CYTOGENETIC EVALUATION OF MICROSPOROCYTES OF *SETCREASEA PURPUREA*(BOOM) FLOWER

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ABSTRACT
Setcreasea purpurea Boom (Wandering Jews) belongs to the family Commelinaceae of the order Commelinales. The family, made up of 38 genera and about 600 species, occurs mostly in the tropical and warm regions of the world. Many are popular as garden or house ornamentals. It is a flower-bearing plant yet it is mostly propagated vegetatively due to the formation of sterile pollen grains thereby making pollination impossible. Cytogenetic study was carried out to observe the chromosome behaviour in the microsporocytes during meiosis with the aim of understanding the reasons behind the sterility of the pollen grains. Immature flower buds of Setcreasea purpurea were dissected in a Petri dish containing tap water. The microsporocytes were squashed using lactic acetic-orcein stain. Most microsporocytes observed from slides contained 12 bivalents. However, a few contained 10 bivalents and one quadrivalent or 8 bivalents and 2 quadrivalents. This shows that 2n = 24 in the microsporocytes. Other irregularities observed included laggards, abnormal disjunction, chromosome bridges and chromosome fragments all at anaphase-1 and micronuclei at telophase-1. Occurrence of laggards, chromosome bridge formation and micronuclei were low ranging from 1 to 3 in the population of S. purpurea studied. The consequence of these irregular chromosomes is their loss and non-inclusion in the daughter nuclei at telophase-I. The loss of these segments would result in loss of genes on them resulting in the unviable microspores at the end of meiosis. These microspores are the pollen grains in which a large number of them (about 90%) have been found to be sterile thereby making the plant to reproduce asexually by stem-tip cuttings.

KEY WORDS: *Setcreasea purpurea*, Cytogenetic, Quadrivalents, Microspores, Laggards.

INTRODUCTION

Setcreasea purpurea Boom (Wandering Jews), a monocot plant, belongs to the family Commelinaceae of order Commelinales. The family is made of 38 genera and about 600 species (Taylor, 1985). They occur mostly in the tropical and warm regions of the world, many of which are popular as garden or house ornamentals (Taylor, 1985).*Setcreasea purpurea*, a perennial herb and highly branched plant has a height of about 50cm or even more with elliptic lanceolate and purple

leaves. The leaves measure between 4.5 — 13cm long and 2.5 to 3.2 cm broad. It has a terminal inflorescence subtended by two leaf-like bracts which are shorter than the leaves. Three sepals make up the calyx and the corolla is made of three pink petals with six epipetalous stamens. The ovary is glamorous and the fruit is a thin-walled dehiscent capsule (Mehra *et al.*, 1965).

Anderson and Sax (1936) suggested that the differentiation of species or even of genera may be dependent upon gene mutation without necessarily changing the chromosome morphology. However, Ugborogho (1980), working on the genus *Sida*, found out that such differentiation leads to structural and numerical changes in chromosome. Ugborogho (1980) stated further that the changes, which mainly provide the initial isolation permitting independent development of genic changes, include the duplication of similar genomes (autopolyploidy); fragmentation and loss of chromosome segment in polyploids; segmental interchange between non—homologous chromosome which may change the morphology of the genome; translocation and inversion of segments; change in chromosome size; and the union and duplication of unlike genomes involving a simultaneous change in genetic constitution and chromosome number (allopolyploidy).

Taxonomists employ various systems in classifying Commelinaceae into different genera. They first divided it into two subfamilies or tribes namely Tradescantiae and Commelinea, based on a number of possible phylogenetic trends (Brenam, 1966). Jones and Jopling (1972) later reviewed the classification using cytological studies as their basis for the classification. In their studies, they found that the family embraces a very wide range of variation in chromosome number, morphology and meiotic behaviour. They concluded that no particular member could be singled out as typical or representative of the family. This finding was in support of earlier studies of Morton (1967) who had stated that many genera of the Commelinaceae found in West Africa are in a state of active evolutionary change and even many of their species show more considerable variation.

Karyotypic studies of the genus *Setcreasea* revealed chromosome numbers of 2n = 12, 23, 24, 25 and 26 (Handlos, 1970). In *Setcreaseapurpurea*, Bose (1958) observed 2n=12; Mehra *et al.*, (1961) observed 2n = 24. Mehra *et al.*,(1963) alsoobserved 2n = 18 for the hybrids of *S. purpurea* x *S. pallida*. Mehra *et al.*, (1965) detected meiotic aberrations such as quadrivalents, trivalents and univalents at metaphase-I in *S. purpurea*. He concluded, therefore, that aregular

feature in many genera of Commelinaceae is the irregular chromosome behaviour during meiosis.

Setcreasea purpurea is a flowering plant that produces a thin-walled dehiscent fruit in a capsule (plate I). This notwithstanding, it is mostly propagated vegetatively by stem-tip cuttings. Therefore, this study is aimed at studying meiosis in the microsporocytes of the pollen mother cells of the plant in order to determine the types and frequencies of meiotic aberrations responsible for the formation of sterile pollen grains which made it impossible for the plant to undergo sexual reproduction.

MATERIALS AND METHODS

The *Setcreasea purpurea* plant materials(complete flowers) used were obtained from various locations on the University of Lagos campus, Akoka, Lagos. Immature flowers were collected and placed in water in a beaker, to prevent drying up of the flowers. In a careful manner and one at a time, the fresh immature flowers were dissected in a clean petridish containing water to release the anthers in them. Thereafter, two or three suitable anthers, which were slightly creamy or whitish were placed on a clean glass slide and punctured. Puncturing releases the content of the anther. No discernable meiotic stages were observed when a yellowish anther was used.

The debris of the punctured anthers was removed and a drop of lactic-acetic orcein stain was applied to the remaining content and smeared on the glass slide. The original concentration of lactic acetic orcein solution contained 1g orcein in a mixture of 25ml lactic acid and 25ml acetic acid diluted in a proportion of 1:1 with distilled water. The essence of lactic acid in the stain is to prevent the acetic-orcein from drying quickly. The slide and its content was left for about 15 to 20 minutes after which a clean cover slip was placed on the preparation. The cover slip was firmly held down and tapped gently with the blunt end of a dissecting needle. This dispersed the cells and prevented clumping. Excess stain was removed from the preparation by placing the slide between folds of firmly held filter paper and a gentle pressure applied on the cover slip.

Prepared slides were then examined under the microscope fordifferent stages of meiosis. Thiswas done at different magnifications --low power (x10) and high power (x40); and necessary data were scored for selected good slides. More pressure was applied to each slide when necessary. The selected good ones were then sealed along the edges of the cover slip with colourless and transparent nail

varnish. This reduces the rate of drying up of the slide, and as such make them to remain in good condition(for about 3 months of storage) pending the time when photomicrographs will be taken. They were then kept in a slide box in the laboratory for further examination and scoring. Photomicrographs were later taken under oil immersion (x100 objective) with the aid of Wild Photomicroscope MPS 55.

RESULTS

Meiosis was observed in the microsporocytes of *Setcreasea purpurea's* immature flower buds. However, no single flower bud showed all the division stages of both meiosis-I and meiosis-II. Several abnormalities were equally observed in the chromosome behaviour in the course of meiosis.

In prophase- I, the chromosomes appeared thin with bead – like chromomeres visible along their length. At zygotene, the chromosomes appear thicker as a result of condensation, (plateII). Presence of one quadrivalent is also shown in plate II. At diplotene, chromosomes further contract and thicken.Also, there appears to be a mutual repulsion of the paired homologues, which remain held together at one or more chiasmata along their length (plate III); plate III equally shows 2 quadrivalents. At diakinesis, coiling and contraction of chromosomes reach their maximum and become highly visible. They become thick, heavily dark-stained bodies, with three types of chiasmata easily seen and distinguished: Terminal, Sub-terminal and Interstitial chiasmata (plateIV). Most bivalents also appear in a ring form as a result of chiasma terminalization. Terminal chiasmata were observed to have the highest occurrence.

At metaphase-I, the twelve bivalents aligned at the equatorial plate of the cell, but one bivalent is separated from the rest (plate V). At anaphase-I, homologous chromosomes separate from one another and move to opposite poles of the spindle (plate VI).

Some irregular chromosome behaviour was observed at anaphase – I and include the following:

a. Chromosomes moving to the poles at different rates after normal disjunction with centromeres leading (plate VI).
b. The occurrence of laggards at late anaphase - I at the centre (plate VII). They are not distinct enough to show their centromeres.
c. Movements of chromosomes within the cell, not particularly to any pole (plate VIII).

d. Occurrence of dicentric chromatid bridge and acentric chromosome fragments (plate IX).

At telophase - I, cell plate cut across, dividing the cell into two daughter cells (plate X b).However, some irregularities were also observed at telophase — I:

a. Presence of micronuclei in the daughter cells' nuclei after cytokinesis (plate XI a).
b. Presence of chromatid fragments within the daughter cells. This is as a result of cell plates cutting the chromatid bridges leaving them as fragments within the daughter cells' cytoplasm (plate XII) or as projecting arms of broken chromatid bridges in the daughter nuclei (plate XII).

During anaphase - II, the chromatids, which are both V - shaped and J-shaped due to the position of their centromeres, separate and move to the opposite poles (plate XIII). Plate XIV shows late anaphase-Il chromosomes becoming non-distinct. At early telophase-Il, two daughter cells showing four microspores are produced(plate XV). These will later on at late telophase - II be divided to form four daughter cells or microspores. The chromosomes in each daughter nucleus are not distinct enough to be counted to ascertain whether their haploid number is normal or not.

DISCUSSION

Prophase-I is of extremely long duration compared with mitotic prophase. This is because specific functions are performed by the chromosomes at meiotic prophase — I (e.g. synapsis, crossing over, etc) which they do not have to carry out during mitotic prophase. The structure of chromosome of *Setcreasea purpurea* at prophase-I in this study is similar to those Taylor (1931) observed in *Gasteria*, and to those Anderson and Sax (1936) reported in *Tradescantia* species during microsporogenesis. During diplotene, chiasmata, which are the points of crossing over become visible and appear as X-shaped attachments, in this study.The occurrence of few interstitial chiasmata in *S. purpurea* in this report corroborate the observation of Richardson (1935) in *Tradescantia virginiana* and *Setcreasea brevifolia*. He suggested that the low frequency of interstitial chiasmata probably indicates the presence of reciprocal translocation between chromosomes as a result of non-homology of parts of pairing chromosomes.

The meiotic data in this study do not show that *S. purpurea* is an autotetraploid. This is because in an autotetraploid, pairing or synapsis may result in four homologues forming a quadrivalent. But in this report, only one or two

quadrivalents were found in each microsporocyte. This low frequency of quadrivalent in *S. purpurea* suggests that it is not an autotetraploid. Another reason for *S. purpurea* not an autotetraploid is the presence of trivalents in it, which are lacking in autotetraploids such as *Primula sinensis* (Darlington, 1931) and *Avena* (Darlington, 1933). This study, though, did not observe univalent or trivalent, it makes it different from the report of Mehra *et al.,* (1965) who observed trivalents in some microsporocytes of *S. purpurea*. The presence of univalent or trivalent probably indicates the presence of structural hybridity in *S. purpurea*.

High frequency of bivalents and the occurrence of few multivalents was also observed in this report. This supports the findings of Richardson (1935) and Mehra *et al.,* (1963) on *S. brevifolia*. This is equally similar to that of Giles (1942) *on Cuthbertia graminae*. This report did not observe chains and rings of chromosomes. When present they are the results of reciprocal translocation. Darlington (1929) had suggested that the origin of chains from rings was as a result of breakage in terminalized chiasmata rather than to a failure of pairing and chiasmata in such segments. However, the absence of rings in this study corroborates the observation of Ndare (1990). The occurrence of few quadrivalents, few trivalents and mostly bivalents probably suggests that *S. purpurea* is more of an allopolyploid than an autotetraploid.

This present study observed many irregularities in chromosome behaviour especially during meiosis I. This is in accordance with the observations of Islam and Baten (1942) on such other Commelinaceae as *Tradescantia*, *Rhoeo*, *Cyanotis* and *Zebrina*. Differential chromosome movement observed at anaphase I may be as a result of different rates of movement on the spindle due to differential separation of bivalents, as observed in the genus *Tripogandra* by Handlos (1970). Although chromosomes in *S. purpurea* do not show marked difference in size, it was seen that the small bivalents separated first and started moving to the poles while the large ones were still aligned on the metaphase plate. The observation of one or two chromosome fragments in a few microsporocytes in this report was due to their non-movement to either pole. They were probably acentric (i.e. without centromeres), therefore their non-movement was not surprising because they did not have any interaction with the spindle fibres. Islam and Baten (1942) had earlier found chromosome fragments in some species of *Cyanotis*, and *T. virginiana*. Acentric chromosome fragments are the products of crossing over within paracentric inversions resulting in the formation of Chromosome Bridge. It follows, therefore, that the higher the

frequency of paracentric inversion, the more the fragments. In *S. purpurea*, therefore, occurrence of paracentric inversion is low and as such, few fragments were observed.

The laggards observed in this study possess constrictions along their lengths signifying the presence of a centromere. Therefore, they are not chromosome fragments. Their occurrence is an evidence of abnormal disjunction. At anaphase-I, their centromeres do not appear to move towards either poles; even when present at a pole, they were not included in the nuclei of the daughter cells formed at telophase—I. Sax and Anderson (1933) gave reason for these lagging chromosomes as improper functioning of the spindle apparatus, based on their findings in *Tradescantia* where they observed that during division, some chromosomes were ejected into the cytoplasm outside the spindle. Such chromosomes become laggards while others are pole-bound. Improper functioning of the spindle apparatus could also be a reason for different rates of chromosome movements to the poles.

Chromosome fragments not included in the nuclei of the daughter cells condensed and formed micronuclei at the end of telophase — I. The occurrence of micronuclei in this report is very low ranging from 0-2 per microsporocyte. This observation supports the low occurrence thereby stretching the chromosome across the metaphase plate. The breakage of this bridge during telophase-I may be due to the opposing forces between the two centromeres at opposite poles at anaphase-I or cell plate cutting across the bridge during telophase – I. The result is the daughter nuclei having projecting arms of broken chromosome bridges. No chromosome bridge was observed at anaphase — II in this study. Its occurrence in telophase – I would probably be due to non-disjunction of a bivalent containing a dicentric chromosome.

The consequence of this and other irregularities is the formation of deficient or duplicated chromosomes at telophase leading to the occurrence of aneuploid and euploid nuclei. The occurrence of anomalies in this study during meiosis — I indicates non-inclusion in the daughter nuclei at telophase — I of such irregular chromosomes as fragments, laggards and broken dicentric chromosome bridges. The loss of these segments would result in loss of genes on them resulting in the unviable microspores at the end of meiosis. These microspores are the pollen grains in *S. purpurea* and other angiosperms, in which a large number of them have been found to be sterile (Taylor, 1985). This is similar to the earlier findings of Choudhuri (1975) on *Solarium* and Islam and Baten (1942) on *Cyanotis*

axillaris. As a result of the sterility of the pollen grains of *S. purpurea*, and many other Commelinaceae, they are mostly propagated vegetatively, as pollination that leads to fertilization in most microspores is impossible.

However, Mehra *et al.*, (2014) observed viable seeds in *S. purpurea*. This was made possible by cross pollination with another species of the genus *Setcreasea*, as the egg or ovum of *S. purpurea* is viable. This again supports the findings that most Commelinaceae are allopolyploids. The vegetative propagation of different species of Commelinaceae is a major factor that maintains the structural and numerical alterations in different species.

LITERATURE CITED

Anderson, E. and Sax, K. (1936). A cytological monograph of the American Species of Tradescantiae. *Botany Gazette*, **97:** 433 - 476.

Bose, S. (1958). Cytological studies in *Setcreasea purpurea* Boom and *S. brevifolia* (Tour) Rose. *Virginia Journal of Science*, **9:** 392-393.

Brenam, J.M.M. (1966). The Classification of the Commelinaceae. *Journal of Linnaeus Society (Botany),***59:** 349-370.

Choudhuri, R.P. (1975). Cytological studies in West African *Solanum melongena* L. Var. Zaria. *Nucleus*, **18:** 33-39.

Darlington, C.D. (1929). Chromosome behaviour and structural hybridity in the Tradescantiae. *Journal of Genetics*, **21:** 207—286.

Darlington, C.D. (1931). Meiosis in a diploid and tetraploid *Primula sinensis*. *Journal of Genetics*, **24:** 65-69.

Darlington, C.D. (1933). The origin and behaviour of chiasmata IX. Diploid and tetraploid Avena. *Cytologia*, **5:** 128-34.

Giles, N. (1942). Autopolyploidy and geographical distribution in *Cuthbertia graminae* small. *American Journal of Botany*, **29:** 637-645.

Handlos, W.L. (1970). Cytological investigations of some commelinaceae from Mexico. *Baileya*, **17:** 6-34.

Islam, A. S. and Baten, A. (1942). Cytology of *Cyanotis*. *Nature*, **169:** 457-458.

Jones, K. and Jopling, C. (1972). Chromosomes and the classification of the Commelinaceae. *Journal of Linnaeus Society (Botany),***65:** 129-162.

Mehra, K.L, Faruqi, S.A. and Celarier, R.P. (1961). A cytotaxonomical studies of five species of *Setcreasea*. *Phyton*, **17:** 133-140.

Mehra, K.L., Faruqi, S.A. and Celarier, R.P. (1963). Cytogenetical studies in the interspecific hybrid of *Setcreasea brevifolia* (Torr) Sch. and Sydow X *S. pallida* Rose. *Caryologia*, **16:** 693-700.

Mehra, K.L., Faruqi, S.A. and Celarier, R.P. (1965). Cytogenetical studies in the interspecific hybrid of *Setcreasea brevifolia* (Torr) Sch. and Sydow X *S. purpurea* Boom. I. Fl Hybrids. *Caryologia*, **18:** 127-138.

Mehra, K. L., S. A. Faruqi and Late R. P. Celarier. (2014). Cytogenetical Studies in the Interspecific Hybrid of *Setcreasea Purpurea* Boom × *S. Pallida* Rose, *Caryologia: International Journal of Cytology, Cytosystematics and Cytogenetics*, **14** (3): 429-437.

Morton, J. K. (1967). The Commelinaceae of West Africa: A biosystematic survey. *TOC***60** (382): 167–221.

Ndare, V.A. (1990). A cytological study of *S. purpurea* Boom. M.Sc. Project. University of Lagos. 61pp.

Richardson, M.M. (1935). *Setcreasea brevifolia*, a further example of polyploidy and structural hybridity in the Tradescantieae. *Botany Gazette*, **97:** 399-407.

Sax, K and Anderson, E. (1933). Segmental interchange in chromosomes of Tradescantia. *Genetics*, **18:** 53-67.

Taylor, G. (1985). *Flowering plants of the world*. Croom Helm and Sydney, London. 330 pp.

Taylor, M.R. (1931). Chromosomes studies on *Gasteria* III. Chromosome structure during microsporogenesis and the post meiotic mitosis. *American Journal of Botany*, **36:** 367-386.

Ugborogho, R.E. (1980). The significance of environmental changes on the taxonomy of *Sida* in Nigeria. *The Nigerian Field*. **45**:83-90.

FIGURE CAPTIONS

Plate I: *Setcreasea purpurea* plant with flowers (x 1/4)

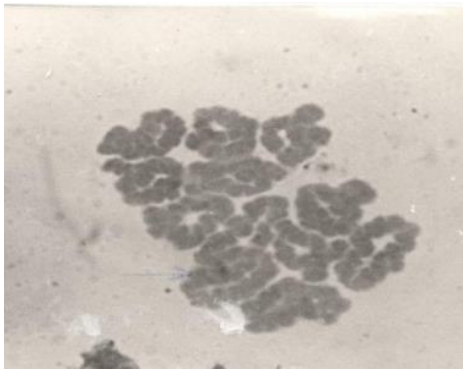

Plate II: Prophase I; zygotene sub-stage showing 10 bivalents and 1 tetravalent.* Arrow points to the tetravalent (x 2000)

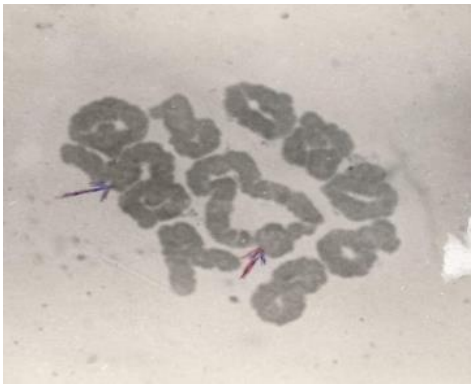

Plate III: Prophase I; zygotene sub-stage; 8 bivalents and 2 quadrivalents. Arrows point to the 2 quadrivalents (x 2000)

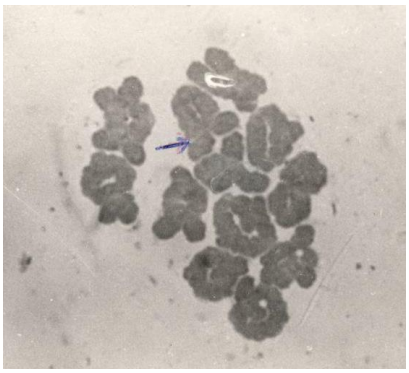

Plate IV: Diakinesis; bivalents showing terminalization of chiasmata. Arrow shows the position of centromere (x 2000)

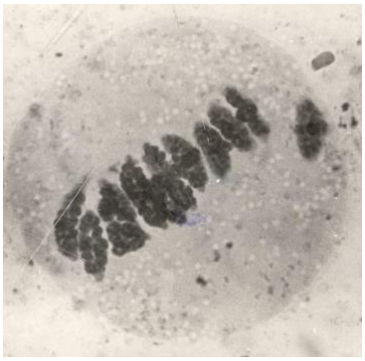

Plate V: Metaphase I; bivalents aligned at the equatorial plate (x 2000)

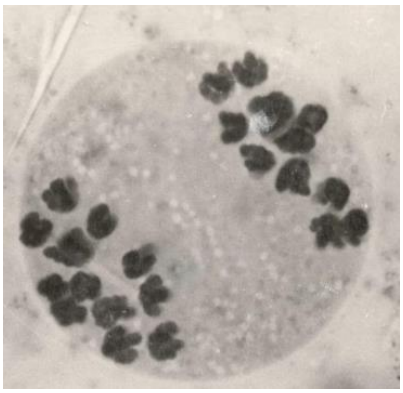

Plate VI: Early anaphase I; Normal disjunction (x 2000)

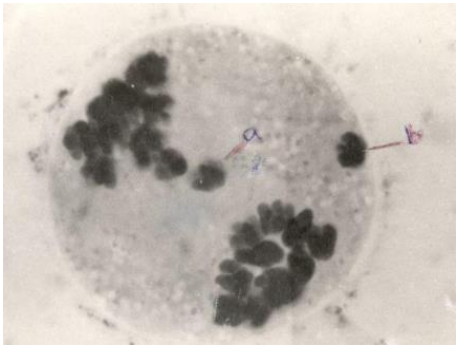

Plate VII: Late anaphase I; Abnormal disjunction; a = Laggard; b = Chromosome fragment (x 2000)

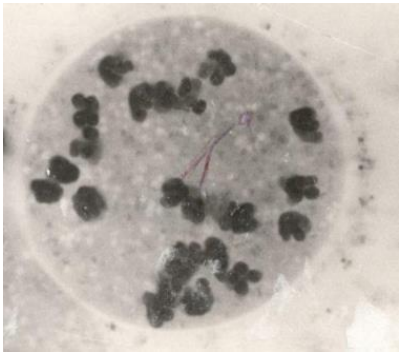

Plate VIII: Chromosomes moving within the cell not to any particular pole; a = Laggards at the centre (x 2000)

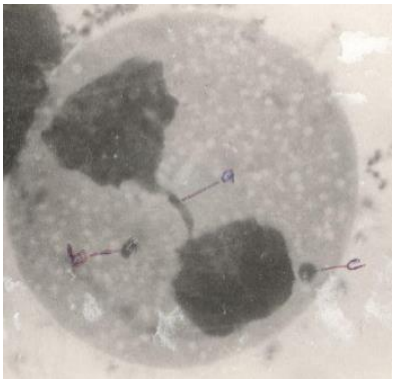

Plate IX: Late anaphase I; a = chromatid bridge; b and c = chromosome fragments (x 2000)

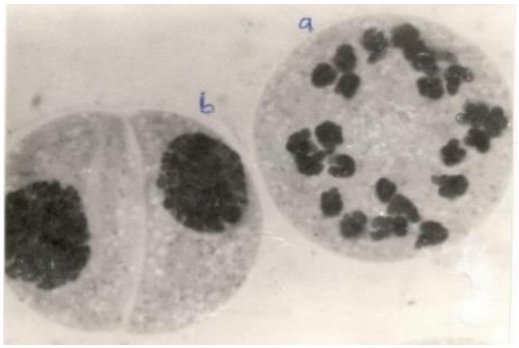

Plate X: Cell a shows chromosomes moving within the cell not to any particular pole. Cell b shows normal telophase (x 2000)

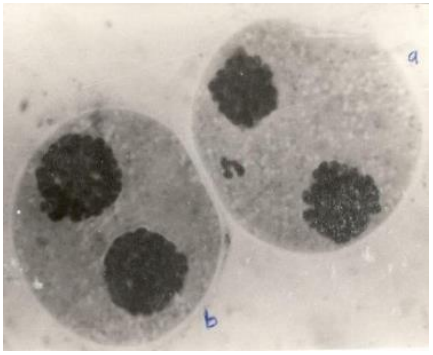

Plate XI: Early telophase I; cell a shows chromosome fragment; cell b shows normal disjunction (x 2000)

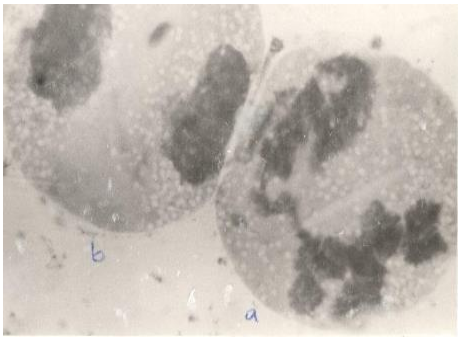

Plate XII: Early telophase I; cell a shows cell plate cutting across the chromatid bridge. Cell b shows a chromosome fragment (x 2000)

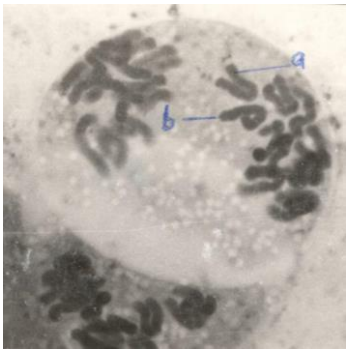

Plate XIII: Anaphase II chromosomes;
a = V-Shaped chromosome;
b = J-shaped chromosome (x 2000)

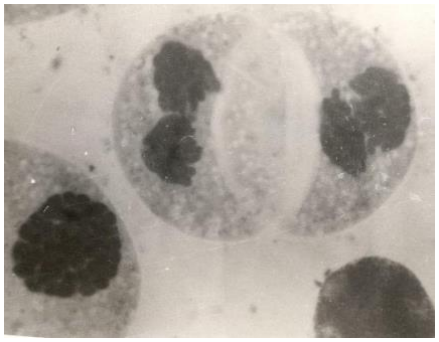

Plate XIV: Late anaphase II; chromosomes become undistinguishable (x 2000)

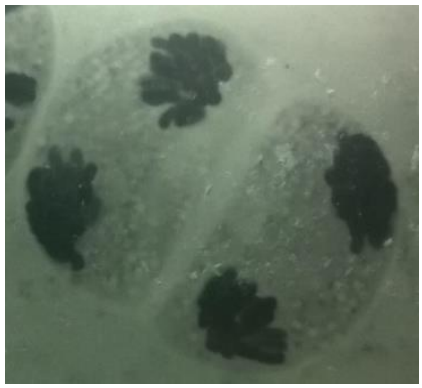

Plate XV: Early telophase II; two daughter cells showing four microspores (x 2000)