Cytological Studies for Date Palm (*Phoenix Dactylifera* L) Tissue Culture Derived Plants

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Abstract: The cytological studies on the micropropagated date palm (*phoenix dactylifera* var. Karama) revealed that no chromosomal changes occurred during micropropagation and there were 36 chromosomes arranged in 18 bivalents of chromosomes in c-meta phase profile, seventeen bivalents are autosomal chromosomes and XY bivalent in male or XX in female. Chromosome No.2, 3, 4, 5 and 6 were metacentric, chromosomes No. 1,7,8,9 and 10 were submetacentric and chromosomes from No.11 to No17 were subtelocentric. While X chromosome was submetacentric and Y chromosome was subtelocentric. There were no significant differences in the chromosome length, area and centromer positions between the micropropagated plantlets and the mother plants.
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1. Introduction:

Date palm (*Phoenix dactylifera* L.) is a tree crop of economic importance in Egypt. It is finding or represents an income to the oases inhabitants, protects the under-crops from the effects of the climate and reduces the speed and damage from sand storms and wind erosion. Date palm (*Phoenix dactylifera* var. Karama) grew in Siwa Oasis. Plantlets were produced through the micropropagation of this variety (*Phoenix dactylifera* var. Karama) in the Desert Research Centre, Genetic Resources Department, Tissue Culture Lab. It is a dioecious, perennial, monocotyledon, diploid (2n = 36) with long generation time, Almaarry (1995). Somaclonal variations can occur through utilization of tissue culture technique, one of these variations is chromosomal apparitions (duplication. deletion. translocation and ploidy). The progress in computer sciences enabled scientists to improve researches. Vra´na, *et al*. (2000) develops an improved procedure for preparation of chromosome suspensions, and to evaluate the potential of flow cytometry for chromosome sorting in wheat. Torrell *et al* (2001) determinated the chromosome number in the metaphase stage of root tip meristems obtained from 12 *Artemisia spp.* from Armenia and Iran. Mártonfiová (2004), studied Karyology of *Pulsatilla zimmermannii* SOÓ, a pannonian endemic in details according to the position of centromere. Hussein (2005), mentioned that C-banding analysis of plant chromosomes has various applications including construction of karyotype to identify lines with polymorphic banding patterns, to study structural apparitions and other cytogenetics studies. Madon *et al* (2005) studied the cytology of pollen mother cells of oil palm during meioses division. Fregonezi *et al.* (2006) studied the karyotypes of four Brazilian *Cestrum* species (*C. amictum*, *C. intermedium*, *C. sendtnerianum* and *C. strigilatum*) using conventional Feulgen staining, C-Giemsa and C-CMA_3/DAPI banding, induction of cold-sensitive regions (CSRs) and fluorescent *in situ* hybridization (FISH) with rDNA probes. Lanzone and De Souza (2006) used Orcein staining of spermatocytes to study the meiotic behavior of holocentric chromosomes in three member of the genus *Antiteuchus* (commonly known as stink bugs). They describe and illustrate the karyotype of some species. Chengqi (2008), determined somatic chromosome numbers individuals of *Allium przewalskianum* from the Qinghai-Tibet Plateau and five populations were selected for karyotype analysis based on the available chromosome data. Fernandes *et al*. (2009) studied the karyotypes of four South American species of *Cestrum* using conventional staining. Las Peñas *et al* (2009), performed karyotype analyses in members of the four Cactaceae subfamilies. Karyotypes can be used for many purposes; such as, to study chromosomal apparations, cellular function, taxonomic relationships, and to gather information about past evolutionary events. This study aims to assess the chromosomal abberations (number. length. centromer position and size) and to make karyotype for *Phoenix dactylifera* var. Karama.

2. Materials and methods

Plant material

This study was carried out in Tissue Culture Laboratory, Genetic Resources Department, Desert Research Center, Cairo, Egypt; through the years 2006-2009. The micropropagated date palm plantlets (*Phoenix dactylifera* var. Karama) were produced through the micropropagation study for this variety (*Phoenix dactylifera* var. Karama). Some plantlets of this variety grew in Siwa Oasis and other are still in the micropropagation process. Root samples from the *in vitro* plantlets were collected to assess the cytological changes within the micropropagated plantlets through micropropagation process and the mother plants. Embryos of immature seeds from the mother plant were germinated in Petri dishes to get the root tips. Cytological studies and karyotype are based on the morphological characteristics of chromosomes visualization. Karyotype analysis is a well established method (Fukui and Kakeda, 1994). The excised root tips were chemically treated before microscope utilization as follow:

1- Germination of the immature date palm mother plant seeds in Petri dishes at 26-30° C.
2- Collection of lateral root tips from both (tissue culture rooted plantlets and mother plant germinated seeds) when they are (3-4 cm) long.
3- Pretreatment with colchicines (0.25%) for 2h at room temperature.
4- Root tips were fixed in fixation solution (Ethanol alcohol and Glacial acetic acid 3:1) at 4° C for 5 min. then were washed with distilled water.
5- The root tips (2-3mm) were then incubated in enzyme mixture of (4% cellulose and 1% pectenase, 75 mM KCl and 7.5 mM EDTA) on the glass slides at 37ºC for 40 min.
6- The root tips were washed with distilled water to remove enzymatic mixture and then root tips were squashed with a drop of aceto orcein stain after they were flamed.
7- The prepared samples were then examined on microscope using Image Processing Analysis System (Mac-Type).

The cytological profiles of the divided root tips cells of the tissue cultured plantlets and the mother plant samples were imaged by digital camera in the c-metaphase and analyzed using Image Processing analysis system (Video Test Karyotype). The chromosomal characteristics data for each chromosome of the three replicates (R1. R2 and R3) of *Phoenix dactylifera* var. Karama root samples were: (chromosomes number, chromosome lengths (µ), Chromosomes area (µ²), centromeric index percentage(length of short arm/ chromosome length) for each chromosome and chromosomes were arranged according their lengths in a karyotype according to Hussein (2005). For karyotype analysis of *Phoenix dactylifera* the two homologues chromosomes (a and b) of each chromosome pair were judged according to length similarity of short arm, long arm, the total length and centromeric index percentage. An average length, area and centromeric index were calculated (a+b)/2 for each chromosome pair was determined and arranged in descending order and were given their number from 1 to 18.

Means were calculated using the obtained data from each of the three replicates of the tissue cultured plantlets and the mother plants. Mean values of the samples were evaluated by using t.test for paired observation, according to formula: tc = (d`- Mo) / sd`. tc = t calculated value, d`= mean of differences

3. Results and Discussion:

The cytological analysis data at the c-metaphase profile in Table (1) for both of the mother plants and the tissue culture derived plantlets of *Phoenix dactylifera* var. Karama showed the mean length of chromosome of the prepared root tips. There were about 36 chromosomes arranged in 18 pairs of chromosomes according to their lengths for each of the tissue cultured plantlets and the mother plants. There were no numerical changes observed in the c-metaphase of the cytological profile of any of the tissue cultured plantlets or the mother plants. This is in agreement with that of Almaarry (1995) he reported that date palm *Phoenix dactylifera* contains 36 chromosomes (2n). The highest mean length of the micropropagated plantlets chromosomes in c-metaphase profile was 9.93 µ for the chromosome No. 1 and 9.73 µ for the mother plants while lowest mean length was 3.37 µ in tissue culture samples and 3.73 µ in the mother plants for chromosome No. 17. The data in Table (1) revealed that there were no significant differences between the tissue cultured derived samples and the mother plants in the chromosome lengths at the c-metaphase profile, but some insignificant increases within the chromosomes lengths in the c-metaphase profile were recorded from chromosomes No. 1- 14.

This difference may be due to the differences in cell size between the tissue culture samples and the mother plant whereas the tissue culture cells are larger than the mother plant cells. The tissue culture plantlets grew in highly moisture medium. The last chromosome was sex chromosome in which the mean length (6.17 **µ** and 6.61 **µ**) was non significance, and the mean length of Y chromosome (4.17**µ)** was smaller than X

chromosome. These results are in agreement with that of Changqi (2008) used the available chromosome data to make karyotype.

Table (1).The chromosomal data of *Phoenix dactylifera* var. karama mother plant and the tissue culture plantlets at c- metaphase profile.

Chromosome length (μ)					Chromosome area(μ²)				Centromer position %			
Ch no.	M1	M2	t.c	SN	M1	M2	t.c	SN	M1	M2	t.c	SN
1	9.73	9.93	**1.262**	Ns	25.36	26.86	**4.23**	Ns	36.0	35.33	**0.244**	Ns
2	8.00	8.13	**0.994**	Ns	22.33	23.02	**2.561**	Ns	41.33	42.33	**2.212**	Ns
3	7.7	7.8	**0.789**	Ns	20.5	21.14	**2.459**	Ns	44.67	44.33	**3.00**	Ns
4	7.53	7.6	**0.764**	Ns	19.6	20.17	**2.309**	Ns	41.0	42.67	**3.08**	Ns
5	7.07	7.13	**0.726**	Ns	19.4	19.98	**2.336**	Ns	45.33	45.33	**0.91**	Ns
6	6.73	6.8	**0.764**	Ns	19.03	19.5	**2.109**	Ns	44.0	42.67	**2.64**	Ns
7	6.47	6.6	**0.994**	Ns	17.73	18.22	**2.150**	Ns	38.33	37.66	**0.244**	Ns
8	6.1	6.16	**1.071**	Ns	16.56	16.92	**1.88**	Ns	31.67	32.67	**2.213**	Ns
9	6.03	6.1	**0.764**	Ns	15.6	16.33	**2.645**	Ns	27.33	26.67	**0.325**	Ns
10	5.9	5.96	**0.726**	Ns	15.13	15.34	**1.574**	Ns	30.0	29.33	**0.244**	Ns
11	5.57	5.63	**0.726**	Ns	13.8	14.69	**2.973**	Ns	9.00	8.33	**0.123**	Ns
12	5.44	5.5	**0.917**	Ns	13.4	13.9	**2.171**	Ns	8.33	7.67	**0.123**	Ns
13	5.17	5.3	**0.994**	Ns	13.16	14.09	**3.06**	Ns	4.63	4.67	**0.962**	Ns
14	4.97	5.13	**1.109**	Ns	12.8	13.4	**2.38**	Ns	4.53	4.33	**4.06**	Ns
15	4.83	4.87	**0.648**	Ns	11.93	12.1	**1.493**	Ns	4.33	6.33	**3.512**	Ns
16	4.03	4.17	**1.031**	Ns	8.93	9.2	**1.7**	Ns	4.67	4.67	**0.91**	Ns
17	3.73	3.38	**-0.22**	Ns	8.5	8.33	**1.493**	Ns	6.33	6.33	**0.91**	Ns
x	6.61	6.17	**1.19**	Ns	15.06	15.3	**1.653**	Ns	34.65	35.67	**1.04**	Ns
y	4.17				11.3				5.67			
d`			**0.1294**				**0.556**				**0.7**	

Sn=significance level Ns =not significance
t.c= M 1=mean of mother M 2= mean of tissue culture samples
Tabulated t=4.303 calculated t plant chromosome chromosomes

The chromosomes as they were arranged in Table (1) according to the value of chromosome area. The chromosome area of the tissue culture plantlets is high than it in the mother plants. The highest mean area was 26.86 μ² in chromosome No.1 for the tissue culture plantlets and decreased to 8.33 μ² in chromosome No. 17, and in the same trend for the mother plant, the mean of chromosome area was 25.36 μ² and also decreased to 8.5 μ². As it is obvious from Table (1) there is a positive relation between chromosome length and chromosome area, it decreased with decreasing of chromosome length. Data in Table (1) revealed that no significant differences in chromosome area of the 18 bivalents of chromosomes, some increases in chromosome area were observed within tissue culture samples and mother plant; these differences were affected by chromosome length and the cells size of tissue culture samples.

Figure (1) shows that there are 36 chromosomes in the c-metaphase of date palm chromosome *(Phoenix dactylifera* var. Karama), the tissue culture sample (left) and the mother plant (right).The chromosomes are arranged in 17 bivalents and sex chromosome bivalent (XX) appeared at the left corner down of the tissue culture profile, while in the mother plant profile the XY chromosomes are at the right corner down the profile . There were 18 pairs of chromosomes appeared in the profile and no chromosomal apparitions were observed in the c-metaphase profile.

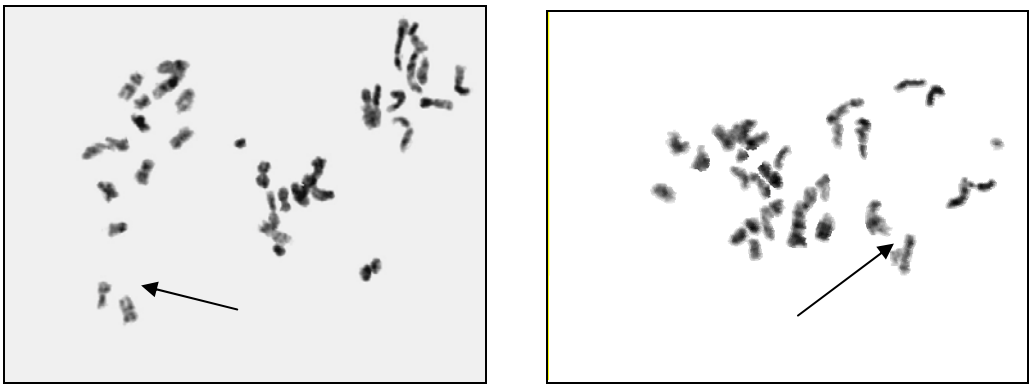


Figure (1) C- metaphase profile date palm *Phoenix dactylifera* var. karama for tissue culture derived plantlets (left) and mother plant (right).

The data in Table (1) illustrated the differences in the centromer positions in c-metaphase of the 18 bivalents of chromosomes. The centromer position was calculated by dividing the length of the short arm / chromosome length. Therefore some chromosomes are metacentric in which the centromer is near the chromosome center. Regarding to the centromer positions of the tissue culture plantlets and the mother plant, chromosome No.2, 3, 4, 5 and 6 their centromer position values were ranging between 45.33 and 41.0 %, probably they are metacentric chromosomes as it obvious in Table (1) and Figure (2). The chromosomes No. 1, 7, 8, 9 and 10 their centromer position values were ranging from 38.33 to 21.67%, their centromer were submetacentric while chromosomes from No.11 to No17, their position values were ranging from 9.0% to 4.33% and subsequently their centromers were subtelocentric Table (1) and Figure (2).

The centromer position of Chromosome X is sub-metacentric, it was existed at 35%, while chromosome Y is subtelocentric, its centromer was existed at 6.0% of the chromosome Table (1) and Figure (2). The karyotype profile of date palm *Phoenix dactylifera* var. karama for tissue culture derived plantlets illustrated the chromosomes according their lengths. The results showed that there were no significant and high similarity within the tissue culture derived plantlets and the mother plant of *Phoenix dactylifera* encarage to make karyotype using tissue culture samples whereas tissue culture plantlets cells still in high dividing state and the cells are larger in size, that enables to study chromosome behavior, characterizations (number, length and centromer position) and the structural apparitions and subsequently to study karyotype. These results are agreement with Terrell *et al* (2001) they determined the chromosome number in metaphase stage of root tips, and Martonfiova (2004) studied the karyotype in details according to the centromer position. Hussein (2005) used the c.banding analysis of plant chromosomes to construct karyotype and to study structural apparitions. Changqi (2008) used the chromosome data to study karyotype in Allium sp.

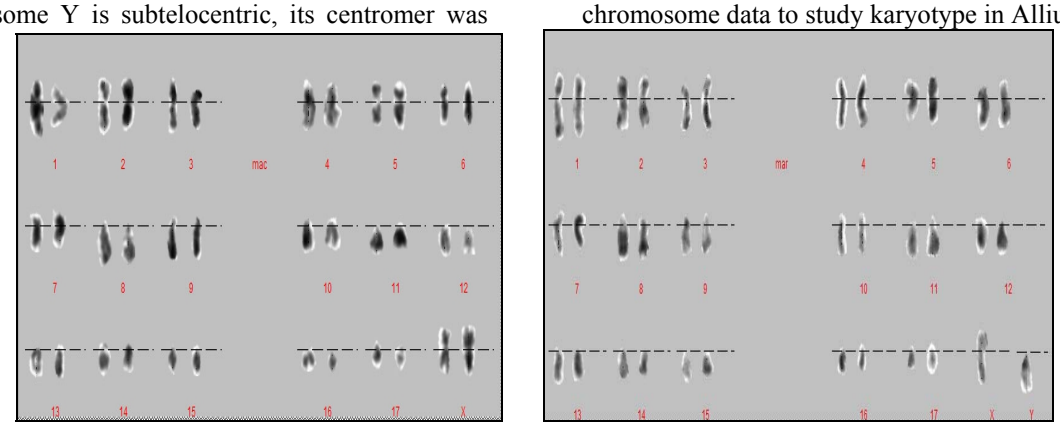
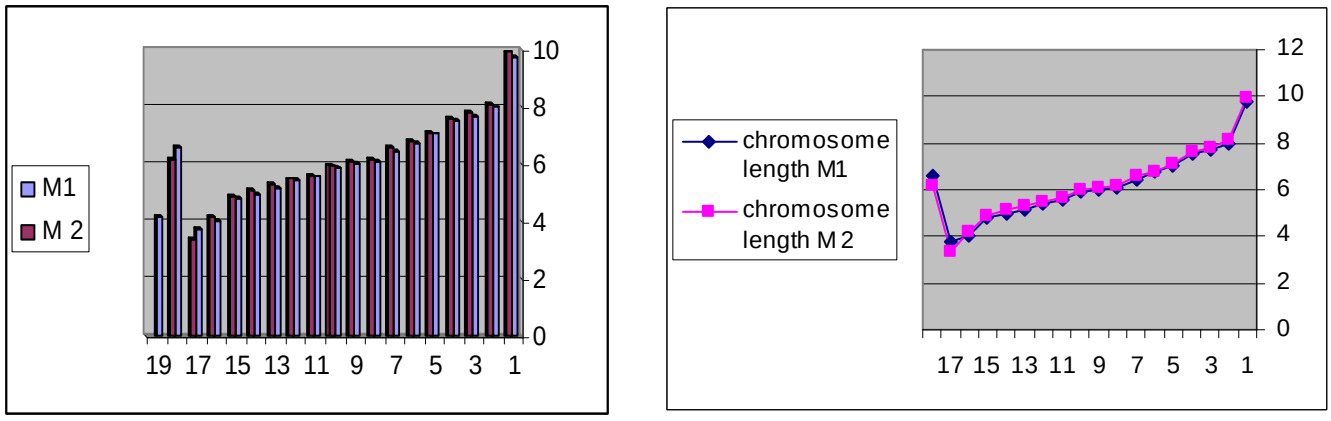


Figure (2) The karyo type profile of date palm *Phoenix dactylifera* var. karama for tissue culture derived plantlets (left) Mother plant (right).



a b

Figure (3) Histogram for chromosome length of tissue cultured plantlets and their mother plant (a and b).

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