

# بسم الله الرحمن الرحيم



-C-02-50-2-





شبكة المعلومات الجامعية التوثيق الالكتروني والميكرونيلم





# 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 يعيدا عن الغيار













بالرسالة صفحات لم ترد بالأصل



BIVCTV

2

## BIOTECHNOLOGICAL STUDIES ON THE STABILITY OF TISSUE CULTURED DATE PALM PLANTS

By

#### Mohamed Mohamed AbdAlla Saad

B.Sc. (Genetics), Ain Shams Univ. 1977 M.Sc. (Botany) Al-Azhar Univ., 1988.

A thesis submitted in partial fulfilment of the requirement for the degree of DOCTOR OF PHILOSOPHY in

Agricultural Science (Genetics)

Department of Genetics Faculty of Agriculture Ain Shams University

#### APPROVAL SHEET

### BIOTECHNOLOGICAL STUDIES ON THE STABILITY OF TISSUE CULTURED DATE PALM PLANTS

By

Mohamed Mohamed Abd-Alla Saad B.Sc. (Genetics), Ain Shams Univ., 1977 M. Sc. (Botany) Al-Azhar Univ., 1988.

This thesis for Ph. D. has been approved by:

Prof. Dr. Ibrahim I. El- Shawaf
Prof. of Genetics and Vice Dean,
Faculty of Agric. Moshtohor, Zagazig Univ.

Prof. Dr. Abdel Fattah A. Awad Prof. of Genetics, Dept. of Genetics Faculty of Agric. Ain Shams Univ. A.A.Awad

Prof. Dr. Mohamed A. Rashed

Prof. of Genetics, Dept. of Genetics
Faculty of Agric. Ain Shams Univ.

**Date:** / / 2000

### BIOTECHNOLOGICAL STUDIES ON THE STABILITY OF TISSUE CULTURED DATE PALM PLANTS

#### By

#### Mohamed Mohamed AbdAlla Saad

B.Sc. (Genetics), Ain Shams Univ. 1977 M.Sc. (Botany), Al-Azhar Univ., 1988.

#### **Under Supervision:**

# **Prof. Dr. Mohamed Abd El-Salam Rashed**Professor of Genetics

Faculty of Agricuture, Ain Shams Univ.,

#### Prof. Dr. Mahdia Farid Gabr

Professor of Tissue culture and Head Dept. of Plant Genetic Resources. Desert Research Center.

#### **ABSTRACT**

Mohamed Mohamed Abd Alla Saad, Biotechnological studies on the stability of tissue cultured date palm plants. Unpublished Doctor of Philosophy Dissertation, Ain Shams University, Faculty of Agriculture, Genetics Department, 2000.

The main aim of this study was to produce true to of plantlets date palm (Zaghloul cv.) using micropropagation techniques by either axillary proliferation(organogenesis) or somatic embryogenesis through callus, and to typefying these plantlets to the origin mother plants by means of molecular marker (Protien. isozymes and DNA RAPD.

The establishment stage results showed that the contamination percent decresed to 4.7% when explants were resterilized in 2% sodium hypochlorite (NaOCl) for 15min, followed deeping in sodium hypochlorite (6%) for 10sec. before culturing on media (53.4%). The highest survival percent was 95.3% while the lowest was 29%.

The browning percent was high in Autumn (36.1%0 while the lowest was (11.1%) in Summer for the shoot tip explants and with leaf explants the highest browning percent was 76.3% in winter while the lowest was 51.7% in Summer.

The best shoot tip explant growth (6 cm) was observed with media No. 3, 5 and 8 respectively, followed by media contained (0.2 NAA + 0.2 BA + 0.2 2ip) and (0.2NAA + 10.0 BA + 5.0 2ip), which produced (4 cm) in shoot tip length the average of length was 4.8 cm/shoot in Summer, and in Autumn, the best media for shoot growth

(6.8 , 6 cm) were contained (0.2 NAA + 0.2 BA + 0.2 2ip) and (2.0NAA + 0.2 BA + 0.2 2ip) and 5. (5NAA + 2BA+ 2 2ip) The best budding was on media contained (1mg NAA, in Summer and Autumn. The best multiplication of shoot tip was in the presence of 0.5 NAA/l + 0.5 mg BA/l + 0.5 mg IAA/l + 0.5 mg 2ip/l.

The callus formation and growth was best on media containing 100 mg 2,4-D + 3 mg BA or 2ip. The presence of high cytokinin 20 mg/l and low auxin (3-5 mg/l) resulted in

formation of somatic embryos.

The multiplication of these somatic embryos was best on media supplemented with (2-3) mg BA/l and 0.5 mg/NAA/l.

The elongation of the embryos was best in the

presence of 1:2 mg BA/l + 0.5 mg NAA/l.

The rooting was best on media supplemented with 5 mg NAA/l + (1-3) mg IBA/l and the best root length was (34.3 kg) on media containing 5 mg NAA/l + 3 mg IBA/l. The acclamatization percent reached 76% when plantlets cultured on mixture of 1:1 sand and peatmose with high humidity.

The finger printing for the extracted soluble and non soluble proteins for the mother plants and the subcultures revealed that there were no differences in banding patterns. The five isozymes (Est, Px, GOT, Acph and Lap) banding patterns revealed that there were no differences in banding

between the mother plants and the subcultures.

The DNA finger printing using RAPD-PCR revealed that the *in vitro* plants of date palm Zaghloul resulted by somatic embryogenesis were genetically identical.

**Key words**: Date palm, Micropropagation, Genetic Stability.

#### **ACKNOWLEDGEMENT**

Great thanks for Allah for his gifts to us and for every thing.

I feel deep appreciation towards **Prof. Dr.**Mohamed Abd El Salam Rashed, Prof. of genetic,
Faculty of Agriculture Ain Shams Univ. for his supervision,
great helps and continuos support.

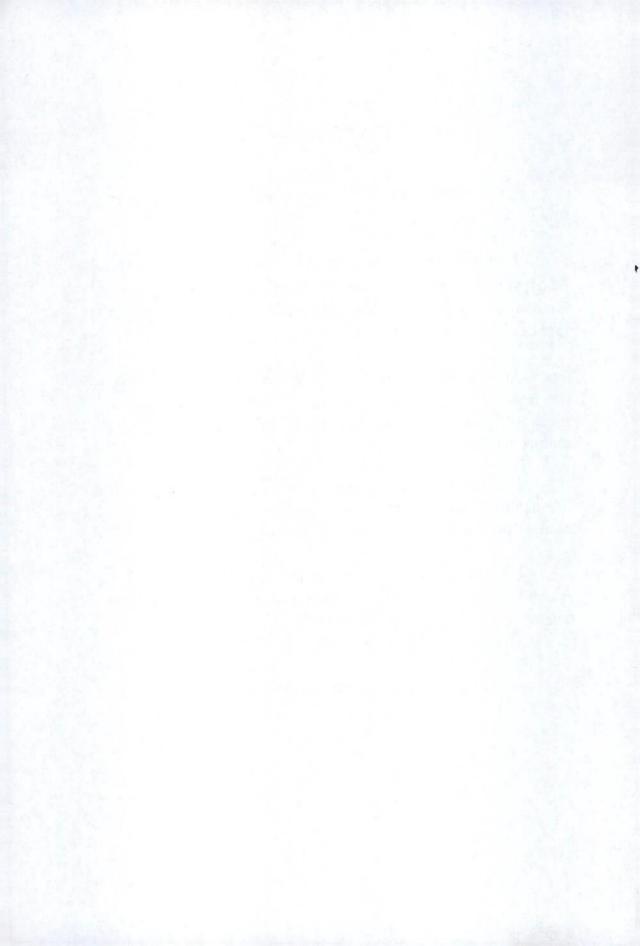
Special thanks for Prof. Dr. **Mahdia Farid Gabr** the head of Genetic resources Dept. Desert Research Center, for her supervision, encouragement and help during tissue culture part of this work.

I wish to produce my thanks to my colleagues in Molecular genetic lab and tissue culture lab for their help and their effort throughout this investigation

I am very grateful to my mother, my wife and my kids, Ahmed, Alaa El Rahman and Aiatul Rahman for their encouragement to me and their patient.

Thanks for all friends

Mohamed Mohamed Abd Alla



## **CONTENTS**

PAG	E
1. INTRODUCTION	1
2. REVIEW OF LITERATURES	3
I. Micropropagation	
1-Type of explant.	3
2 Micropropagation problem	4
2.1- surface sterilization.	
2.2- Browing and seasonal effect	
3- Nutrient media	6
3.1- Basal media	7
3.2- Growth regulators	8
3.2.1. The effect of growth regulators via	
organogenesis	8
3.2.2. The effect of growth regulators	
via somatic embryogenesis	10
a) Initiation of callus and growth	
b) Embryogenesis and regeneration	
4-Rooting of the regenerated plantlets	15
5-Production of Free-Living date palm plantlets	16
II. Genetic stability	19
1-Protein electrophoresis	19
2- Isozymes: electrophoresis	20
3-DNA-RAPD of DATE PALM	25
B-MATERIALS AND METHODS	27
A. Materials:	27
1. Plant material	
2. Plant material for genetic stability	
B- Methods:	28
1. Micropropagation studies	
2- Experimental studies	
a- Nutrient media	

b - Browning	
c - Growth regulators	
1- The effect of growth regulators on	
organogenesis 29	)
a- The effect of auxin and cytokinin	
combination on shoot tips	
b- The effect of auxin and cytokinin	
combination on multiplication stage	
2-The effect of growth regulators on callus	
induction: 30	
a- Callus induction and growth.	
Embryogenesis and regeneration	
3 - Rooting of plantlets	2
4- Production of free -living	2
II – Genetic stability 33	3
1- SDS-PAGE:	
Stock solution.	
a- 1M tris (tris hydroxymethy1) amino	
methane (ph8-8).	
b- 0.25M EDTA (Ethylediamine tetra acetic	
acid).	
C- Sodium dodecyl sulfate (SDS-10% w/v):	
Sample buffer.	
water soluble buffer(ox).	
Water non- soluble buffer (1x).	
Lan's buffer modified form;	•
Gel buffers.	
Resolving gel buffer (4x tris, pH 8.4, 4c):	
Stacking gel buffer pH 6.8.	
2-Isozyme electrophoresis:	)
3- Polymerase Chain Reaction (PCR)	3
3 -1- Stock solutions.	
3-1-1- DNA Extraction.	

3-1-2- Polymerase Chain Reaction (PCR)	
conditions;	
3-1-3-Sample preparation.	
3-1-4- Gel preparations.	
3-1-5- statistical analysis.	
RESULTS AND DISCUSSIONS	47
I- Tissue culture studies	• /
1- Contamination problem.	47
2- Micripropagation Studies:	49
2-A – Browning and season effect	17
2-B- Growth regulators	52
1-The effect of growth substances on	J <u>L</u>
organogensis	52
a- Starting stage.	JL
b- Multiplication stage.	57
2-The effect of growth regulators on somatic	
embryogenesis	60
2-1- Callus induction.	00
2-2- Embryogenesis and regeneration	67
3-Rooting of the regenerated plantlets	74
4-Production of free living plantlets	77
II- Genetic stability:	79
1-Protein electrophoresis.	79
1-1-Water soluble protein	, ,
1-2- Water non-soluble protein	
2-Isozyme electrophoresis	83
/_ L Historian	86
2-2-Peroxides	
2-3-G.O.T.(Glutomate oxaloactate tarns aminase).	89
2-4- Acid phosphates (Acph).	-
2-5- Lucine amino peptidase (LAP).	
3- Random amplified polymorphic DAN of	
the polymerase chain reaction (RAPD –PCR) 9	4
PRIMER OPB- 11	