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MICROSPOROGENESIS in Luffa aegyptiaca, Mill.

BY

J. J. ASANA AND R. N. SUTARIA,

M. R. Science Institute, Gujarat College, Ahmedabad.

Introduction.

Cucurbitaceæ is well represented in India. Among its numerous representatives in this country there are several plants of economic importance. We are endeavouring to gather some cytological data by studying a few representative plants of this large order with the hope that the information thus secured may perhaps prove of some interest to geneticists working in India. Kirkwood (4), Castetter (1), Heimlich (3) and Passmore (7) have studied the microsporogenesis of some common plants of this order.

The present paper gives an account of the microsporogenesis of *Luffa aegyptiaca*, which is one of the common cultivated representatives of the cucurbitaceæ at Ahmedabad.

There is a good deal of controversy as regards the number of chromosomes in some genera of this family. For instance in *Cucurbita maxima* according to Castetter (1) and Passmore (7) there are 20 haploid and 40 diploid chromosomes; whereas Rau (8) records in the same plant 12 pairs as the diploid number of the chromosomes. It will perhaps be profitable to reproduce here the lists of chromosome numbers in the Cucurbitaceæ as reported by McKay (5) and Passmore (7), and to bring these lists up-to-date in a tabular form as in table I.

Material and Methods.

Staminate flowers were collected in the monsoon of 1928 and again in 1931 and were fixed in Allen's modification of Bouin's fluid. To ensure quick penetration of the fixative, the perianth was removed and an exhaust pump was used. Collections of material were usually made between 8 a.m. and 1-30 p.m., on bright sunny days. The time

Table I.

Reported Chromosome Numbers in the Cucurbitaceae.

Species	N	2N	Reported by
Bryonia alba ...	10	...	Von Bonnicke, Mourman.
B. dioica ...	10	...	Strasburger, Mourman.
Benincasa hispada ...	...	24	J. W. McKay.
Bryonopsis ...	...	24	" "
Citrullus vulgaris ...	11	...	Kozhukhow, McKay.
Coccinia hirtella ...	...	24	J. W. McKay.
Cucumis dipsaceus ...	...	24	" "
C. melo ...	12	24	Passmore, Kozhukhow, McKay.
C. metuliferus ...	...	24	J. W. McKay.
C. myriocarpus ...	...	24	" "
C. sativus ...	7	14	Heinlich, Kozhukhow, Passmore.
Cucurbita ficifolia ...	...	42	J. W. McKay.
C. foetidissima ...	...	42	" "
C. maxima ...	24	44–48	Kozhukhow, Ran.
C. maxima ...	20	40	Castetter, Passmore.
C. moschata ...	24	48	Kozhukhow.
C. palmata ...	...	42	J. W. McKay.
C. pepo ...	12	24	Lundegardh.
C. pepo var. pomiformis ...	20	40	Kozhukhow.
C. pepo var. citrullina ...	21	42	"
C. pepo (Jersey white Bush) ...	20	...	Passmore.
C. pepo (English vegetable marrow) ...	20	...	"
Cyclanthera pedata ...	...	32	J. W. McKay.
Echallium elaterium ...	...	24	" "
Echinocystis (micrampelis) lobata ...	16	...	Kirkwood.
Lagenaria vulgaris ...	...	24	J. W. McKay.
Lagenaria ...	17	...	Morinaga and others.
Luffa acutangula ...	...	26	J. W. McKay.
L. aegyptiaca ...	13	...	Asana and Sutaria, Morinaga and others.
L. cylindrica ...	11	...	Passmore.
L. marylandica ...	...	26	J. W. McKay.
Melothria punctata ...	...	24	" "
Momordica charantia ...	...	22	" "
Sicyos angulata ...	...	24	" "
Trichosanthes Japonicus ...	11	22	Y. Sinoto.

of fixation was between two and three hours. The material was dehydrated starting with 5 per cent alcohol, was gradually brought to 70 per cent alcohol drop by drop method and kept in 70 per cent alcohol.

Then gradually 70 per cent alcohol was replaced by freshly distilled aniline oil by drop method and finally cleared in synthetic oil of wintergreen. The material was embedded in paraffin 52.C and sectioned at a thickness of 9 microns. Heidenhain's iron-alum haematoxylin was used as the stain, and gave good results.

Observations.

In a longitudinal section of very young flower buds of *Luffa*, a small number of pollen mother-cells appears in each loculus. The pollen mother-cells are closely packed together. The tapetum forms a compact and continuous layer in which several cells are binucleate. The chromatin of the pollen mother-cells is very meagre and stains faintly. In many cells there is a single large deeply stained nucleolus but in several other cells of the same category besides the large nucleolus a very small nucleolus is also present (Pl. I, Fig. 1). The cytoplasm of the pollen mother-cells is dense and granular.

The chromatin, a little later, forms a fine network of irregular masses which soon resolves into a convoluted thread. The thread shows a faint linin ground-work thickly beaded with chromatin granules (Pl. I, Fig. 2). Gradually the convoluted thread forms a contracted mass which together with the nucleolus moves to one side of the nucleus, where it comes in contact with the nuclear membrane. This is the typical synizesis stage (Pl. I, Fig. 3). The synizetic knot now loosens and loops are thrown out from it. The threads, which become looped and coiled throughout the nuclear cavity, vary in thickness. Some of the threads of the spireme show a distinct split in them (Pl. I, Fig. 4). The method of chromosome pairing seems to fall in line with that of typical parasynapsis. Such a longitudinal split in the spireme is also recorded both by Heimlich (3) and Kirkwood (4) in the Cucurbitaceae.

The spireme soon breaks up into segments of various lengths. Some of the segments are in the form of loops whose sides then twist round each other. The segments have not as yet lost their beaded appearance altogether. It is interesting to note that the nucleolus of *Luffa* displays a papilla-like outgrowth at this stage (Pl. I, Fig. 5). Passmore (7) and Heimlich (3) also refer to such a budding nucleolus, the former author in *Luffa cylindrica* and the latter in *Cucumis sativus*.

Fig. 6 represents a typical early diakinesis stage. The nucleus has now attained its maximum size. The chromosomes contract into short, thick, irregular masses. In our preparations we find the

nucleolus at this stage giving out some material in the form of irregular masses from the bud-like projection. Heimlich (3), who has made a very careful study of the peculiar nucleolar disappearance in Cucumber, is of opinion that since these nucleolar fragments agree with the chromosomes in their staining reactions, it is quite possible that they may easily be mistaken for chromosomes.

The bivalents now condense further and lie scattered throughout the nuclear cavity. Some of the bivalents may be seen connected by delicate strands (Pl. I, Fig. 7). The nucleolus here is of a peculiar triangular shape with three bud-like projections to which some of the bivalents are attached with delicate fibres. Such a peculiarly-shaped nucleolus is not infrequently found in the Cucurbitaceae. Heimlich (3) in *Cucumis sativus* observes that the nucleolus in that species has a much elongated or vermiform structure, while Frew and Bowen (2) record a dumb-bell-shaped structure in *Cucurbita maxima*.

The nuclear wall now becomes irregular and soon in its place a number of very fine delicate fibres appears. The fragments of the nucleolus now completely disappear and the bivalent chromosomes migrate from the periphery to the centre of the nucleus where they are caught by some intervening fibres (Pl. I, Fig. 8).

The observations on the spindle formation in *Luffa* give no clear evidence as to the intra or extra-nuclear origin of the fibres. At the time when the felt-like web is formed by the delicate radiating fibres in the cytoplasm, some intervening fibres also appear in the nuclear cavity. From these observations one may infer that the formation of the spindle is partly of nuclear and partly of cytoplasmic origin.

No clear multipolar or tripolar spindle was seen in *Luffa* as described by Heimlich (3) in *Cucumis sativus* and Castetter (1) in *Cucurbita maxima*. As the bipolar spindle, which is very broad at both the poles, is formed, the bivalents are distributed along the equatorial plate of the metaphase (Pl. I, Fig. 9). The shape and arrangement of these bivalents is quite peculiar. They are beaded or slightly three-lobed in outline and are elongated in the direction of the axis of the spindle. Passmore (7) also states that there seem to be a tendency for some of the chromosomes to assume a trivalent formation at anaphase in *Cucumis sativus*.

Fig. 10 represents a typical polar view of the metaphase. A very dense perinuclear zone is quite conspicuous at this stage. There are thirteen bivalent chromosomes. Although the chromosomes are very small, they can be counted with ease during diakinesis, metaphase and anaphase stages, since they do not clump together but stand well apart. According to Passmore (7) there are only eleven bivalent chromosomes

in *Luffa cylindrica*. Morinaga and others (6) and McKay (5) report thirteen as the haploid number of chromosomes both in *Luffa ægyptiaca* and *Luffa acutangula*.

In the early anaphase as the two univalents separate, the ends attached to the spindle fibres are drawn out into fine points (Pl. II, Fig. 11). A typical heterotypic anaphase is represented by (Pl. II, Fig. 12. where many of the chromosomes are angular in outline. A polar view of thirteen anaphase chromosomes nearing a pole is seen in (Pl. II, Fig. 13). During late anaphase the chromosomes are closely massed together and begin to fuse (Pl. II, Fig. 14). Before the chromosomes form a dense mass at the poles and lose their identity, the nuclear membrane begins to appear so as to organise the daughter nuclei (Pl. II, Fig. 15). Fig. 16 shows the polar view of the late telophase nucleus.

Fig. 17 represents a typical interkinesis stage which is of a comparatively longer duration. A large barrel-shaped spindle is seen between the two daughter nuclei and no cell plate is observed as reported by Castetter (1). Each daughter nucleus possesses several nucleoli and irregular masses of chromatin connected with many radiating fibres. No further stages after interkinesis were observed and the nuclei pass very rapidly into the homœotypic metaphase.

It is very difficult to find the various homœotypic stages as this division process is passed through very quickly. The two broad spindles at the metaphase are commonly found lying parallel to each other (Pl. II, Fig. 18). Fig. 19 represents a typical polar view of both the metaphase plates. The chromosomes are small and lie close together but their number can be counted as thirteen. We have observed relatively few stages of the homœotypic anaphase, which as usual is passed through quickly (Pl. III, Fig. 20).

The reconstruction of the grand-daughter nuclei seems to be very rapid. Each of the grand-daughter nuclei shows many small nucleoli and the chromatin is distributed in the form of a beaded network which persists long into the development of the spores. Unlike Castetter (1) who states that no spindles consisting of true spindle fibres are formed after the homœotypic division, the writers have observed as Heimlich (3) has done that the spindles are made up of true fibres between any two of the nuclei (Pl. III, Fig. 21). A tetrahedral arrangement of the grand-daughter nuclei is usually seen at this stage, but occasionally all four nuclei may occur in one plane (Pl. III, Fig. 22).

Quadripartition of the pollen mother-cell in Luffa takes place by furrowing (Pl. III, Fig. 23).

Summary.

1. Resting pollen mother-cell nucleus contains one or two nucleoli together with a delicate faintly stained network of chromatin.

2. The chromosome pairing seems to be according to the scheme of parasynapsis.

3. The nucleolus shows a tendency to budding and gives out an outflow of some material.

4. In diakinesis delicate radiating fibres are seen in the cytoplasm.

5. The formation of the spindle seems to be partly of nuclear and partly of cytoplasmic origin.

6. When the bivalent chromosomes are arranged on the equatorial plate of the metaphase stage, they are beaded or three-lobed in outline.

7. The haploid number of chromosomes is thirteen.

8. No cell plates were observed on the spindles both at the heterotypic and homœotypic divisions.

9. At the time of the reconstruction of the grand-daughter nuclei spindles made up of true fibres were observed.

10. Quadripartition of the pollen mother-cell is brought about by furrowing.

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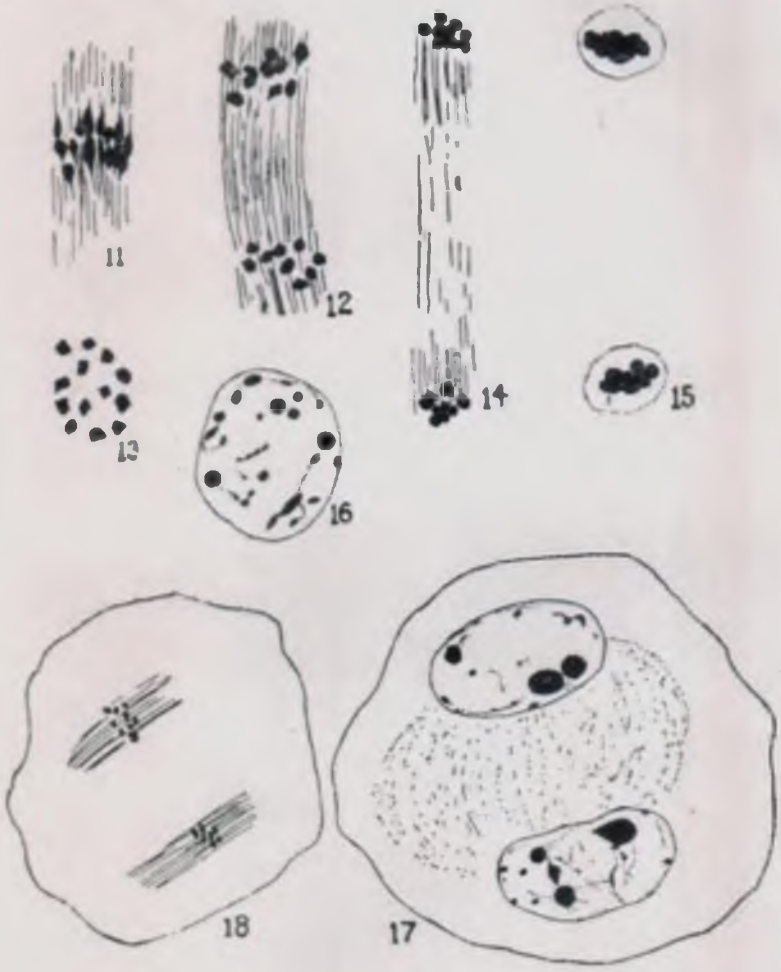
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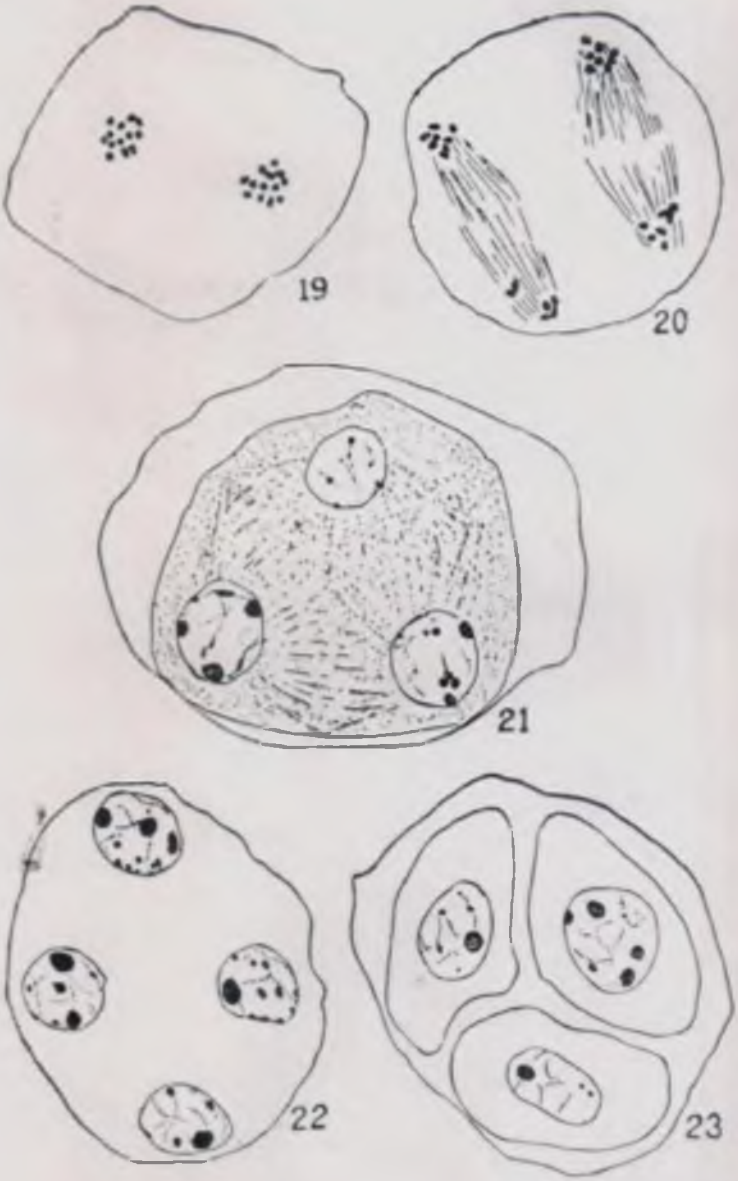
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Explanation of Plates.

All figures except 21, 22 and 23 were drawn with the aid of the Abbe Camera lucida at stage level, under a 2 m.m. imm. Zeiss N. A. 1·4, with Zeiss Comy. Oc. 20 and Leitz Aplanatic oil immersion sub-stage Condenser, N. A. 1·40. Figures 21–23 were drawn with the same objective and Leitz Huyghenian Oc. 5.

Plate I.

Fig. 1. Resting nucleus of the pollen mother-cell.
Fig. 2. Prosynizetic nucleus, with beaded convoluted threads.
Fig. 3. Synizesis.
Fig. 4. Loosening of the synizetic knot, showing the chromosome pairing.
Fig. 5. Segmentation of the spireme, showing twisted chromosomes. Nucleolus budding.
Fig. 6. Early diakinesis. Nucleolus giving out some material.
Fig. 7. Late diakinesis. Nucleolus giving out three buds.
Fig. 8. Formation of the spindle fibres with bivalent chromosomes.
Fig. 9. Heterotypic metaphase with three-lobed bivalent chromosomes on the broad bipolar spindle.
Fig. 10. Polar view of metaphase showing thirteen bivalent chromosomes.

Plate II.

Fig. 11. Early heterotypic anaphase.
Fig. 12. Heterotypic anaphase, chromosomes passing to the poles.
Fig. 13. Polar view of anaphase showing thirteen angular chromosomes.
Fig. 14. Late heterotypic anaphase.
Fig. 15. Early heterotypic telophase.
Fig. 16. Polar view of the late telophase nucleus.
Fig. 17. Interkinesis.
Fig. 18. Homœotypic metaphase with parallel spindles.

Plate III.

Fig. 19. Polar view of the heterotypic metaphase.
Fig. 20. Homœotypic anaphase.
Fig. 21. Late homœotypic telophase, showing the tetrahedral arrangement of grand-daughter nuclei. Spindles fibres are clearly seen.
Fig. 22. Four grand-daughter nuclei in one plane.
Fig. 23 Pollen tetrad with three members.