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Syncytial nuclei formation and development in coconut fruits

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ABSTRACT

Coconut endosperm is unique because of its early liquid syncytial stages, which forms a hard matured kernal at later stages of fruit development. Many free floating, naked nuclei devoid of cell membrane could be observed in the liquid syncytium. Different types of morphologically distinct syncytial nuclei were observed during the course of coconut fruit development. In two months old coconut fruits, the syncytial nuclei of 10-20 µm size in diameter were formed from cytoplasmic aggregations. Two types of syncytial nuclei 20-40 µm and >60 µm in diameter suddenly appear in 3-month old fruits. These early stage synctial nuclei were present in less than 1000 nuclei/ml. The four-month old coconut fruits were characterized by the appearance of numerous 1-2 µm sized bodies which aggregate together in definite numbers forming a dense cluster around which nuclear membrane is synthesized to form syncytial nuclei of 10-30 µm in diameter. During the fifth month of coconut fruit development, several membranous spherical bodies seeded with few 1-2 µm actively moving bodies inside appear. These bodies continuously multiply and condense inside the spherical membrane leading to a dense syncytial nucleus. The syncytial nuclei at this stage are highly active and it merges with one another thus increasing in size to form syncytial nuclei of 40-60 µm in diameter. The individual syncytial nucleus then becomes highly compact and several such compact nuclei organize themselves into a sheet like structure and proceed to settle on the lower regions of the endothelial layer. The settled syncytial nuclei cellularize and grow along the entire endothelial surface which then hardens to form the matured kernel.

Key words: Coconut, endosperm development, syncytial nuclei.

INTRODUCTION

Coconut (*Cocos nucifera*) is a major food and oil seed crop cultivated mainly in the tropical developing countries. In India, around 8.1 mha of land are under coconut and contribute substantially to rural employment generation throughout the year. Perhaps, the greatest gift of the coconut to humankind is its tender water, a naturally fine refreshment drink balanced in sugar, protein and mineral content. It is a recommended nourishment drink in sickness and in health. The World Health Organization (WHO) has reported that the potassium rich coconut water is very suitable as a sports drink and it is better balanced in nutrient content than any of the commercially available sports drinks in the markets of developed countries. Consumption of coconut water accounts for roughly 2-5 % of the total coconut production in India, which makes it a viable product for value-addition in terms of health and energy supplements. Recent developments in the coconut biotechnology studies offer scope for synthesizing high value recombinant therapeutic proteins using the syncytial nuclei of coconut water as an expression system (Bustamante, 3, 4). The kernel of the nut in the coconut fruit consists of two types of endosperm *viz.,* liquid and solid endosperm. In young coconut fruits, the liquid endosperm is formed first, and has numerous free nuclei that share a common cytoplasm. This stage of free nuclei is referred as syncytial phase. The liquid syncytial endosperm consists of large number of free floating nuclei suspended in syncytial fluid. (Cutter and Freeman, 5).The syncytial fluid maintains a unique balance and composition of minerals and other biochemicals for the free nuclei formation, proliferation and development. The liquid syncytium is a dynamic tissue constantly remodeling and changing its contents in accordance with the developmental stages of the coconut fruit. The main function of this liquid syncytium is to support the free nuclei that develop into the solid endosperm by cellularisation. Various types of cytokinins in the coconut water contribute to the cellularisation of the free nuclei after deposition on the endothelial surface of the coconut fruit, (Berger *et al*., 2).

Cutter *et al.* (6) reported the presence of 10-90 µm long amoeboid nuclei in 4cm long coconut fruits and the sudden appearance of large spherical endosperm vesicles in 10cm long coconut fruits. Cutter and Freeman (7) observed gradual increase in number of nuclei in 5-10cm long coconut fruits and reported the presence of distorted nuclei. Both of their articles concentrated mainly on syncytial nuclear stages just prior to settlement and their subsequent cellularisation. However, there was no detailed study on the dynamics

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of syncytial nuclei formations, types of syncytial nuclei, stages of development and their count and clearance from the syncytial liquid. Hence, the present study was undertaken to observe the process of syncytial nuclei formation and to identify developmental stages of the coconut syncytial nuclei.

MATERIALS AND METHODS

Young coconuts in five size ranges, 3.0-5 cm, 5-10 cm, 10-15cm, 15-20 cm and above 20 cm were collected from the eleven experimental coconut palms marked for this study. Eleven coconut palms of 10-20 years old, located at the Central Institute of Cotton Research (CICR), ICAR Sub-Station, Coimbatore were selected for this study. These palms were planted on the bunds of the cotton experimental plots and were not subjected to any kind of fertilizer treatment. Of the ten coconut palms, eight coconut palms were of WCT cultivar type, two were hybrids (WCT × CGD) and one Chowghat Orange Dwarf (COD). These coconuts were collected during the noontime at 2.00 to 2.30 PM, which is the hottest time of the day in Coimbatore. Only one coconut was sampled from each tree in a week. Only healthy nuts were used for this study and eriophyid mite infested nuts were discarded. Since coconut produces one spadix every month, the age of the nut was calculated by counting the number of inflorescence bearing the nut of interest with reference to the inflorescence containing just pollinated female flowers. Additional information on the age of the nut was also obtained by observing the white colouration on the epicarp near the perianth region, since nuts above six months old lack this colouration. Volumes between 3.0 to 600 ml of liquid syncytium could be collected from these different stages. A total of 150 nuts of different developmental stages from the selected coconut palms were observed. For convenience, the observation of the coconut syncytium was divided into three stages of coconut fruit development; early syncytium – 1-3 months; mid-syncytium – 4-5 months; and late syncytium – 6-7 months.

Light microscopy studies on the stained syncytial nuclei from coconut water were carried out with Aristoplan microscope (Leitz, Germany) at 100X magnification with 2X intermediate magnification facility provided in the microscope. The observations on the presence and activity of live syncytial nuclei were done in a Fluovert inverted microscope (Leitz, Germany) by placing a drop of coconut water in a clean microscopic slide. The microscopic observations were carried out with highest magnification of 32X with 2X intermediate magnification facility provided in the microscope. The video images were taken with Sony Cybershot H5 camera (Sony, Japan) fitted with MM-58 adaptor (Martin microscope, USA) at maximum optical zoom of 12X. The size of the syncytial nuclei was calculated by using a stage and ocular micrometer (Erma, Japan). The syncytial nuclei were counted using an inverted microscope (Fluovert FS, Leitz, Germany) by mounting 20µl of coconut water onto a slide and covering it with a round cover slip (22 cm diameter) ensuring an even spread of the sample. The thickness of the sample was taken as 0.1 mm. The volume of the sample covered under the field of vision was calculated using the formula $\pi r^2 h$.

The diameter (1.77 divisions) of the field of view under the 100X magnification was estimated by stage micrometer (0.01 mm/division, Erma, Japan). The number of nuclei from each field of view was counted and the average of 10 such counts from different fields of view was divided by the volume to get the number of nuclei/ml.

$$\frac{22 \times 0.885 \times 0.885 \times 0.1}{7 \times 1000} = 0.000246 \text{ cm}^3 \ (1 \text{ cm}^3 = 1\text{ml})$$

If there are X number of nuclei, then X/0.000246 = Y nuclei/ml

RESULTS AND DISCUSSION

Our observations show that at least three mechanisms contribute to the formation of syncytial nuclei in coconut water: 1. Cytoplasmic aggregations that buds off spherical syncytial nuclei, 2. Spherical membranous structures appear around cytoplasmic granular bodies which multiply and condense to form the syncytial nuclei, and 3. Particle aggregations that synthesize membrane around them.

The just fertilized female coconut flower is 3.0 ± 0.1 cm long and 2.9 ± 0.1 cm broad (n = 11 fruits). The stigmatic tip of the flower starts to turn from white to black and the flower is covered three [quarters/fourths] of its length by the perianth, leaving only about 0.5 cm of young fruit exposed. The embryo sac cavity has not yet developed at this stage. When the young coconut fruit attains 4.5 ± 0.3 cm in length and 4.0 ± 0.1 cm (n = 11 fruits) in breadth, a pinhead sized embryo sac was present, containing a minute amount of liquid. At this stage nearly 1.0 cm of the fruit is exposed outside the perianth cover and the stigmatic tip becomes round, hard and blackened.

Young coconuts 7.0 ± 0.25 cm long and 4.5 ± 0.2 cm broad contained 240 ± 10 µl (n = 11 fruits) of liquid endosperm. At this stage, the fruit has grown 5 cm from the perianth cover and has a well developed embryo sac. A large number of cytoplasmic aggregations, linear filaments and some spherical bodies could be observed (Fig. 1a). The cytoplasmic aggregations appear dense in the small quantity of liquid endosperm. As the nut/embryo sac grows the infusion of liquid makes the aggregations appear more dispersed.In 8.0 ± 0.5 cm long coconut fruits, the cytoplasmic aggregations were more dispersed in the 4-5 ml of liquid syncytium and 10-20 µm sized spherical syncytial nuclei could be observed forming from these aggregations.

The 9 ± 0.25 cm long coconut fruits (corresponding to two months of fruit development) contains 10 ± 0.5 ml of coconut water (n = 11 fruits). The nuclei were spherical and 20-60µm in size. Two types of nuclei, differing in morphology, could be observed. One type of nuclei is about 20-30µm in size with a well developed nuclear membrane. The nuclear membrane is intact and several 1-2 µm bodies could be observed actively moving inside the nucleus. Similar granular particles could also be observed moving outside the nucleus *i.e.,* in the syncytial fluid or liquid cytoplasm. The other type of nuclei is larger and >60µm in length and millions of 1µm sized small particles could be observed actively moving inside it (Fig. 1b). These particles were different from the ones observed in the 20-30 µm sized nuclei. The nuclear membrane is very thin and consequently it lyses upon addition of aceto-orcein stain. Both the types of nuclei are present at less than 1000 nuclei/ml. The 20-30 µm diameter nuclei appear first in young coconuts. Cutter *et al.* (6) reported the presence of 10-90 µm in diameter amoeboid nuclei in early stages of coconut fruit development. In spite of intensive search in young coconut fruits, very few amoeboid nuclei were observed, and could be the result of occasional distortion in the shape of the spherical nuclei. The appearance of two types of syncytial nuclei at the young stages of coconut fruit and their role in early fruit development is not clear. These two types of syncytial nuclei last till the end of the third month of coconut fruit development and they probably settle on the endothelial surface, to leave a clear syncytial fluid. Regarding the source of syncytial nuclei, Cutter *et al.* (6) proposed that the syncytial nuclei may have been either derived from the disintegrated nucellar tissue during fertilization or from division of the primary endosperm nucleus. However, in our studies, in very young coconut fruits, the earliest detected syncytial nuclei were formed as a result of cytoplasmic aggregations.

As the coconut fruit reaches 10.5 ± 0.4 cm long, 6.5 ± 0.2 cm broad, it contains about 18.5 ± 0.5 ml of coconut water. The cytoplasmic aggregations are more dispersed at this stage and clumps of aggregations are observed. The large spherical nuclei of the previous stage could not be found. At this stage very few spherical nuclei (<100 nuclei/ml) of size 10-20 µm could be observed (Fig. 1c). As the fruit reaches 12 ± 1 cm length (at the 3-4 month stage of coconut fruit development), numerous individual cytoplasmic aggregations or coalescences were observed along with the syncytial nuclei. There was 50-60 ml of coconut water and less than 1000 nuclei/ml. When the fruit reaches 14 ± 1.5 cm in length and 10 ± 1 cm breadth, it has 70 ± 5 ml water, cytoplasmic aggregations and few 10-20 µm sized nuclei. Some aggregations could be observed synthesizing surrounding membranes.

During the mid-syncytial stages, as the nut reaches 15 ± 1.25 cm in length (at four months development), the coconut water is clear of cytoplasmic aggregations and numerous translucent, highly active free granular bodies (2 µm in diameter) could be observed (Fig. 1d). These bodies aggregate to form many small nuclei, 10-45µm in diameter. The syncytial nuclei count at this stage varied between 9×10^3 and 1×10^4 per ml. Table 1. These bodies aggregate and synthesize the nuclear membrane around them. One or few granular bodies form the nucleus around which the formation of spherical membranous structures takes place. The origin and nature of these granular bodies and the initiation of the spherical membrane around them is not clear. The granular bodies fill the entire spherical structure of the nuclei and could be observed as independently moving bodies inside the nucleus. This stage ends with a clear liquid syncytium.

In coconut fruits 18 ± 0.5 cm long and 12 ± 0.2 cm broad, free nuclei could be observed budding from the cytoplasmic aggregations. These 1-2 µm bodies inside the nucleus are highly active. There were less than 1000 nuclei/ml at this stage. One week, thereafter, at this stage, there was a sudden appearance of large numbers of free nuclei, 20-30µm in size. A large number of empty spherical membranous nuclei could be observed and many nuclei contained one to two 2 µm diameter actively moving bodies (Fig. 1e). These 2µm diameter bodies stained purple with aceto-orcein stain and the nucleoplasm of the spherical membranous nuclei also stained light purple. (Fig. 2a). The bright staining 2µm bodies multiply and gradually fill the entire spherical nuclei turning into a dense compact structure. Numerous similar types immediately followed this type of syncytial nuclei, where the free bodies were 1µm in diameter. In a further week, both these free nuclei increase in size to 30-60 µm diameters, probably by merging together, and become dense syncytial nuclei of oval to spherical shape (Fig. 1f).

These dense nuclei, which are not active at this stage, show no internal movements, stain bright red with acetocarmine stain (Fig. 3i and 3j). The further condensation of these nuclei leads to highly compact nuclei. Some spherical structures, as large as 90 µm, could also be observed at this stage. A few of the previous stage nuclei (*i.e.,* membranous structures with actively moving bodies) could also be observed. These condensed spherical nuclei are most active at this stage and shows both internal and external movements. The chromatin of these nuclei shows movements and the syncytial nuclei were also found to be throwing out short filaments to merge with other nuclei. The syncytial nuclei

Table 1. Synctial nuclei at various stages of coconut fruit development.

Sl. No.	Stage of coconut fruit development	Types of nuclei	Volume of coconut water	Type of syncytial nuclei formation	No of syncytial nuclei/ml
1.	Early syncytium (1-3 months)	i) 20-30 μm sized ii) > 60 μm sized. iii) 10-20 μm sized.	200 μl to 75 ml.	Spherical membranous bodies. Cytoplasmic aggregations.	1000 -2000
2.	Mid-syncytium (4-5 months)	i) 10-20μm sized.	90 - 420 ml	Intense cytoplasmic aggregations.	1×10^4
		ii) 10-30μm sized.		Granular aggregation.	0.5×10^4-3.4×10^4
		iii) 20-40μm sized.		Spherical membranous bodies	2.5×10^4-4×10^4
		iv) 40-60 μm large, dense syncytial nuclei.		Merging with other nuclei	2×10^4-4.6×10^4
3.	Late syncytium (6-7 months)	i) 20-30 μm sized.	300 450 ml	Few cytoplasmic and particle aggregation	5×10^3 – 1×10^4

were 30-40μm in diameter. These syncytial nuclei then become compact and organize themselves by crowding together (Fig. 1g). A mucilaginous substance could be seen forming around the nuclei at this stage (Fig. 2j). The sheet like structure compacts further before settling and attaching on to the endothelium (Fig. 1h). However, when the initial syncytial deposits were washed with a jet of coconut water and the contents observed, no organized sheet like structures were found. This may probably be due to the loose binding of the syncytial nuclei by the cytoplasmic mucilaginous substances.

The syncytial nuclei initially settles down as a loose deposition at the stigmatic end of the coconut fruits. It then grows upwards as a thin layer of soft jelly endosperm along the endothelial walls and covers the entire surface of the endothelium. Meanwhile a thin layer of soft jelly endosperm immediately adjacent to the endothelial layer start turning into white endosperm which then turns hard and progresses upwards into matured coconut meat at later stages.

The late syncytial stages occurs when the gelatinous mass of syncytial nuclei start forming at the antipodal end of the embryo sac (endothelium) to its maturation into a firm white endoperm, 5 mm thick. In 20.5 cm long and 12 cm broad coconut fruits, the 1-2 mm of thin jelly endosperm has already formed. The free nuclei could still be observed in the syncytial fluid. Most of the free nuclei at this stage develop one to three 10 μm bodies, probably nucleoli inside (Fig. 2k, 2l, 2m, 2n and 2o). There were also a few spherical membranous structures with 10μm bodies inside. The nuclei of this type were in different stages of development (Fig. 3a, 3b, 3c, 3d and 3e). Rarely, some particle aggregation was found at this stage (Fig. 3k). Some nuclei of globular compact mass could also be observed in various stages of development (Fig. 3k, 3l, 3m, 3n, 3o and 3p).

In 24 ± 2 cm long coconut fruits, the jelly endosperm had turned hard into a white solid endosperm. Many 20-40 μm sized spherical nuclei at different stages of development could be observed (Fig. 3f, 3g, 3h and 3i). Many particle aggregates could also be seen. The particles that aggregate at this stage are smaller than the ones seen in previous stages (Fig. 3k). The syncytial nuclei resembling a compact mass like structure (Fig. 3l,3m and 3o) and dense nuclei (Fig. 3o,3p) could also be observed at this stage. Beyond this stage, the liquid syncytium is free of any syncytial nuclei but some oily bodies were noticed.

The coconut syncytial nuclei showed external and internal movements (The video images will be sent upon request). The external movements were in the form of tilting to left and right. The internal movements were orchestrated by the 1-2 μm granular bodies, which showed high activity. The dense syncytial nuclei showed chromatin movements.

The coconut syncytial nuclei also projects filaments from the nuclear membrane which can grow to a distance of 100μm to catch free nuclei (Fig. 4a). The tip of the projection establishes contact with the other nuclei and ingestion of the contents of the nuclei takes place through the filaments in a manner that resembles peristaltic movements. The size of the syncytial nuclei increases by merging other small nuclei. Filamentous structures bridging two large sized syncytial nuclei were also observed (Fig. 4b). Sometimes even multiple filaments were projected to merge other free nuclei (Fig. 4c). A series of images showing the merging of coconut syncytial nuclei is depicted in Fig. 5. It took 20 minutes from initiation of the long filamentous structure for the two nuclei to complete merging.

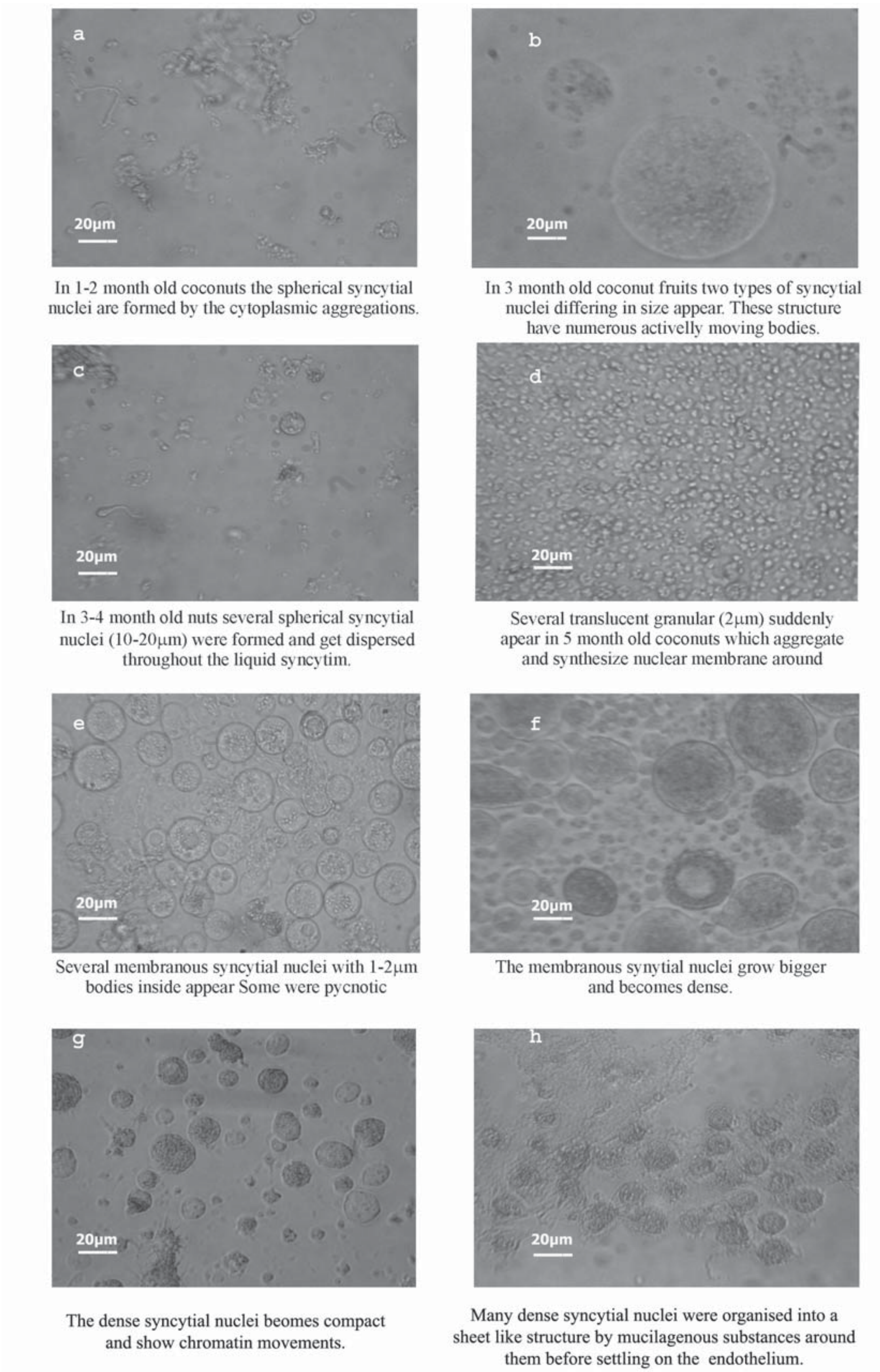
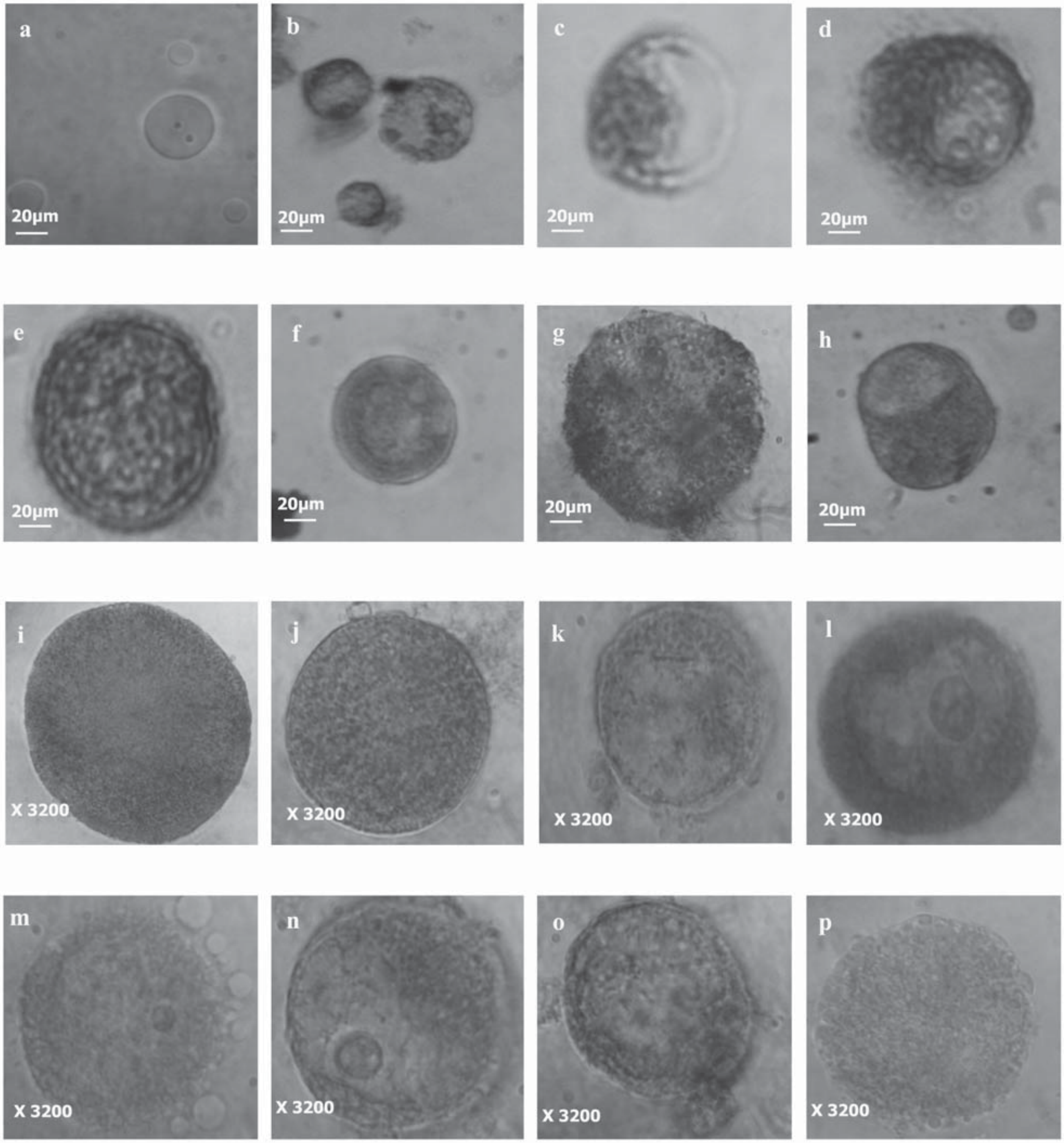
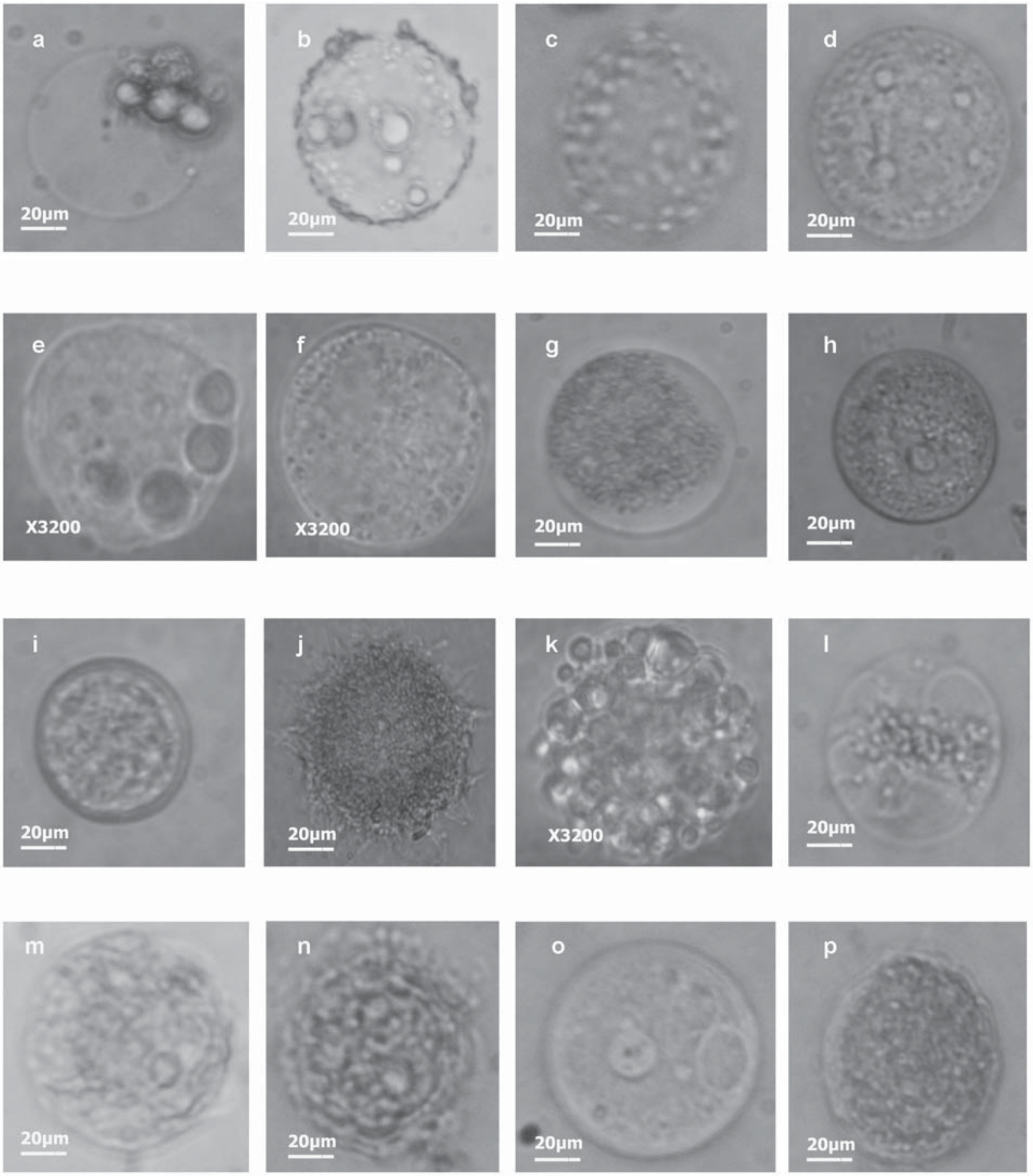


Fig. 1. Syncytial nuclei formation and development in coconut liquid syncytium.



a) Purple staining pycnotic nuclei and 2 µm granular bodies in 4-5 month old coconuts (Acetoorcein). b) Acetocarmine stained syncytial nuclei with 2 µm granular bodies. c) The syncytial nuclei progressively filled by condensation of the granular bodies. d) Some large spherical bodies probably nucleolus appear inside the condensing nucleus. e) A fully formed syncytial nuclei. f) A compact syncytial nuclei. g) A aggregate without membrane. h) Syncytial nucleus undergoing structural change probably for merging with another. i) and j). Dense staining structures suddenly appear in the liquid syncytium in 5 month old coconuts. k),l),m)n) and o). Acetocarmine stained syncytial nuclei when soft jelly endosperm has formed. p) Aggregation of cytoplasmic particles towards the end of late syncytium when white hard endosperm is 4-5 mm in thickness.

Fig. 2. Acetocarmine and acetoorcein stained syncytial nuclei in mid and late syncytial stages.



Syncytial nuclei with two different sized particles appear in late syncytial stages. a), b), c), d) and e) depicts the formation and developemnt of this type of syncytial nuclei.f), g), h) and i): The 20-40µm sized syncytial nuclei similiar to that observed in early syncytial stages undergoing maturation. j) A syncytial nuclei with numerous filamentous structures on its surface probably caried over from the mid syncytial stages. k) A particle aggregate. l), m) and n) Syncytial nuclei wih vacuole developing into a compact mass. o) Syncytial nucleus with a nucleoli and vacuoles. p) A dense syncytial nulcei.

Fig. 3. Light microscopy images of various types of syncytial nuclei in later stages of syncytium development.

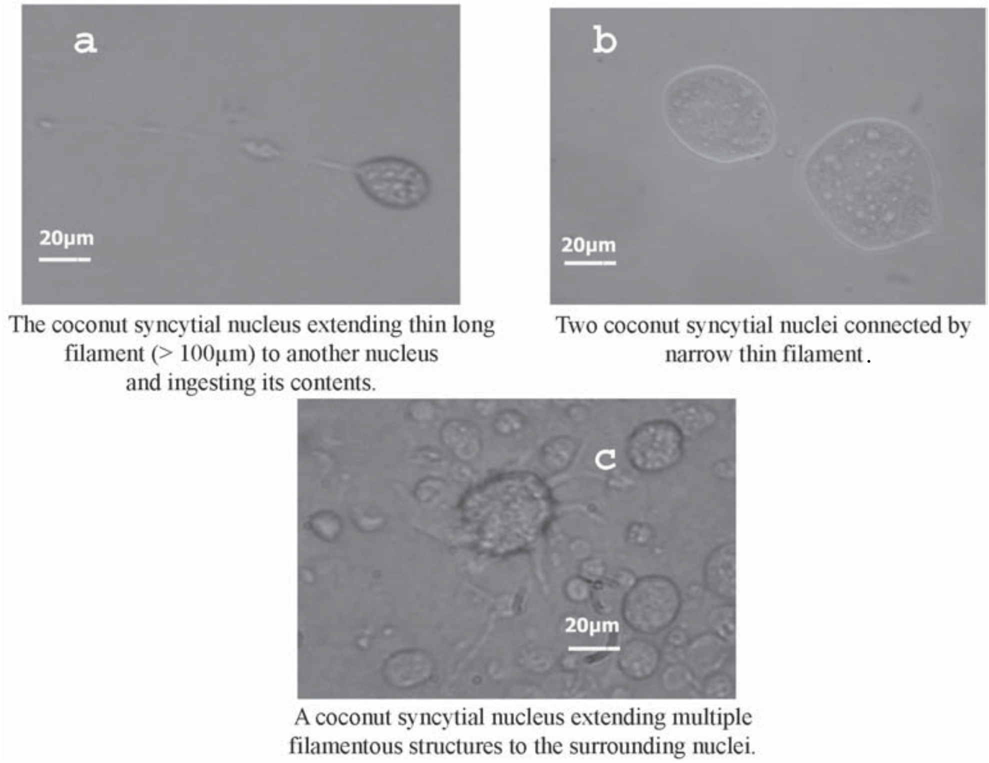


Fig. 4. Activity of syncytial nuclei in coconut (a, b and c) and palmyra fruits.

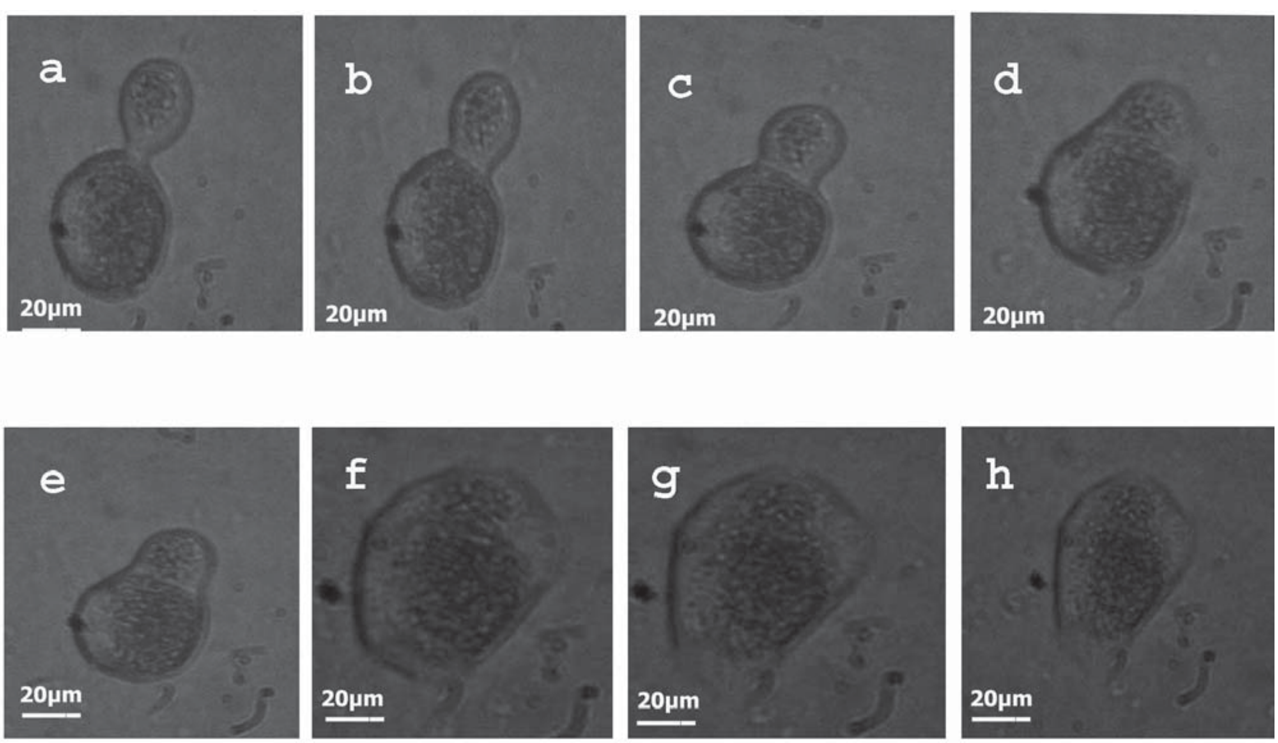


Fig. 5. Series of images depicting merging of syncytial nuclei.

Rare forms of coconut syncytial nuclei

A very less percentage of syncytial nuclei displayed abnormal shapes (Fig.6a-p). Some of the syncytial nuclei were abnormally shaped extending filaments like clusters on its nuclear membrane, (Fig.6a), pointed ends (Fig.6b and c), barrel shape (Fig.6d), oval shaped (Fig.6e), dark compact mass of aggregate, (Fig.6f), Syncytial nucleus with a large vacuole, (Fig.6g), syncytial nucleus with constriction in the middle, (Fig.6h) and linear shaped. (Fig. 6i).

A pair of syncytial nuclei with no intervening nuclear membrane was found. The syncytial nuclei may either be dividing or merging together (Fig.6j). A damaged syncytial nucleus with a long extended nuclear membrane, (Fig.6k) was also observed. Some syncytial nuclei had a dense mass inside a syncytial nucleus (Fig.6l). Four syncytial nuclei were found either dividing or merging together (Fig.6m). Three syncytial nuclei clubbed together (Fig.6n). syncytial nucleus, without well defined nuclear membrane (Fig.6o) and syncytial nucleus with a mass of cytoplasmic aggregation (Fig.6p).

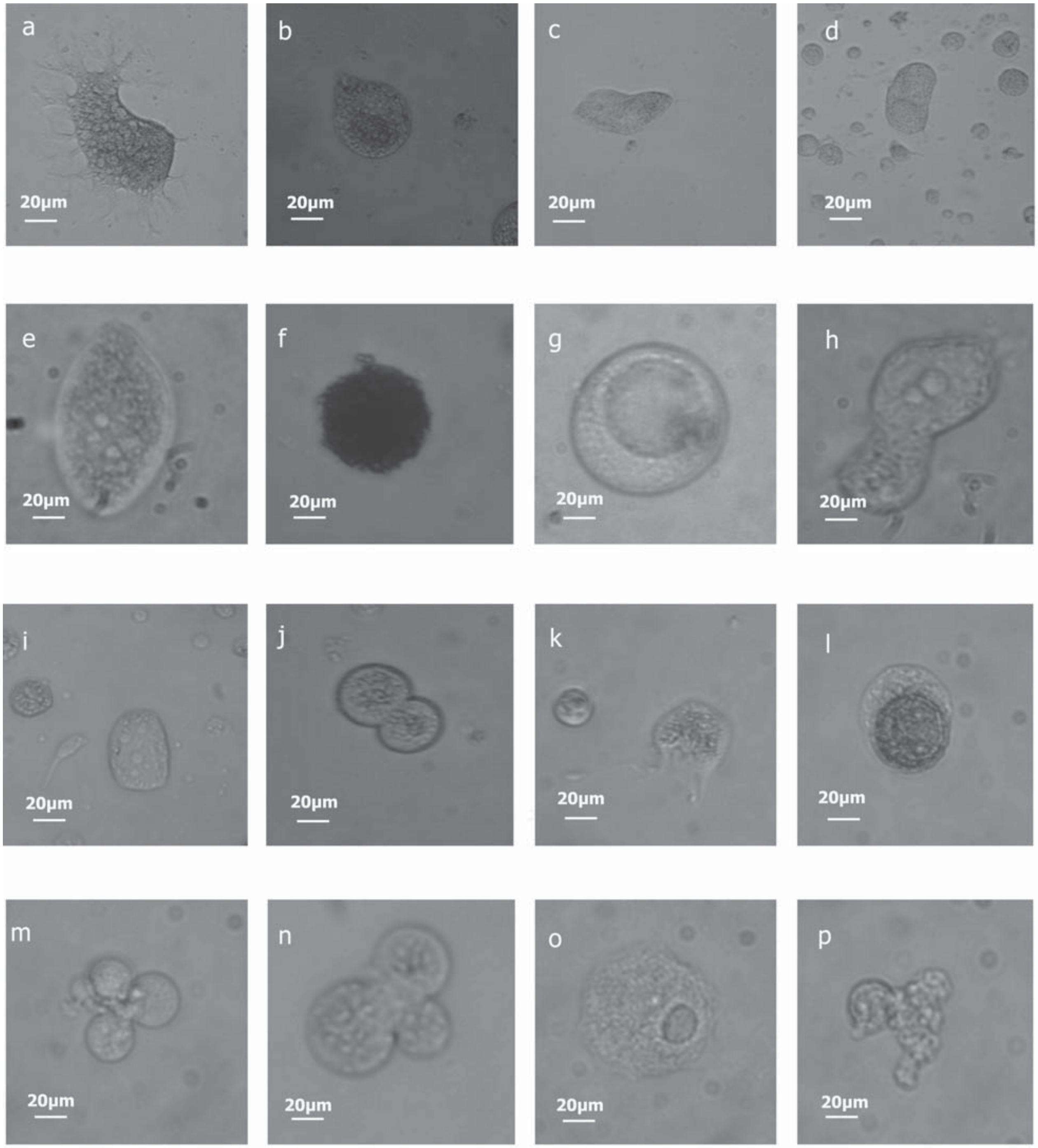
Cutter and Freeman (7) could not detect any mitotic behaviour of nuclei and concluded that amitosis as the mode of multiplication in coconut syncytial nuclei. The observations on coconut syncytial nuclei by Bustamante (3, 4), also suggests that nuclear divisions takes place by amitosis. In this study, evidences of syncytial nuclei merging to form larger ones were obtained. Two and three syncytial nuclei were clumped together without any intervening nuclear membrane (Fig.6j and 6n) suggest evidences for amitotic divisions. However, clear evidence for syncytial nuclei division was lacking. Our *in-vitro* observation with more than 200 coconuts, suggest, amitosis if any present, as a minor mode of multiplication of the coconut syncytial nuclei.

Our observations show that syncytial nuclei of varying types are formed during the different stages of coconut fruit development. These different types of syncytial nuclei were cleared from the liquid syncytium after undergoing maturation. The regular pattern in which the syncytial nuclei of different stages were formed and cleared from the liquid syncytium suggests that the coconut meat development may takes place by a combined activity of these different syncytial nuclei types. The outermost layer of the coconut meat contains the testa, which provides a firm bonding of the coconut meat with the endothelium. Seigerman (11) reported that the layer of cells immediately adjacent to the testa were isodiametric in shape followed by cell types that were variable in shape. The brown testa covers the coconut meat on the outer surface providing a firm bonding of the meat with the endothelial layer. While cellularization of the syncytial nuclei was aided by the cytokinins present in coconut water, it is not known whether the syncytial nuclei derive its entire nutrient from the coconut water for hardening and maturation. The testa can probably act as a support tissue and also aid in the maturation of the cellular endosperm.

The free floating coconut syncytial nuclei are an excellent model system for understanding the nuclei and nucleolar structural organization and the nature of nucleoplasm. The assembly of nuclear pore complexes, a large 125-MDa multiprotein structure with a pore diameter of 25 nm (Paine *et al.,* 10) and the diffusion of macromolcular structures and gene signaling and expression studies can be understood with this model system. Further, it offers insight into the processes involved and the inter-relationship between the free floating nuclei and its incorporation into the cellular nuclei.

The normal coconut contains three eyes and out of which two eyes were covered with hard shell on maturity and one soft eye through which nutrients and large volume of water enter the nut. The maximum amount of nut water was recorded during the seventh month of coconut development (Jeyalakshmi *et al., 9*) and after which it starts decreasing. The sucrose content of the coconut water was maximal during 7-8th month of coconut development and decreases with maturity which indicates that it is either metabolized or probably got incorporated in the coconut meat. (Balasubramaniam and Alles, 1). The mechanism of coconut water entry into the nut is not known but the nut remains filled with pressure till the 6th month of development, *i.e.,* before the hard shell begins to form. Below the soft eye, the rudimentary embryo lies embedded in the soft meat which then develops into matured embryo during 11th month of nut development. The soft mesocarp tissues that lies above the soft eye acts as a nurse cell for the embryo.

The hybrids and the dwarf coconut types contained 20-30% more volume of liquid endosperm than the WCT type. The syncytial nuclei were also 10-20% lower than that observed in WCT (Data not shown). The low syncytial nuclei count in hybrids and dwarf may be due dilution effect. It is not known whether the syncytial nuclei count differs between nuts of various sizes. Our data is insufficient to explain this observation. The observation carried out in an endosperm mutant coconut tree, in which the early coconut meat development takes place unattached to the endothelial layer, i.e. found scattered in the liquid endosperm shows three types of cells. i) Globular cells, ii) elongated meat particles, and iii) syncytial nuclei with a condensed centre (Devakumar and Prabakaran, 8). The spherical globular cells resemble the isodiamteric cells mentioned by Seigermann (11), which were followed by elongated cells



a. Abnormally shaped syncytial nucleus with clusters of extended filaments; b. and c. Syncytial nuclei with abnormal pointed ends; d. Barrel shaped syncytial nucleus; e. Syncytial nucleus with oval shape; f. Dark compact mass of aggregate g. Syncytial nucleus with a large vacuole; h. Syncytial nucleus with constriction in the middle; i. linear shaped syncytial nucleus; j. Two syncytial nuclei either dividing or merging together; k. Syncytial nucleus with torn membrane; l. Dense mass inside a syncytial nucleus; m. Four syncytial nuclei either dividing or mergin together; n. Three syncytial nuclei clubbed together o. Syncytial nucle without a well defined nuclear membrane; p. Syncytial nucleus with a mass of cytoplasmic aggregation.

Fig. 6. Rate forms and shapes of syncytial nuclei observed during coconut fruit development.

during the course of meat development. While the syncytial nuclei formed in 5-6 month old coconuts play a definitive role in the kernal formation, the fate and role of the early and middle stage syncytial nuclei before the formation of visible deposition on the endothelium needs further study.

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