

شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلو

بسم الله الرحمن الرحيم





MONA MAGHRABY



شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلو



شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



MONA MAGHRABY



شبكة المعلومات الجامعية التوثيق الإلكترونى والميكروفيلم

جامعة عين شمس التوثيق الإلكتروني والميكروفيلم قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



MONA MAGHRABY

GENETIC IMPROVEMENT OF SOME ORNAMENTAL PLANTS THROUGH MUTATION INDUCTION

By

EMAN AHMED MOHAMED EL-MENBAWY

B.Sc. Agric. Sc.(Genetics), Fac. Agric. Ain Shamus Univ.(2000) M.Sc. Agric. Sc.(Genetics), Fac. Agric. Ain Shamus Univ.(2006)

> A Thesis Submitted in Partial Fulfillment Of The Requirement for the Degree of

in
Agricultural Sciences
(Genetics)

Department of Genetics Faculty of Agriculture Ain Shams University

Approval Sheet

GENETIC IMPROVEMENT OF SOME ORNAMENTAL PLANTS THROUGH MUTATION INDUCTION

By

EMAN AHMED MOHAMED EL-MENBAWY

B.Sc. Agric. Sc.(Genetics), Fac. Agric. Ain Shamus Univ.(2000) M.Sc. Agric. Sc.(Genetics), Fac. Agric. Ain Shamus Univ.(2006)

This thesis for Ph.D. degree has been approved by:	
Dr. Mohammed Abd EL-Salam Rashed Prof. Emeritus of Molecular Genetics and Dean of the Higher Institute for Agriculture Co-operation	
Dr. Fatthy Abdel – Tawab Prof. Emeritus of Molecular Genetics, Faculty of Agriculture, A Shams University	in
Dr. Eman Mahmoud Fahmy Prof. Emeritus of Molecular Genetics, Faculty of Agriculture, A Shams University	in

Date of Examination: 23 / 9 / 2020

GENETIC IMPROVEMENT OF SOME ORNAMENTAL PLANTS THROUGH MUTATION INDUCTION

By

EMAN AHMED MOHAMED EL-MENBAWY

B.Sc. Agric. Sc.(Genetics), Fac. Agric. Ain Shamus Univ.(2000) M.Sc. Agric. Sc.(Genetics), Fac. Agric. Ain Shamus Univ.(2006)

Under the supervision of:

Dr. Eman Mahmoud Fahmy

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

Dr. Sawsan Youssef Youness Mohamed Elateek

Lecturer of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University

Dr. Nahla Abd EL-Fattah Awad

Head Research of Fruit, Fruit, Ornamental and Woody Plant Breeding Research Department, Horticulture Institute. Agricultural Research Center

ABSTRACT

Eman Ahmed Mohamed ELMenbawy: Genetic Improvement of Some Ornamental Plants through Mutation Induction. Unpublished Ph.D. Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2020.

Calendula officinalis and Antirrhinum majus are the basic plants of ornamentals. They are used for various purposes as cut flowers, pot plants and in gardens. They are rich in active compounds, thus are used as medicinal plants. The present study used mutation induction to improve the two plants. Seeds were treated with three doses of gamma ray; 25, 50, 70 Gy in Calendula and 40, 60, 80 Gy in Antirrhinum. Two chemical mutagens colchicine and EMS (Ethyl Methane Sulfonate) were applied in Calendula at concentrations of 1000, 3000, 10000 ppm for each one. Seeds without treatment were used as control. The yield-related traits, active compounds, SDS-PAGE profiling and DNA banding patterns with degenerative primer for gene CYC were detected. The data of Calendula plants showed that the highest plant height (50.60) in M_2 was obtained by 70 Gy and 10000 ppm of EMS, while the lowest dose of colchicine (1000 ppm) made the most impact on the plant height (47.0) in M₂. The highest number of leaves was obtained at 50 Gy (83.3) in M₂ compared with the control. The number of flowers/plant was not affected by the gamma radiation and colchicine but increased significantly by 10000 ppm of EMS. The flower diameter decreased at 25 Gy (3.5) and 3000 ppm of colchicine (3.0) in M₂ compared with the control while no effect was observed by EMS. Both Chl-a, Chl-b and carotenoids contents increased at 50 Gy, while flavonoid increased at 25 Gy. All doses of gamma ray eliminated the phenols content. While the lowest concentration of colchicine; 1000 ppm increased both of Chl-a, Chl-b, flavonoid and carotenoids, even though the 10000 ppm of colchicine increased the phenol. On the other hand, the highest concentration of EMS 10000 ppm increased Chl-a and Chl-b (0.80, 0.97), while carotenoids, flavonoid and phenol increased at 3000 ppm of EMS compared with the control.

The data of M₂ Antirrhinum plant showed the highest plant height in M₂ at 60 Gy. The highest number of leaves was obtained at 80 Gy. The number of flowers per spike and spike length was not affect by gamma rays. The Chl-a, Chl-b and anthocyanin increased at 80 Gy. Flavonoids decreased with increasing gamma doses and phenol increased at 40 Gy. The SDS-protein electrophoresis showed fluctuation in the gene expression based on the type of mutagen and its dose. Due to the morphological changes in the flower shape obtained through this study, the flower symmetry CYC gene was investigated using degenerative primers DeCYC-1/DeCYC-2. A specific band with MS ~270 bp was detected in Calendula in four treated plants that gain flower change at; 50 Gy of gamma rays, 1000 and10000 ppm cochicine, 3000 ppm EMS, and in both treated and untreated plants (control) in Antirrhinum.

Key words: Calendula officinalis, Antirrhinum majus, mutation, gamma ray, EMS, colchicine, active compounds, SDS-protein electrophoresis, CYC gene.

ACKNOWLEDGMENT

First of all I would like to thank Allah the Almighty for helping me to accomplish and finishing this manuscript.

I would like to express my deep gratitude to my principal supervisor prof. Dr. **Eman Mahmoud Fahmy**, Professor of Molecular Genetics, Dept. of Genetics, Faculty of Agriculture, Ain Shams University, for her supervision, guidance, encouragement and writing this manuscript.

Heartfelt thanks to Dr. **Sawsan Youssef ELateek,** Lecturer of Genetics, Dept. of Genetics, Faculty of Agriculture, Ain Shams University, for her supervision, advice, patience through all the stages of this thesis, facilitating the lab work and writing this manuscript.

My sincere thanks also due to Prof. Dr. Nahla Abd El-fattah Awad, Horticulture Institute, Agriculture Research Center for her supervision and suggesting the idea of this thesis. She also supported the part of germination of the work.

I don't forget my supervisor Prof. Dr. **Fatma Mohamed Badawy,** my Allah bless her soul.

Thanks are also due to all **staff member of the Dept. of Genetics,** Faculty of Agriculture for their great help.

In addition, my thanks to my friends in the **Department of Botanical gardening, Horticulture Research Institute**, **ARC** for their encouragement through this study.

I would also like to thank the **Biotechnology Lab** at Cairo University Research Park, Faculty of Agriculture for carrying out the molecular analysis of this study.

Finally, many thanks for **my husband, sons and my family** for their help and encouragement.

CONTENTS

Title	page
LIST OF TABLE	IV
LIST OF FIGURES	V
I. INTRODUCTION	I
II. REVIEW OF LITERATURE	5
2.1. Calendula officianalis	5
2.2. Antirrhinum majus	6
2.3. Phytochemical studies	. 6
2.4. Induced mutagenesis	. 7
2.4.1. Physical mutagenesis	8
2.4.2. Chemical mutagenesis	9
2.5. Molecular genetics studies	. 11
2.5.1. SDS-Protein electrophoresis	. 11
2.5.2. Gene studies on flower symmetry	. 10
III. MATERIALS AND METHODS	. 16
3.1. Plant materials	. 16
3.2. Methods	16
3.2.1. Plant growth	. 16
3.2.2. Irradiation treatments	. 17
3.2.2.1. Irradiation treatment in <i>Calendula</i>	. 17
3.2.2.2. Irradiation treatment in <i>Antirrhinum</i>	17
3.2.3. Chemical treatments	. 17
3.2.4. Plant measurements	18

3.2.4.1. Yield-related traits	18
3.2.4.2. Phytochemical measurements	18
3.2.5. Statistical analysis	19
3.2.6. Molecular genetic analysis	19
3.2.6.1. SDS-protein electrophoresis	19
3.2.6.2. <i>CYC</i> gene detection	22
IV. RESULTS AND DISCUSSION	27
4.1. Yield-related traits	27
4.1.1. <i>Calendula</i> parameters in γ-irradiation plants	27
4.1.1.1 Plant height	27
4.1.1.2. Number of leaves per plant	28
4.1.1.3. Number of flower per plant	28
4.1.1.4. Flower diameter	28
4.1.1.5. Flower shape	29
4.1.2. <i>Calendula</i> with colchicine treatments	30
4.1.2.1. Plant height	30
4.1.2.2. Number of leaves per plant	30
4.1.2.3. Number of inflorescences per plant	31
4.1.2.4. Flower diameter	31
4.1.2.5. Flower shape	31
4.1.3. Calendula using EMS	33
4.1.3.1. Plant height	33
4.1.3.2. Number of leaves per plant	33
4.1.3.3. Number of inflorescences per plant	33
4.1.3.4. Flower diameter	34
4.1.3.5. Flower shape	34
4.1.4. <i>Antirrhinum</i> parameters in γ -irradiation plants	37

4.1.4.1. Plant height	37
4.1.4.2. Number of leaves per plant	37
4.1.4.3. Number of florets per spike	37
4.1.4.4. Spike length	37
4.1.4.5. Flower shape	38
4.1.5. Antirrhinum using chemical mutagenesis of colchicine	
and EMS	38
4.2. Phytochemical measurements	39
4.2.1. Calendula plants	39
4.2.1.1. <i>Calendula</i> using γ-irradiation plants	39
4.2.1.2. Colchicine treatment in <i>Calendula</i>	41
4.2.1.3. EMS treatment in <i>Calendula</i>	43
4.2.2. Antirrhinum using gamma rays	45
4.3. Molecular genetic analysis	46
4.3.1. SDS-protein pattern in <i>Calendula</i>	46
4.3.1.1. Gamma irradiation experiment	47
4.3.1.2. Colchicine treatment	48
4.3.1.3. EMS treatment	49
4.3.2. SDS-protein contant in <i>Antirrhinum</i> using γ -irradiation plants	. 51
4.3.3. DNA molecular analysis	52
V. SUMMARY	58
VI. REFERANCES	62
ARABIC SUMMARY	

LIST OF TABLES

Table		page
Table (1)	Sequences of the specific degenerative primers	
	to detect flower symmetry	25
Table (2)	Mean performance of the yield-related traits of	
	Calendula officinalis L. plants as affected by	
	gamma rays	29
Table (3)	Mean performance of the yield-related traits of	
	Calendula officinalis L. plants as affected by	
	colchicine	32
Table (4)	Mean performance of the yield-related traits of	
	Calendula plants as affected by EMS	34
Table (5)	Mean performance of the yield-related traits in	
	Calendula officinalis in two generations	
	affected by gamma ray and the two chemical	
	mutagenesis of cholchicine and EMS	36
Table (6)	Mean performance of the yield-related of	
	Antirrhinum majus plants in the two	
	generations affected by gamma rays	38
Table (7)	The active compounds of Calendula officinalis	
	extracts as affected by gamma rays	40

Table (8)	The active compounds of Calendula extracts as	
	affected by cholchicine	42
Table (9)	The active compounds of Calendula extracts as	
	affected by EMS.	44
Table (10)	The active compounds of Antirrhinum extracts	
	as affected by gamma rays	45
Table (11)	Protein intensity for the effect of gamma	
	irradiation on Calendula seeds in M_1 and M_2	
	generation	48
Table (12)	Protein intensity for the effect of colchicine on	
	Calendula seeds in M ₁ and M ₂ generation	50
Table (13)	Protein intensity for the effect of EMS on	
	Calendula seeds in M ₁ and M ₂ generation	51
Table (14)	Protein intensity for the effect of gamma	
	irradiation on Antirrhinum seeds	53

LIST OF FIGURES

Figure		page
Figure (1)	Flowers of wild type and mutants in Calendula using Gamma rays	30
Figure (2)	Flower shape in <i>Calendula</i> using colchicine	32
Figure (3)	Flower shape in <i>Calendula</i> using EMS	35
Figure (4)	Inflorescence shape in <i>Antirrhinum</i> using gamma ray	39
Figure (5)	Dendrogram for the active compounds of <i>Calendula officinalis</i> extracts as affected by gamma rays.	41
Figure (6)	Dendrogram for the active compounds of <i>Calendula officinalis</i> extracts as affected by cholchicine	43
Figure (7)	Dendrogram for the active compounds of Calendula officinalis extracts as affected by EMS	44
Figure (8)	Dendrogram for the active compounds of of <i>Antirrhinum</i> extracts as affected by gamma rays	46
Figure (9)	Polyacrylamide gel electrophoresis for the effect of gamma rays on the SDS- protein of <i>Calendula</i> seeds	48
Figure (10)	Polyacrylamide gel electrophoresis for the effect of colchicine on the SDS- protein of <i>Calendula</i> seeds	49
Figure (11)	Polyacrylamide gel electrophoresis for the effect of EMS on the SDS- protein of <i>Calendula</i> seeds	51