



شبكة المعلومات الجامعية

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ





شبكة المعلومات الجامعية



شبكة المعلومات الجامعية

التوثيق الالكتروني والميكرو فيلم

جامعة عين شمس

التوثيق الالكتروني والميكرو فيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
على هذه الأفلام قد اعدت دون أية تغيرات



يجب أن

تحفظ هذه الأفلام بعيداً عن الغبار

في درجة حرارة من 15 – 20 مئوية ورطوبة نسبية من 20-40 %

To be kept away from dust in dry cool place of
15 – 25c and relative humidity 20-40 %



شبكة المعلومات الجامعية



بعض الوثائق الأصلية تالفة



شبكة المعلومات الجامعية



بالرسالة صفحات

لم ترد بالأصل



Faculty of Science

Evaluation of P-element Mediated Transformation Vectors for The Expression of Foreign Genes in Eukaryotes

A thesis submitted in partial fulfillment of the requirement
for the degree of

Master of science

by

Sayed Mohamed Sayed

B. Sc. (Biochemistry / Chemistry)

Supervised by

Nadia

Dr. Nadia Mohamed Abdallah
Professor and Head of
Biochemistry Department
Faculty of Science
Ain Shams University

Salama

Dr. Mohamed Sayed Salama
Associate professor -
Entomology Department
Faculty of Science
Ain Shams University

Department of Biochemistry
Faculty of Science
Ain Shams University
(1998)

B
2077

BIOGRAPHY

Date and Place of Birth : 16/ 7 / 1967. Cairo.

Date of Graduation : May, 1989.

Degree Awarded : B. Sc. (Biochemistry /-Chemistry).

Grade : V. G. with Honor Degree.

Occupation : Research Assistant,
Agricultural Genetic Engineering
Research Institute (AGERI).
Agricultural Research Center.

I declare that this thesis has been composed by myself and has not been submitted for a degree at this or any other university.

Sayed Mohamed Sayed

22

23

24

25

26

27

28

29

30

31

Acknowledgement

I would like to thank, Prof. Dr. **NADIA M. ABDALLAH**, Professor and Head of Biochemistry Department, Faculty of Science, Ain Shams University, for her help, encouragement, advice and valuable criticism for the manuscript.

I wish to express my sincere appreciation and gratitude to, Dr. **MOHAMMED S. SALAMA**, Associate Professor - Entomology Department, Faculty of Science, Ain Shams University, for suggesting this point of research, reading the manuscript, his care, endless help, advice through the hard times that I passed in this study.

My deepest gratitude to Prof. Dr. **MAGDY A. MADKOUR**, Director of Agricultural Genetic Engineering Research Institute, for supporting this work.

My deep thanks to my colleague **AHMED M. AHMED** for his kind, warm support and encouragement. I would like also to thank my Colleagues in AGERI, specially, **TAMER, ZOMOROD** and **RABAB**.

CONTENTS

	Page
Abstract.....	i
List of abbreviation.....	ii
List of figures.....	iv
Introduction and Aim of the work.....	vi
Review of Literature.....	1
I. Animal transformation.....	1
1. Early trials for gene transfer into insects.....	1
2. P-element mediated transformation.....	2
3. Mariner transposable element.....	6
4. Hobo transposable element	7
5. Minos transposable element.....	9
II. Particle bombardment.....	10
Materials and Methods.....	17
1. Preparation of the transformation vector pJKP2 for gene bombardment.....	17
1.1. Introduction of plasmid DNA into <i>E. Coli</i> cells.....	17
1.1.1. Preparation of competent cells.....	17
1.1.2. Transformation of competent cells.....	18
1.2. Plasmid purification.....	19
1.2.1. Miniprep.....	19
1.2.2. Maxiprep.....	20
1.3. Agarose gel electrophoresis.....	21
1.3.1. Preparation of agarose gel and electrophoresis.....	21
1.3.2. Visualization and photography of the gel.....	22
2. Genetic transformation of plants.....	23
2.1. Preparation and coating of particles with plasmid DNA.....	23
2.2. Particle bombardment.....	24

3. Genetic transformation of insect embryos.....	24
3.1. Preparation and coating of particles with plasmid DNA.....	24
3.2. Particle bombardment.....	24
3.3. Rearing of transformed insects.....	25
3.3.1. Rearing of pink bollworm.....	25
3.3.2. Rearing of cotton leafworm.....	25
3.3.3. Rearing of eri silkworm.....	26
4. Detection of transforming DNA into genetically modified samples.....	26
4.1. Isolation of DNA from plant tissues.....	26
4.2. Isolation of DNA from animal tissues.....	27
4.3. polymerase chain reaction (PCR).....	28
4.4. Southern blot.....	29
4.5. Isolation of DNA for hybridization.....	30
4.5.1. Restriction enzyme digestion of pJKP2 vector.....	30
4.5.2. Elution of DNA from agarose.....	31
4.5.3. Nucleic acid labeling.....	32
4.5.4. DNA hybridization.....	32
5. β - Galactosidase assay.....	33
Results.....	34
1. Preparation of the transformation vector pJKP2 for gene bombardment.....	34
1.1. Introduction of plasmid DNA into <i>E. Coli</i> cells.....	35
1.2. Plasmid purification.....	35
2. Genetic transformation of plants.....	38
2.1. β - Galactosidase assay.....	39
2.1.1. Faba bean.....	39
2.1.2. Squash.....	42
2.1.3. Pepper.....	42
2.2. PCR.....	47
2.3. Southern blot hybridization analysis.....	47

3. Genetic transformation of insects.....	51
3.1. PCR.....	51
3.2. Southern blot hybridization analysis.....	52
Discussion	53
1. Genetic transformation of insect embryos.....	53
1.1. <i>P</i> -element mediated insect transformation.....	54
1.2. Particle bombardment and insect transformation.....	55
2. Genetic transformation of plant tissues.....	57
2.1. <i>P</i> -element and plant transformation.....	58
2.2. Particle bombardment and plant transformation....	58
Summary	62
Recommendations.....	66
References	67
Arabic summary	83

