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Karyological Study of "Khudari" Date Palm (*Phoenix dactylifera* L.) Seedlings Chromosomes

M.A. SHAHEEN* and M.K. YOUSSEF
Dept. of Arid Land Agriculture, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Saudi Arabia; and Department of Genetics, Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

ABSTRACT. Using the present technique, clear prometaphase figures have been obtained from the primary root tips of "Khudari" date palm (*Phoenix dactylifera* L.) seedlings. The diploid number of chromosomes confirmed the previously reported $2n = 36$. Colchicine treatment for only one hour showed more accurate measurements of total chromosome length and arm ratio than three hours. Karyological analysis of the largest nine pairs of chromosomes revealed that pairs 1, 2 and 4 are submetacentric, pair 3 is metacentric, pairs 6 and 7 are subtelocentric, while pairs, 5, 8 and 9 are acrocentric. The discrepency between these results and those previously reported was attributed to the duration of colchicine treatment. Because of the consistency of results in all preparations, none of the largest nine chromosome pairs was considered to have a role in sex determination in "Khudari" date palm.

Introduction

The date palm *Phoenix dactylifera* L. constitutes an important fruit tree in the Kingdom of Saudi Arabia as in many other Arab countries.

Although many research work have been carried out concerning the physiological aspects of this plant, those dealing with the cytological features are very limited

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(Sharma and Sharma 1976). The first chromosome count in date palm was introduced as $2n = 36$ (Darlington and Wylie 1955). This number was later confirmed by Murin and Chaudhri as cited in "Chromosome number reports" (Love 1970). Report in the context was reconfirmed the findings of Soliman and Al-Mayah 1978. They considered that the lack of a reliable method of producing well spread chromosomes, their small size and relatively large number constitute an obstacle in studying the Karyotype analysis in date palm. Since a precise Karyological study of date palm chromosomes is not available, the present study was carried out to characterize "Khudari" date palm chromosomes.

Material and Methods

Seeds from the date palm cultivar "Khudari" were obtained from the College of Agriculture Research Station, King Saud University, Riyadh. The seeds were germinated according to Said and Murashige (1979) for 25-30 days. At the end of this period, only primary roots of the seedlings were collected and pretreated with 0.02% colchicine for 1 or 3 hours at room temperature. The roots were then washed with distilled water and fixed in ethanol acetic acid (3:1) for at least 48 hours. The fixed roots were then hydrolyzed in 1*N* hydrochloric acid at 60°C for 12 minutes after which they were thoroughly washed with distilled water. The treated roots were immerged in 45% acetic acid for 10 minutes and then stained in 2% acetocaramine solution for 12-18 hours at room temperature.

Slides were prepared by squashing root tips in a drop of 45% acetic acid on a clean slide, covering the squashed material with a coverslip then tapping over gently with an eraser to obtain equal distribution of material. When needed, slides were gently heated to promote maximum absorbance of the dye.

Preparations were examined using a heitz research microscope equipped with a calibrated micrometer eye piece and a built-in camera. For each slide, three micrographs representing different microscopic fields of well spread polar view prometaphase figures were obtained using the 100 × objective piece. A number of 20 preparations were examined for chromosome counts and Karyological characterization.

Karyotypes were then applied using the micrographed chromosome complements by direct measurements by the mean of a thread.

Average chromosome lengths were calculated as the mean total length of each chromosome pair over all measurements obtained for this pair, while standard error was calculated according to the variance obtained between these measurements. Numerical labelling of chromosomes has been set according to their total lengths in descending sequence. Arm ratio denotes the ratio between the right and the left arm, while in other words denote the long to the short arm.

Results

In the present study, good chromosome preparations were obtained from the primary root tips of "Khudari" date palm seeds germinated over a period of 30 days.

Although the great majority of the mitotic figures observed were in the stages of prophase and full metaphase, a proportion of the dividing cells were found in the prometaphase. Colchicine treatment for three hours showed rather short and thick chromosomes with defined chromatid structure, while the cells treated with colchicine for only one hour showed thin and elongated chromosomes. Thus, colchicine treatment for one hour is more suitable for revealing the structure of prometaphase chromosomes. Therefore, the following descriptions are based upon cells in the prometaphase stages treated with colchicine for only one hour.

Table 1 and Fig. 1 present the average total length and standard error, arm ratios (*R/L*) and the Karyotype of the largest nine chromosome pairs of "Khudari" date palm seedlings.

TABLE 1. Average chromosome length (in μm) and arm ratios of the largest nine pairs of date palm chromosomes.

Pair no.	Characteristics	Chromosome length (μm) $\bar{X} \pm S.E.$	Arm ratio *R/L*
1	Macro	5.64 ± 0.17	1.68
2	Macro	5.51 ± 0.12	2.16
3	Macro	3.74 ± 0.88	1.07
4	Macro	3.52 ± 0.14	2.52
5	Macro	2.71 ± 0.17	–
6	Micro	1.81 ± 0.08	3.85
7	Micro	1.53 ± 0.11	4.47
8	Micro	1.27 ± 0.15	–
9	Micro	0.89 ± 0.13	–

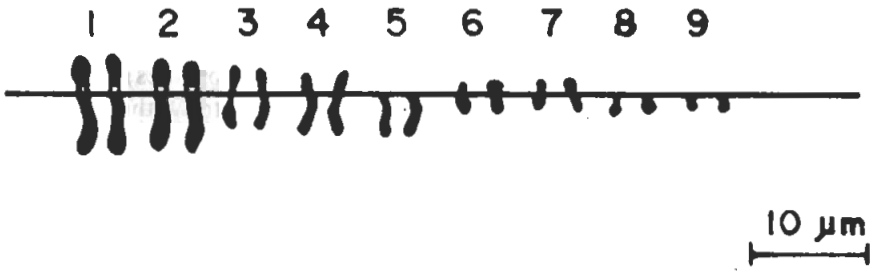

FIG. 1. Karyotype of the largest nine pairs of date palm chromosomes.

The first five pairs are large and distinctive enough to permit the individual identification of these chromosomes (macrochromosomes). The first and second pairs are the longest, and are submetacentric. The arm ratios for these two chromosomes are 1.68 and 2.16, respectively. Pairs three and four are chromosomes of similar length, but the third is metacentric (arm ratio 1.07), while the fourth pair has a dis-

tinct short arm (arm ratio 2.52). In addition, the average total length, arm ratio, and the Karyotype of the largest 4 microchromosomes of "Khudari" date palm seedlings are also presented in Table 1 and Fig. 1. Pairs six and seven are almost of similar length, and both are subtelocentric (arm ratios 3.85 and 4.47, respectively). The eighth pair is obviously acrocentric similar in shape to pair five, but is about the half of its size. Pair nine is also acrocentric. They are followed by nine further pairs of microchromosomes.

Discussions

Using the present technique, clear prometaphase figures have been obtained from the primary root tips of "Khudari" date palm seedlings. The total chromosome number found in this study ($2n = 36$) is similar to that previously reported by Darlington and Wylie (1955), and confirmed by Love (1970), and Soliman and Al-Mayah (1978). However, it was found in the present investigation that the most suitable time for colchicine treatment is only one hour. Other colchicine treatments may affect the accurate measurement of total chromosome length or arm ratio. It has been emphasized that long colchicine treatment causes the contraction of chromosomes, making it difficult to examine the prometaphase figures for Karyological studies (Clement 1971, El-Metainy *et al.* 1980).

Details of the Karyotype of "Khudari" date palm seedlings macro- and microchromosomes have been tabulated in Table 1. The first five pairs are large and can be identified. This agrees with Soliman and Al-Mayah (1978) who reported that chromosome pairs from 1 to 5 are, in particular, much larger than the rest of the chromosome. However, the precise measurement of their arm ratio in this study indicated that chromosomes 1, 2 and 4 are submetacentric and not metacentric as reported by them. This difference may be attributed to the long time of colchicine treatment (5 hours) applied in their study. The Karyological determination of the remaining 2 pairs of macrochromosomes, *i.e.* 3 and 5, are in good agreement with those previously reported in date palm (Soliman and Al-Mayah, 1978). The following thirteen pairs of chromosomes are very small ones (microchromosomes), only four pairs of these microchromosomes were examined in the present study; their Karyological identification revealed that chromosomes eight and nine are acrocentric and not subtelocentric as reported by Soliman and Al-Mayah (1978). This difference may be attributed, again, to the time of treatment with colchicine.

The consistency of results observed in regard to absolute length of chromosomes and arm ratio in all prometaphase figures examined may indicate that none of the nine chromosome pairs studied is involved in sex determination mechanism in "Khudari" date palm.

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دراسات على الهيئة الكروموسومية لبادرات نخيل البلح صنف الخضرى

محمد عبد الرحيم شاهين* ومحمد خليل يوسف

قسم زراعة المناطق الجافة ، كلية الأرصاد والبيئة وزراعة المناطق الجافة – جامعة الملك عبد العزيز ، جدة – المملكة العربية السعودية ، وقسم الوراثة ، كلية الزراعة ، جامعة الإسكندرية – الإسكندرية – مصر .

استخدمت القمم الجذرية النامية لبذور النخيل من صنف « الخضرى » فى عمل تحضيرات سيتولوجية تحتوى على أشكال واضحة من الخلايا فى دور الوضع المتوسط . وقد وجد أن العدد الثنائى للكروموسومات يطابق ماوجد قبل وهو ٢ن = ٣٦ ، إلا أنه وجدت علاقة بين زمن المعاملة (ساعة واحدة) بالكولشيسين ودقة القياسات المتعلقة بالطول الكلى للكروموسومات ومعامل الذراعين لكل منهما .

وقد أوضحت قياسات الهيئة الكروموسومية – بالنسبة لوضع السنترومير – لأطول تسعة أزواج من كروموسومات النخيل أن السنترومير فى الأزواج الأول والثانى والرابع كان قريبا من الوسط ، في حين كان السنترومير فى الزوج الثالث وسطيا . وفى الزوجين السادس والسابع كان السنترومير قريبا من الطرف ، فى حين أنه فى الأزواج الخامس والثامن والتاسع كان طرفيا .

وقد نوقشت تلك النتائج فى ضوء المعلومات السابقة عن كروموسومات النخيل ، وأرجعت الاختلافات بينها إلى التباين فى زمن المعاملة بالكولشيسين . كما اتضح عدم وجود علاقة للأزواج التسعة الكبرى من كروموسومات النخيل الخاصة بتحديد الجنس فى صنف الخضرى وذلك بسبب ثبات النتائج فى جميع التحضيرات . إذ يمثل كل تحضير تركيباً وراثياً مختلفاً .

* أجرى هذا البحث في جامعة الملك سعود بالرياض أثناء عمل المؤلف بها .