

بسم الله الرحمن الرحيم





شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم



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



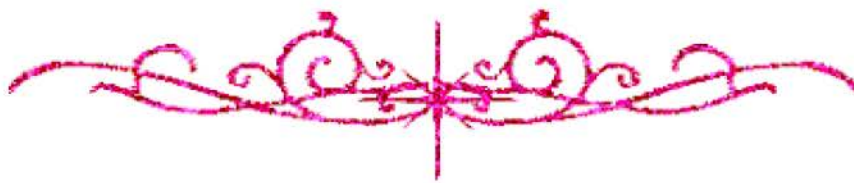
يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار





بعض الوثائق الأصلية تالفة





بالرسالة صفحات
لم ترد بالأصل



B17A7

**ADVANCED STUDIES ON *IN*
VITRO FORMATION OF SYNTHETIC
SEEDS OF DATE PALM**

By
Amany Mostafa Mohammed

B.Sc. Agric., Cairo Univ., 1992
M.Sc. Pomology, Cairo Univ. 1997

THESIS

Submitted in Partial Fulfillment of the Requirements
For The Degree
Of

Doctor of Philosophy

In

Pomology

Pomology Department
Faculty of Agriculture
Cairo University

2002

ADVANCED STUDIES ON *IN VITRO* FORMATION OF SYNTHETIC SEEDS OF DATE PALM

By
Amany Mostafa Mohammed
B.Sc. Agric., Cairo Univ., 1992
M.Sc. Pomology, Cairo Univ. 1997

Supervisors :

Prof. Dr. Amina Hamed Gomaa

*Professor of Pomology,
Faculty of Agriculture,
Cairo University*

Prof. Dr. Ibrahim Abd El-Maksoud

*Vice Dean of Genetic
Engineering and Biotechnology
Research Institute,
Menofya University*

APPROVAL SHEET

Advanced Studies on *in vitro* Formation of Synthetic Seeds of Date Palm

By

Amany Mostafa Mohamed

B.Sc. Agric., Cario Univ., 1992

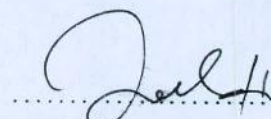
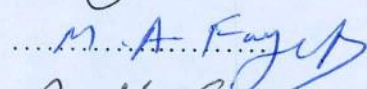
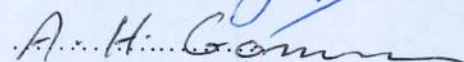
M.Sc. Pomology, Cairo Univ., 1997

This Dissertation for Ph.D. Degree has been approved by:

Prof. Dr. F. H. Mohamed

Prof. Dr. M. A. Fayek

Prof. Dr. A. H. Gomaa


.....

.....

.....

Committee in charge

Date: 4/6/2002

**Faculty of Agriculture
Cairo University**

2002

Name of Candidate Amany mostafa mohamed Degree Ph.D.
Title of Thesis Advanced Studies on *in vitro* Formation of Synthetic Seeds of
Date Palm

Supervisors Prof. Dr. Amins Hamed Gomaa – Prof. Dr. Ibrahim Abd El s
maksoud Ibrahim

Department Pomology
Branch

Approval

ABSTRACT

Conversion frequency after encapsulation in a sodium alginate matrix of somatic embryogenesis (SEs) of date palm (*Phoenix dactylifera* L.) C. V. Samani was evaluate. SEs were hydrogel encapsulate using 4% sodium alginate dissolved in distilled water and 100mM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ within an ion exchange duration for 30 min. to produce synthetic seeds. Beads of uniform size, sufficient firm kept their viability and ability to convert to shoots and roots with considerable delay in conversion frequency when incubated at $20 \pm 1^\circ\text{C}$ day and night temperature under dark condition.

The frequency of *in vitro* conversion into plantlets of synthetic seeds was affected by different nutrient additives included in the encapsulating matrix. The synthetic seeds with MS nutrient and 0.1M sucrose in the capsule had highest conversion frequency. Less resistance to rupture of gel beads resulted from adding gel rite to the sodium alginate.

The highest plantlets formation frequency was observed on half-strength of MS basal nutrient medium solidified with 6 or 8 g/l agar. Liquid media were unsuitable to prolong conversion frequency duration all encapsulated SEs failed to occur conversion frequency on mannitol conversion medium, will encapsulated SEs maintain their viability and regrowth ability through 12 weeks on sorbitol conversion medium. An excellent condition for encapsulated SEs to get a highest significant conversion frequency and maintain them for medium term storage was applied by adding GA_3 to conversion medium with reduction strength to eighth or quarter MS medium. While mannitol combined with sucrose or sorbitol lead to reduce the percentages of conversion frequency but offer prolong duration of conversion frequency. Addition of ABA to either gel matrix or conversion medium decreased significantly the conversion frequency(%).

The artificial endosperm supplied to the capsule serve as a reservoir of nutrients to the SEs allowed storage tolerance and retrained their viability to convert into shoots and roots after 6 months storage duration at low temperature ($4 \pm 1^\circ\text{C}$). Encapsulated SEs with double testa by rinsed within ion exchange duration of 10. min during the first testa formed and rinsed with sterilized distilled water between the formation of the first and second testa extended conversion frequency duration up to 6 months with highest conversion frequency(%) compared with storage at $4 \pm 1^\circ\text{C}$.

Keywords: Synthetic seed, Somatic embryos, Date palm, Artificial endosperm.

A. H. Gomaa

ACKNOWLEDGEMENT

The author's utmost indebtedness and appreciation to *Prof. Dr. Amina Hamed Gomaa*, Professor of Pomology, Department of Pomology, Faculty of Agriculture Cairo University for the great honor of working under her administration, suggestion the research project and all the contributions in the field of facilities, advice and guidance.

I would like to express my appreciation to *Prof. Dr. Ibrahim Abdel-Maksoud Ibrahim* Vice Dean of Genetic Engineering and Biotechnology Research Institute Sadat City, Monofya University and the team leader of the Tissue Culture Activity, Agriculture Development System Project(ADS) for his supervision, valuable assistance, great support during all the steps of the research project.

Special thanks are due to *Prof. Dr. Fayek Badawi* Professor of Pomology, Department of Tropical Fruit, Horticulture Research Institute Agricultural Research Center, for his great help and guidance.

Thanks to *Dr. Abdel-Halim Seif El-Din* researcher of Tissue Culture Central Laboratory for Date Palm Research and Development, Agriculture Research Center, for all his attributions.

My earnest salute and respect to my best friends *Tareq Abdel-Monem* and *Mona Hassan*. Also my greatest respect is paid to my close friends *Sameh El Sarrwy* and *Ezz El-Din Gad. Houda, Rana, Awatif, Enas, Dina, Assem, Ibrahim* and *Ahmed* to whom I owe thanks for their help.

Also my thanks to all my dearest friends and members of the agriculture development system project for their true co-operation and for providing facilities needed to make this work possible.

TABLE OF CONTENTS

SUBJECT	PAGE
1- INTRODUCTION.	1
2- REVIEW OF LITERATURE.	3
3- MATERIALS AND METHODS.	40
4- RESULTS.	57
4-1 Experiment 1: Production of hydrate encapsulated somatic embryos (SEs).	57
4-1-1 Effect of sodium alginate (%w/v) and Ca-salts.	57
4-1-2 Effect of exposure duration to Ca- salt.	71
4-2 Experiment 2 :Effect of incubation conditions.	74
4-2-1 Effect of incubation temperature.	74
4-2-2 Effect of lightness condition.	77
4-3 Experiment 3 : Effect of gel matrix.	79
4-3-1 Effect of gel matrix composition.	79
4-3-2 Effect of sterilized physical form of sodium alginate and the addition of gelrite.	83
4-4 Experiment 4 : Effect of conversion medium.	86
4-4-1 Effect of different concentrations of agar.	86
4-4-2 Effect of physical form.	89
4-4-3 Effect of carbon source.	92
4-4-4 Effect of different concentrations of mannitol and mannitol combined with 0.05 M of sucrose or sorbitol.	95

4-4-5 Effect of different growth regulators concentrations (mg/l).	98
4-5 Experiment 5: Effect of nutrient diffuse between the gel matrix and conversion medium.	105
4-5-1 Effect of different sugars.	105
4-5-2 Effect of different concentrations of sucrose (M).	108
4-5-3 Effect of dissolved sodium alginate with different strength of MS basal nutrient medium or with distilled water.	121
4-5-4 Effect of different concentration of ABA (μ M) added to gel matrix and to conversion medium.	124
4-6 Experiment 6 : Effect of SEs capsules storage.	126
4-6-1 Storage at low temperature ($4\pm 1^{\circ}\text{C}$).	126
4-6-2 Effect of different methods for encapsulated SEs with double testa.	129
5- DISCUSSION.	133
6- SUMMARY AND CONCLUSION.	156
7- LITAREATURE CITED.	176
8- ARABIC SUMMARY.	

1- INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is one of the most Important members of the Areaceae family which contains over 200 genera and over 2500 species (**Corner , 1966; Tomlinson , 1961**).

The exact origin or gene center of the date palm has been lost in history , but evidence of date palm cultivation goes as far back as 4000 B.C. in what is now southern Iraq . The present situation in the world shows a wide belt from the Atlantic Ocean through the Sahara, the Arabian Peninsula , into Iran and the Indus Valley in Pakistan with their main centers of production . Outside this belt the concentrations are much more localized and except for the U.S.A. of less importance worldwide (**FAO,1993**) . The total Egypt area harvest (Ha) of dates is approximately 34.500 . The total production (MT) is approximately 890.340 (**FAO, 1999**). The date palm not only provided a concentrated energy food , due to the nutritious nature of the fruit (**Branton and Blake ,1983**) but also all parts of the palm had a useful purpose . As many as 800 uses have been recorded in the Middle East (**Purseglove, 1972**). Indeed, in many instance date palms are now only grown for the fruit they produce, with little or no use for the secondary palm products (**FAO , 1993**).

Date palm is a dioecious monocotyledonous fruit tree seed propagated palms do not bear true to type due to heterozygosity (**Munier, 1973**) , so propagated vegetatively by offshoot .Offshoot production is limited in date palm , depending on plant vigor and variety consequently, clonal multiplication rate is slow (**Pareek , 1984**).

Somatic embryogenesis (SEs) from vegetative material of Juvenile and adult palm has created many hopes in the field of *in vitro* plant culture . Therefore, high volume propagation potential of SEs combined with formation of synthetic seed which to encapsulated them in an artificial matrix

can be potentially serve as testa , a reservoir for nutrients that may aid the survival and speed the growth of embryo, development control agent and other components necessary for germination and conversion for low-cost delivery would open a new field for clonal propagation (Redenbaugh *et al.*, 1987a).

Synthetic seed technology will have a significant impact on crop production , in both vegetatively propagated and seed propagated crops. For the vegetatively propagated plants, synthetic seed would allow direct planting of clonal varieties and may provide a means for maintenance of elite germplasm (Ganapathi *et al.*,2001). In addition, synthetic seeds thus may provide the only technology realistically amenable to the extensive scale – up required for the commercial production of elite clones (Bornman, 1993). The advantages of using artificial seeds is that they could be easily handled , transportation and potential storage, higher scale-up potential and low cost of production and subsequent propagation (Redenbaugh *et al.*, 1988; Redenbaugh 1990; Rao *et al .*, 1998), is ideally suitable for germplasm exchange between laboratory (Sakamoto *et al .*, 1995).Also, synthetic seed technology may be a value in breeding programs and allow the propagation of many elite genotype derived plant in a short time(Nieves *et al .*, 1998). Synthetic seed of *Phoenix dactylifera* L. somatic embryogenesis would enable easier and more rapid replanting of diseased plantation them propagation by suckers (Daikh and Demarly, 1987).

This investigation would have the way to create the actual form of synthetic seed of *Phoenix dactylifera* L. somatic embryogenesis cv. Samani may allow a new possibilities for handling , potential long-term storage, transportation and delivery of *in vitro* tissue culture of this date palm cv. Samani . Also how far the storage duration of encapsulated somatic embryos could be extend by using *in vitro* storage through minimal growth technique.

2- REVIEW OF LITERATURE

Since the beginning of agriculture plants have been propagated when ever possible by seeds as a convenient means of multiplication, storage and distribution. In many respects, seeds are an ideal propagation system. Propagation of most species in the palm family (Arecaceae) is dependent on seed germination and development (**Kiem, 1958**), but date palm is a dioecious monocotyledonous fruit tree, so sexual propagation by seeds is unsatisfactory because heterozygosity and about half will be females which are not true to type (**Mater, 1986**). Also, the sex cannot determined before flowering within the rang of four to six years (**Shaheen, 1990**).

Date palms are propagated commercially through offshoots, which develop from the base of leave usually during the juvenile phase. This is a difficult and laborious process, but also a very slow way of multiplying the crop, as only a limited number of offshoot are produced in the lifetime of a palm (**Belal and El-Deeb, 1997**). In addition, the method of excision is complicated, time consuming and the percentage of offshoots successfully established in soil is highly variable (30-80 %) (**Veramendi and Navarro, 1996**).

In vitro tissue culture propagation of the date palm (*Phoenix dactylifera* L.) is the most promising method for large-scale clonal propagation of this economically important plant species (**Leary et al., 1986**). Two approaches to *in vitro* propagation are possible for date palm. The first is to exploit the ability of juvenile shoots to produce axillary vegetative buds, but the process is slow and the extend of proliferation is limited. The secondary approach is to use the process of somatic embryogenesis, the asexual differentiation embryo-like structures from diploid plant cells and their subsequent 'germination' and development to give seedling-like plants. Somatic embryos are, in principle, identical genetic