# دراسات كيميائيه حيويه على زراعة الانسجه لقصب السكر

رسالة مقدمة من الطالبه حاليا ابراهيم حنهى الجدامي

بكالوريوس العلوم الزراعية - (كميياء حيوية) - كلية الزراعة - جامعة القاهرة (1998) ماجستير الكيمياء الحيويه - كلية الزراعة - جامعة القاهرة (2002)

للحصول على درجةالدكتوراه في العلوم الزراعية (كيمياء حيوية) قسم الكيمياء الحيوية كلية الزراعة كلية الزراعة جامعة القاهرة 2006

## دراسات كيميائيه حيويه على زراعة الانسجه لقصب السكر رسالة مقدمة من الطالبه

#### داليا ابراميم حنهى الجداوي

بكالوريوس العلوم الزراعية – (كميياء حيوية) – كلية الزراعة – جامعة القاهرة (1998) ماجستير الكيمياء الحيويه – كلية الزراعة – جامعة القاهرة (2002) للحصول على درجةالدكتوراه في العلوم الزراعية (كيمياء حيوية)

<u>جسه الإشراف العلمي</u> .	
د/ حسن محمد سالم	•••••
ستاذ الكيمياء الحيوية-كلية الزراعة- جامعة القاهر	
. د/ عبد المنعم محمد نجيب	
ستاذ الكيمياء الحيوية-كلية الزراعة- جامعة القاهر	
/ سعید محمد خلیل	
احث أول– معهد بحوث الهندسه الوراثيه– مركز اا	وث الزراعيه

#### APPROVAL SHEET

Name: DALIA IBRAHIM HANFEY EL-GEDDAWY

Title: BIOCHEMICAL STUDIES ON TISSUE CULTURE OF SUGARCANE

Prof. Dr: Hassan Mohamed Salem

Prof. Dr: Abd-Elmonem Mohamed Naguib

Prof. Dr: Abd El-Wahab Ismaiel Eissa

Prof. Dr: Abd- El Kader Moursy Abd El-Samad

Committee in charge

This thesis has been approved by:

**Date**: 4/11/2006

#### <u>ACKNOWLEDGEMENT</u>

Many thanks and praise be to great Allah most gracious who shined my way and supported me with patience and perseverance to fulfill this humble work.

I wish to express my deep gratitude to *Prof. Dr. Hassan Mohamed Salem*, professor of Biochemistry, Biochemistry department, Faculty of Agric. Cairo University, for his efforts and supervision to fulfill this work.

Many thanks to *Prof. Dr. Abd El-moneim Mohamed Naguib*, professor of Biochemistry, Biochemistry department, Faculty of Agric. Cairo University, for his help and supervision throughout this work.

My sincere appreciation and deep thanks to Dr. Saed Mohamed Khalil Prof assistant, Agricultural Genetic Engineering Research Inistitute, Agric. Research Center, Ministry of Agric. for his supervision, continuous encouragement, valuable advice, guidance, supporting, constructive criticism, writing and reviewing thesis.

I wish to express my appreciation and deep thanks to **DR. Ahmed Abd El-Fatah**, a researcher in Sugar Cane crop Research Institute, Agric. Research Center, Ministry of

Agric. for his help and kind assistance in the green house stage.

Many thanks to *Dr Ahmed El-Sherief and Dr Clara Azam* Prof. assistant in Cell Division-Agronomy Research institute for their advices and great help in many ways.

Special thanks for **Prof DR Abd El-Moneem El-Bana**, Director of Central Laboratory for Palm Research and Development for his generosity and kind help in providing the needed facilities at tissue culture laboratory in El-Zohrya Botanical Garden, Unit of Horticultural Services, Ministry of Agric.-Egypt during his charge and also my deep gratitude to all staff members who aided me a lot.

I wish to express my appreciation and deep thanks to my colleague Heisham Abd El-monein a research assistant in Plant Disease Institute for his great help in directing the thesis presentation.

Finally my sincere thanks that I feel indebted to my family and my precious son "Marwan".

## List of Figures

Figure No.	Title	Page no.
1	The three types of callus.	105
2	The response % of the four varieties to the different concentrations of 2,4-D and coconut water application.	108
3	The ex-plants response for the cultivar Phil8013.	108
4	The ECI % of the four varieties under the different concentrations of 2,4-D and coconut water application.	113
5	The establishment of somatic embryogenesis in the cultivar Phil8013.	115
6	The number of globular embryos/ ex-plant of the four varieties under the different concentrations of 2,4-D and coconut water application.	120
7	The number of torpedo embryos/ ex-plant of the four varieties under the different concentrations of 2,4-D and coconut water application.	124
8	The stages of the somatic embryogenesis under binocular.	125
9	The germination % of the four varieties under the different concentrations of 2,4-D and coconut	129

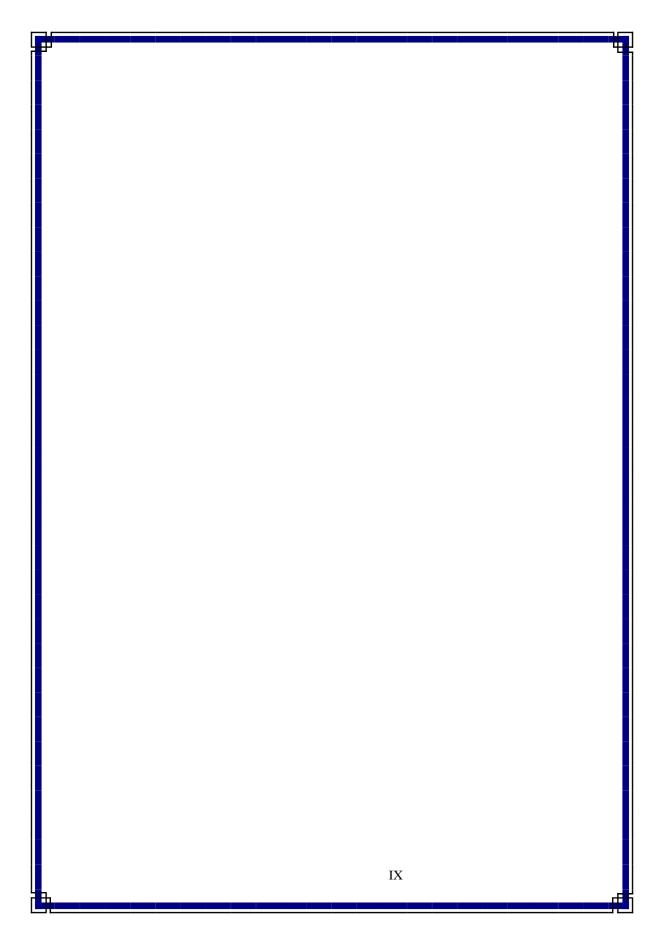
#### water application.

10	The rooting % of the four varieties under the different concentrations of NAA and sucrose application.	134
11	The shooting and rooting elongation stage for the cultivar Phil8013.	134
12	The root length (cm) of the four varieties under the different concentrations of NAA and sucrose application.	138
13	The leaves number of the four varieties under the different concentrations of NAA and sucrose application.	142
14	The shoot length (cm) of the four varieties under the different concentrations of NAA and sucrose application.	146
15	The shoot number of the four varieties under the different concentrations of NAA and sucrose application.	150
16	The growth regulators content in the different cultivar used as ex-plant at the age of 8 months.	152
17	The Acclimatization steps under green house	155

18	Vegetative characters of the examined parent cultivars.	157
19	Juice quality of the parent examined varieties.	159
20	DNA polymorphism of the four sugarcane varieties and the three regenerated varieties amplified with primer (2).	161
21	DNA polymorphism of the four sugarcane varieties and the three regenerated varieties amplified with primer (3).	163
22	DNA polymorphism of the four sugarcane varieties and the three regenerated varieties amplified with primer (4).	165
23	DNA polymorphism of the four sugarcane varieties and the three regenerated varieties amplified with primer (5).	167
24	DNA polymorphism of the four sugarcane varieties and the three regenerated varieties amplified with primer (6).	169
25	Dendrogram for the genetic distances between the four sugarcane varieties and their three regenerated varieties based on RAPD-PCR analysis.	173
26	Dendrogram for the genetic distances between the four sugarcane varieties based on RAPD-PCR analysis.	174

## List of ABBREVIATIONS

ABA	Abscisic acid
<b>AFLP</b>	Amplified Fragment Length Polymorphism
<b>AGERI</b>	Agricultural Genetic Engineering Research Institute
bp	Base pair
СН	Casein Hydrolysate
CM	Coconut Milk
CRD	Complete randomized Design
CV.	Cultivar
$\mathbf{CW}$	Coconut water
2,4-D	2,4 Dichlorophenoxyacetic acid
D.W or dist. H <sub>2</sub> O	Distilled water
ECI	Embryonic Calli Induction
EDTA	
ER	Ex-plant response
$\mathbf{g}$	Gram
$GA_3$	Gibbrellic acid
IAA	Indole Acetic Acid
IBA	Indole Butaric Acid
ISSR	Inter Simple Sequence Repeats
LSD	Least significant differences
$\mathbf{M}$	Mole
$\mathbf{M}\mathbf{g}$	Milligram
Mm	Millimole
ng	nanogram
NAA	Naphthalene acetic acid
NS	Non significant
p	pico
PCR	Polymerase Chain Reaction
PPM	Part per million
RAPD	Random Amplified Polymorhic DNA
SCARI	Sugar Crop Research Institute
SDS	Sodium Dedosyl Sulphate
SDS-PAGE	SDS Polyacrylamide Gel Electrophoresies
SSR	Simple Sequence Repeats
TSS	Total Soluble Solids
u	Unit



## List of Tables

Table No.	Title	Page
1	The Pedi degree and origin of the studied varieties	83
2	The basic constituents of Murashige and Skoog, 1962 used in culturing Sugarcane different varieties.	87
3	The primers sequence	97
4	The suitable concentrations of the commercial Clorox® for sterilization of sugarcane ex-plants.	104
5	The ex-plants response percentage for the four varieties under the different concentrations of 2,4-D and coconut water application.	107
6	The embryonic callus induction (ECI) percentage for the four varieties under the different concentrations of 2,4-D and coconut water application.	114
7	The number of globular embryos / ex-plant for the four varieties under the different concentrations of 2,4-D and coconut water application.	119
8	The number of torpedo embryos / ex-plant for the four varieties under the different concentrations of 2,4-D and coconut water application.	123

9	The germination percentage for the four varieties under the different concentrations of 2,4-D and coconut water application.	128
10	The rooting percentages for the four varieties under the different concentrations of NAA and sucrose application.	133
11	The rooting length (cm) for the four varieties under the different concentrations of NAA and sucrose application.	137
12	The leaves number of the four varieties under the application of different concentrations of NAA and sucrose.	141
13	The shoot number for the four varieties under the different concentrations of NAA and sucrose application	145
14	The shoot length (cm) for the four varieties under the different concentrations of NAA and sucrose application.	149
15	The growth regulators content in the different cultivar used as ex-plant at the age of 8 months	151
16	The relationship between auxin content in media and ex-plant source on ex-plant response (ER) and ECI best combination from 2,4-D and CW	153
17	Vegetative characters of the examined parent varieties	157
18	Juice quality of the examined parent varieties	159
19	DNA polymorphism using randomly amplified polymorphic DNA (RAPD) for the	162

	four sugarcane varieties and the three	
	regenerated varieties with primer (2).	
20	DNA polymorphism using randomly amplified polymorphic DNA (RAPD) for the four sugarcane varieties and the three	164
	regenerated varieties with primer (3).	
21	DNA polymorphism using randomly amplified polymorphic DNA (RAPD) for the four sugarcane varieties and the three regenerated varieties with primer (4).	166
22	DNA polymorphism using randomly amplified polymorphic DNA (RAPD) for the four sugarcane varieties and the three regenerated varieties with primer (5).	168
23	DNA polymorphism using randomly amplified polymorphic DNA (RAPD) for the four sugarcane varieties and the three regenerated varieties with primer (6).	170
24	Similarity index as percentage (Pairwise comparison) among the four sugarcane varieties and three regenerated varieties based on RAPD-PCR analysis.	171
25	Similarity index as percentage (Pairwise comparison) among the four sugarcane varieties based on RAPD-PCR analysis.	172

### **Contents**

1	Introduction	1
2	Review of literatures	6
2.1	General information on sugarcane	6
2.2	Effect of different growth regulators and media composition	14
	on callus and somatic embryogenesis	
	induction	
2.3	Effect of different growth regulators and media composition	59
	on root and shoot	
	elongation	
2.4	Some molecular studies on the sugarcane plants obtained by	<b>73</b>
	tissue culture	
3	Materials and Methods	82
3.1	The optimization of regeneration system for the studied	82
	varieties	
3.1.1	Plant materials	83
3.1.2	Ex-plant isolation and	83
	disinfections	
3.1.3	The culture	84
	media	
3.1.4	Procedures and experiments layout	85
3.1.4.	Effect of Clorox® concentrations with respect to different time	85
1	on free contaminated % ex-plants	
3.1.4.	Effect of different concentrations of 2,4-D combined with	86
2	three concentrations of coconut water in inducing the somatic	
	embryogenesis	
3.1.4.	Effect of different concentrations of NAA combined with five	88
3	concentrations of sucrose on root and shoot induction	
3.1.4.	Chemical analysis and vegetative parameters	90
4	envinent miniges and regenerate parameters.	- 0
3.1.4.	The effect of the regeneration system used on the four varieties	94
5	at the molecular level	•
3.2	Statistical	10
	analysis	1
4	Results and Discussion.	10
=		2

4.1	The best concentration of the commercial Clorox® in ex-plant sterilization with respect to the time
4.2	The effect of using different concentrations of 2,4-D in combination with different coconut water concentration on somatic embryogenesis induction
4.2.1	Callus initiation
4.2.2	Response percentage
4.2.3	The percentage of embryonic calli induction
4.2.4	Number of globular embryos/ex-plant 1
4.2.5	Number of torpedo embryos/ ex-plant 1
4.2.6	Germination percentage
<b>4.3 4.3.1</b>	The effect of using different concentrations of NAA in combination with different sucrose concentration on root and plantlets elongation
4.3.2	Root length
<b>4.3.3 4.3.4</b>	Leaves 1 number Shoot number 1
4.3.5	Shoot length
4.4	Plant Growth Regulators
4.5	Acclimatization
4.6	The Vegetative Characters and juice quality. 1
4.7	Molecular 1 studies
5	Summary 1