Cytology of Coconut Endosperm

BY

A. ABRAHAM AND P. M. MATHEW

Department of Botany, Kerala University, Trivandrum, India

With one Plate and four Figures in the Text

ABSTRACT

The cytology of coconut endosperm was studied from tender nuts from an ordinary tall variety of coconut palm. Cellular endosperm development becomes visible in nuts about 6 months old, at which stage a thin coating of jelly-like endosperm tissue is seen around the periphery of the large embryo-sac cavity. The endosperm tissue is thicker at the antipodal end. The nuclei in the young coconut embryos are diploid ($2n = 32$), and they divide by normal mitosis. Nuclei of varying sizes were observed in the endosperm tissue, and they showed very active mitosis, the frequency of division being higher in regions nearer to the micropyle. Three different chromosome counts were made in the dividing nuclei, 48 ($3n$), 96 ($6n$), and 192 ($12n$). This shows that increase in size of nuclei is due to euploid increase in chromosome number. In addition to normal mitosis, a C-mitotic type of division was also observed in the $3n$ and $6n$ nuclei, and it is suggested that this type of aberrant mitosis is responsible for the attainment of higher levels of ploidy in coconut endosperm.

INTRODUCTION

THE work reported here deals with the cytology of the endosperm of *Cocos nucifera* L. (coconut), the most important cultivated palm. While practically all parts of this tropical palm are useful to man, the most valuable product is the white endosperm obtained from the nut, which is very rich in oil.

As early as 1927 Quisumbing and Juliano described the development of the ovule and the formation of the embryo-sac in *Cocos*. Cutter et al. (1952) gave a generalized account of the development of the major tissues in the ripening coconut, and later described the orientation of the developing embryo-sac (Cutter and Freeman, 1954). Cutter et al. (1955) have also made some interesting observations on the occurrence and behaviour of the free nuclei in the liquid syncytial endosperm (milk), and of their fate during the maturation of the cellular endosperm. Though the endosperm has been shown to possess nuclei with striking variation in size (Cutter et al., 1955) and ploidy (Dutt, 1953), how this happens has not been clearly understood.

The object of the present study was to find out the pattern of nuclear behaviour at various stages in the development of the endosperm in coconut.

MATERIAL AND METHODS

The coconuts used in this study were collected from a healthy tall variety of coconut palm grown in the experiment station attached to the College of Agriculture, Vellayani, Trivandrum. This tree bore 18 bunches of nuts (in

February 1962), and each had about 25–30 nuts. Nuts of various developmental stages, beginning from the youngest bunch, were carefully plucked and examined, and up to the eighth bunch (from the top downwards) they were completely devoid of visible fleshy endosperm, the presence of which, however, was observed from the ninth bunch. For cytological observation, endosperm from the nuts belonging to the ninth and tenth bunches was used, and for this fresh nuts were plucked between 9 and 11 a.m., and portions of the integuments with attached endosperm were removed from various positions around the periphery of the cavity and fixed separately in Carnoy's fluid. Young embryos of these tender nuts were also fixed in the same fluid for cytological study. The fixed materials were kept in a refrigerator for about 1 week before making cytological preparations. Slides were made using the simple aceto-carmine technique. Addition of a trace of iron-acetate to the fixative helped to increase the staining of the chromosomes. For taking microtome sections, blocks of endothelium (Cutter et al., 1955) with attached endosperm from various positions in relation to the micropyle were fixed in Craf.

RESULTS

Embryos of the tender nuts (from the ninth and tenth bunches) were small in size, measuring about 1–1·5 mm in diameter. The embryonic tissue showed very active division during the forenoon hours and 32 chromosomes were clearly counted at metaphase (Plate 1 A). This count agrees with all the previous reports on the cytology of *Cocos nucifera* (Santos, 1928; Janaki Ammal, 1945; Venkatasubhan, 1945; Sharma and Sarkar, 1956; Nambiar and Swaminathan, 1960; and Abraham, Mathew and Ninan, 1961). The chromosomes were medium sized, ranging from 2·2 to 5·5 μ in length, and their morphology corresponds in general with the earlier report of Nambiar and Swaminathan (1960) from the root tip cells of an ordinary tall variety of coconut. The longer chromosomes showed submedian constrictions, while in the smaller ones the centromeres occupied nearly median position. One of the longer pairs of chromosomes showed two constrictions. A pair of the smaller chromosomes also appeared to possess two constrictions. In late prophase each chromosome of this pair had a small heterochromatic segment and a tiny euchromatic satellite beyond the secondary constriction. However, in metaphase, when the chromosomes attain maximum contraction, these satellites were not clearly visible (see the chromosomes at 10 o'clock in Plate 1 A and Fig. 1).

In the material examined by us, solid endosperm development on the inner face of the endothelium begins in nuts about six months old (ninth bunch). At this stage the nuts showed a thin coating of a jelly-like solid or semi-solid endosperm tissue around the periphery of the cavity. Visual observation as well as paraffin sections have shown this tissue to be thicker at the antipodal end, and it gradually diminished towards the micropylar end (Fig. 4). The nuclei in the endosperm tissue showed remarkable variation in size, some being several times as large as the normal $3n$ endosperm nucleus. Data of chromosome counts of this tissue from the basal, middle and top regions of two nuts each in bunches 9 and 10 are given in Table 1.

Nuclei possessing three different chromosome numbers have been observed in the endosperm. The majority of these, especially in endosperm tissue

FIGS. 1 and 2. Explanatory diagrams drawn from photographs of Plate 1, A and B. Fig. 1. Mitosis in a developing embryo in a 6-month-old coconut showing 32 chromosomes at metaphase. Fig. 2. Mitosis in a normal triploid nucleus in coconut endosperm showing 48 chromosomes at metaphase. × 1,500.

adjacent to the endothelium, are the normally expected triploid ($3n = 48$; Plate 1 B). The next in frequency are hexaploid nuclei ($6n = 96$; Plate 1 C). A still smaller number of $12n$ nuclei with 192 chromosomes have also been observed (Plate 1 D).

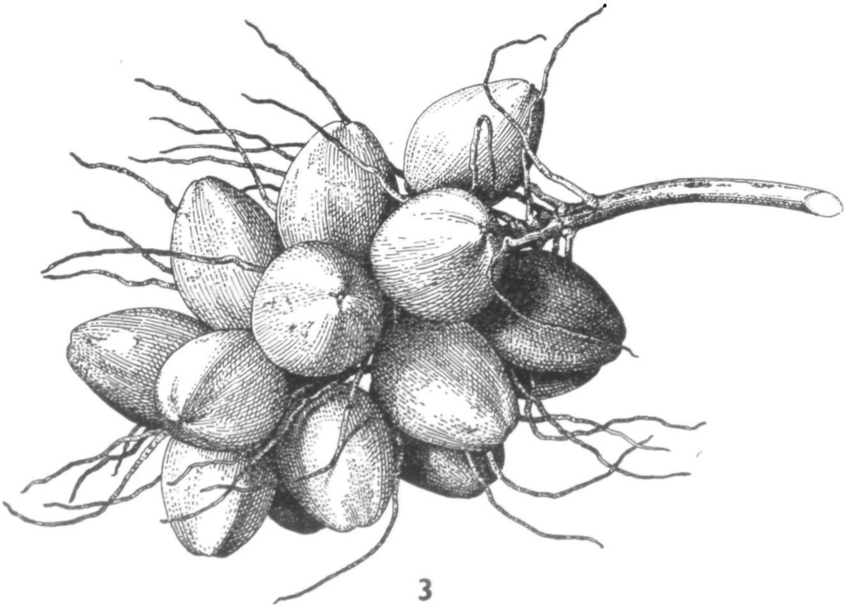
An interesting observation was the presence of a number of divisions exactly corresponding to C-mitosis. In Plate 1 E is illustrated one such

TABLE I

Chromosome Numbers in the Endosperm of Cocos nucifera

No. of slides examined	Bunch no.	Region	Ploidy 3n N	3n C-m	6n N	6n C-m	12n N	12n C-m	Total
10	9	Top	193	15	95	6	13	..	322
10	9	Middle	79	15	34	8	7	..	143
10	9	Base (antipodal end)	40	4	23	3	11	..	81
10	10	Top	45	5	19	2	8	..	79
10	10	Middle	18	2	12	..	4	..	36
10	10	Base (antipodal end)	10	1	6	..	1	..	18

(N = normal mitosis; C-m = C-mitosis)



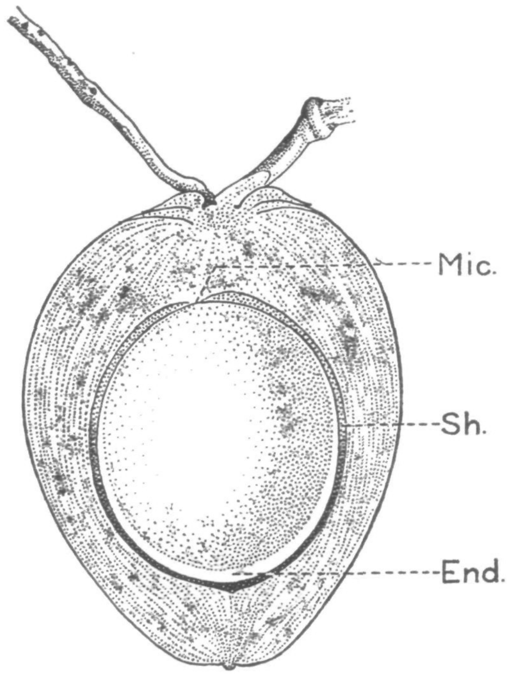
3

FIG. 3. A 6-month-old coconut bunch, bearing 18 nuts. Most of the nuts are hanging down; some are held in a horizontal position, and a few others are held in such a way that the antipodal end points upwards.

metaphase showing 96 'double chromosomes'. This type of division is evidently responsible for the increase in chromosome numbers from the normal triploid to $6n$ and $12n$. Though accurate counts have not been made of higher levels of polyploidy in the endosperm tissue, in the present material there is evidence that $24n$ nuclei are also very occasionally produced. It would be of considerable interest to find out how C-mitosis is induced in certain cells in the endosperm. This and related problems are under investigation.

Discussion

The endosperm in angiosperms has been reported by many workers to constitute favourable material for cytological study, and investigations in plants like maize (Duncan and Ross, 1950), *Allium*, &c. (Kato, 1957) have yielded very interesting information concerning the occurrence of mosaics of



4

FIG. 4. Longitudinal section of a 6-month-old coconut. Note the thin coating of endosperm around the periphery of the cavity; the tissue is thicker at the antipodal end. Mic.—micropylar region. End.—endosperm. Sh.—the endothelium which becomes hard in the mature nut and is known as the shell.

different cells having different degrees of ploidy, endomitosis, &c. Compared to other plants, the endosperm in coconut has a unique structure associated with the peculiar mode of development of the embryo-sac. In a developing nut, the large cavity of the embryo-sac is filled with a watery fluid in which numerous free nuclei of varying sizes are suspended (Cutter et al., 1955; Dutt, 1953; and Abraham and Thomas, 1962). The reports of Cutter and Dutt, however, are at variance in regard to the relation these free nuclei have with the solid endosperm, and their behaviour during division. Cutter holds the view that (1) development of the cellular endosperm is initiated by the deposition of the suspended free nuclei in the milk at the basal region of the cavity (antipodal end) and (2) the division of the free nuclei is exclusively by amitosis, and that in the early cell layers of the endosperm also amitosis is frequent, regular mitotic type of division coming in only at a later stage of cellular endosperm development. Dutt (1953), on the other hand, has observed regular mitosis in the free nuclei, and believes mitosis to be the usual mode of division throughout the stages of endosperm development in coconut. She also considers that endosperm development starts first on the wall, and from the loose jelly of the syncytium free nuclei get detached and become suspended in the milk. In this connexion it is to be noted that in a thick bunch, not all the nuts are hanging (Fig. 3). Some are held in a horizontal position, and still others occupy a position which keeps the antipodal end of the nut pointing upwards. Even in such cases endosperm development first becomes evident at the antipodal end, so that Cutter's view of initiation of endosperm development by settling down and deposition of suspended free nuclei does not seem acceptable. It is possible that, to start with, an initial layer of endosperm cells is formed throughout the embryo sac cavity, and further development by division of cells is more marked at the antipodal region, irrespective of the position of the fruit on the bunch.

Both Cutter and Dutt have reported striking size variation of nuclei in coconut endosperm. The former believes this to be the result of amitosis and nuclear fusion, and cites Dutt's report of $2n$, $3n$ and $10n$ nuclei as evidence in support of this, though she reported no case of amitosis in her material. In the material examined by us we have not come across any nuclei in the cellular endosperm dividing amitotically, nor have we seen any dividing nucleus possessing a chromosome number equal to or close to the $2n$ or $5n$ numbers or their multiples. Cytological observations made on materials transported long distances, as in the case of nuts examined by Cutter, may not always reveal the true state of affairs.

Table 1 shows that the frequency of division is higher in the regions nearer to the micropyle, gradually decreasing towards the basal (antipodal) region at the stages of development examined. As the tissue grows mature the frequency of nuclear division diminishes still further, and a stage is reached when there is little or no division at all in the endosperm. Paraffin sections have shown that in the 6-month-old nuts the endothelial wall at the micropylar region possess only a few endosperm cell layers which are highly meristematic, while in regions farther away towards the antipodal end the rate of division gradually decreases, only a few cell layers adjacent to the wall being in the state of division. Irrespective of the region of the endosperm, $3n$ nuclei predominate in the innermost cell layers, and the level of ploidy becomes higher in layers away from the endothelium and towards the cavity containing the milk.

In maize, increase in the size of endosperm nuclei has been reported to be due to an endomitotic process (Duncan and Ross, 1950) in which each chromosome divided to give a multistranded appearance (polyteny). However, in the same material, Punnet (1953) has reported from actual chromosome counts the occurrence of hexaploid nuclei. In the endosperm of *Gagea* and some dicotyledonous plants also increase in the size of nuclei has been reported to be due to euploid increase in chromosome number (endopolyploidy) (Tandon and Kapoor, 1962). Coconut endosperm evidently presents a similar case since polyploid nuclei, up to the 12-ploid level, have been recognized in the present study from accurate chromosome counts at mitotic metaphase. Apart from the normal mitotic type of nuclear division in $3n$, $6n$, and $12n$ nuclei, an appreciable frequency of C-mitosis has been noticed in the $3n$ as well as $6n$ nuclei (Table 1). The C-mitotic type of division becomes clear only at the metaphase stage. This is followed by the formation of restitution nuclei and at the following division the chromosome number becomes doubled. This type of aberrant mitosis, here reported for the first time in coconut, readily explains how nuclei with higher levels of ploidy originate in the endosperm tissue. However, it is not clear what exactly causes the divisions leading to endopolyploidy in this tissue. In this connexion it is interesting to note that in the young embryos, which remain in direct contact with the developing endosperm, all the cells are diploid ($2n = 32$) and the several hundred divisions examined were all found to be of the normal type.

Literature Cited

ABRAHAM, A., MATHEW, P. M., and NINAN, C. A., 1961: Cytology of *Cocos nucifera* L. and *Areca catechu* L. *Cytologia*, **26**, 327–32.

—— and THOMAS, K. J., 1962: A Note on the *in vitro* Culture of Excised Coconut Embryos. *Indian Coconut J.* **15**, 84–88.

CUTTER, V. M., WILSON, K. S., and DUBE, J. F., 1952: The Endogenous Oxygen Uptake of Tissues in the Developing Fruit of *Cocos nucifera*. *Amer. J. Bot.* **39**, 51–56.

—— and FREEMAN, B., 1954: Development of the Syncytial Endosperm of *Cocos nucifera*. *Nature*, **173**, 827–8.

—— WILSON, K. S., and FREEMAN, B., 1955: Nuclear Behaviour and Cell Formation in the Developing Endosperm of *Cocos nucifera*. *Amer. J. Bot.* **42**, 109–15.

DUTT, M., 1953: Dividing Nuclei in Coconut Milk. *Nature*, **171**, 799–800.

DUNCAN, R. E., and ROSS, J. G., 1950: The Nucleus in Differentiation and Development. III. Nuclei of Maize Endosperm. *J. Hered.* **41**, 259–68.

JANAKI AMMAL, E. K., 1945: *Chromosome Atlas of Cultivated Plants*, by Darlington, C. D., and Janaki Ammal, E. K. George Allen & Unwin Ltd., London.

KATO, Y., 1957: Cytological Abnormalities in the Embryo Sac Mother Cell and Endosperm Tissue of *Lilium formolongo* Hort. and *Allium fistulosum* L. *Cytologia*, **22**, 69.

NAMBIAR, M. C., and SWAMINATHAN, M. S., 1960: Chromosome Morphology, Microsporogenesis and Pollen Fertility in Some Varieties of Coconut. *Indian J. Genet.* **20**, 200–11.

PUNNET, H. H., 1953: Cytological Evidence of Hexaploid Cells in Maize Endosperm. *J. Hered.* **44**, 257–9.

QUISUMBING, E., and JULIANO, J. B., 1927: Development of the Ovule and Embryo Sac of *Cocos nucifera*. *Bot. Gaz.* **84**, 279–93.

SANTOS, J. K., 1928: A Cytological Study of *Cocos nucifera* L. *Philippine Jour. Sci.* **37**, 417–37.

SHARMA, A. K., and SARKAR, S. K., 1956: Cytology of Different Species of Palms and its Bearing on the Solution of Problems of Phylogeny and Speciation. *Genetica*, **28**, 361–488.

TANDON, S. L., and KAPOOR, B. M., 1962: Cytology of Endosperm in Angiosperms. *Sci. & Cult.* **28**, 114–16.

VENKATASUBHAN, K. R., 1945: Cytological Studies in Palmae. Chromosome Numbers in a Few Species of Palms of British India and Ceylon. *Proc. Ind. Acad. Sci.* **22**, 193–207.

EXPLANATION OF PLATE

(All photographs at a magnification of 800)

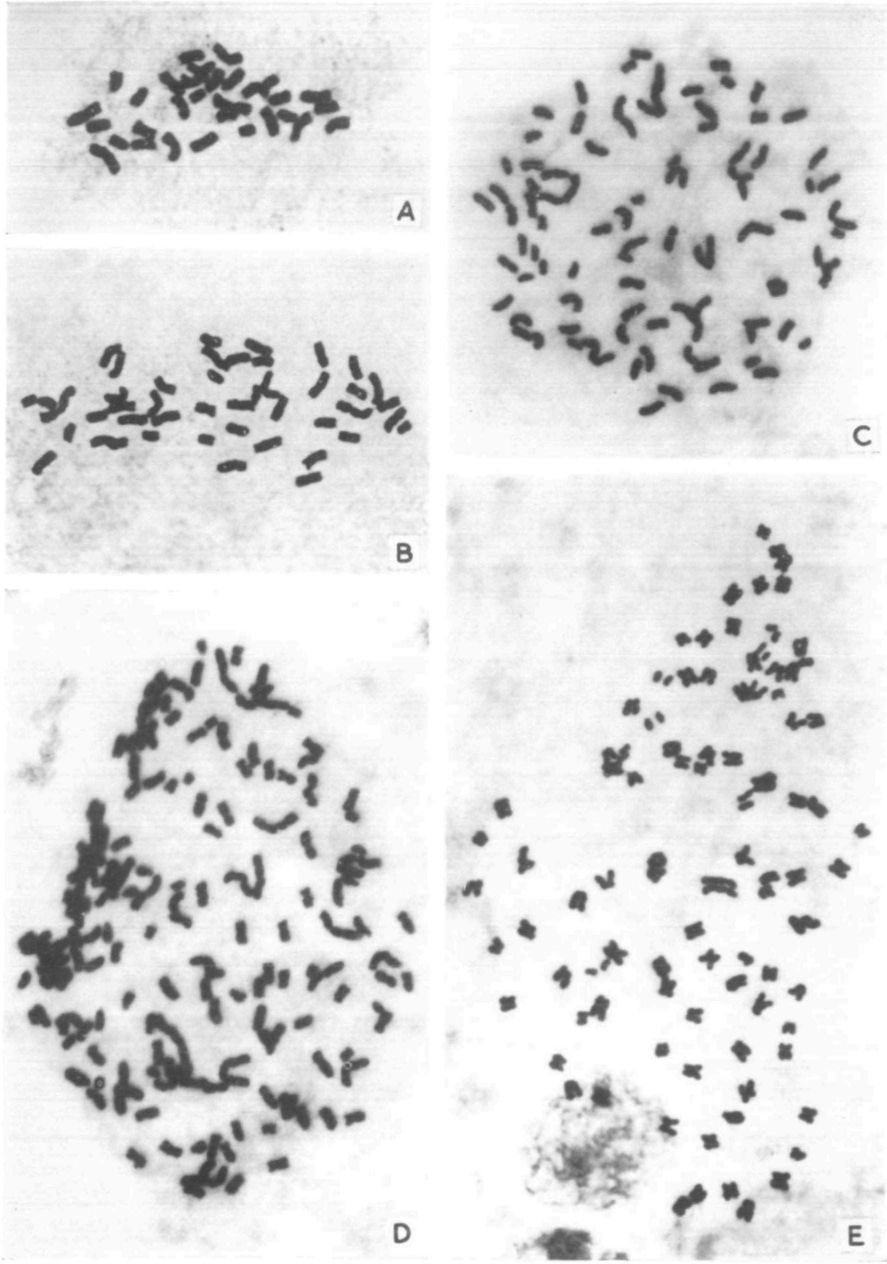
A. Mitosis in the developing embryo in a 6-month-old coconut showing $2n = 32$ at metaphase.

B. Mitosis in a normal triploid nucleus in coconut endosperm showing 48 chromosomes at metaphase.

C. Mitosis in a hexaploid nucleus in coconut endosperm showing 96 chromosomes at metaphase.

D. Normal mitosis in a $12n$ nucleus in coconut endosperm showing 192 chromosomes at metaphase.

E. C-mitosis in a $6n$ nucleus of coconut endosperm showing 96 'double chromosomes' at metaphase.



A. ABRAHAM and P. M. MATHEW