

**GENETIC POLYMORPHISM OF SOME
MEDICINAL PLANTS BELONGING
TO BRASSICACEAE**

By

RASHA MOUSTAFA ABU SEREAI KHALIL

B.Sc. Agric. Sc. (Genetics), Ain Shams University, 1997

M. Sc. Agric. Sc. (Genetics), Ain Shams University, 2005

A thesis submitted in partial fulfillment

of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

**Agricultural Science
(Genetics)**

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

2010

Approval Sheet

**GENETIC POLYMORPHISM OF SOME
MEDICINAL PLANTS BELONGING
TO BRASSICACEAE**

By

RASHA MOUSTAFA ABU SEREI KHALIL

B.Sc. Agric. Sc. (Genetics), Ain Shams University, 1997

M.Sc. Agric. Sc. (Genetics), Ain Shams University, 2005

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

Dr. Hassan Mohamed Zaky Mahmoud Allam

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

Dr. Alia Ahmed Mohamed El-Seoudy

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

Dr. Khaled Abdel- Alziz Soliman

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

Dr. Samir Abdel- Aziz Ibrahim

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

Date of Examination: 13 / 3 / 2010

GENETIC POLYMORPHISM OF SOME MEDICINAL PLANTS BELONGING TO BRASSICACEAE

By

RASHA MOUSTAFA ABU SEREAI KHALIL

B.Sc. Agric. Sc. (Genetics), Ain Shams University, 1997

M. Sc. Agric. Sc. (Genetics), Ain Shams University, 2005

Under the supervision of:

Dr. Samir Abdel-Aziz Ibrahim

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

Dr. Khaled Abdel-Aziz Soliman

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

Dr. Nahed Ahmed Kamel Fahmey Rashed

Associate Research Prof. of Genetics, Department of
Genetic Resources, Desert Research Center

ABSTRACT

Rasha Moustafa Abu-Sereai: Genetic Polymorphism of Some Medicinal Plants Belonging to Brassicaceae. Unpublished Ph.D. Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2010.

Brassicaceae family has large number of species with a major economic, medicinal importance and great genetic diversity in the flora of Egypt. The genetic analysis was carried out on 11 Egyptian samples of **Brassicaceae** representing ten **Brassicaceae** species collected from different localities in Egypt. These species were characterized by SDS-PAGE, isozymes, RAPD and ISSR. SDS-PAGE of total proteins showed variation among ten species and indicate the relationship among them. Five isozyme systems including and *Est* (esterases), *Prx* (peroxidase), *Acp* (acid phosphatase), *Adh* (alcohol dehydrogenase), *Mdh* (malate dehydrogenase) were carried out to test the genetic variability among the studied species. The results obtained revealed a significant level of polymorphism among **Brassicaceae** species. ISSR and RAPD-PCR primers were more informative than protein, isozyme in fingerprints. Fourteen reselected ISSR primers were used to evaluate genetic variability among **Brassicaceae** species under investigation. The results indicated that there was some variation in banding patterns among ten species. The genetic relationship based on SDS-PAGE, isozyme, RAPD and ISSR clearly indicated the genetic relationships among the ten species.

Key words: *Brassicaceae*, SDS-PAGE, ISSR-PCR, genetic polymorphism.

ACKNOWLEDGMENT

I wish to express my great gratitude and deep thanks to **Prof.Dr. Samir Abdel-Aziz Ibrahim** Prof. of Genetics, Fac. of Agric. Ain Shams University, for his supervision, useful suggestions, continuous guidance throughout the course of this investigation, helping me to solve any technical problem and his useful and faithful guidance in work.

Sincere appreciation is due to **Prof.Dr. Khaled Abdel-Aziz Soliman** .Prof. of Genetics, Faculty of Agric., Ain Shams University, for his supervision and his efforts, following up the stages of laboratory work and his help in writing the manuscript.

Deep thanks are extended to **Dr. Nahed Ahmed Kamel**, Assoc. Prof. of cytological Genetics, Desert Research Center, for her supervision, patience in following up the stages, useful suggestions, continuous guidance of laboratory work, and the great efforts of collecting the material of the study and her help in writing the manuscript.

I wish to express my profound gratitude, sincere appreciation, deep thanks and honorable respect to **Prof.Dr. Ismail Abdel- Galil** previous president of Desert Research Center and **Prof.Dr. Hassan Mohamed Zaky Allam** Prof. Emeritus of Genetics, Faculty of Agriculture, Minia University for helping me to accomplish this work.

Finally, many thanks to all the staff members of molecular genetic lab in North Sinai Station and to all the staff members of Ain Shams Center for Genetic Engineering and Biotechnology Branch of Faculty of Agriculture (**ACGEBFAG**) for their help.

CONTENTS

List of Tables	ii
List of Figures	iv
List of abbreviations	vi
I. Introduction	1
II. Review of Literature	
1. Importance of cruciferae	4
2. Biochemical genetic studies	6
1.1. SDS-PAGE electrophoresis	6
1.2. Isozyme electrophoresis	8
3. Molecular genetic studies based on PCR	11
3.1. RAPD analysis	
3.2. ISSR analysis	
III. Materials and Methods	25
III. A. Materials	25
III. B. Methods	26
1. Biochemical genetic studies	26
2.1. SDS-PAGE electrophoresis	26
2.2. Isozyme electrophoresis	28
2. Molecular genetic studies based on PCR	32
2.1. RAPD analysis	32
2.2. ISSR analysis	36
IV. Results and Discussion	37
1. Biochemical genetic studies	39
2.1. SDS-PAGE	39
2.2. Isozyme	41
2. Molecular genetic studies based on PCR	50
2.1. RAPD analysis	
2.2. ISSR analysis	
V. Summary	98
VI. References	92
VII. Arabic summary	

LIST OF TABLES

No.	Title	Page
1.	Sources of the studied ten Brassicaceae species	25
2.	RAPD Primer codes and nucleotide sequences	34
3.	ISSR primer codes and their nucleotide sequences	36
4.	Protein banding pattern of the studied Brassicaceae species as revealed by SDS-PAGE for total protein	40
5.	The presence (1) and absence (0) of bands in five isozyme systems among the eleven Brassicaceae samples	47
6.	Polymorphism percentages generated by five isozyme systems in the eleven Brassicaceae Samples.	48
7.	Similarity matrix among the eleven Brassicaceae samples based on five isozyme analysis	49
8.	The presence (1) and absence (0) of bands in RAPD profiles of primer OPB1 among eleven Brassicaceae samples	51
9.	The presence (1) and absence (0) of bands in RAPD profiles of primer OPB2 among eleven Brassicaceae samples	52
10.	The presence (1) and absence (0) of bands in RAPD profiles of primer OPB5 among eleven Brassicaceae samples	54
11.	The presence (1) and absence (0) of bands in RAPD profiles of primer OPB6 among eleven Brassicaceae samples	55
12.	The presence (1) and absence (0) of bands in RAPD profiles of primer OPB17 among eleven Brassicaceae samples	57

13.	The presence (1) and absence (0) of bands in RAPD profiles of primer OPA3 among eleven Brassicaceae samples	58
14.	The presence (1) and absence (0) of bands in RAPD profiles of primer OPA6 among eleven Brassicaceae samples	59
15.	The presence (1) and absence (0) of bands in RAPD profiles of primer OPA9 among eleven Brassicaceae samples	60
16.	The presence (1) and absence (0) of bands in RAPD profiles of primer OPA10 among eleven Brassicaceae samples	62
17.	The presence (1) and absence (0) of bands in RAPD profiles of primer OPD1 among eleven Brassicaceae samples	63
18.	The presence (1) and absence (0) of bands in RAPD profiles of primer OPZ18 among eleven Brassicaceae samples	64
19.	Polymorphism percentages, monomorphic and polymorphic number of bands detected by each primer (Kits A, B, D and Z) among eleven Brassicaceae samples.	66
20.	Similarity matrix based on RAPD- PCR among the eleven Brassicaceae samples.	67
21.	Positive marker molecular sizes among the eleven Brassicaceae samples Based on RAPD-PCR	68
22.	The presence (1) and absence (0) of bands in ISSR profiles of primer A17898 among eleven Brassicaceae samples	70

23. The presence (1) and absence (0) of bands in ISSR profiles of primer B17898 among eleven **Brassicaceae** samples 71
24. The presence (1) and absence (0) of bands in ISSR profiles of primer A17899 among eleven **Brassicaceae** samples 72
25. The presence (1) and absence (0) of bands in ISSR profiles of primer B17899 among eleven **Brassicaceae** samples 73
26. The presence (1) and absence (0) of bands in ISSR profiles of primer HB10 among eleven **Brassicaceae** samples 74
27. The presence (1) and absence (0) of bands in ISSR profiles of primer HB12 among eleven **Brassicaceae** samples 75
28. The presence (1) and absence (0) of bands in ISSR profiles of primer HB15 among eleven **Brassicaceae** samples 76
29. The presence (1) and absence (0) of bands in ISSR profiles of primer IS-01 among eleven **Brassicaceae** samples 77
30. The presence (1) and absence (0) of bands in ISSR profiles of primer IS-02 among eleven **Brassicaceae** samples 78
31. The presence (1) and absence (0) of bands in ISSR profiles of primer IS-05 among eleven **Brassicaceae** samples 79
32. The presence (1) and absence (0) of bands in ISSR profiles of primer IS-06 among eleven **Brassicaceae** samples 80

- 33. The presence (1) and absence (0) of bands in ISSR profiles of primer IS-012 among eleven **Brassicaceae** samples 81
- 34. The presence (1) and absence (0) of bands in ISSR profiles of primer IS-015 among eleven **Brassicaceae** samples 82
- 35. Polymorphism percentages, number of monomorphic and polymorphic bands detected with each ISSR primers among **Brassicaceae** species. 84
- 36. Positive marker molecular sizes among the eleven **Brassicaceae** samples Based on ISSR- PCR 85
- 37. Similarity matrix among the eleven **Brassicaceae** Samples based on ISSR-PCR. 87

LIST OF FIGURES

No.	Title	Page
1.	Egypt map showing the locations (1-11) as in table (1) of investigated Brassicaceae species	27
2.	Photographs representing the morphological variations of the ten Brassicaceae species.	38
3.	SDS-PAGE profile of leaf total-protein among the eleven Brassicaceae species.	40
4(a).	Zymogram of -Est banding patterns among the eleven Brassicaceae samples.	44
4(b).	Zymogram of -Est patterns among the eleven Brassicaceae samples	44
5.	Zymogram of Prox banding patterns among the eleven Brassicaceae samples.	45
6.	Zymogram of Adh banding patterns among the eleven Brassicaceae samples.	45
7.	Zymogram of Mdh banding patterns among the eleven Brassicaceae samples	46
8.	Zymogram of Acph banding patterns among the eleven Brassicaceae samples.	46
9.	Dendrogram based on five isozyme analysis among eleven Brassicaceae samples	49
10.	DNA profile generated by primer OPB1 among the eleven Brassicaceae samples.	50
11.	DNA profile generated by primer OPB2 among the eleven Brassicaceae samples.	52
12.	DNA profile generated by primer OPB5 among the eleven Brassicaceae samples.	53
13.	DNA profile generated by primer OPB6 among the eleven Brassicaceae samples.	55

14.	DNA profile generated by primer OPB17 among the eleven Brassicaceae samples.	56
15.	DNA profile generated by primer OPA3 among the eleven Brassicaceae samples.	58
16.	DNA profile generated by primer OPA6 among the eleven Brassicaceae samples.	59
17.	DNA profile generated by primer OPA9 among the eleven Brassicaceae samples.	60
18.	DNA profile generated by primer OPA10 the eleven Brassicaceae samples.	61
19.	DNA profile generated by primer OPD1 the eleven Brassicaceae samples.	63
20.	DNA profile generated by primer OPZ18 the eleven Brassicaceae samples.	64
21.	Dendrogram based on ten RAPD primer profiles among eleven Brassicaceae samples	67
22.	DNA profile generated by primer A17898 among the eleven Brassicaceae samples.	69
23.	DNA profile generated by primer B17898 among the eleven Brassicaceae samples	71
24.	DNA profile generated by primer A17899 among the eleven Brassicaceae samples.	72
25.	DNA profile generated by primer B17899 among the eleven Brassicaceae samples.	73
26.	DNA profile generated by primer HB10 among the eleven Brassicaceae samples.	74
27.	DNA profile generated by primer HB12 among the eleven Brassicaceae samples.	75
28.	DNA profile generated by primer HB15 among the eleven Brassicaceae samples.	76
29.	DNA profile generated by primer IS-01 among the	77

eleven **Brassicaceae** samples.

- 30. DNA profile generated by primer IS-02 among the eleven **Brassicaceae** samples. 78
- 30. DNA profile generated by primer IS-05 among the eleven **Brassicaceae** samples. 79
- 32. DNA profile generated by primer IS-06 among the eleven **Brassicaceae** samples. 80
- 33. DNA profile generated by primer IS-012 among the eleven **Brassicaceae** samples. 81
- 34. DNA profile generated by primer IS-015 among the eleven **Brassicaceae** samples. 82
- 35. Dendrogram based on ISSR-PCR of **Brassicaceae** 88
- 36. Dendrogram based on overall systems (protein, isozymes, RAPD and ISSR). 88

LIST OF ABBREVIATIONS

AcpH	Acid phosphatase
10 mer-primer	10 - oligonucleotide primer
AFLP	Amplified fragment length polymorphism
APS	Ammonium persulfate
EDTA	Ethylenediamine- tetra acetic acid
EST	Esterase
L	Liter
MW	Molecular weight
Mdh	Malate dehydrogenase
mg	Milligram
ml	Milliliter
NAD	Nicotine amid adinine dinucleotide
NADH	NADH dehydrogenase
NBT	Nitroblue tetrazolium salt
PCR	Polymerase chain reaction
PMS	Phenazine methosulfate
Prx	Peroxidase
RAPD	Random Amplified polymorphic DNA
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SPSS	Statistical package for social science
ISSR	Inter simple sequence repeat