

**GENETIC STUDIES AND PROPAGATION OF
DATE PALM SEEDLINGS USING
TISSUE CULTURE**

By

AHMED GAMAL EL DIN MOHIE EL DIN HASSAN

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2004

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Approval Committee

Dr. MOHAMED SAFWAT A. MOHAMED.....
Professor of Genetics, Head of Microbial Genetics Dept., NRC, Giza

Dr. AHMED MOHAMED ELSHARKAWY.....
Emeritus Professor of Genetics, Fac. Agric., Cairo University

Dr. AHMED NAGIB SHARAF.....
Professor of Genetics, Fac. Agric., Cairo University

Dr. REDA ELWANY A. MOGHAIEB
Assistant Professor of Genetics, Fac. Agric., Cairo University

Date: 28 /10/ 2010

SUPERVISION SHEET

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SUPERVISION COMMITTEE

Dr. AHMED NAGIB SHARAF
Professor of Genetics, Fac. Agric., Cairo University

Dr. REDA ELWANY A. MOGHAIEB
Assistant Professor of Genetics, Fac. Agric., Cairo University

Dr. MOHAMED REDA A. AHMED
Researcher, Plant Genetic Resources, Desert Research Center, Cairo

Name of Candidate: Ahmed Gamal ElDin Mohie ElDin Hassan **Degree:** M.Sc.
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Dr. Reda Elwany A. Moghaieb
Dr. Mohamed Reda A. Ahmed
Department: Genetics **Approval:** 28 /10/ ٢٠١٠

ABSTRACT

Genetic diversity and the degree of relatedness were measured among six date palm varieties (*Phoenix dactylifera L.*) by means of protein banding pattern, isozyme, RAPD, and inter-simple sequence repeat (ISSR) analyses. The levels of polymorphism among the six genotypes were 83.3, 62.5, 60, and 50% as revealed by protein, esterase, GOT and peroxidase banding patterns, respectively. While, 60.2% and 73% genetic polymorphism percentage were obtained by RAPD and ISSR, respectively. Based on the data obtained, it was possible to discriminate among the different genotypes used and to identify the unknown genotype collected from Matroh Governorate. The highest number of RAPD specific markers was scored for the unknown genotype (5 markers) while the cultivar Karamah and Frehi scored three and two markers, respectively. Four ISSR markers characterized the cultivar Karamah while Frehi and Oshkingbil cultivars were characterized by only one marker each. The cluster analysis indicates that the unknown cultivar was closely related to the cultivar Frehi and Oshkingbil. The potential uses of the biochemical and molecular markers in sex identification of *in vivo* grown date palm were investigated. Two male-specific protein bands with the molecular weights of 72 and 47 kDa and another two female-specific bands with the molecular weights of 135 and 55kDa were detected. Female-specific peroxidase band (RF of 0.4) and male-specific esterase band (RF of 0.5) were also detected. Random amplified polymorphic DNA (RAPD) technique was used to compare genetic material from male and female date palm trees. The data indicate that 13 and 5 RAPD markers were found to be female-and male-specific, respectively and can be applied for breeding programs aiming to improve date palms. An efficient protocol for date palm regeneration from petiole explants was developed. The genetic similarity among the regenerated plantlets was determined by analyzing several regenerated plantlets and their mother plant by RAPD analysis which indicates 68% similarity among them.

Key words: Date palm, ISSR, RAPD, sex-specific markers.

دراسات وراثية وإكثار لبعض الأصول البذرية لنخيل البلح
باستخدام تقنية زراعة الأنسجة

رسالة ماجستير
في العلوم الزراعية
(وراثة)

مقدمة من

أحمد جمال الدين محي الدين حسن

بكالوريوس في العلوم الزراعية (تكنولوجيا حيوية) كلية الزراعة - جامعة القاهرة، ٢٠٠٤

لجنة الإشراف

الدكتور/ أحمد نجيب السيد شرف
أستاذ الوراثة - كلية الزراعة - جامعة القاهرة

الدكتور/ رضا علواني عبد الحليم مغيب
أستاذ الوراثة المساعد - كلية الزراعة - جامعة القاهرة

الدكتور/ محمد رضا عبد المجيد أحمد
باحث- قسم الاصول الوراثية- مركز بحوث الصحراء

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لجنة الحكم

دكتور/ محمد صفوت عبد السلام محمد
أستاذ الوراثة – رئيس قسم الوراثة الميكروبية – المركز القومي للبحوث

دكتور/ أحمد محمد الشرقاوي
أستاذ الوراثة غير المتفرغ – كلية الزراعة – جامعة القاهرة

دكتور/ أحمد نجيب السيد شرف
أستاذ الوراثة – كلية الزراعة – جامعة القاهرة

دكتور/ رضا علوانى عبد الحليم مغيب
أستاذ الوراثة المساعد – كلية الزراعة – جامعة القاهرة

التاريخ: ٢٨ / ١٠ / ٢٠١٠ م

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رسالة مقدمة من

أحمد جمال الدين محي الدين حسن

بكالوريوس في العلوم الزراعية (تكنولوجيا حيوية) كلية الزراعة – جامعة القاهرة، ٢٠٠٤

للحصول على درجة

الماجستير

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العلوم الزراعية
(وراثة)

قسم الوراثة
كلية الزراعة
جامعة القاهرة
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المشرفون : دكتور: أحمد نجيب السيد شرف
دكتور: رضا علوانى عبد الحليم مغيب
دكتور: محمد رضا عبد المجيد أحمد

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المستخلص العربي

تم دراسة التباين الوراثي ودرجة القرابة بين ستة من أصناف نخيل البلح على مستوى تحليل كل من البروتينات، المشابهات الإنزيمية، والRAPD وكذلك ISSR. أوضحت النتائج أن نسبة التباين الوراثي المتحصل عليها وصلت إلى ٨٣.٣، ٦٢.٥، ٦٠ و ٥٠% على مستوى كلا من البروتين، الاستيريز، GOT و البيروكسيديز على التوالي بينما كانت النسبة ٦٠.٢ و ٧٣% على مستوى كل من RAPD و ISSR على التوالي. طبقاً للنتائج المتحصل عليها يكون من السهل التمييز بين مختلف التركيب الوراثية المستخدمة وكذلك تعريف الصنف مجهول الهوية وراثياً. ووصلت عدد الواسمات المحددة للصنف المجهول إلى خمسة واسمات (RAPD) بينما كانت هناك ثلاثة واسمات محددة للصنف كرامة و اثنين آخرين محددة للصنف فريحي. و اتضح من نتائج الISSR أن ٤ واسمات تحدد الصنف كرامة وواسم واحد يحدد كل من الصنف فريحي والصنف اوشكينجيل. و أوضحت شجرة القرابة أن الصنف المجهول التركيب الوراثي شديد القرابة للصنفين فريحي و اوشكينجيل. وتم استخدام كل من الواسمات البيوكيميائية والجزيئية في تحديد الجنس في نخيل البلح وأوضحت النتائج أن هناك اثنين من شرائط البروتين عند الوزن الجزيئي ٧٢،٤٧ كيلو دالتون مميزة للذكور واثان أخران عند الوزن الجزيئي ١٣٥،٥٥ كيلو دالتون مميزة للإناث. وتم تحديد شريط بيروكسيديز مميز للإناث عند الRF المساوية لـ ٠.٤ و آخر خاص بالاستيريز عند الRF المساوية ٠.٥ مميز للذكور. وأوضحت نتائج الRAPD أن ١٣ واسم مميز للإناث بينما خمسة واسمات فقط مميزة للذكور ويمكن استخدام هذه الواسمات في برامج التربية لتحسين صفات البلح. وبهدف المحافظة على الصنف مجهول الهوية الوراثية تم إكثاره معملياً باستخدام كل من القمم النامية وكذلك الأجزاء القاعدية لأوراق البادرات الصغيرة وتم التوصل إلى طريقة ذات كفاءة عالية لإعادة الكشف في نباتات نخيل البلح. وكذلك تم تحديد درجة التماثل الوراثي بين النباتات الناتجة من زراعة الأنسجة مقارنة بالنبات الأم عن طريق تحليل هذه النباتات بواسطة الRAPD الذي أوضح وجود درجة تشابه تصل إلى ٦٨%.

الكلمات الدالة: نخيل البلح - الواسمات الوراثية الجزيئية - تحديد الجنس - إعادة الكشف.

DEDICATION

I dedicate this work to whom my heart felt thanks: to my great father who I wish to be a good man like him, mother, sister, Precious wife and my sons Mohamed and Yassin for all the support and encouragement they continually offered along the period of my post-graduate studies. And to every one want that world to be a better place to live.

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INTRODUCTION

The botanical name of the date palm, *Phoenix dactylifera* L., is presumably derived from a Phoenician name "phoenix" which means date palm, and "dactylifera" derived from a Greek word "daktulos" meaning a finger, illustrating the fruit's form (Linné, 1734). Another source refers this botanical name to the legendary Egyptian bird, "Phoenix" which lived to be 500 years old, and cast itself into a fire from which it rose with renewed growth (Pliny, 1489 and Van Zyl; 1983). This resemblance to the date palm, which can also re-grow after fire damage, makes the bird and the date palm share this name, while "dactylifera" originates from the Hebrew word "dachel" which describes the fruit's shape (Popenoe, 1938).

Belonging to the angiosperms-monocotyledones, *Palmaceae* a family of about 200 genera and 1,500 species (Dowson, 1982). Phoenix (*Coryphoideae phoeniceae*) is one of the genera which contains a dozen species, all native to the tropical or subtropical regions of Africa or Southern Asia, including *Phoenix dactylifera* L. (Munier, 1973). According to Dransfield and Uhl, (1986) date palm is classified as follows:

Group:	Spadiciflora
Order:	Palmea
Family:	Palmaceae
Sub-family:	Coryphoideae
Tribe:	Phoeniceae
Genus:	Phoenix
Species:	Dactylifera L.