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CYTOLOGICAL STUDIES IN *ARECA CATECHU* LINN. AND *ARECA TRIANDRA* ROXB.

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A detailed account of the morphology of the pachytene and somatic chromosomes of *A. catechu* is presented below. The somatic chromosome morphology of *A. triandra* has also been investigated and compared with that of *A. catechu.* The latest records of the chromosome number of the two species are by Abraham *et al.* (1961) and Sharma and Sarkar (1956).

Materials and Methods

Flower-buds from palms grown in four major arecanut growing tracts of South India, *viz.*, South Kanara and Shimoga Districts of Mysore State, Palghat of Kerala State and Coimbatore of Madras State were collected in Carnoy's fluid (9 : 6 : 1 mixture) and utilised for the study. Flower buds of *A. triandra* were fixed from palms grown at the Museum Gardens, Trivandrum. The course of meiosis was studied in temporary acetocarmine mounts. Root-tips were pretreated with 0·002 8-hydroxyquinoline for four hours at 17° C.

In comparing the size of pachytene and somatic chromosomes, "Relative length" which represents the ratio in percentage of the length of individual chromosome to that of the longest (Huziwara, 1956) was used. For classifying the chromosomes based on centromeric position, the arm ratios were taken into consideration and chromosomes were classed as median (1 : 1·25), sub-median (1 : 2·00) and sub-terminal (1 : over 2·00).

Results

The variation in respect of morphological characters between the two species of *Areca* investigated are as follows:—

	A. catechu	*A. triandra*
Trunk—Length (in metres)	12–30—single	3–7 in clusters consisting of 4–6 suckers
Circumference (cm.)	50	18
Number of leaves	6–11	3–7
Length of spadix (cm.)	65	30
Male flowers (cm.)	0·5×0·3	0·3×0·1
Female flowers (cm.)	Many, 1·2×0·8	Fewer, 0·8×0·3
Fruits (cm.)	4–6×3–4	2–3×0·8–1·5

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The nature of chromosome association and aberrations at meiosis are detailed in Table I.

Morphology of the Somatic Chromosomes

Depending upon the length of the chromosomes and the centromeric position, the complement of *A. catechu* can be divided into the following seven types:—

Type A.—A pair of long chromosomes (I) generally the longest in the complement with median centromere.

Type B.—Two pairs of fairly long chromosomes (II and III) having sub-median centromere. One of the two pairs (II) has secondary constriction in its short arm.

Type C.—Two pairs of medium-sized chromosomes (IV and V), one having sub-terminal and another having sub-median centromeres.

Type D.—Two pairs of medium-sized chromosomes (VI and VII) having sub-terminal centromeric constriction.

Type E.—Two pairs of medium-sized chromosomes (VIII and IX) with sub-median centromere.

Type F.—Three pairs of medium-sized chromosomes (X, XI and XII) having sub-median centromeric constriction.

Type G.—Four pairs of short chromosomes (XIII, XIV, XV and XVI) which have either median, sub-median or sub-terminal centromere. Chromosome XV has secondary constriction in its short arm.

In *A. triandra* there is greater chromatin than in *A. catechu.* In the complement of the former, about 44 per cent of the chromosomes show median centromere and eight per cent with sub-terminal constrictions; the corresponding values for *A. catechu* are 12·5 and 25 per cent respectively. Two of the satellite chromosomes of *A. triandra* are characterised by the presence of a single trabant. The idiograms of the two species are illustrated in Text-Fig. 1. The length of individual arms, relative length, arm ratios and centromeric position of the somatic chromosomes of the two species are presented in Table II.

Morphology of Pachytene Chromosomes

The individual pachytene bivalents of *A. catechu* drawn from the same nucleus are illustrated in Text-Fig. 2. Based on the characteristics detailed below, the pachytene idiogram has been constructed (Text-Fig. 3) to bring out the diagnostic features of the complement.

Chromosome I.—This is the longest of the complement. The centromere is flanked on either side by fairly large and deeply stained chromatic regions; the long arm is intercepted by a very lightly stained achromatic region at about one-third of the total distance from the

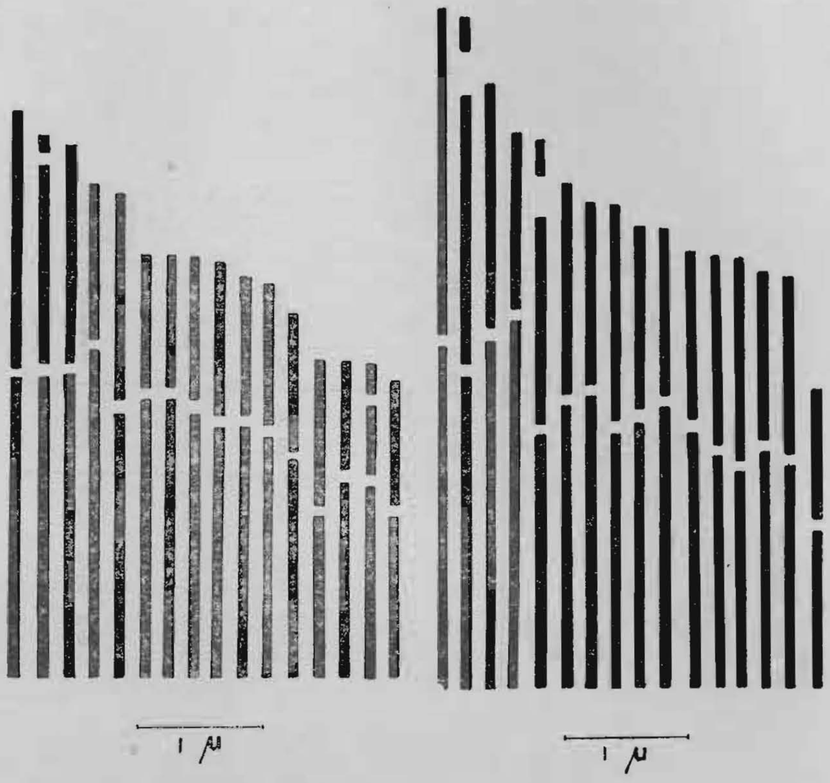
TABLE I

Chromosome association and meiotic aberrations

TABLE I

Chromosome association and meiotic aberrations

	No. of PMCs studied	Mean frequency per cell			Mean No. of Xta.		Percentage of cells (Anaphase I and II) with				Pollen stainability (in per cent)	Pollen diameter (in μ)	Aberrations in meiosis	
		IV	II	I	Per cell	Per bivalent	Inversion bridge	Delayed disjunction bridge	Laggards	Micro nuclei				
A. catechu "South Kanara" type	57	..	16·00	..	29·8	1·86	..	..	..	2·9	97·7	8·40±0·82	Non-synchronised orientation of the bivalents and a tendency or clumping. Delayed disjunction of bivalent at anaphase I.	Pl. I, Figs. 1 to 4
"Shimoga" type	48	0·041	15·875	0·083	25·2	1·58	..	..	11·1	7·1	95·0	7·07±1·01	Precocious separation of a bivalent at diakinesis.	Pl. I, Figs. 5 and 6
"Palghat" type	52	..	16·00	..	28·9	1·30	7·4	41·3	14·3	33·9	88·3	7·41±1·35	Bridge formation, lagging chromosomes at anaphase, chromosome mosaic cells and supernumerary spores.	Pl. I, Figs. 7 to 10; Pl. II Figs. 11 to 14
"Coimbatore" type	67	0·044	15·91	..	26·8	1·68	5·7	4·3	27·1	32·9	56·2	6·74±1·14		
A. triandra	42	..	16·00	..	..	..	..	..	..	..	..	..	..	..



TEXT-FIG. 1. Idiograms of the somatic chromosomes of *A. catechu* (left) and *A. triandra* (right).

centromere, followed again by a chromomere and moderately stained chromatic portion. In the short arm, the chromatic region gradually decreases in staining intensity and ends in an achromatic region.

Chromosome II.—This has the nucleolar organizing region (in the short arm, lying at the distal end from the centromere) and is the only pair which does not have a deeply stained large chromatic region on either side of the centromere. The short arm has a number of chromomeres in addition to the large and deeply stained distal end.

Chromosome III.—Almost equidistant from the centromere, there is an achromatic region on each arm. On the long arm between the achromatic region and the centromere are three small achromatic segments.

Chromosome IV.—The chromatic region in the short arm has one lightly stained portion almost in the middle. A similar lightly stained

Comparison o

Chromosome	*A. catec*
I	4·4
II	4·2
III	4·1
IV	3·8
V	3·78
VI	3·41
VII	3·31
VIII	3·28
IX	3·21
X	3·11
XI	3·06
XII	2·80
XIII	2·47
XIV	2·44
XV	2·40
XVI	2·29
Total ..	52·31

M = Median;

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TABLE II

Comparison of the somatic complements of A. catechu *and* A. triandra

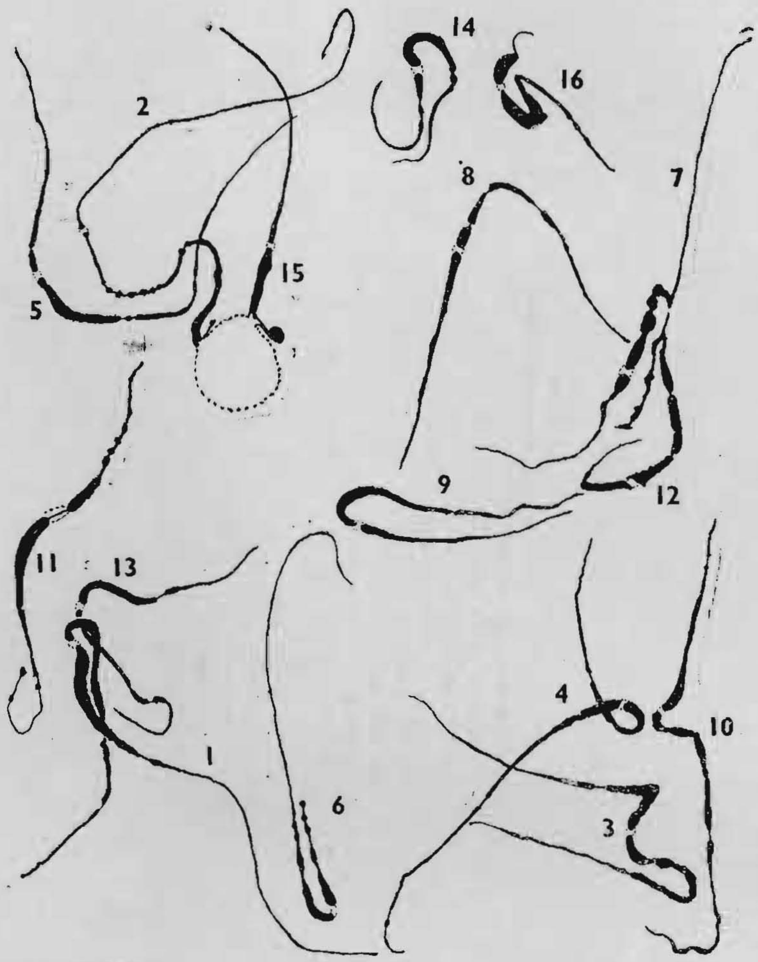
Chromosome	Length (in μ)		Relative length		Arm ratio		Centromere position	
	A. catechu	*A. triandra*	*A. catechu*	*A. triandra*	*A. catechu*	*A. triandra*	*A. catechu*	*A. triandra*
I	4·41	5·33	100·00	100·00	1 : 1·20	1 : 1·05	M	M
II	4·28	5·32	97·00	99·81	1 : 1·46	1 : 1·46	Sm	M
III	4·17	4·73	94·50	88·74	1 : 1·45	1 : 1·46	Sm	Sm
IV	3·89	4·34	88·20	81·43	1 : 2·19	1 : 2·10	St	St
V	3·78	4·31	85·70	80·86	1 : 1·29	1 : 1·28	Sm	Sm
VI	3·41	3·92	77·30	73·56	1 : 2·09	1 : 1·39	St	Sm
VII	3·31	3·82	75·10	71·67	1 : 2·09	1 : 1·56	St	Sm
VIII	3·28	3·75	74·40	70·36	1 : 1·88	1 : 1·21	Sm	M
IX	3·21	3·60	72·70	67·54	1 : 1·83	1 : 1·42	Sm	Sm
X	3·11	3·59	70·50	67·35	1 : 1·83	1 : 1·68	Sm	Sm
XI	3·06	3·39	69·30	63·60	1 : 1·78	1 : 1·53	Sm	Sm
XII	2·80	3·35	63·50	62·85	1 : 1·62	1 : 1·23	Sm	M
XIII	2·47	3·34	56·00	62·66	1 : 1·11	1 : 1·06	M	M
XIV	2·44	3·22	55·30	60·41	1 : 1·87	1 : 1·40	Sm	Sm
XV	2·40	3·18	54·40	59·66	1 : 3·00	1 : 1·21	St	M
XVI	2·29	2·29	51·90	42·96	1 : 1·31	1 : 1·14	Sm	M
Total ..	52·31	61·48	..	..	..	..	..	..

M = Median; Sm = Sub-median; St = Sub-terminal.

portion is also present in the chromatic region of the long arm and its location is about one-third the distance from the centromere.

Chromosome V.—This can be distinguished both by the differential arm ratio and from the presence of a large chromatic region on the long arm with a chromomere in the centre.

Chromosome VI.—This has a telomere on the short arm. On the same arm there are three chromomeres located between the telomere and the large chromatic region. The chromatic and achromatic regions of both the short and long arms are almost similar.



TEXT-FIG. 2. Pachytene bivalents of *A. catechu*, ×2,000.

Chromosome VII.—On either side of the centromere there is a deeply stained chromatic region on each arm. These regions are followed by three chromomeres on the long arm and one on the short arm.

Chromosome VIII.—It can be distinguished by the presence of a short deeply stained chromatic region on the long arm followed by three larger deeply stained regions. On the short arm the heavily stained chromatic region is about half the length of this arm.

Chromosome IX.—It can be identified by the fairly long and deeply stained chromatic regions flanking the centromere. The lightly stained achromatic regions are present only at the distal ends of both the arms.

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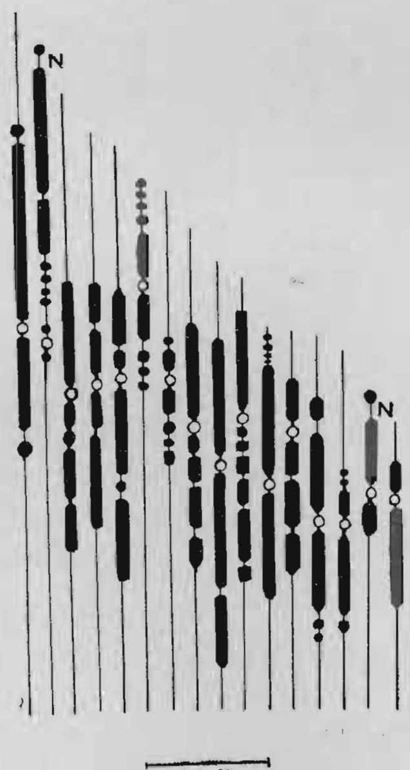
On the long arm the chromatic region has a lightly stained segment located almost centrally.

Chromosome X.—The deeply stained chromatic region on the short arm extends about three-fourths in length but the one in the long arm is intercepted by achromatic segments in five places. The presence of a deeply stained distal end in the long arm is a character of diagnostic value.



TEXT-FIG. 3. Pachytene idiogram of *A. catechu*.

Chromosome XI.—The chromosome is characterized by the long and deeply stained chromatic region in the long arm and a short chromatic region followed by four chromomeres located in the short arm. The long arm terminates in a telomere.

Chromosome XII.—The short arm has a deeply stained chromatic region which extends to nearly half the length of the arm. The long arm is chromatic along its entire length and has two lightly stained regions which divide this into three segments.

Chromosome XIII.—In the short arm, the chromatic segment is intercepted by an achromatic region and followed by a lightly stained chromatic region; in the long arm, there are two chromomeres following the chromatic region.

TABLE III

Comparative analysis of the somatic and pachytene chromosomes of A. catechu

Chromosome	Length (in μ)		Relative length		Arm ratio		Centromere position	
	Somatic	Pachytene	Somatic	Pachytene	Somatic	Pachytene	Somatic	Pachytene
I	4·41	56·63	100·0	100·0	1 : 1·20	1 : 1·33	M	Sm
II	4·28	53·89	97·0	95·2	1 : 1·46	1 : 1·40	Sm	Sm
III	4·17	49·91	94·5	88·1	1 . 1·45	1 : 1·09	Sm	M
IV	3·89	46·86	88·2	82·7	1 : 2·19	1 : 1·35	St	Sm
V	3·78	46·00	85·7	75·9	1 : 1·29	1 : 1·54	Sm	Sm
VI	3·41	42·63	77·3	75·5	1 ; 2·09	1 : 4·26	St	St
VII	3·31	42·04	75·1	74·2	1 : 2·09	1 : 1·79	St	Sm
VIII	3·28	39·00	74·4	68·9	1 : 1·88	1 : 1·45	Sm	Sm
IX	3·21	36·36	72·7	64·2	1 : 1·83	1 : 1·25	Sm	M
X	3·11	34·31	70·5	60·2	1 : 1·83	1 : 2·21	Sm	St
XI	3·06	30·99	69·3	54·7	1 ; 1·78	1 : 1·45	Sm	Sm
XII	2·80	30·77	63·5	54·3	1 : 1·62	1 : 2·76	Sm	St
XIII	2·47	30·22	56·0	53·4	1 : 1·11	1 : 1·04	M	M
XIV	2·44	28·49	56·3	50·3	1 : 1·87	1 ; 1·14	Sm	M
XV	2·40	25·63	54·4	45·3	1 : 3·00	1 : 3·50	St	St
XVI	2·29	22·67	51·9	40·0	1 : 1·31	1 : 3·02	Sm	St
Total ..	52·31	616·40	..	..	..	..	..	..

M = Median; Sm = Sub-median; St = Sub-terminal.

Chromosome XIV.—It can be distinguished by the very deeply stained chromatic region which occupies about half the length of the long arm. There is a single chromomere immediately next to the chromatic region. The chromatic region on the short arm is rather small and is followed by two chromomeres.

Chromosome XV.—This chromosome characterized by the chromatic short arm has a nucleolar organizing region located at the distal end.

Chromosome XVI.—This is the shortest chromosome of the complement. The deeply stained chromatic region of the short arm is characteristically semi-circular in shape and ends in a small achromatic region. The chromatic region of the long arm is also deeply stained.

A comparison of the somatic and pachytene chromosomes of *A. catechu* is presented in Table III.

Discussion

Varying degrees of pollen sterility have been reported in arecanut (Sarkar, 1956). The sterility appears to be chromosomal as judged from the meiotic aberrations. It has been suggested that the dwarf coconut, which possesses characters associated with inbreeding depression, owes its origin to inbreeding facilitated by the imposition of a self-pollinating device (Nambiar and Swaminathan, 1960). A similar mode of evolution may be said to exist in *A. catechu* also. A reduction in the size of fruit, endosperm development and seedling vigour (in a descending order from 'South Kanara' → 'Palghat' → 'Coimbatore' to 'Shimoga' types) and the nature of meiotic irregularities suggest that the efficiency of chromosome mechanism is affected in somatic, gametic and endosperm cells. This view is supported by the finding that in arecanut which is largely cross-fertilized, self-pollination also takes place (Bhat, 1961). The recent report of a dwarf arecanut palm (Naidu, 1963) occurring in the Mysore State lends further support. It would appear that the 'Palghat', 'Coimbatore' and 'Shimoga' types acclimatized to different ecological conditions are differentially inbred ones from an original 'South Kanara' type. In this connection, it is of interest to record that the big-sized 'South Kanara' type is under large-scale cultivation. The basic chromosome number of $x = 7$ inferred from secondary association also derives support from the karyomorphological data. The somatic and pachytene chromosomes could be assembled into seven groups based on their length and morphology. Brown (1949) found that the range in differentiation of pachytene chromosomes extends in an overlapping series from quite undifferentiated chromosomes to highly differentiated ones. The pachytene chromosomes of *Areca* stand in between these two extremities like those of *Sorghum ankolib* (Magoon and Shambulingappa, 1960). Reddy (1958) found that in *Sorghum purpureosericeum*, the chromomeres showed a close gradient in size from the proximal to the distal end. A similar differentiation into deeply stained proximal and unstained distal parts has been noted in *Areca catechu*. A comparative study of the somatic and pachytene chromosomes show fairly close agreement. The second and fifteenth chromosomes as nucleolar organisers are confirmed. In general, the pachytene chromosomes are about ten times longer than the somatic chromosomes. From a study of karyotype symmetry—asymmetry in relation to species evolution, Stebbins (1958) observed that the first chromosome to be affected is usually the satellite-chromosome. This observation holds good in the case of the material under study. The first pair of satellite-chromosomes is median in *A. triandra*, and sub-median in *A. catechu*. In respect of the second satellite pair it is sub-terminal in *A. catechu* and sub-median in *A. triandra*. A gradual reduction in chromatin matter from primitive to advanced forms has been made in the different genera and tribes of palms (Sharma and Sarkar, 1956). That *A. catechu* is more advanced than *A. triandra* is in conformity with the above findings.

Summary

The diploid and haploid chromosome numbers of *A. catechu* and *A. triandra* have been confirmed to be 32 and 16 respectively. Evidences from karyomorphological features, nucleolar chromosomes and secondary association indicate a secondary allotetraploid origin. The chromosome complements of the two species have been analysed and found to possess differences with regard to the size of chromosomes, total chromatin, position of primary and secondary constrictions, nucleolar organizer, etc.

Acknowledgments

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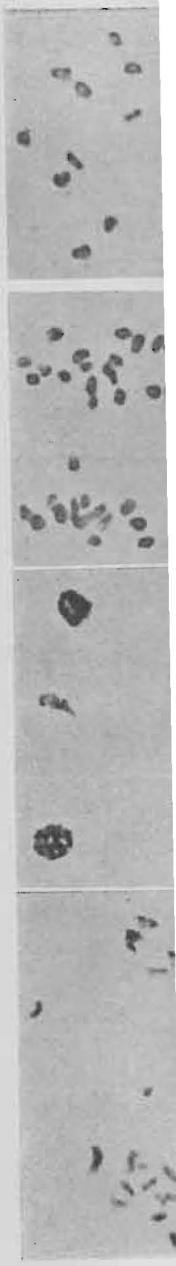

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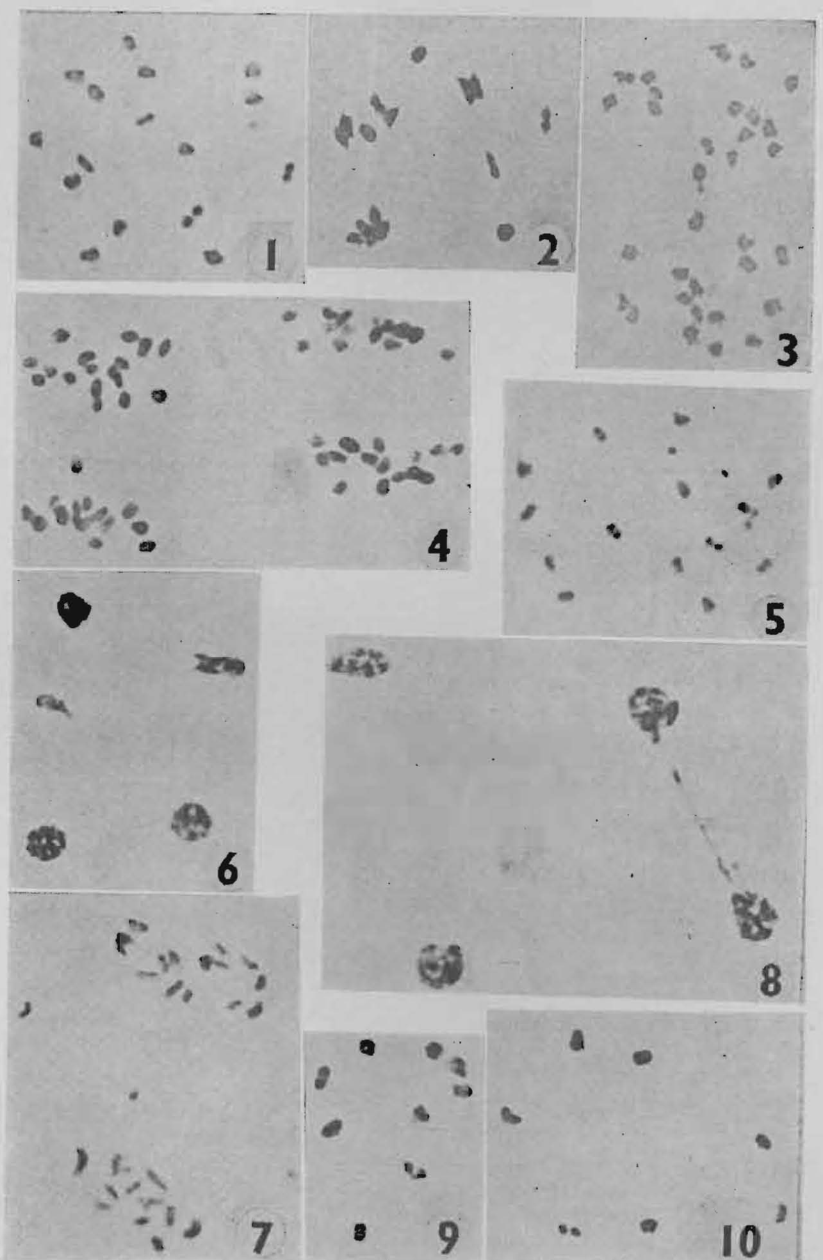

FIGS. 1–10

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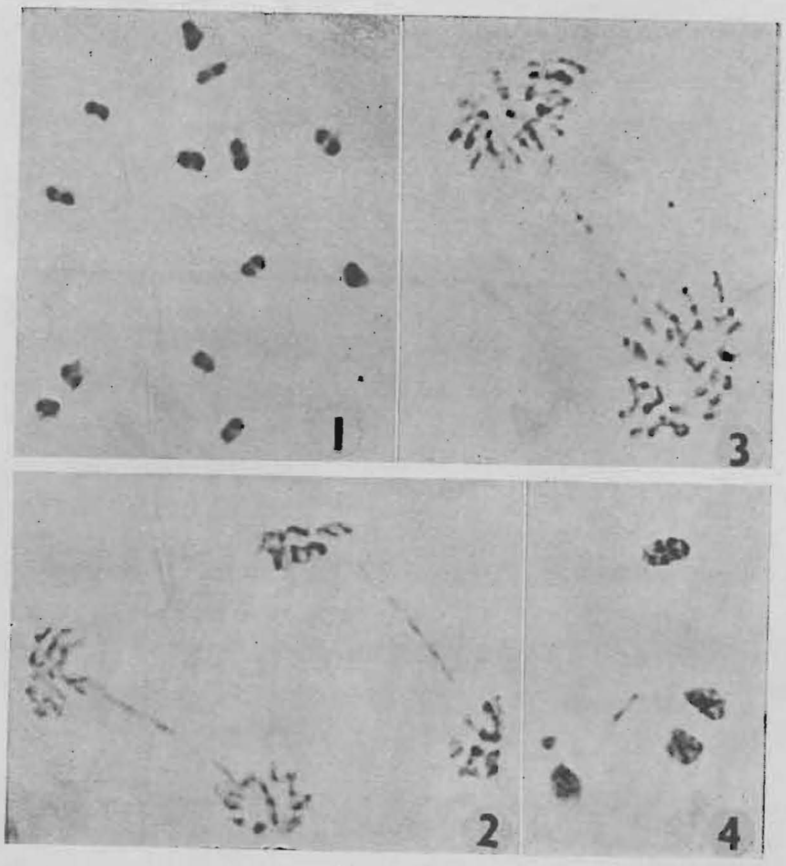

FIGS. 1-4

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EXPLANATION OF PLATES

PLATE I

Meiosis in *Areca catechu*

FIG. 1. Late diakinesis showing 16 bivalents.

FIG. 2. Prometaphase showing clumping of the scattered bivalents.

FIG. 3. Anaphase I. Note the delayed disjunction of a bivalent.

FIG. 4. Anaphase II with normal distribution of chromosomes.

FIG. 5. Late diakinesis ($15_{II} + 2_{I}$).

FIG. 6. Late telophase II showing a micronucleus.

FIG. 7. Laggards at telophase I.

FIG. 8. Telophase II with a bridge and fragment.

FIGS. 9 AND 10. Mosaic PM cells with 9 and 7 bivalents respectively (Figs. 1–10, × 1,000).

PLATE II

FIG. 1. Chromosome mosaic cell with 13 bivalents.

FIG. 2. Telophase I. Note the dicentric bridge and fragment.

FIG. 3. Telophase II showing persistent bridges.

FIG. 4. A sporad with four microspores, one micronucleus and excluded chromosomes (Figs. 1–4, × 1,000).