

سامية محمد مصطفى



شبكة المعلومات الجامعية

بسم الله الرحمن الرحيم



سامية محمد مصطفى



شبكة المعلومات الجامعية



شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



سامية محمد مصطفى



شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

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



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



سامية محمد مصطفى



شبكة المعلومات الجامعية



بعض الوثائق الأصلية تالفة



سامية محمد مصطفى



شبكة المعلومات الجامعية



بالرسالة صفحات لم ترد بالأصل



Biotechnological Studies of Date Palm: Micropropagation of Inflorescence, Molecular Biology and Secondary Metabolites

By
Adel Ahmed Mahmoud Ahmed Abo-El-Soaud

*B.Sc. Agric., Cairo Univ., 1993
M.Sc. Pomology, Cairo Univ., 1999*

Dissertation

Submitted in Partial Fulfillment of the Requirements for the Degree

***Of
Doctor of Philosophy***

***In
Agricultural Sciences
(Pomology)***

***Pomology Department
Faculty of Agriculture
Cairo University***

2003

B

١٣٤٨٠

Biotechnological Studies of Date Palm: Micropropagation of Inflorescence, Molecular Biology and Secondary Metabolites

By

Adel Ahmed Mahmoud Ahmed Abo-El-Soaud

*B.Sc. Agric., Cairo Univ., 1993
M.Sc. Pomology, Cairo Univ., 1999*

Supervision Committee :

Prof. Dr. / **El-Sayed Ibrahim Bakr**

Professor of Pomology-Faculty of Agriculture-Cairo University.

Prof. Dr. / **Nabeil Raafat El-Sherbeny**

Professor of Pomology-Faculty of Agriculture-Cairo University.

APPROVAL SHEET

Biotechnological Studies of Date Palm: Micropropagation of Inflorescence, Molecular Biology and Secondary Metabolites

By

Adel Ahmed Mahmoud Ahmed Abo-El-Soand

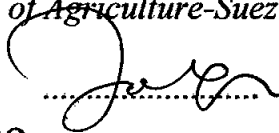
B.Sc. Agric., Cairo Univ., 1993

M.Sc. Pomology, Cairo Univ., 1999

**This dissertation for Ph.D. degree has been
approved by:**

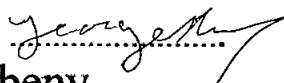
1. Prof. Dr/ Foad Hasan Mohammed

*Professor of Pomology-Faculty of Agriculture-Suez Canal
University.*



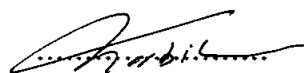
2. Prof. Dr/ George Ramsy Stino

*Professor emeritus of Pomology-Faculty of Agriculture-Cairo
University.*



3. Prof. Dr/ Nabeil Raaft El-Sherbeny

Professor of Pomology-Faculty of Agriculture-Cairo University.



Committee in charge

Date : 18/8/2003

*Pomology Department
Faculty of Agriculture
Cairo University
2003*

Name of Candidate: **Abo-El-Soaud, Adel Ahmed Mahmoud Ahmed** Degree: **Ph.D.**
Title of Dissertation: **Biotechnological Studies of Date Palm: Micropropagation of Inflorescence, Molecular Biology and Secondary Metabolites**
Supervisors: **Prof. Dr / El-Sayed Ibrahim Baker,** **Prof. Dr / Nabeil Raafat El-Sherbeny**

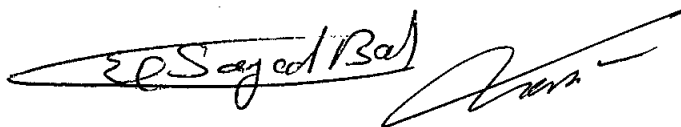
Department: **Pomology Horticulture**
Branch: **Pomology**

Approval: **18/ 8 /2003**

ABSTRACT

The study was performed during the period of 1999 to 2003 in the laboratory of plant tissue culture, Agriculture Development Systems project (ADS), Ministry of Agriculture. The best method to surface sterilize the female spathe of date palm 'Zaghloul' was using HgCl_2 solution at 0.1% for 5 min. The morphological survey of different types of buds within the date palm head was carried out. It is recommended that date palm spikes at 2.5 cm in length would be oriented to somatic embryogenesis as a main target. Nutrient medium ($\frac{3}{4}$ MS and 40 gm l^{-1} sucrose) supplemented with 2.5 mg l^{-1} 2,4-D + 0.5 mg l^{-1} IBA + 0.2 mg l^{-1} 2ip, some of the small florets which existed on the spikes formed direct shoots or roots. At the same time, some florets swelled and formed ball-like structures. The direct proembryos were formed within these structures. Generally, 73.0 % of spike segments were able to form embryos whether directly or indirectly after subculture 3. In respect to date palm spikes at 7.0 cm in length, it is suspected that they must instruct to direct shoot formation (caulogenesis). Only 33.0 % of middle spikes produced direct shoots (vegetative buds) after subculture 2 when they were cultured onto the basal nutrient medium (MS and 50 gm l^{-1} sucrose) supplemented with 2.5 mg l^{-1} 2,4-D + 0.5 mg l^{-1} IBA + 0.2 mg l^{-1} 2ip. Also, the same result was obtained with the nutrient medium which contained 0.5 mg l^{-1} 2,4-D + 0.5 mg l^{-1} IBA + 0.2 mg l^{-1} 2ip. Moreover, the majority of spike explants tend to form direct proembryos better than preceding age. Regarding the spikes in late age of growth and development (20.0 cm in length), no considerable response was recorded. Subsequently, it is not preferable to use an inflorescence as a desirable source for explant in date palm micropropagation at later age of growth (29.0 cm in length). All major amplified DNA fragments of date palm cv. Zaghloul, generated by PCR amplification using the random primers OPD2; OPB18 and OPC14, were detected in all samples, to ensure the genetic stability in the three samples (donor mother plant; *in vitro* propagated plantlets and *ex vitro* plants grown in the greenhouse). The growth of embryogenic callus that used to produce the flavonoids was steadily decreased by increasing the flavonoids content as indicated by the increasing value. It is suggested that spraying with particular substances including ethrel and other promoting substances after traditional offshoots separation is beneficial to secrete more amounts of flavonoids and their relative compounds to improve the natural defense within the palm tree against pathogens invading. Administration rats with 40 mg ethanolic extract of flavonoids incorporated into 100 gm of a diet caused considerable responses in the gain of body weight, organs weight, total lipids, total cholesterol, serum glucose level, and liver and kidney functions as a result of increasing the health of hyperglycemic rats.

Key words: *Phoenix dactylifera* L., Inflorescence, Tissue culture, Somatic embryogenesis, Secondary metabolites, Hyperglycemia.



Dedication

To my parents, you are the unique truth in my life that I love it. You are rare jewels, an ever-flowing river of love, dedication and wisdom. No words can express my feelings towards you. To you I dedicate my love, my life and my work, God blesses you.

Acknowledgement

Firstly my unlimited thanks to **Allah** who always help me and give me the strength and patience to achieve this research.

The author wishes to express his deepest gratitude and appreciation to **Prof. Dr /El-Sayed Ibrahim Baker** and **Prof. Dr /Nabeil Raafat El-Sherbeny** Professors of Pomology, Department of Pomology, Faculty of Agriculture, Cairo University, for giving me many helpful comments and their supervision and encouragement.

Special thanks to **Prof. Dr /Ibrahim Abd-El-Maksoud** Professor of biotechnology, Genetic Engineering and Biotechnology Research Institute, Menofya University, for providing the facilities throughout the course of this study and continuous help.

I am grateful to all members of the department of Tropical Fruit, Horticulture Research Institute, Agriculture Research Center, especially **Prof. Dr /Asem shaltot; Prof. Dr /Galela Ahmed** Chairman of Department **Prof. Dr /Asma Sabour** and **Prof. Dr /Hamdy El-Massry**.

I thank my dearest friends and all members of the Agriculture Development System project (ADS), in fact my teachers who first made me aware of this fascinating subject (tissue culture and biotechnology), particularly **Zeinab, Amal, Rehab and Amera** for their truly co-operation and for valuable advice. You are sunshine in my sky.

Sincere thanks to **Prof. Dr /Emam Abd-El-Rihem** and **prof. Dr/ Omar Abd-El-Aziz** Professors of biochemistry, Department of Chemistry, Faculty of Agriculture, Cairo University, for his worthy advice in part III.

I would like to appreciate all members of Plant Cell and Tissue Culture Department, National Research Center (N.R.C), particularly **Prof. Dr./Mohamed Kamal El-Bahr, Dr /saker; Dr /Hussein Taha** and **Mr /Hatem** for their help in the genetic part.

Table of Contents

	Page
Abbreviations -----	IX
List of Tables -----	XI
List of Figures -----	XVI
1. Introduction -----	1
2. Review of Literature -----	6
2.1. Micropropagation -----	6
2.1.1. Establishment of the inflorescence cultures -----	6
2.1.2. Proliferation stage -----	8
2.1.3. Enlargement and rooting stage -----	9
2.1.4. Weaning and acclimatization stage -----	10
2.2. Genetic Stability -----	12
2.3. Secondary metabolites -----	16
2.3.1. What is secondary metabolism, and what are secondary compounds? -----	16
2.3.2. The function of secondary products -----	17
2.3.3. Accumulation -----	17
2.3.4. Synthesis -----	18
2.3.5. Current trends for in vitro secondary metabolite production -----	18
2.3.6. Flavonoids -----	20
2.3.7. Elicitation -----	20
2.3.8. Factors affecting callus growth and flavonoids content -----	22
3. Materials and Methods -----	26
3.1.1. Micropropagation -----	26
3.1.2. Establishment Stage -----	26
3.1.3. Proliferation stage -----	30
3.1.4. Enlargement; rooting and weaning stage -----	32
3.1.5. Acclimatization stage -----	33
3.1.6. Genetic Stability -----	34
3.1.7. DNA Extraction from the plant materials (Walbot, 1988) -----	34
3.1.8. Gel electrophoresis and DNA visualization -----	36
3.1.9. RAPD analysis -----	38

3.2. Secondary Metabolites -----	38
3.2.1. Chemical constituents -----	39
3.2.2. Preliminary phytochemical screening -----	47
3.2.3. Pharmacological studies: -----	48
3.2.4. Factors affecting callus growth and flavonoids content -----	59
4. Results and Discussion -----	61
4.1. Micropropagation -----	61
4.1.1. Establishment Stage -----	61
4.1.1.1. Sterilization -----	61
4.1.1.2. Inflorescence age -----	62
4.1.1.3. Plant growth regulators -----	63
4.1.2. Proliferation stage -----	115
4.1.3. Enlargement; rooting and weaning stage: -----	118
4.1.4. Acclimatization stage -----	122
4.2. Genetic Stability -----	125
4.3. Secondary Metabolites -----	134
4.3.1. Chemical constituents in source and callus tissues -----	134
4.3.2. Preliminary phytochemical screening -----	143
4.3.3. Pharmacological studies -----	144
4.3.3.1. Effect of flavonoids on hyperglycemia -----	144
4.3.4. Factors affecting callus growth and flavonoids content -----	155
4.3.4.1. Plant growth regulators -----	155
4.3.4.2. Signal molecules -----	162
4.3.4.3. Salt stress -----	167
4.3.4.4. Amino acids -----	174
4.3.4.5. Heavy metals -----	181
4.3.4.6. Carbon source -----	185
4.3.4.7. Fungal and bacterial elicitation -----	195
5. Conclusions and Outlook -----	207
6. Literature Cited -----	214
7. Arabic Summary -----	

Abbreviations

A.O.A.C-----	Association of Official Agriculture Chemists.
°C-----	degrees Celsius.
cDNA-----	complementary DNA.
Cm-----	centimeter.
Conc.-----	concentration.
2,4-D-----	2,4-dichlorophenoxyacetic acid.
dATP-----	deoxyribose adenine triphosphate
dCTP-----	deoxyribose cytosine triphosphate
dGTP-----	deoxyribose guanine triphosphate
dTTP-----	deoxyribose thiamine triphosphate
DNA-----	deoxyribonucleic acid.
dsDNA-----	double strands of DNA.
DW-----	dry weight.
EcoR1-----	<i>Echrichia coli</i> restriction enzyme 1.
Fig-----	figure.
FW-----	fresh weight.
2ip-----	N ⁶ -(Δ^2 - isopentenyl) adenine
g-----	gram(s).
GLC-----	Gas-liquid chromatography.
h-----	hour(s).
GA ₃ -----	gibberellic acid.
Kg-----	kilogram.
l-----	liters.
M-----	molar.
mg-----	milligrams.
min-----	minutes.
ml-----	milliliter.
µg-----	micro gram.
µl-----	micro liter.
mM-----	millimolar.
MS-----	Murashige & Skoog (1962).
N-----	normal.
NAA-----	naphthaleneacetic acid.
ng-----	nano gram.
nm-----	nano meter(10 ⁻⁹).
No-----	number.
NRE-----	non repeated embryos.
PGRs-----	plant growth regulators.
RAPD-----	Random Amplified polymorphic DNA .
rDNA-----	ribosomal DNA.
RE-----	repeated embryo.

RFLPs	-----	Restriction Fragment Length Polymorphisms.
RNAs	-----	RNA types.
RNAse	-----	RNA analysis enzyme.
rpm	-----	revolution per minute.
Rt	-----	retention time.
S ₁ ; S ₂ and S ₃	-----	subcultures 1;2 and 3.
SDS	-----	sodium dodecyl sulphate.
ssDNA	-----	single strands of DNA.
0.5x TBE buffer	-----	Tris Borate EDTA buffer.
2,4,5-T	-----	2,4,5-Trichlorophenoxyacetic acid.
Taq	-----	<i>Thermus aquaticus</i> .
TBE buffer	-----	Tris borate EDTA.