سامية محمد مصطفى



شبكة المعلومات الحامعية

بسم الله الرحمن الرحيم



-Caro-

سامية محمد مصطفي



شبكة العلومات الحامعية



شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم





سامية محمد مصطفى

شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسو

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



يجب أن

تحفظ هذه الأقراص المدمجة يعيدا عن الغيار



سامية محمد مصطفي



شبكة المعلومات الجامعية



المسلمة عين شعور المسلمة عين شعور المسلمة عين شعور المسلمة عين شعور المسلمة ا

سامية محمد مصطفى

شبكة المعلومات الحامعية



بالرسالة صفحات لم ترد بالأصل



Alexandria University
Faculty of Agriculture
(Saba Basha)
Plant Production Dept.

Phenotypic and Genotypic Variation of (Solanum tuberosum, L.) Regenerated Via Tissue Culture

A THESIS

Submitted to the Post Graduate Division of

Faculty of Agriculture (Saba Basha) Alexandria University

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURAL SCIENCES

ln

HORTICULTURE

By

EMAN YOUSSEF MOHAMED HASSAN

2000

B VOVCV 2)Ms

5.0

seg:

3)Ms

 $(R_{\rm F})$



PHENOTYPIC AND GENOTYPIC VARIATION OF (SOLANUM TUBEROSUM, L.) REGENERATED VIA TISSUE CULTURE

Presented By

EMAN YOUSSEF MOHAMED HASSAN

Submitted on Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy in Agricultural Sciences in (HORTICULTURE)

Examiner's Committee:

Approved

A.N.F.

Prof. Dr. HOSSAM EL-DIN MOHAMED FATHY EL-WAKIL Prof. of Genetics, and Dean of Faculty of Agriculture, (Saba Basha), Alexandria University.

Prof. Dr. AHMED YOUSSEF EL-METAINY
Prof. of Genetics, Faculty of Agriculture (El-Shatby),
Alexandria University.

Prof. Dr. ALY IBRAHIM ALY EBIDA
Prof. of Vegetable Crops, Plant Production Department
Fac. of Agric. (Saba Basha), Alexandria University.

Prof. Dr. AHMED NABIL FAYAD
Chief Researches, and Head of Potato Research
Department, Horticulture Research Institute,
Agriculture Researches Center.

SUPERVISOR'S COMMITTEE

Prof. Dr. HOUSSAM EL DEEN MOHAMED FATHY EL-WAKIL

Professor of Genetics, and Dean of Faculty of Agriculture (Saba Basha), Alexandria University

Prof. Dr. AHMED MAHMOUD EL-GAMAL

Professor of Vegetable Crops and Head of Plant Production Department Faculty of Agriculture (Saba Basha), Alexandria University

Prof. Dr. ALY IBRAHIM ALY EBIDA

Professor of Vegetable Crops,
Plant Production Dept.,
Faculty of Agriculture (Saba Basha),
Alexandria University

TABLE OF CONTENTS

INTRODUCTION 1	age
REVIEW OF LITERATURE	
1-Regeneration from shoot tip3	;
2- Regeneration from different callus tissues	
3- Regeneration from tuber discs	
4- Effect of plant growth regulators9	
5- Somaclonal variation	
6- Phenotypic variation1	
7- Isozyme analysis1	
8- Cytological analysis1	
9- Acclimatization of neoformed regenerants	
MATERIALS AND METHODS2	
I. Plant material	
II. Basal Medium2	
III. De novo regeneration from shoot tip culture	23
1. Material's preparation2	
2. Culture media	
2.1. Initiation of leafy stem2	
2.2. Multiplication stage	
2.3. Rhizogenesis stage	25
3. Culture conditions and plantlet acclimatization	25
IV. Organogenesis from leaf and stem calli2	26
1. Plant material explants2	26
2. Culture medium	27
3. Culture conditions and plantlets acclimatization2	!7
V. Organogensis from potato tuber's discs2	28
1. Plant material explants2	28

2. Culture medium	1			•••••
3. Culture condition				
VI. Somaclonal varia	ation		· · · · · · · · · · · · · · · · · · ·	•••••
1. Morphological	evaluation			************
2. Biochemical stu	ıdy (isozyme a	nalysis)		
2.1. Source of sa	mples and prep	paration		
2.2. Starch gel pi	reparation			
2.3. Buffers				
2.3.1. Gel buff	fer			
2.3.2. Electrod	le buffer			
2.4. Electrophore	esis	··········		
2.5. Gel staining				
3. Cytological stud	dies			
RESULTS ÅND DI	SCUSSION		* * * * * * * * * * * * * * * * * * * *	
I. Proliferation from	shoot tip cultu	res		
1.1 Leafy stem in	itiation and noc	lal cutting cu	ılture	
1.2 Rhizogenesis				
II. Regeneration from	m leaf and sten	ı calli		,
III. Regeneration att	empts from po	ato tuber dis	scs	, , , , , , , , , , , , , , , , , , ,
IV. Acclimatization	of regenerants	in greenhou	se	
V. Somaclonal varia				
V.1. Morphologic	cal evaluation		· · · · · · · · · · · · · · · · · · ·	
V.2. Biochemical	study			,
V.3. Cytological s	_			
SUMMARY		••••		
REFERENCES				
ARABIC SUMMAI	RY			
				•
	•			

ACKNOWLEDGEMENT

Sincere thanks are due to **Prof. Dr. Houssam E.M.F. El-Wakil,** Professor of Genetics, and Dean of Faculty of Agriculture (Saba Basha), Alexandria University for his encouragement, valuable advice and continuous help throughout the course of this study.

It gives me great pleasure to express my profound and deep sense of gratitude to **Prof. Dr. A.M. El_Gamal**, Professor of Vegetable Crops and Head of Plant Production Department, Faculty of Agriculture (Saba Basha), Alexandria University for his guidance, advice and encouragement throughout the course of this investigation.

I am highly appreciate to **Prof. Dr. Aly I.A. Ebida**, Professor of Vegetable Crops, Plant Production Department, Faculty of Agriculture (Saba Basha), Alexandria University for suggesting the problem, valuable guidance, encouragement, kind advice, continuous assistance, helpful and creative discussion, and his friendly supervision.

The author wishes to express her sincere appreciation and deep gratitude thankful to **Prof. Dr. Osama M. Badawi**, Director of Sugar Crop Research Station, Sugar Crop Research Institute, Agriculture Research Center, for continuous help during the investigation.

My thanks are due to my colleagues and friends, especially the stuff members of Genetic Engineering Institute, who have helped me in one way or the other to complete this research. My thanks are due to my colleagues and friends, especially the stuff members at the Sabahia Station who have helped or sported me in one way or the other to complete this research.

Finally, my gratitude to my dear husband and my beloved children for their patience and sustained encouragement throughout the course of this study.

NIRODUCTION

INTRODUCTION

Potato (Solanum tuberosum L.) belongs to the family Solanaceae. It is the most important non-cereal food crop in the world. In Egypt, potato is one of the most important vegetable crop grown on a large-scale for either local consumption or exportation abroad to obtain hard currency. However, the total cultivated area was 211,461 feddans in 1998. The reciprocally yield was about 1,984,013 tons with an average of 9.4 tons/ Feddan, (Economical Report of Ministry of Agriculture, 1998). Potato is a crop plant to which in vitro culture technology has extensively been used. Cultures have been raised from shoots and meristems, leaf and stem segments, anther and pollen, tuber explants and from mesophyll and callus protoplasts. Tissue culture techniques can be applied not only to increase propagation rates, but also to modify germplasm (Dodds, 1989). Meristem, shoot tip and nodal segment cultures are used, in general, for production of pathogen free material and rapid propagation too. On the other hand, in vitro regeneration of plantlets from somatic cells is a useful tool in the improvement of potato.

The ultimate goal of the present study is to evaluate the plants derived through different *in vitro* techniques for two potato cultivars grown, widely, in Egypt, phenotypically and genetically.

Therefore, the investigation could be summarized in the following items:

- 1. Regeneration plants from different in vitro techniques, using different explants.
- 2. Investigating the somaclonal variation that might be occur as a result of *in vitro* cultures.

3. Evaluate the somaclonal variation by using evaluational parameters; morphological, biochemical and cytological studies.