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with best wishes.

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**CYTOLOGY AND SEX DETERMINATION  
IN PALMYRAH PALM (BORASSUS FLABELLIFER Linn.)**

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- S.R. RANGASAMY & P. DEVASAHAYAM (Coimbatore, India) : Cytology and sex determination in Palmyrah Palm (*Borassus flabellifer* L.) (avec 1 planche).
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- Z. SWIDERSKI (Genève) : La structure fine de l'oncosphère du cestode *Catenotaenia pusilla* (GOEZE, 1782) (*Cyclophyllidea*, *Catenotaeniidae*) (avec 10 planches, 3 fig. de texte et 1 tableau).

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## INTRODUCTION

The genus *Borassus* includes four to seven species and is dioecious in sex expression. The palmyrah palm, *Borassus flabellifer* Linn. is a tropical one and is widely distributed perhaps next to *Cocos nucifera*, the coconut palm. The palmyrah palm is an economically important one and is the best known for the sweet, jelly like mucilagenous edible and highly palatable endosperm that is rich in sugar. The dioecious nature in sex is prevalent in plants but the sex expression may or may not be associated with the presence of sex chromosomes in a convincing manner. In the present report, an evaluation has been presented on the cytology of male palms, the chromosome behaviour in endosperm cells and the phenotypic distribution of both the sexes in natural population of palmyrah and conclusions had been drawn as also related to the evolutionary trend.

## MATERIALS AND METHODS

The branches of male inflorescence were cut and the young developing male flowers were dissected out from the axils of bracts and fixed in Carnoy's mixture of alcohol : Chloroform : acetic acid in 6 : 3 : 1 proportion. The fixation was done between 9 and 10 a.m. during February-March. The endosperm was also dissected out intact from each carpel from young developing fruits on female spikes at various stages of maturity. The endosperm was cut into pieces and then fixed in Carnoy's mixture. The pollen mother cells were removed from anthers by gentle tapping of the dissected out anthers and stained with iron-acetocarmine by squashing. The procedure adapted for study of cytology of endosperm cells was as follows : each small bit of endosperm (2-4 mm) devoid of endothelium sampled out from different portions of endosperm was placed on the slide and warmed for 30 to 60 seconds with three to four drops of iron-acetocarmine added to it. The stained material was subsequently transferred to another slide and squashed with 2 to 3 drops of iron-acetocarmine. The cover slip was then placed and pressed repeatedly with intermittent warming and then sealed with sealing wax for examination. Figure on karyogram was prepared by arranging the metaphase chromosomes from mitotic cells of endosperm.

## OBSERVATIONS

### 1. Cytology

1. MEIOSIS : Eighteen bivalents are regularly noticed at diplotene, diakinesis and metaphase-I of meiosis on P.M. cells. Of the eighteen bivalents, two are frequently associated with nucleolus. The bivalents exhibited size differences from large to small, seven to nine

being large, five medium and the rest small in size (FIG. 7 and 8). Seldom bivalents disjoin into univalents at diakinesis due to early disjunction. At prometaphase and metaphase-I of the first division of meiosis, pronounced secondary association of bivalents into seven or eight groups is frequently noticed. The group of seven consists of either 1 (4) + 4 (3) + 2 (1) or 1 (4) + 2 (3) + 3 (2) + 2 (1). Among the 5 to 6 small bivalents, one bivalent was characteristically the smallest and rod like (FIG. 10-12). The rod shaped bivalent is also heteromorphic and much attenuated and it is seen often off the plate. The disjunction of chromosomes is regular at anaphase I and II. The pollen fertility was 95 per cent.

2. MITOSIS : The endosperm cells are uniform in size from all parts of endosperm. Two nucleoli are frequently seen in each cell with two to three chromosomes attached to each (FIG. 1). Many of the mitotic chromosomes exhibited clearly the centric constrictions at prophase. The metaphase stage of mitosis in the endosperm cells revealed well spread out and highly condensed chromosomes (FIG. 2-4). The chromosomes at this stage also exhibited distinct size differences. The diplophase is characteristic in the endosperm cells with 54 chromosomes in each cell in the divided stage with centric constriction discernable (FIG. 5). The anaphase division in the endosperm cells is normal and regular. All the endosperm cells showed the presence of 45 chromosomes. Secondary constrictions have been noticed in many of the chromosomes. The arm ratio exhibited differences and the chromosomes are either median or submedian or sub-terminal (FIG. 2). Among the small size chromosomes in the endosperm, one is perhaps the smallest. The squash preparations containing endosperm from all positions and also that portion including embryo and adjoining micropylar region were examined for the size differences of nuclei in embryo as well as endosperm cells. The frequency distribution of size of cells in embryo and endosperm is given in TABLE I. The size of nucleus varied from 7.3 to 15.3  $\mu$  and from 15.3 to 26.3  $\mu$  respectively in embryo and endosperm cells at various developmental stages. The mean size of nucleus of embryo is 10.34  $\mu$  while that of endosperm is 20.32  $\mu$ . There is uniformity in size of cells in endosperm from all positions and perhaps indicating that all cells are uniformly triploid and diploid in status respectively.

TABLE I

*Frequency distribution of size of nucleus in embryo and endosperm cells in Palmyrah*

(Ocular divisions : 1 division = 0.73 $\mu$ )																
<i>Embryo</i>	10	11	12	13	14	15	16	17	18	19	20	21				
<i>No. of cells</i>	3	15	20	18	29	33	15	3	2	6	3	3				
(Ocular divisions : 1 division = 0.75 $\mu$ )																
<i>Endosperm</i>	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
<i>No. of cells</i>	4	3	5	5	32	15	15	13	10	13	11	7	3	3	9	2

TABLE II

*The sex distribution and sex ratio in Palmyrah*

Locations	Pistillate	Staminate	Chisquare value (1 : 1)	P. value
1.	302	280	0.8300	30-50
2.	24	21	0.1467	70-80
3.	60	71	0.9204	30-50
4.	38	31	0.7003	70-80
5.	125	135	0.3800	50-70
6.	969	1049	3.1700	5-10
7.	59	62	0.7439	30-50
8.	34	36	0.0590	80-90
9.	69	73	0.1110	70-80
10.	34	30	1.0000	30-50
11.	290	302	0.2432	50-70
12.	42	49	0.5400	30-50
13.	64	69	0.1900	50-70
14.	75	67	0.4500	50-70
15.	215	187	1.9000	10-20
16.	31	33	0.0600	80-90
	2431	2195	0.0750	70-80

## 2. Sex Expression

The distribution of sex in palms in the grooves from natural population was studied during March-April, in different localities and the data are given in TABLE II. The distribution of male and female sex exhibits equal proportion in nature. The chisquare test also showed a good fit for the sex ratio of 1 : 1.

## DISCUSSION

In the family *Palmae*, only a few genera namely, *Borassus*, *Phoenix* and *Trachycarpus* exhibit dioecious nature. A study of meiosis in palm *Trachycarpus* by SINOTO (1929), indicated the presence of allosomes in male sex. Similarly indications of allosomes in date and palmyrah palms had been also made by SARKAR (1957) the constitution of male sex being XY. BEAL (1937) made cytological studies in *Phoenix* but made no indication of sex chromosomes in date palm. The criterion for the establishment of presence of allosomes or sex chromosomes is on the basis of (1) the heterogametic sex possessing unequal pairs of chromosomes, (2) absence of unequal pairs in the homogametic sex and (3) the identification of sex chromosomes in both the sexes (WESTERGAARD, 1958).

The course of meiosis in PM cells of *B. flabellifer* in the present study indicated the heteromorphic nature in one of the pairs. The male sex is possibly XY and female is XX. The endosperm is in triploid condition possessing 18 sets, each set with three chromosomes. The endosperm cells from each developing carpel may be either all X chromosomes (XXX) or XX + Y chromosomes depending on whether the egg is fertilised by male gametes carrying either X or Y chromosome. The karyotype as arranged from endosperm cells also

possibly show that the last group of three is perhaps composed of all X chromosomes, or 2 X and the Y chromosomes.

The presence of sex chromosomes or indications of the presence of the same in either one sex alone is not sufficient to understand sex determination and hence an evaluation of the sex distribution is also essential. The estimation on sex distribution in the present study showed that the sex ratio was 1 : 1 (TABLE II) and thereby confirmed that the sex in palmyrah is due to one sex being heterogametic and probably controlled by one pair of heteromorphic sex chromosomes and the mechanism of sex determination is associated with that pair of sex chromosomes. The male sex in palmyrah is heterogametic and possesses heteromorphic chromosomes. It is considered that mostly male sex alone is in general heterogametic in plants. (WESTERGAARD, 1958). Rarely other mechanism will also operate as in *Myristica* (FLACH, 1966). There are also cases of dioecism in plants with no heteromorphic sex chromosomes (ALLEN, 1940) of the distinction of the sex chromosomes is not highly distinct and always not convincing owing to smallness in size (WESTERGAARD, 1958, DRONAMRAJU, 1965). The segregation of staminate and pistillate palms in palmyrah in 1 : 1 sex ratio along with the evidence from meiosis in male palms, however, establishes the type of XX-XY mechanism of sex determination in palmyrah. A critical study of karyotypes in both sexes and pollen mitosis may perhaps render further evidences on the mechanism that operates in this tropical dioecious palm.

The somatic (2n) chromosomes in palmyrah is 36 and the present study confirmed the counts on chromosomes previously made (DARLINGTON & WYLIE 1955). The ancestors of *Borassus* and *Phoenix* were considered originally as monoecious with  $n = 9$  without sex chromosomes. The trend of evolution was thus hypothesised from bisexual flowers to unisexual but monoecious and finally dioecious (SHARMA & SARKAR, 1956, CORNER, 1966). It is now considered that the sex distinction in the palms arose only after doubling of chromosomes (SARKAR, 1957, CORNER, 1966) and also involving alteration in a single pair of chromosomes into heteromorphic one.

The secondary association which is generally relied on to infer basic chromosome number revealed the presence of seven or eight groups in meiosis in Palmyrah and thereby 7 or 8 to be the probable primary base chromosome number. A survey of published counts of chromosome numbers in Palms indicates either  $n = 8, 14$  or  $16$ , or presence of  $n = 16$  and  $18$ . Thus it may be that the evolution of the present day palmyrah palm with its tetraploid status and  $2n : 36$  is by tetraploidy probably through secondarily balanced allopolyploidy.

### SUMMARY

The course of meiosis in the P.M. cells of palmyrah palm, *Borassus flabellifer* Linn. was regular and exhibited eighteen bivalents. Among the bivalents, one pair was characteristically small and heteromorphic and related to the sex determination in this dioecious palm. The small pair comprises X and Y chromosomes. This was elaborated by the cytology of endosperm cells which showed a normal mitotic division besides being either XXX or XXY in constitution of sex chromosomes corresponding to the zygote being either male or female in sex. Size variations in nucleus was noticed in mitotic division in embryo and endosperm

and was in consonance with the level of ploidy. The sex determination was further confirmed and corroborated by data on segregation of male and female sex in natural population showing a marked fit to 1 : 1 sex ratio. The existence of heterogametic nature in one sex namely in male through the differentiation into heteromorphic sex chromosomes in the palmyrah palms is thus brought out in the present study. The probable evolution of genus *Borassus* is also indicated to be from the primary base number of  $X = 7$  or 8 to the present day status through the allotetraploid level.

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## EXPLANATION OF PLATE

FIG. 1-6 and 9. Cytology of endosperm in palmyrah palm

FIG. 1. Prophase.

FIG. 2,3 and 4 : Prometaphase stage showing 54 chromosomes.

FIG. 5. A cell showing diplo-chromosomes ( $2n : 54$ )

FIG. 6. Division of chromosomes in early anaphase.

FIG. 9. Karyogram of palmyrah palm showing 18 sets of mitotic chromosomes each set with three chromosomes as prepared from an endosperm cell at metaphase.

FIG. 7-8 and 10-12 : Meiosis in PM cells of palmyrah palm.

FIG. 7 and 8. Cells showing eighteen bivalents at diakinesis.

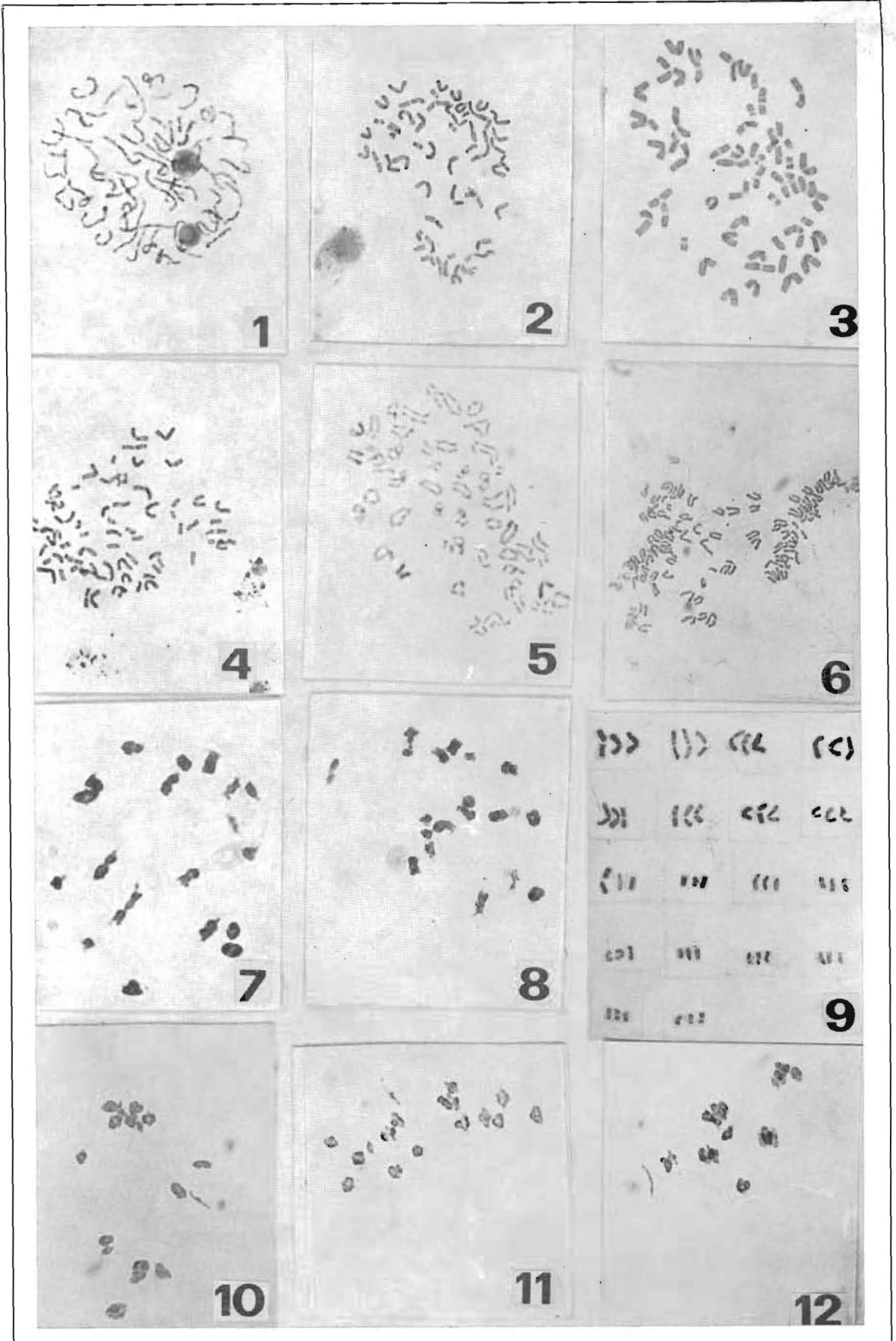
FIG. 10. Prometaphase.

FIG. 11 and 12. Metaphase-I showing 18 II and secondary association.

(FIG. 1, 2 and 4.  $\times 1800$ ; FIG. 3 :  $\times 1900$ ; FIG. 5.  $\times 1400$ ;

FIG. 6 :  $\times 900$ ; FIG. 7, 8, 10-12 :  $\times 1400$ ).

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