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PREMATURATION STUDIES ON THE MICROPROPAGATED DATE PALM PLANTS

BY

Abd-Alla, M.M.
Tissue Culture Unite, Genetic Resources Department, Desert Research Center (DRC) Egypt.

ABSTRACT

This study was achieved through 2001-2004 in Tissue Culture Unit of Desert Research Center on some tissue culture derived plants of semi dry *Phoenix dactylifera* cv. Karama. The plants were cultured in Khemesa Farm at Siwa Research Station. The objective of this study is to determine the beginning of sex maturation of date palm tissue culture derived plants and the related marker using SDS-PAGE.

The results showed that the plants exhibited high growth rate after transplanting. The offshoot production was four offshoots/ plant. After five years of culture in field the first fluorescence bud was observed in some plants. At the retrospective and prospective levels, plants showed biochemical marker using SDS –PAGE. The related protein band No. 4 and molecular weight 51 kda were detected in early fluorescenced plants.

Key words: tissue culture, date palm, growth rate, sex maturation protein marker.

INTRODUCTION

Date palm is the most important fruit crop in the Arab countries. Micropropagation provides a practical mean to clone desired palm trees and obtain a large number of high quality and disease-free propagules (Reuveni, 1979; Reynolds and Murashige, 1979; Tisserat, 1981, 1982; Omar, 1988; and Omar *et al.*, 1992). Somatic embryogenesis has been the most successful mean to propagate date palm *in vitro*. Abd Alla (1988) measured the growth rate of callus by using the differences in callus weight. Abd-elrahman (1999) found that tissue culture derived date palm plants were more vigorous than the conventionally propagated ones, and the tissue culture derived plants were uniform and produced significantly much more primary, secondary and aerial off-shoots in comparison to the traditionally propagated ones. Melouk *et al.* (1999) determined the relative growth rate in date palm trunk length. Date palm is monocotyledonous and dioecous plant in which there is two types of sex i.e, male and female plants. So there is a suggestion that may be different expressions between female plants until maturity. The gene expression will appear at a certain time of plant age through the production of flower buds. At this time, the plant reaches sex maturity age. In other plant species, there are three sex types i.e., male, female and hermaphrodite. Hagagy *et al.* (1999) found that finger print of *in vitro Carica papaya* L plantlets assured the possibility of early sex identification at early stages. Pablito and Charles (2003) determined the sex of papaya spp using RAPD PCR. They found variations between male, female and hermaphrodite plant.

Thereupon this study was conducted to study the behavior of date palm tissue culture derived plants after they had been cultured in field, and to determine the time at which plants reach sex maturity with the appearance of the first flower bud, and also to detect the related protein marker by SDS-PAGE. Of date palm plants.

MATERIAL AND METHODS

This work was carried out in the Tissue Culture Unit. Genetic Resources Department. Date palm (*Phoenix dactylifera*, L) tissue culture derived plants were produced via somatic embryogenesis through tissue culture propagation during 2001-2004. *Date palm (Phoenix dactylifera)* tissue culture derived plants were cultured in Sewa Research Station at Khemesa Research Farm, after they were cultured in green house in tissue culture unit in DRC in order to determine the date of sex maturation of plants and the related protein marker by SDS-PAGE methods. Plants height was measured at the first culture and periodically at six months intervals.

The initial lengths (L1)of these plants (sample of 18 plants) were measured at beginning of culture in the farm (termed P1) and successfully every 6 months (termed P2 up to P8) to determine the growth rate of the date palm (*Phoenix dactylifera* L) cv. Karama tissue culture derived plants. Samples of these plants were collected at culture time and after 6 months until appearance of the first flower bud. At this time the changes in proteins profiles using SDS-PAGE at retrospective level and prospective level was detected. The collected samples were stored at - 80°C until use. The differences in lengths (P1, P2, P3...P8) were calculated. The number of offshoot / plant and the growth rate of plants were calculated using plant height according to Abd Alla (1988) and Melouk *et al.* (1999) method as follows:

Gr =P2-P1 /P1. where:

G = growth rate

P1= initial plant height

P2 =second reading of plant height after six months.

For biochemical and genetic studies, SDS-poly accrylamide gel electrophoresis (SDS-PAGE) was performed according to the method modified by Studier(1973) .

RESULTS AND DISCUSSIONS

Data in Table (1) show the differences in plant height through seven periods (six months each) after the initial height of *Phoenix dactylifera* cv. Karama tissue culture derived plants cultured in field (18 plants). The differences between P2 and P1 were measured and between P3 and P2 were measured and so on. There were increasing in the mean height of these plants throughout these periods from 28.22cm to156.67 cm. The highest growth rate of these plants was 55.51% in P2 followed by 37.09%, 29.73%, 32.38% and then decreased till reached 11.02% in P8. This decrease was observed in the latest period. At this time, plants started to produce new offshoots so the growth decreased. The mean differences of the plant height between initial and consecutive periods were 15.67, 16.27, 16.89, 24.77, 16.11, 21.67 and 15.56 cm/plant respectively. The rapid and vigorous growth of tissue culture derived plants encourages plants to produce offshoots and to reach sex maturity rapidly. From these results it is clear that tissue culture derived plants showed rapid growth *In vivo* and there were uniformity between plants in the general view in the farm. These results are agree with those mentioned by Abd-elrahman (1999) who found that, tissue culture derived plants were more vigorous than the conventionally propagated ones, and tissue culture derived plants were uniform and produced significantly much more primary, secondary and aerial off-shoots in the comparison to the normal plants. Melouk *et al.* (1999) determined the relative growth rate in date palm trunk and leaf length. Beginning of the second period of transplanting (12 months) plants started to produce offshoots (12 offshoots) and then at the third period they reached 20 offshoots at P4 followed by 38 offshoots at P5, 42 offshoots at P6, 65 offshoots at P7 and 73 offshoots at the latest period P8. The mean number of the offshoots / plant was increasing from 0.67 offshoots/plant at P3, 1.11 offshoots at P4 and 2.11 offshoots at P5. At period P6 the mean number of offshoot was 3.33 offshoots/ plant followed by 3.61 offshoots at P7 and 4.06 offshoots/ plant at P8. The rapid growth may encourage the growing plants to reach sex maturation early than vegetatively propagated offshoot plants.

Table (1): The increase in high rate and No of shoots of *Phoenix dactylifera* cv. karama tissue culture plants cultured in Sewa every six months

Periods	The differences in height every 6 months/plant over initial height (cm)							
	P1	P2	P3	P4	P5	P6	P7	P8
Average of plant height (cm)	28.222	43.889	60.167	78.056	103.33	119.44	141.11	156.67
Differences (P2-P1)		15.67	16.27	16.89	24.77	16.11	21.67	15.56
Growth rate (P2-P1/p1)		0.555	0.371	0.297	0.324	0.156	0.181	0.110
Growth rate %		55.51	37.09	29.73	32.38	15.59	18.14	11.02
Total of offshoot number		-	12	20	38	42	65	73
Average of offshoot number / plant		-	0.67	1.11	2.11	2.33	3.61	4.06

P1= Plant height at transplanting **P2 – P8**= Plant height every 6 months

At the fifth year of plant age, some plants produced fluorescence buds, which were the first indicator to sex maturity. At this time protein samples of leaves from these plants and their previous samples of these plants were extracted (retrospective level) and also protein samples of leaves from of the unflowered plants at the same time were achieved by SDS-PAGE electrophoresis apparatus with protein marker to calibrate the molecular weight of protein (Table, 2 and Figure, 1). At the retrospective level, there was difference between the protein samples during and before fluorescence in one band appeared at molecular weight of 51 kda . The same difference was found between the flowered plant and the unflowered ones at the same line (prospective unflowered). Data by Hagagy *et al.* (1999), indicated that there are factors involved in sex determination in some plants (*Carica papaya*, L). They studied the finger print of leaves from *In vitro Carica papaya* L plantlets, male, female and herma-phrodite plant using SDS-PAGE for early sex identification. They found that finger print of *in vitro Carica papaya* L plantlets assured the possibility of early sex identification at early stages. Pablito and Charles (2003) determined the sex of papaya. The female plants showed one band in PCR product. Their results are referring that, DNA factors should translate and express into proteins which appear as protein band in SDS-PAGE. This result insures that date palm has the same factors involved in sex maturation. This will be done in other studies (molecular, cytological and chromosomal).

Table (2): Densitometer analysis of SDS-PAGE .band number, Molecular Weight band and intensity as present band or absent.

Band mol. W.	14	13	12	11	10	9	8	7	6	5	4	3	2	1	Band no.
98kda	+	+	+	+	+	-	-	-	-	-	-	-	-	-	1
90kda	-	-	+	+	+	+	+	-	+	+	+	+	+	+	2
80 kda	+	+	+	+	+	+	+	+	+	+	+	+	+	+	3
51 kda	++	+	-	-	-	-	-	-	+	-	-	-	+	-	4
20 kda	+	+	+	+	+	+	+	+	+	+	+	+	+	+	5
15 kda	-	+	+	+	+	+	+	+	+	+	+	+	+	+	6
11 kda	+	+	+	+	+	-	-	-	-	-	+	-	+	+	7
	3	5	5	6	6	4	4	3	5	4	5	4	6	5	Total

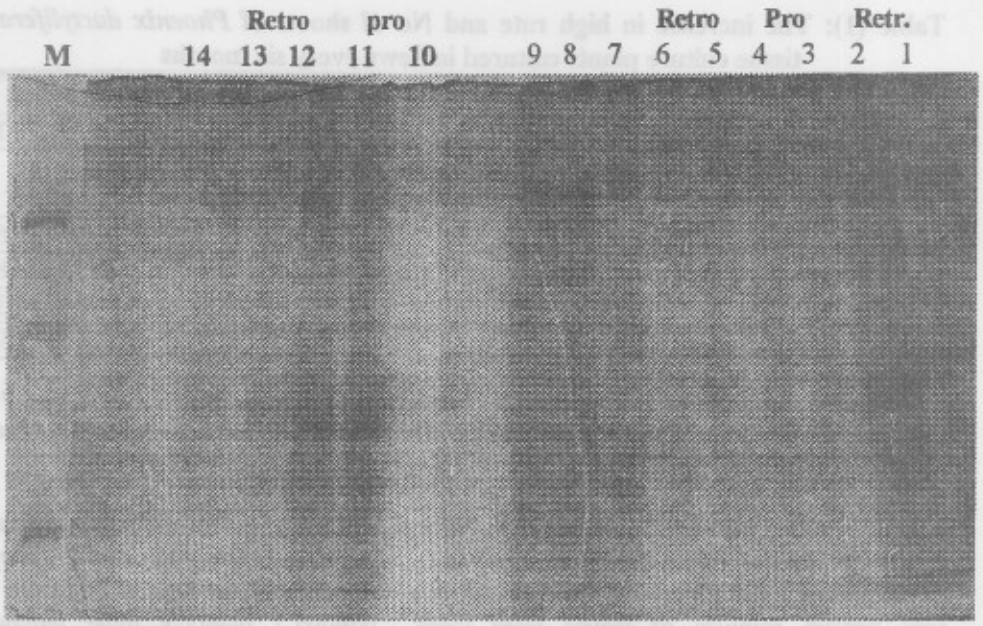

Figure (1): SDS-PAGE profile of date plam leaves protein for the tissue culture derived plants cultured at the prospective and retrospective levels.

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دراسات ما قبل بلوغ الأثمار علىنباتات النخيل المنتجة معمليا

محمد محمد عبدا لله

وحدة زراعة الأنسجة، الأصول الو راثية مركز بحوث الصحراء بالمطرية مصر

أجريت هذه الدراسة على نباتات النخيل الناتجة من زراعة الأنسجة بوحدة زراعة الأنسجة بمركز بحوث الصحراء بالمطرية(مصر) في الفترة من عام ٢٠٠١الى ٢٠٠٤ م علـى النخيـل صـنف كرامـة (النصف جاف) . زرعت هذه النباتات بمزرعة خميسة بمحطة سيوة الزراعية بهدف معرفة توقيت وصـوا نباتات النخيل المنتجة معمليا من زراعة الأنسجة لمرحلة البلوغ الجنسي ومعرفة الحزم البروتينية المرافقـة لها.

أعطت النباتات المنزرعة أربعة خلفات لكل نبات . وقد ظهر اول برعم زهري علي النبات بعـد مرور خمس سنوات من الزراعة الحقلية. وأيضا ظهر بالتفريد الكهربي للبروتينات أن هناك حزمة بروتينية عند ٥١ كيلو دالتون في النباتات التى اعطت أزهار مقارنة بالنباتات الغير مزهرة.