
3.2.4. Gel electrophoresis of PCR products	37
3.2.4.1. TAE buffer (50 X), pH 8.0	37
3.2.4.2. Agarose gel preparation	37
3.2.4.3. Separation on agarose	37
3.2.5. Gel imaging and data analyses	37
4. Results and Discussion	39
4.1. Retrotransposons-based technique: IRAP technique "Inter retrotransposon amplified polymorphism"	39
4.1.1. IRAP for yeast strain.	39
4.1.2. IRAP for barley cultivars.	43
4.2. SCoT "Start Codon Targeted Polymorphism" markers	48
4.3. Semi-quantitative analysis of DNA	62
5. Summary	67
6. References	71
Arabic Summary	

LIST OF TABLES

Table	Page
Table (1): The names, codes and sources of the yeast strain and barley cultivars	27
Table (2): The names and sequences of the three barley IRAP primers.	32
Table (3): The names and sequences of the two yeast IRAP primers.	32
Table (4): The primer-combinations of IRAP technique for yeast strain and barley cultivars.	33
Table (5): The names and sequences of the nine SCoT primers.	34
Table (6): The names and sequences of degenerate forward and reverse specific primer	35
Table (7): The names and sequences of forward and reverse Actin primer	36
Table (8): The molecular sizes of the three bands of yeast with IRAP primers under control and salinity treatments	42
Table (9): The molecular sizes of different bands of barley-IRAP primers under the control and salinity treatments	47
Table (10): The presence and absence of the different bands of yeast using nine SCoT primers with their molecular sizes under the control and salinity treatments.	58
Table (11): The presence and absence of the different bands of barley using nine SCoT primers with their molecular sizes under the control and salinity treatments.	60

LIST OF FIGURES

Figure	Page
Fig. (1): Structure of the different types of plant transposable elements	6
Fig. (2): Class I transposable element; retrotransposons	7
Fig. (3): Types of LTRs retrotransposons	9
Fig. (4): Proposed scheme for activation of retrotransposon due to stress	14
Fig. (5): Inter retrotransposon amplified polymorphism (IRAP)	18
Fig. (6): Diagram showing principle of SCoT PCR amplification	21
Fig. (7): Banding patterns of ScM1 IRAP primer for the yeast strain.	40
Fig. (8): Banding patterns of ScM2 IRAP primer for the yeast strain.	41
Fig. (9): Banding patterns of ScM1 and ScM2 IRAP primer combination for the yeast strain	42
Fig. (10): Primer 5'LTR- IRAP reaction with the barley cultivars	44
Fig. (11): Primer of sukkula-IRAP reaction with the barley cultivars	45
Fig. (12): Primer of 5'LTR–sukkula-IRAP combination reaction with the barley cultivars	46
Fig. (13): Primer WLTR2105-IRAP reaction with the barley cultivars	47
Fig. (14): Banding patterns of SCoT-05 primer with the yeast strain	49
Fig. (15): Banding patterns of SCoT-07 primer with the yeast strain	50