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KARYOTYPE ANALYSIS AND MEIOTIC CHROMOSOME BEHAVIOUR IN CORCHORUS OLITORIUS, C. TRIDENS AND C. AESTUANS.

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ABSTRACT

Mitotic and meiotic chromosomes were studied in Corchorous olitorius, varieties "yaya" and "agbadu", Corchorus tridens and Corchorus aestuans using the conventional plant parts and microscopic procedures. The mitotic chromosome length measurements provided evidence of polysomy for all the species studied. Meiotic chromosome associations further suggest that the three species are tetrasomics. The attachment of two bivalents to the nucleolus in C. aestuans suggests that it is also a secondarily balanced polyploid as C. olitorius and C. tridens.

The high percentage pollen viability in the species is consistent with the absence of meiotic irregularities such as laggards, precocious movements, non-disjunction bridges and fragments. Microsporogenesis was therefore normal resulting in high male fertility as shown by pollen grain viability.

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INTRODUCTION

Corchorus olitorius Linn. *C. tridens* Linn. and *C. aestuans* Linn. are three species of the genus *Corchorus* Linn. (Family Tiliaceae) that have wide distribution in Nigeria. These three species are morphologically close, but each is reproductively isolated through pre and post zygotic barriers (Swaminathan *et al.*, 1961; Morakinyo, 1997). However, these authors have also shown that artificial hybridization is possible between *C. olitorius* and *C. Tridens* and between *C. olitorius* and *C. capsularis* through pre-anthesis emasculation and hormonal treatment, respectively. *Corchorus olitorius* and *Corchorus tridens* are eaten as vegetables and the former is a source of industrial fibre (jute). Improving these crops to meet the different need would involve hybridization by which their desirable characters could be combined. The level of success so far achieved in hybridizing *Corchorus olitorius*, *Corchorus tridens* and *Corchorus aestuans* has been rather low, yet these species have the same chromosome number 2n = 14 (Rao and Datta, 1953; Islam and Feroza, 1961; Hague and Ahmad, 1972)

More detailed information on both the karyotype and microsporogenesis is necessary to facilitate the development of an appropriate hybridization programme for these species. The aim of this study therefore is to provide more information on the mitotic and meiotic chromosome in *Corchorus olitorius*, *C. tridens* and *C. aestuans*.

MATERIALS AND METHODS

The three species investigated in this study were collected from the south western parts of Nigeria. They include two varieties of *Corchorus olitorius* (i.e. "agbadu" and "yaya") and the representative types of *Corchorus tridens* and *C. aestuans*, making four groups of plants of known taxonomic identity. Seed germination in each group of plants was effected by dipping the seeds, tied in a piece of cloth, in boiling water for five seconds before either plating or sowing them for mitotic and meiotic studies respectively. Sprouting plated seeds with 1cm long roots were removed from petri-dishes

and put in aqueous solution of α- bromo naphthalene for one hour twenty-five minutes at about mid-morning. The sprouting seeds were then fixed in 1:3 (v/v) glacial acetic acid and absolute alcohol mixture for twenty four hours. After fixation, roots for immediate slide preparation were excised, washed in distilled water, hydrolysed in 1M HCl for twenty minutes at room temperature, washed again in distilled water before the meristematic regions of the root tips were squashed in FLP orcein stain (Olorode, 1973). Chromosome arm lengths were measured in micrometers and the r - values were determined as ratio of long to short chromosome arms. Appropriate meiotic cells were photographed at X1000 using a photomicrographic equipment.

Meiotic studies were carried out by harvesting flower buds from mature plants between 8.00 - 10.00 a.m. The flower buds were split open and fixed in freshly prepared fixative as above for 24 hours. After fixation, anthers were dissected out of the flower buds and used in slide preparation by squash method as above. Chromosome associations at diakinesis and metaphase I were scored and photographed. Pollen grain viability and size were determined by staining in cotton blue-in-lactophenol and making appropriate pollen counts and pollen diameter measurements under a compound microscope.

RESULTS AND DISCUSSION

The chromosome arm lengths, the r-values and the centromeric positions (c.p) for *Corchorus olitorius*, *C. tridens* and *C. aestuans* are summarized in Table 1.The centromeric positions are classified using r-values according to the recommendation of Levan *et al.*, (1961) that c.p. is in the median position (M) if r = 1.0; median region (m) if r = 1.1 - 1.7; submedian region (sm) if r = 1.8 - 3.0. Based on c.p., the chromosomes were either metacentric or submetacentric. The chromosome of *C. olitorius* were 10 metacentric (M and m) + 4 submetacentric (sm); *C. tridens*: 6 metacentric + 8 submetacentric; *C. aestuans*: 14 metacentric, (Table 1, Plate 1). There is variation in chromosome lengths in each species. Chromosome length varied between 2.50 and 3.75μm in *C. olitorius* (Plate 1A), between 1.66 and 3.75μm in *C. tridens* (Plate 1B) and between 1.66 and 3.33μm in *C. aestuans* (Plate 1C).

The total amount of chromatin material measured as total chromosome length per diploid prometaphase cell was 45.02μm for *C. olitorius*, 39.14μm for *C. tridens* and 38.30μm in *C. aestuans* (Table 1). The highest amount of chromatin material observed in *C. Olitorius* (45.02) may be due to selection under cultivation as was observed by Morakinyo and Adebola (1991) in *Pennisetum americanum*. In the largest chromosomes, the c.p. was either in the submedian or median region, none was at the median position while c.p. in the smallest chromosomes of each species was at the median position. The variation in relative arm length accompanying the centromeric positions may be due to non-reciprocal translocation between non-homologous chromosomes in the course of evolution of these species (Stebbins, 1971).

Plate 2 shows diakinesis in *Corchorus olitorius* var. "yaya": 2A shows 7 bivalents: 2B shows 3 ring bivalents and 4 rod bivalent in two groups (arrowed). Plate 3 shows diakinesis, metaphase I, anaphase I and metaphase II in *Corchorus olitorius* var. "agbadu": 3A shows 7 bivalents; 3B shows 3 bivalent rings and 4 bivalent rods in secondary quadrivalent associations (arrowed); 3C shows normal anaphase I, 3D shows normal metaphase II. Plate 4 shows A, pachynema; B, diplonema; C and D, diakinesis in *C. tridens*. 4A shows the nucleolar organizing chromosome; 4B shows evidence of homologous pairing and chaismata formation; 4C shows 7 bivalents, four of which are in groups of two (arrowed). 4D shows 7 bivalents, four of which are in secondary quadrivalent association (arrowed). Plate 5 shows diplonema and metaphase I in *Corchorus aestuans*. 5A-C are diplonema cells showing bivalents at various stages of chiasmata terminalization, 5A shows four bivalents clustering together and two bivalents attached to the nucleolus, and 5B shows secondary

quadrivalent association (arrowed) and two bivalents attached to the nucleolus. 5C shows 3 rod bivalents, 2 ring bivalents and 1 ring quadrivalent (arrowed), 5D is a metaphase cell showing seven bivalents.

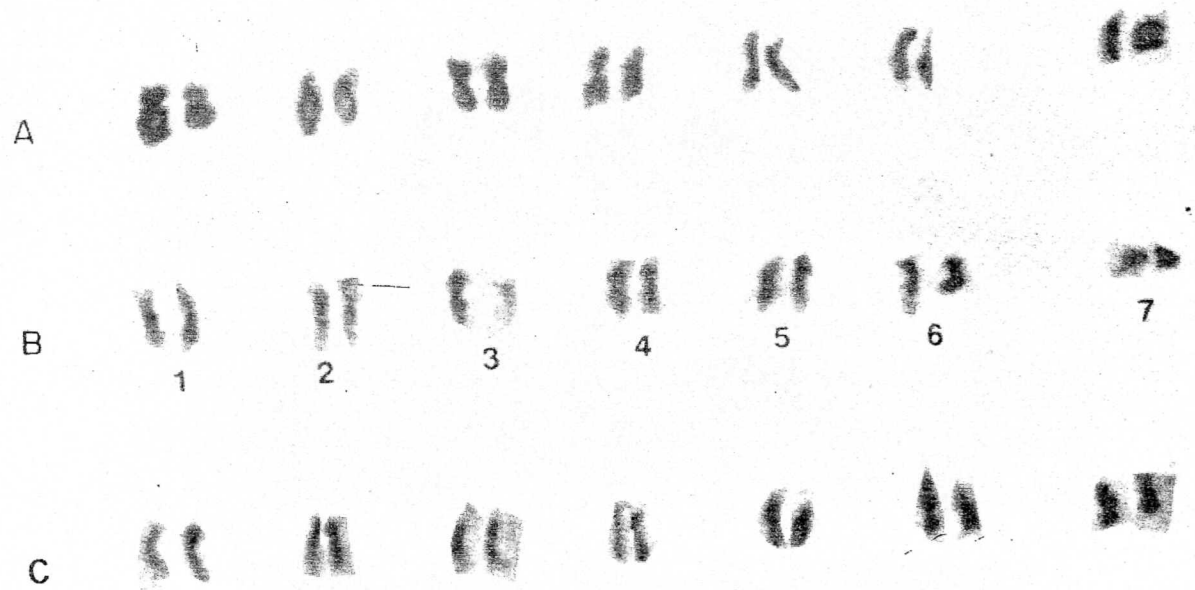

Plate 1: Karyotypes in A, *Corchorus olitorius*; B, *Corchorus tridents*C, *Corchorus aestuans.*

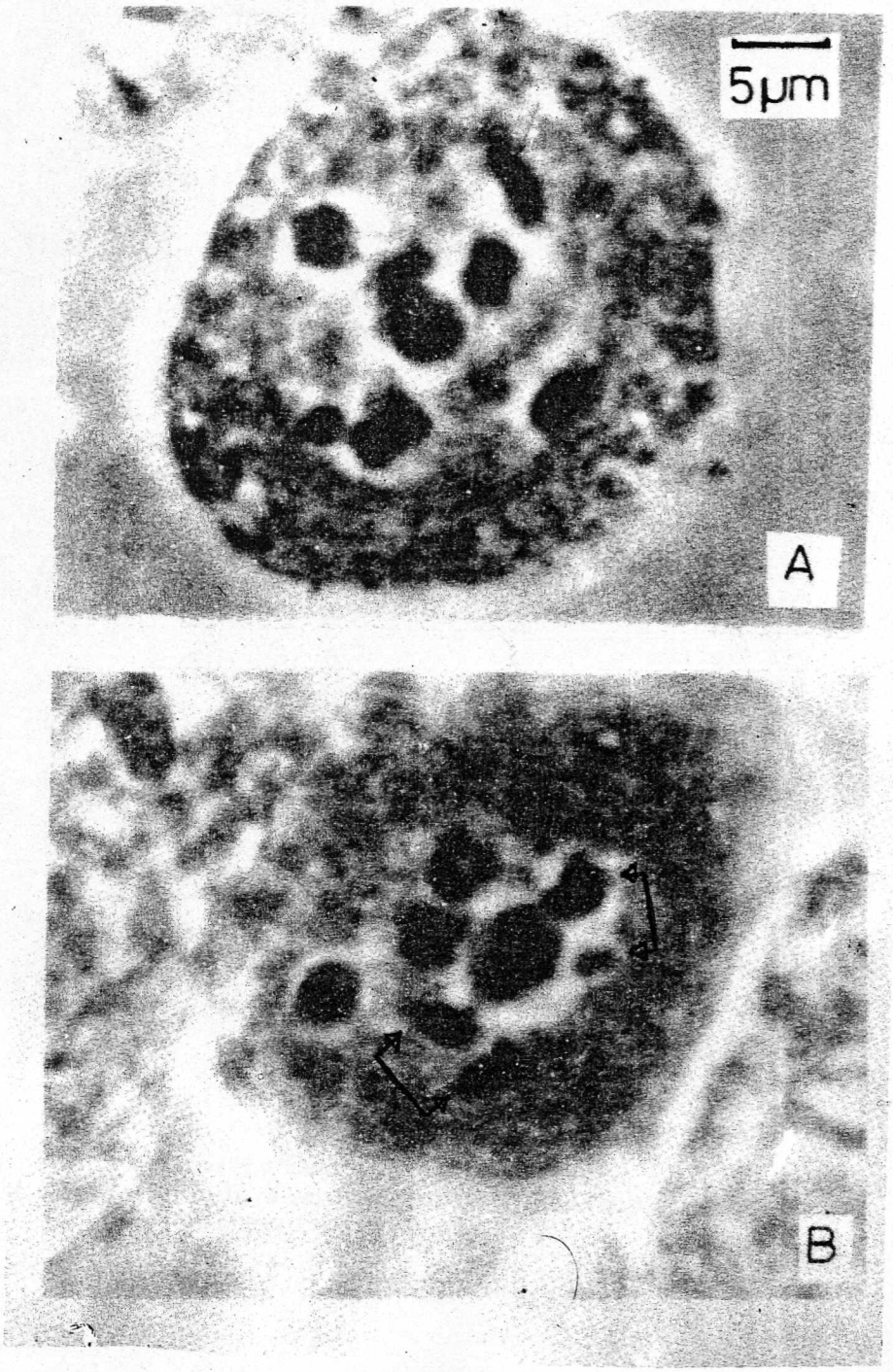

Plate 2: Diakinesis in *Corchorus olitorius* var. "yaya";
A,7 II; B3II rings + 4II rods (arrowed).

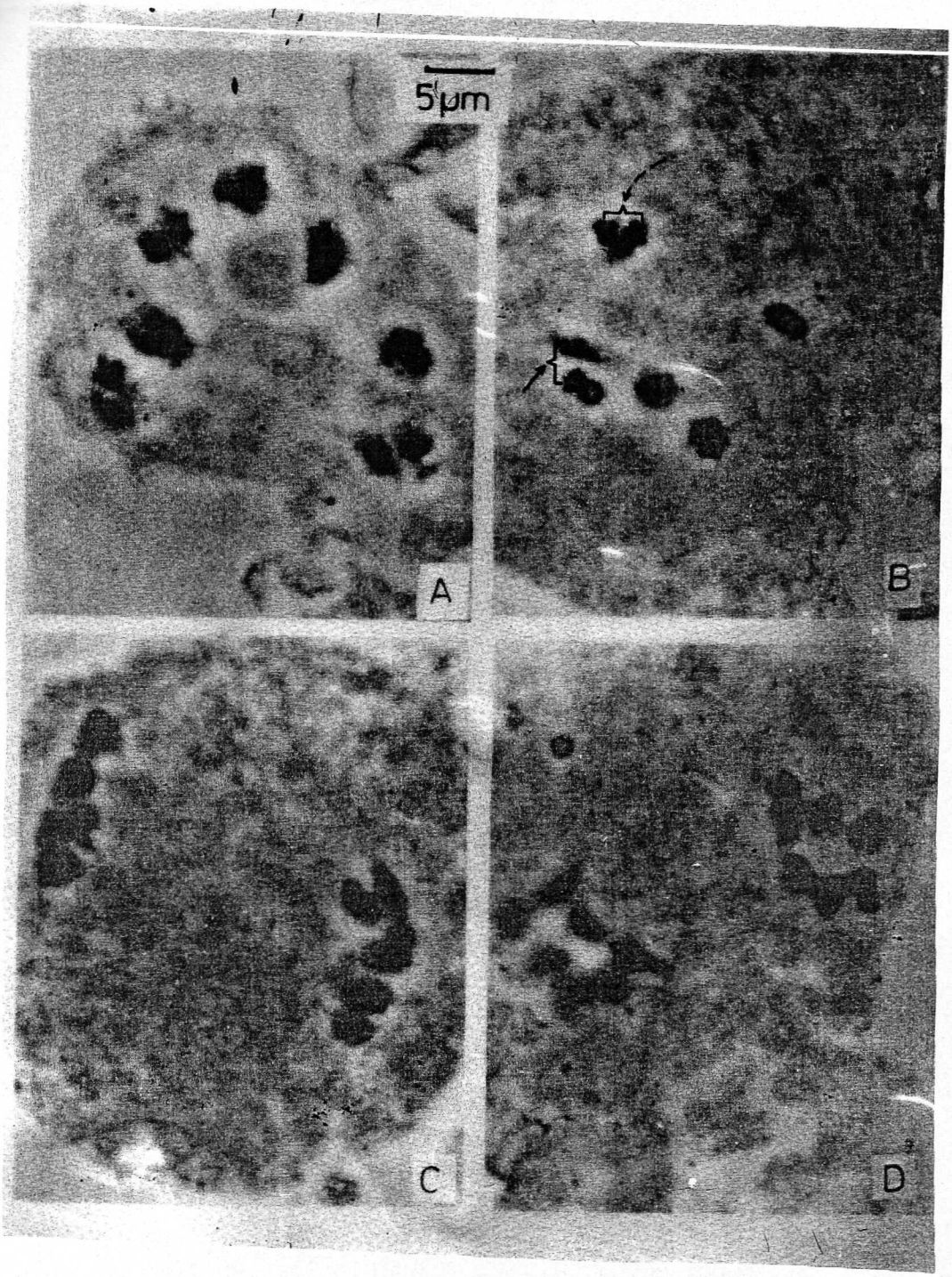

Plate 3: Meiosis in Corchorus olitorius var. "agbadu"
A, Diakinesis cell showing 7II; B, Metaphase I cell showing 3II rings + 4II rods (arrowed).
C, Anaphase I cell ; D, Metaphase II cell.

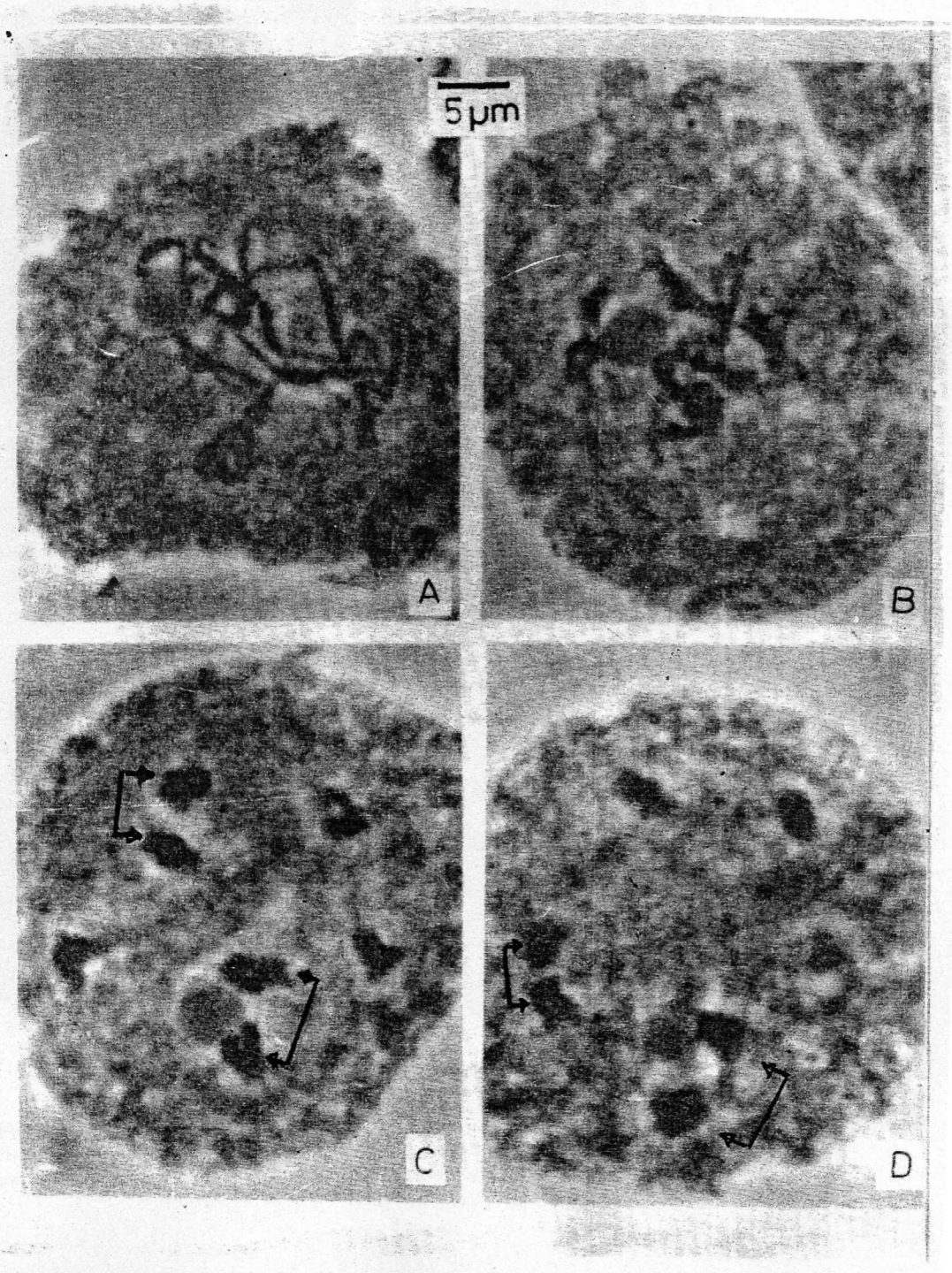

Plate 4: Meiosis in Corchorus tridents; Apachnema cell; B.Diplonema cell, C and D.Diakinesis cells, each showing bivalents in secondary association (arrowed).

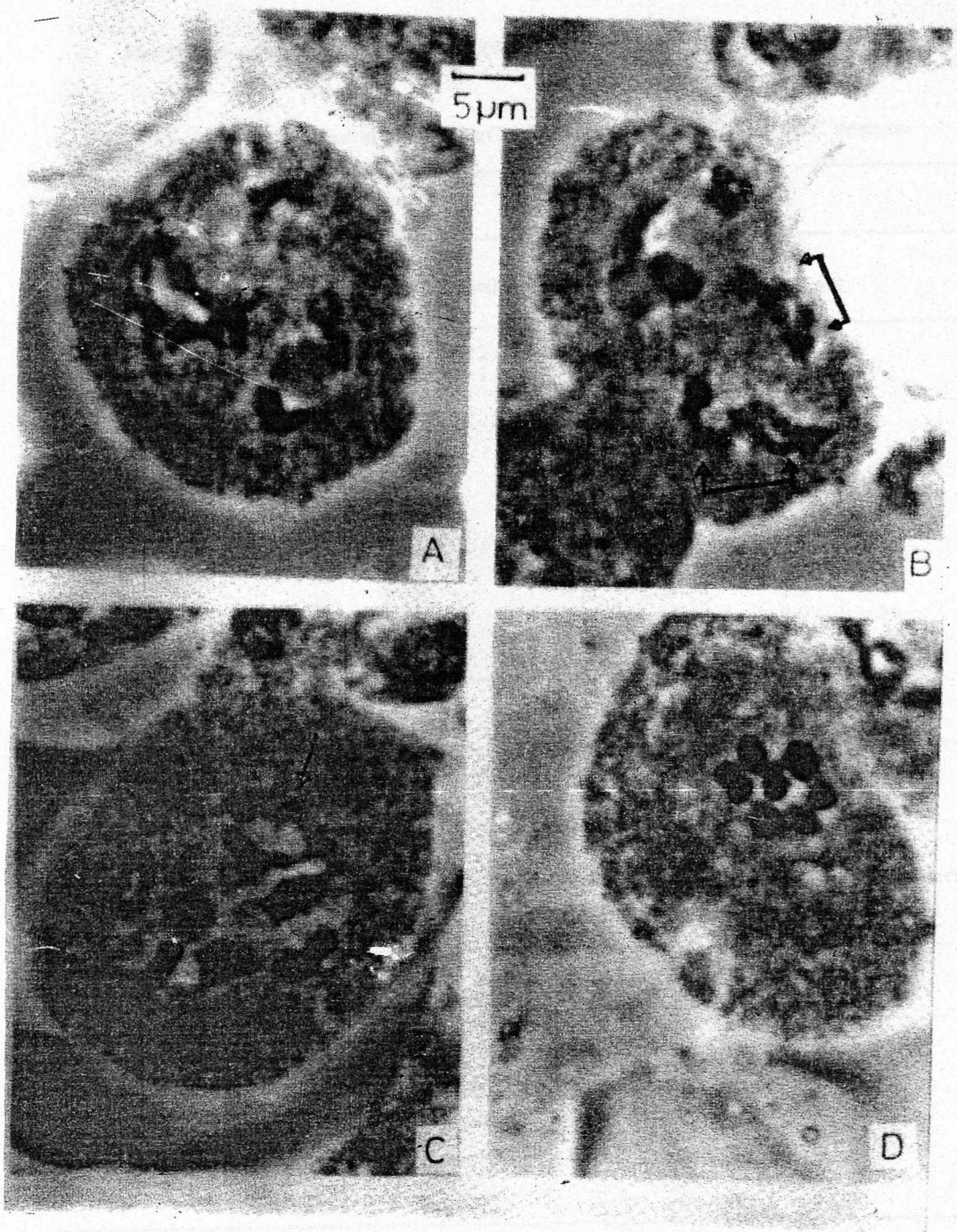

Plate 5: Meiosis in Corchorus aestuans; A and B are Diplonema cells, each showing 21 I's attached to the nucleolus, Arrows in B show secondary association of bivalents; C. Diplonema showing bivalent rings and rods an quadrivalent ring (arrowed); D. Metaphase I cell with 7 N

Table 1: Karyotype data on *Corchorus olitorious, C.tridens* and *C. aestuans*

C. *olitorius* (yaya)			*Ctrindens*			C.*aestuans*		
chromosome length(um)	r-value	c.p	Chromosome length(um)	r-value	c.p	Chromosome length (um)	r-value	c.p
3.75	2.0	Sm	3.75	2.0	Sm	3.33	1.7	m
3.75	2.0	Sm	3.75	2.0	Sm	3.33	1.7	m
3.33	1.7	m	3.75	2.0	Sm	3.33	1.7	m
3.33	1.7	m	3.75	2.0	Sm	3.33	1.7	m
3.33	1.7	m	2.91	2.5	Sm	3.33	1.7	m
3.33	1.7	m	2.91	2.5	Sm	3.33	1.7	m
3.31	1	M	2.92	1.3	m	2.92	1.3	m
3.31	1	M	2.92	1.3	m	2.92	1.3	m
3.31	1	M	2.50	2.0	Sm	2.50	1.0	M
3.31	1	M	2.50	2.0	Sm	2.50	1.0	M
2.92	1.3	m	2.08	1.5	m	2.08	1.5	m
2 92	1.3	m	2.08	1.5	m	2.08	1.5	m
2.50	1	M	1.66	1	M	1.66	1.0	M
2.50	1	M	1.66	1	M	1.66	1.0	M
Total cell	45.02		39.14			38.30		

Legends r- value is ratio of long to short chromosome arm lengths, c.p. is centromeric position,
M- median position when r = 1, m is median region when r = 1.1-1.7,sm is submedian region when r = 1.8 - 3.0.

Table 2: Diakinesis chromosome association in *Corchrous* species with ranges in parenthesis and scores based on 30 cells.

Plant	Ring bivalent II	bRodents II
Corchrous olitorious var. "yaya"	6.6 (5 - 7)	0.4 (0 - 7)
C. *olitorius* var. "agbadu"	5.7 (5 - 7)	1.3 (0 - 7)
C. *aestuans*	6.93 (6 - 7)	0.07 (0 - 1)
C. *tridens*	4.5 (3 - 7)	2.5 (0 - 7)

Table 3: Pollen data of the *Corchorus* species studied.

Plant	Pollen diameter in micrometers based on 100 measurements	Number on which estimation was made.	Percentage pollen variability based on stainability and pollen shape.
C. olitorius var. "yaya"	32.9± 0.20	990	99.0
C. olitorius var. "agbadu"	32.01± 0.33	985	98.5
C. aestuans	23.9± 0.12	995	99.5
C. tridens	32.9± 0.16	993	99.3

Datta (1958) suggested that *Corchorus tridens* and *C. olitorius* are secondarily balanced polyploids because of two bivalents that are frequently found attached to the nucleolus. Observations recorded above also suggest that *Corchorus aestuans* may be a secondarily balance polyploid. The occurrence of secondary association of bivalents in this study and the observed chromosome lengths (Table 1) suggest that these species are tetrasomics. In *Corchorus olitorius*, 4 chromosomes have 3.33µm length, 4 have 3.31µm length; in *C. tridens*, 4 chromosomes have 3.75µm length, 4 have approximately 2.90µm, in *C. aestuans*, 6 chromosomes have 3.33µm and 2 have 2.92µm. In *C. aestuans* 2.92 is sufficiently close to 3.33µm to consider 8 chromosomes as belonging to the same length group making possible the occurrence of two quadrivalents and three bivalents per cell and confirming that the basic chromosome number (x) is 5 and that 2n is 14 which is indicative of double tetrasomy.

Table 2 summarizes chromosome association at diakinesis in all plants studied. All chromosomes were in bivalent association with high frequency of rings indicating frequent occurrence of chiasmata. Quadrivalents were not scored because bivalents tend to maintain their individuality in secondary quadrivalent associations and were therefore scored as individual bivalents. Secondary association of the bivalents shows that the chromosomes are homoeologous, suggesting that they have common origin. Bivalent association of chromosomes may have been encouraged by structural changes in the chromosomes over time.

Table 3 summarizes pollen grain data in the *Corchorus* species studied. Pollen viability is generally high (>98%) confirming that microsporogensis was regular in these plants. The slight drop in percentage pollen viability in *C. olitorius* var. "agbadu" may be due to its more recent hybrid origin as suggested by Morakinyo (1997).

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