



شبكة المعلومات الجامعية

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



شبكة المعلومات الجامعية

# جامعة عين شمس

التوثيق الالكتروني والميكرو فيلم

## قسم

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



## يجب أن

تحفظ هذه الأفلام بعيدا عن الغبار

في درجة حرارة من ١٥-٢٥ مئوية ورطوبة نسبية من ٢٠-٤٠%

To be Kept away from Dust in Dry Cool place of  
15-25- c and relative humidity 20-40%





# شبكة المعلومات الجامعية التوثيق الالكتروني والميكرو فيلم

# بعض الوثائق الأصلية تالفة

# بالرسالة صفحات لم ترد بالاصل



581,151

**Biotechnological studies on  
potato ( *Solanum tuberosum* L.) plants.**

A thesis

Submitted for the degree of Ph.D. of Science in Botany

(Cell and Genetics)

**By**

**Alaa El-din Sayed Sayed Ewase Khodeir**

**B. Sc. (1990)**

Faculty of Science, Cairo University.

**M.Sc. (2002)**

Faculty of Science, Menoufiya University.

**Supervisors**

**Prof.Dr. Mahmoud Imam Nasr**

Prof. of Genetics, former Dean and Founder  
of Genetic Engineering and Biotechnology  
Research Institute (GEBRI),  
Menoufiya University, Sadat city.

**Prof.Dr. Hala Mustafa Habib**

Prof. of Virology, Botany department.  
Faculty of Science- Cairo University


**Botany department**

**Faculty of Science**

**Cairo University**

**(2005)**





بسم الله الرحمن الرحيم  
يسألونك عن الروح قل الروح من أمر ربي وما أوتيتم من العلم إلا قليلا  
صدق الله العظيم

# Approval sheet

Title of Ph.D thesis:

Biotechnological studies on potato (*Solanum tuberosum* L.) plants.

Name of the candidate: Alaa El-din Sayed Sayed Ewase Khodeir

Submitted to the  
Faculty of Science, Cairo University  
Botany department.

## Supervision Committee:

Prof. Dr. Hala Mustafa Habib.

(.....Hala M. Habib.....)

Prof. of Virology, Botany department.

Faculty of Science, Cairo University.

Prof. Dr. Mahmoud Imam Nasr.

(.....M. I. Nasr.....)

Prof. of Genetics, former Dean and Founder  
of Genetic Engineering and Biotechnology

Research Institute (GEBRI), Menoufiya University,  
Sadat city.

Chairman of Botany department,

Prof. Dr. Yosri E. Saleh. (.....Yosri E. Saleh.....)

Faculty of Science, Cairo University.

(2005)



Name of the candidate: Alaa El-din Sayed Sayed Ewase Khodeir

Degree : Ph.D

Title of thesis:

Biotechnological studies on potato ( *Solanum tuberosum* L.) plants.

Supervisors : Prof.Dr. Mahmoud Imam Nasr.... Prof.Dr. Hala Mustafa Habib

Department : Botany department

Branch : Cell and Genetics

*Hala H. Habib* *M. I. Nasr*  
Approval.....

---

## Abstract

Potato plantlets were produced free from : PVA, PVM, PVS PVX, PVY and PLRV viruses, by a combination between different meristem sizes and chemotherapy ( virazole). Three different meristem sizes were used ( 0.3, 0.5 and 1 mm) in a combination with five virazole concentrations ( 0, 80, 100, 120 and 150 mg/l ). The studies showed that the most difficult virus to be removed completely was PLRV , where there was a must to use the highest virazole concentration (150 mg/l ) combined with the smallest meristem size ( 0.3 mm ).Then, Random amplified polymorphic DNA (RAPD) markers were used to characterize and detect the variations which might be occurred as a result of virazole presence in potato tissues. Five treatments were used involving different virazole concentrations (0, 80, 100, 120 and 150 mg/l). Ten primers were run here, only six primers were found to be polymorphic.

Also, the effect of virazole on protein content in potato tissues was studied. This process was performed using SDS-PAGE technique for the five treatments of the same concentrations of virazole (0, 80, 100, 120 and 150 mg/l). The results showed that when virazole concentration increases, protein density also increases up to virazole concentration (150 mg/l ), protein density here decreases. This may be due to plants excrete more protein to overcome the negative effect of virazole which delay potato growth. The effect of virazole on some isozymes activity was studied concerning four isozymes (peroxidase ,esterase ,alkaline phosphatase and acid phosphatase).

There is no any effect of virazole on isozyme peroxidase activity in potato tissues.

In case of esterase, increasing virazole concentration led to an increase in the density and the activity of the esterase isozyme.

The results showed also that virazole has a negative effect on the activity of acid phosphatase isozyme in potato tissues.

The final isozyme was alkaline phosphatase, where its activity was very high in control treatment (0mg/l virazole ) while this activity was decreased in all the other treatments with the same degree ( the higher virazole concentration had the same effect like the lower one), so the effect here of all virazole concentrations is similar which decreases alkaline phosphatase density and activity.

Using virus-free potato plantlets, A trial to make an adjustment for transformation conditions in potato was carried out here. *Agrobacterium tumefaciens* ( LBA4404) clones were used , where results were verified by both GUS Assay and PCR reaction.

Virus-free potao microtubers were produced , while the best treatment for microtubers production was that of (0.5 ) mg/l 2,4-D which gave 2.5 microtuber per plant with an average weight of 627 mg.

Also, Potao minitubers were produced where two treatments were carried out here, the first one was involving plants which were cultured *in vitro* in MS supplemented with 2 mg/l silver thiosulphate (STS) , and the other was involving plants which were cultured in normal MS without STS. It was found that the treatment of silver thiosulphate (STS) { 2mg/l } gave the best results for all the parameters.



## Dedication

To the memory of my sincere  
father,

my fiddle and kind mother,

my sincere uncle Moustafa,

my brother and my sister,

my wife and my daughters:

Sara , Hoda and Menna,

to my great professors,

to my relatives and my

deer friends,

and to my honest students

## Acknowledgement

Firstly, I must thank "*The God*" who grant us many benefits continuously. The author would like to express his sincere appreciation to the senior supervisor of this work *Prof. Dr. Mahmoud Imam Nasr*, Professor of Genetics, former dean and founder of Genetic Engineering & Biotechnology Research Institute (GEBRI), Menoufiya university, Sadat City, for his magnificent supervision, valuable advice, continuous help in carrying out all the parts of this work and many sides in his life.

The author also must thank *Prof. Dr. Hala Mostafa Habib*, Professor of Botany, Faculty of Science, Cairo university, for her kind help, great reinforce and indispensable supervision.

Many great thanks to *Prof. Dr. Elsayed Hassan Hassanein*, Professor of Genetics, faculty of agriculture, Ain Shams university, and former president of Menoufiya university for his kind help and formidable support, also to *Prof. Dr. Foteh Eldomiati* for his very fruitful help and kindest support. Great thanks are also presented to *Prof. Dr. Ahmed Bahai El-Din*, *Dr. Ahmed Abdulkhalek*, and *Dr. Rania Youness* in the same institution for their great help.

I would like also to present my unlimited thanks to *Prof. Dr. Ibrahim Abdel-Maksoud Ibrahim*, Professor of Biotechnology and Dean of GEBRI, for his fruitful assistance, continuous patience, unlimited help and superb encouragement.

My unlimited thanks also to *Prof. Dr. Zeinat Kamel Mohammed*, Professor of Microbiology, Faculty of Science, Cairo university for her great support during all the stages of my academic study.

Special thanks to *Prof. Dr. Salem Abdel-Ghani Hassan*, Professor of Food and Dairy Microbiology, National Research Center, for his great support during all parts of this work.

Many thanks also must be presented to *Dr. Gaber Shihab*, lecturer, and *Mr. Nizar Ali*, assistant researcher in plant biotechnology lab., Faculty of Agriculture, Cairo University.

I must thank also *Dr. Alaa Hemida*, Lecturer of Genetics in GEBRI for his scientific help in carrying out the experiment of protein and isozymes.

Acknowledgement

My thanks must be presented to *Dr. Hadia Hekal*, Lecturer of Genetics in GEBRI for her very strong help.

The author would like to present his thanks to *Mr. Abdulmonaim Sharaf*, head of agricultural section in Potato Chips company (Chipsy) for his admirable help.

Thanks are also presented to all staff members of the laboratory of Tissue Culture in GEBRI, , specially, *Dr. Hamdy Emara*, , *Dr. Moselhi El-Shaaer*, *Dr. Adel Hegazy*, *Mr. Hamid Bastawissy* **and all the workers** for their great assistance.

I can't forget now to thank strongly all the members of my family specially my fidele mother ,my brother , my sister, my wife, my three daughters : Sara, Hoda and Menna, and my sincere friends for their indispensable support.



# **Contents**

<b>Title</b>	<b>Page</b>
<b>I-Introduction</b> .....	<b>1</b>
<b>II- Review of Literature</b> .....	<b>5</b>
<b>III- Materials and Methods</b> .....	<b>38</b>
<b>IV- Results and Discussion</b> .....	<b>52</b>
Production of virus-free potato plantlets .....	<b>52</b>
Effect of meristem size and virazole on the percentage of survivals	<b>55</b>
Effect of meristem size and virazole on elimination of some potato viruses	<b>57</b>
Effect of virazole on DNA in potato tissues using RAPD Markers method	<b>60</b>
Effect of virazole on protein in potato tissues	<b>71</b>
Effect of virazole on isozyme peroxidase activity in potato tissues.	<b>80</b>
Effect of virazole on isozyme esterase activity in potato tissues	<b>82</b>
Effect of virazole on isozyme acid phosphatase activity in potato tissues.	<b>84</b>
Effect of virazole on isozyme alkaline phosphatase activity in potato tissues.	<b>86</b>
Adjustment of transformation conditions in Potato.	<b>88</b>
Production of potato microtubers	<b>93</b>
Production of potato minitubers	<b>98</b>
<b>V -Summary</b> .....	<b>102</b>
<b>VI-References</b> .....	<b>108</b>
<b>VII-Arabic summary</b> .....	

## List of Tables

Table no.		Page
<b>Table (1)</b>	Chemical ingredient of Murashige and Skoog's medium .....	40
<b>Table (2)</b>	Different treatments used for virus elimination.	41
<b>Table (3)</b>	Code and sequence of primers used in the present study	43
<b>Table (4)</b>	Detection of Potato viruses A, M, S X, Y, and PLRV in Potato ( <i>Solanum tuberosum</i> L. cv. Lady Rosetta) plantlets using ELISA test.	54
<b>Table (5)</b>	Summation number of polymorphic PCR banding patterns using six primers with all samples.	62
<b>Table (6)</b>	The total specific polymorphic bands in each sample.	63
<b>Table (7)</b>	Molecular weights of common amplified bands between two or more samples.	64
<b>Table (8)</b>	Number of bands in Primer 1 .	65
<b>Table (9)</b>	Number of bands in Primer 2 .	66
<b>Table (10)</b>	Number of bands in Primer 3 .	67
<b>Table (11)</b>	Number of bands in Primer 4 .	68
<b>Table (12)</b>	Number of bands in Primer 5 .	69
<b>Table (13)</b>	Number of bands in Primer 6 .	70
<b>Table (14)</b>	Protein Zero-One table	73
<b>Table (15)</b>	Bands parameters in all lanes.	76
<b>Table (16)</b>	Total specific polymorphic bands in each protein sample	79
<b>Table (17)</b>	Percentage of calli formation and regeneration	89
<b>Table (18)</b>	Effect of 2,4-D and Silver thiosulphate (STS) on production of Potato ( <i>Solanum tuberosum</i> L. cv. Lady Rosetta) microtubers.	96
<b>Table (19)</b>	Effect of Silver thiosulphate (STS) on production of potato ( <i>Solanum tuberosum</i> L. cv Lady Rosetta) minitubers from micropropagated plants in green house.	100

## List of Figures

Figure no.		Page
Figure (1)	Potato tubers ( <i>Solanum tuberosum</i> L. cv. Lady Rosetta).	50
Figure (2)	Sprouted Potato tubers.	50
Figure (3)	Potato leaflets incubated with Ms liquid media with <i>Agrobacterium tumefaciens</i> .	50
Figure (4)	The binary T1 plasmid pBI121 carrying NPT-II as selectable marker for kanamycin resistance and GUS genes beside the T-DNA left and right borders.	51
Figure (5-10)	RAPD profile generated by primers( 1,2,3,4,5 and 6).....	61-66
Figure (11)	Total protein densitometric scans of potato ( cv. Lady Rosetta ).	74-75
Figure (12)	Different protein bands in potato cv. Lady Rosetta.	78
Figure (13 and 14)	Different bands and Zymogram of peroxidase isozyme patterns in potato leaves.	80
Figure (15 and 16)	Different bands and Zymogram of esterase isozyme patterns in potato leaves.	82
Figure (17 and 18)	Different bands and Zymogram of acid phosphatase isozyme patterns in potato leaves.	84
Figure (19 and 20)	Different bands and Zymogram of alkaline phosphatase isozyme patterns in potato leaves.	86
Figure (21)	Potato formed callus	91
Figure (22)	Potato regenerated callus	91
Figure (23)	GUS-expressing blue spots on callus of potato	92
Figure (24)	Agarose gel (1.5%) in TAE buffer stained with ethidium bromide showed the detection of GUS gene.	92
Figure (25)	Potato microtubers	95
Figure (26)	Histogram represent the effect of 2,4-D and STS on tuberization	97
Figure (27)	Potato plants grown in green house.	99
Figure (28)	Potato minitubers.	100
Figure (29)	Potato plant carrying minirtubers.	101