MOLECULAR AND CYTOGENETIC SOMACLONAL VARIATIONS IN TISSUE CULTURE OF SOME EGYPTIAN DATE PALM (*Phoenix dactylifera* L.) CULTIVARS

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ABSTRACT

Five Egyptian date palm (Phoenix dactylifera L.) varieties were used in this study, i.e. Ammry, Agglani, Zaghloul, Sewi and Ferehi. This work aims to test the biochemical variations and chromosome instabilities and abonrmalities during callus formation in in vitro culture techniques for those five varieties. The study of somaclonal variations were done through: 1- studying peroxidase isoenzymes from callus and primary leaves 2- cytological examination of callus cells suspensions. Results showed some somaclonal variations due to tissue culture exhibited as variablity in band numbers and activities of peroxidase isoenzymes and chromosome numerical aberrations in callus cells.

Key words: *somaclonal variations, peroxidase isoenzymes, tissue culture, chromosome aberrations, Phoenix dactylifera L*

INTRODUCTION

The date palm (*Phoenix dactylifera* L, 2n=36) is the "tree of life" and is just one of many examples of tree crop that benefit immediately from applications of the recent biotechnologies of plant tissue culture. Slow growth, dioecy (separate male and female trees), the slow offshoot-based propagation system and the impossibilities of predicting adult characteristics of the seedlings have severally restricted improvement of this ancient tree crop.

Date palm trees are essential integral components of farming systems in dry and semi-arid regions and can be produced equally well in small farm units or as large scale commercial plantation units. The tremendous advantage of the tree is its resilience, its requirement for limited inputs, its long term productivity and its multiple purposes attributes.

Date palm breeding is hampered by the long generation cycles of the trees. It usually takes more than 30 years to complete three backcrosses and to obtain the first offshoots from an intervarietal cross. To produce sufficient offshoots for testing in the field, other generations are required and if the breeding target is yield or fruit quality even more time will be needed.

Biotechnology tools of tissue culture and genetic engineering can now effectively speed up all of the above processes (improving the characteristics, reduce the periods of breeding programs and improving crop). A tissue culture cycle involves the establishment of a more or less dedifferentiated cell or tissue culture under defined culture conditions (Larkin and Scowcroft 1981) especially hormones concentrations and kinds (Bregitzer *et al.*, 2002and Mousa, *et al.*, 2003), exhibiting somaclonal variations could affect the true-to-type plants produced by tissue culture.

Isoenzymes were used to study relationships between date palm cultivars and also, was used as markers for somatic development in tissue cultured date palm (Baaziz *et al.*, 1994). In this work, isoenzymes, in addition to chromosome examination, were used to identify somaclonal variation in the calli of five Egyptian date palm varieties.

MATERIAL AND METHODS

The present work was carried out in the laboratories of Genetics Department, Faculty of Agriculture, Alexandria University and Nubaria Research Station, Horticulture Research Institute.

Plant Materials:

Five Egyptian date palm (*Phoenix dactylifera* L.) varieties were used in this study, i.e. Ammry, Agglani, Zaghloul, Sewi and Ferehi. Offshoots were collected from different regions of Egypt.

Plant Tissue Culture Medium:

For preparing culture medium, 4.40519 g/l of MS medium (Murashige & Skoog 1962) including vitamins (Duchefa Biochemie BV) + 30 g/l sucrose were dissolved in distilled water pH was adjusted (at 5.6 to 5.8) using 0.1 N of NaOH and HCl solutions. 7 grams (Bacteriological agar agar, European type) was added to the above mentioned solution and autoclaved for 3–5 minutes for melting and dissolving agar completely. Then 20 ml of agar medium were dispensed in suitable culture jars (125 ml volume). Medium was sterilized by autoclaving at 121C° for 20 minutes and stored for 2–3 days in the refrigerator until explants were prepared. Then 1 mg of both growth regulators Kinetin and NAA were added (El-Assar, 2000).

Plant Tissue Culture:

Recent small and healthy offshoots (less than one year old) were separated from vigorous date palm trees of studied varieties. Apical tip (5–10 mm length and 3–6 mm diameter) was excised using sharp sterilized cutter and used as explants for tissue culture (El-Assar, 2000). After culturing the tissues, jars were

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labeled and transferred to aseptic incubation conditions under 27±2°C and 95 % relative humidity in darkness for 3 weeks and then transferred into light conditions for 5 weeks. Cultured tissues were inspected periodically each week and contaminated or dried tissues were removed.

After 8 weeks, the growing tissues were re- or sub-cultured on fresh medium with the same composition. Eight weeks later, tissues were excised for molecular and cytogenetic analysis.

Peroxidase isoenzymes study:

In the present work electrophoresis was used to separate the peroxidase isozymes from the leaves of apical tip and from calli of the five date palm local varieties mentioned before. The buffers, Agar-Starch-PVP gel media, staining solution and the electrophoretic procedure were used according to (Sabrah, 1980 and Yacout *et al.*, 1998). Peroxidase isoenzymes have high nutritive value. Image analysis software " *phoretix*™ TotalLab " version 2003.02, (Nonlinear Dynamics Ltd, U.K), was used to locate isoenzymes bands and to determine enzyme activity in pixel units.

Chromosome Examination:

Numerical chromosome abnormalities were detected in callus cells after 16 weeks from callus initiation. The C-metaphase of callus cells were prepared according to Mousa, (1995) and stained by Giemsa 4%,pH 6.8. Number of aberrant cells was recorded from a total of 150 cells assessed for each variety according to Choi *et al.*, (2000).

RESULTS AND DISCUSSION

Plant Tissue Culture:

After eight weeks from culturing, callus were produced. Tissues were re- or sub- cultured according to its size. Eight weeks later, tissues have been differentiated into different growth shapes (Fig. 1). These growth from one variety to another. Such variations of callus growth shape could be attributed to genetical differences between varieties, since all tissues were cultured under the same conditions.

Peroxidase Isoenzymes:

Peroxidase isoenzymes patterns and activities are shown in Figure (2) and table (1). The band numbers of peroxidase isozymes extracted from the leaves of apical tip and callus for the five studied date palm local varieties ranged between 2 to 4 bands. Those bands could be identified as two cathodal bands (**POX.**C_1 and **POX.**C_2) and two Anodal bands (**POX.**A_1 and **POX.**A_2). The **POX.**C_1 and **POX.**C_2 bands were found in all of the five varieties in either leaves of apical tip or callus, but their stainability, which indicates enzyme activity, varied between and within varieties in some cases. **POX.**C_1 showed higher activity in callus tissues than in leaves. On the other hand, the anodal band **POX.**A_1 appeared in leave extracts for three varieties, i.e. Agglani, Zaghloul and Ferehi, while it was found in all callus extracts. In contrary, **POX.**A_2 band appeared as a new band in the callus of Sewi and Ferehi with low activity and was absent in the other varieties. Concerning **POX.**A_1 activity, Zaghlol callus showed the highest activity in comparison with other calli or leave bands.

Somaclonal variations resulting from tissue culture were observed as variability in band numbers. The band **POX.**A_1 appeared as anew band in the tissue cultures of the varieties Ammry and Sewi, moreover the band **POX.**A_2 appeared also as a new band in the tissue culture of varieties Sewi and Ferehi. Somaclonal variations due to tissue culture have been reported in many plants by Larkin and Scowcroft (1981), Mousa, (1995), Mehta and Angra (2000), and in palm by Tregear *et al.*, (2002) and Gurevich *et al.*, (2005).

Chromosome Examination:

The number of aberrant cells and their percentage in the callus tissues derived from the five date palm in this study are shown in table (2) and Fig. (3). Several types of polyploid and aneuploid cells were observed and scored as aberrant cells. The mean number of aberrant cells was 22.8 and their SD was 7.15. The percentages of numerical chromosome aberrations ranged from 11.3 to 23.3%. This result agreed with previous reports of callus cultures in barley by Orton (1980), Choi *et al.*, (2000) and in potato by Mousa, *et al.*, (2003). Where they reported aberrant cells percentages ranging from 0.3% to 46%. Ferehi showed the highest number of aberrant cells (35 cells) and varied significantly from the rest of the varieties.

Finally, it could be concluded that tissue culture in date palm caused somaclonal variations at the biochemical and cytological levels. Somaclonal variations could be a source of variation for plant breeding and improvement (Larkin and Scowcroft 1981 and Mehta and Angra 2000), On the other hand, to generate normal and true-to-type date palm from tissue culture, somaclonal variation should be screened and eliminated using more accurate genetic patterns by amplification fragment length polymorphism (AFLP) (Gurevich *et al.*, 2005). Also, somaclonal variations could be reduced by using the best hormone concentrations and kinds (Bregitzer *et al.*, 2002and Mousa, *et al.*, 2003).

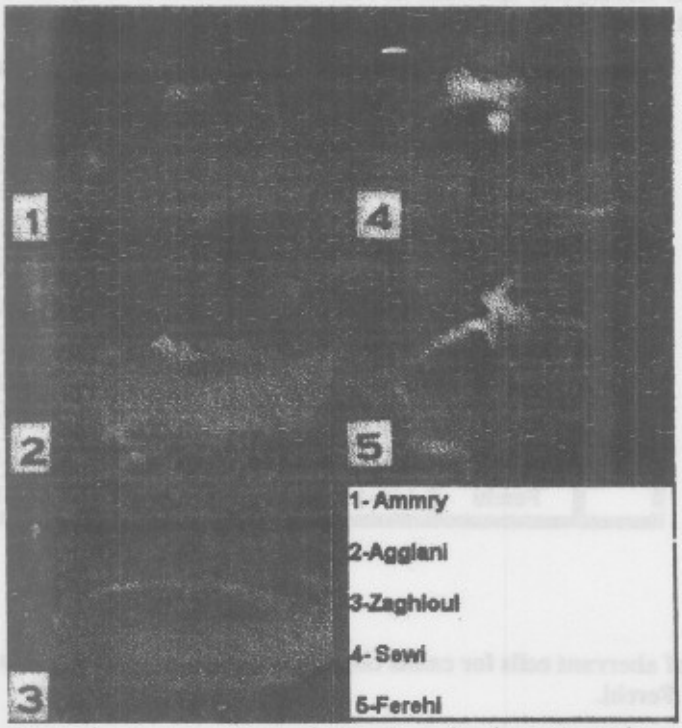

Figure (1): Callus of 16 weeks age for Ammry, Aggiani, Zaghloul, Sewi and Ferehi date palm varieties

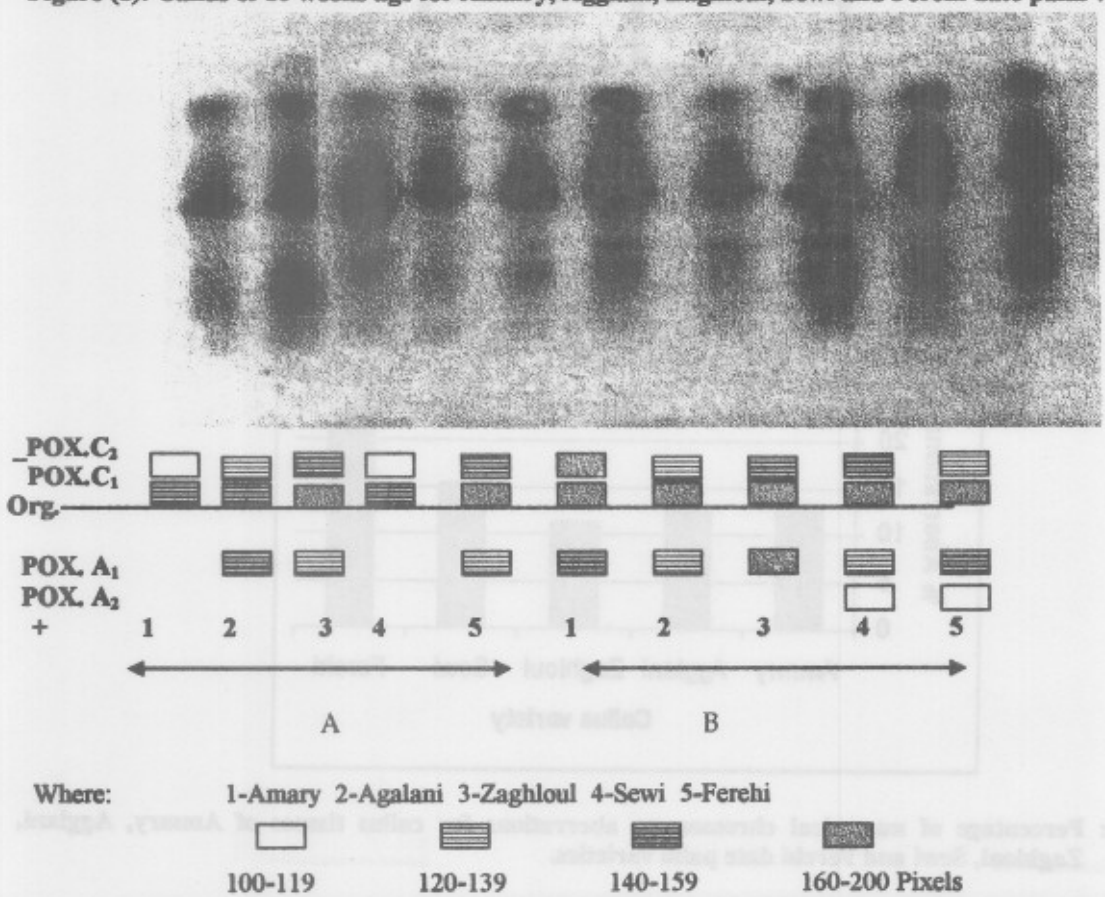

Figure (2): Descriptive diagram of Peroxidase Isoenzymes patterns for leaves of apical tip (A) and callus (B) for five date palm local varieties.

Table (1): Peroxidase isoenzymes activities in Pixel units for leaves of apical tip and callus for five date palm local varieties.

Variety		Peroxidase isoenzymes bands			
		POX. A_1	POX. A_2	POX.C_1	POX.C_2
Leaves of apical tip	Ammry	-	-	140-159	100-119
	Agglani	140-159	-	140-159	120-139
	Zaghloul	120-139	-	160-200	140-159
	Sewi	-	-	140-159	100-119
	Ferehi	120-139	-	160-200	140-159
callus	Ammry	140-159	-	160-200	160-200
	Agglani	120-139	-	160-200	120-139
	Zaghloul	160-200	-	160-200	140-159
	Sewi	120-139	100-119	160-200	140-159
	Ferehi	140-159	100-119	160-200	120-139

Table (2): Number of aberrant cells for callus tissues of date palm varieties; Ammry, Agglani, Zaghloul, Sewi and Ferehi.

Callus variety	Aberrant cells No.± SD
Ammry	20±7.15
Agglani	19±7.15
Zaghloul	17±7.15
Sewi	23±7.15
Ferehi	35±7.15

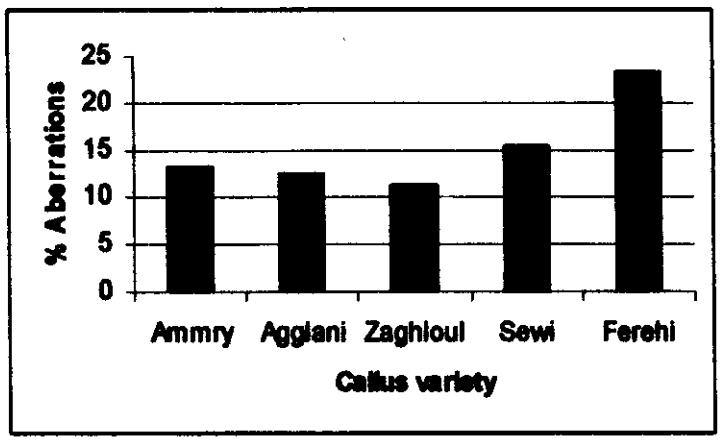

Figure (3): Percentage of numerical chromosome aberrations for callus tissues of Ammary, Agglani, Zaghloul, Sewi and Ferehi date palm varieties.

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الملخص العربى

الاختلافات الجسمية الجزيئية والخلوية فى زراعة الأنسجة لبعض الأصناف المصرية من نخيل البلح (*Phoenix dactylifera* L.)

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تم أستخدام خمسة اصناف مصرية من نخيل البلح فى هذه الدراسة وهى: الزغلول والفريحى والمجهلاتى والمعرى والسيوي. يهدف هذا البحث الى اختبار الاختلافات الكيموحيوية وعدم الثبات والشذوذ الكروموسومى الناتج نتيجة زراعة هذه الاصناف الخمسة فى البيئات الصناعية *in vitro* معمليا.تمت دراسة الاختلافات الجسمية من خلال: ١– دراسة مشابهات انزيم البيروكسيديز فى الكالس ومبادئ الأوراق ٢–الفحص السيتولوجى لمعلق خلايا الكالس. أظهرت النتائج وجود بعض الاختلافات الجسميةالناتجة من زراعة الانسجة على هيئة اختلافات فى عدد ونشاط حزم مشابهات البيروكسيديز وشذوذات عددية لكروموسومات خلايا الكالس.