



# Observations on syncytial nuclei development in a mutant endosperm bearing coconut tree

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## Abstract

An abnormal endosperm forming coconut tree was identified in a farmer's field in Kasaragod District, of Kerala. In a single bunch, this coconut tree regularly bears normal coconuts as well as coconuts containing sparse deposition of the malformed solid endosperm. Several undeveloped kernel detachments were found to be floating in the liquid endosperm. The formation and development of syncytial nuclei was studied to understand the process of endosperm development in this abnormal coconut tree. The syncytial nuclei development was observed to be normal until the fifth month after fertilization. The defects in condensation process that lead to matured syncytial nuclei which then organise into a compact sheet like structure before settling on the endothelium or the defects on the endothelial surface to provide adherence for the settling of the syncytial nuclei was hypothesized as the possible cause for this abnormality.

**Keywords:** Coconut, Endosperm abnormality, Syncytial nuclei

## Introduction

Abnormalities in growth, leaf and floral morphology and nut characteristics are very rare in coconut and have been reported from time to time. Menon and Pandalai (1957) have described many abnormalities in coconut. The coconut endosperm mutants are quite popular with consumers because of their peculiar properties as compared to normal coconuts. Two such endosperm mutants, Makapuno and Kopyor are now important coconut cultivars in South East Asian nations where they are widely cultivated for their distinct taste and texture as compared to normal nuts. In Makapuno type, widely propagated and cultivated in the Philippines and Thailand, the meat develops into a spongy, amorphous soft jelly and there is either absence of the liquid endosperm or the presence of highly viscous liquid endosperm. Kopyor is another type of coconut growing in the Java islands of Indonesia, whose mature kernel is soft and uncompact, some parts becoming detached from the shell and filling the watery endosperm. Kopyor water has meat particles floating in the liquid endosperm. (Santoso *et al.*, 1996).

The embryos of both Makapuno and Kopyor will not germinate due to abnormality of the kernel which

normally supports its growth. In normal nuts, the  $\alpha$  galactosidase enzyme hydrolyses the predominant polysaccharide of the endosperm, galactomannans into mannans. The deficiency of  $\alpha$  galactosidase activity in the makapuno coconut results in higher content of galactomannans leading to loss of cell adhesion, highly elongated cells and amitosis in them (Mujer *et al.*, 1984). Balasubramaniam (1976) suggested that galactomannans play a structural role in the formation of the primary cell wall in normal coconut endosperm. During the transition of the endosperm from the hydrated, gelatinous phase to the dehydrated, solid mature state, most of the galactose groups are removed from the galactomannans present in the cell wall of jelly endosperm giving a hard texture to the matured coconut meat (Kooiman, 1971). The cytological observations by Abraham *et al.*, (1965) in the abnormal makapuno endosperm revealed a higher ploidy level of 48 and above. Two types of cells, normal cells and micro cells were reported by Del Cruz and Ramirez (1968) in the makapuno endosperm. The identification and propagation of makapuno mutant coconut palms have spawned a new export industry in some South East Asian nations like Thailand and Philippines. The wasted viscous liquid endosperm of

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macapuno is also finding new application as body moisturizer and soothener and the galactomannans isolated from it is used as stabilizing agent in ice cream and other preparations. The cytological investigations have been carried out in these coconuts, but there are no reports on the study of syncytial nuclei morphology and development in these abnormal coconuts.

Coconuts are among the few of the plant species, where the early fruit development take place as a liquid syncytial phase, the others predominantly taking place in a gelatinous syncytium. Cutter *et al.* (1955a) studied the syncytial nuclear morphology in normal coconuts and their subsequent cellularization to form the hard matured kernel. They reported the presence of 10-90  $\mu\text{m}$  long amoeboid nuclei in 4 cm long coconut fruits and the sudden appearance of large spherical endosperm vesicles in 10 cm long coconut fruits. Cutter and Freeman (1955b) observed gradual increase in number of nuclei in 5-10 cm long coconut fruits and reported the presence of distortions or aberrations in syncytial nuclei in normal nuts. Recently, the live observations on syncytial nuclei formation and development in normal coconuts were studied by Devakumar and Prabakaran (In Press).

In this study, an abnormal endosperm containing coconut was observed for aberrations if any, in syncytial nuclei that hamper the deposition and initiation of the cellularisation process that leads to the development of the solid kernel. In this mutant, the solid endosperm developed only as a rudimentary deposition that never hardened even in fully matured coconut (Fig. 1a) and the endothelial veins of this mutant coconut showed an abnormal development. (Fig. 1b, 1c). The coconut water was sweeter than in normal coconuts and some endosperm detachments were seen floating in coconut water. The nuts were examined in order to seek answer to the basic questions like whether the abnormality arises from either defective syncytial nuclei or defects in the endothelial surface that prevent the syncytial nuclei to get attached to it and develop normally. This work was undertaken to understand the nature of defects in syncytial nuclei formation and development that lead to the abnormal endosperm development in this coconut.

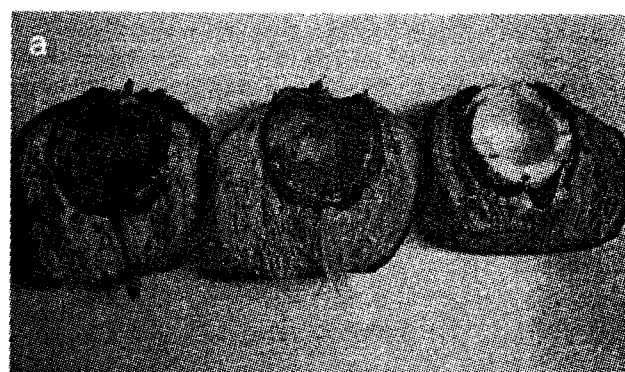
### Materials and Methods

The coconuts from a palm bearing abnormal endosperm were collected from a tree located in a farmer's field at Chakkunnige, near Seethangoli in Kasaragod District of Kerala. The coconuts from the abnormal palm (2-5 nuts each at different stages of growth based on their availability) were collected and transported to Coimbatore. The nuts were observed

within 3 days after reaching Coimbatore. According to the farmer, nearly 50% of the nuts from this palm failed to develop solid endosperm while the rest developed normally. A total of 12 coconuts from five to eight months of development were observed for the presence of syncytial nuclear aberrations. These include, 3, 4, 2 and 3 coconuts from 5, 6, 7 and 8 months, respectively.

### Results and Discussion

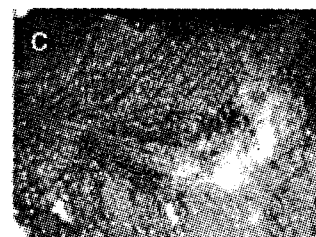
In 5 month-old mutant coconuts, the endothelium showed abnormal development of veins (Fig. 1b). All the three nuts studied had spherical syncytial nuclei and the maturation stage of the syncytial nuclei varied between these nuts. In one of the nuts, the syncytial nuclei could be observed to be forming from the granular aggregation (Fig. 2a).



Endosperm mutant coconut and normal coconut from the same coconut bunch



Five month old endosperm mutant coconut abnormal veins

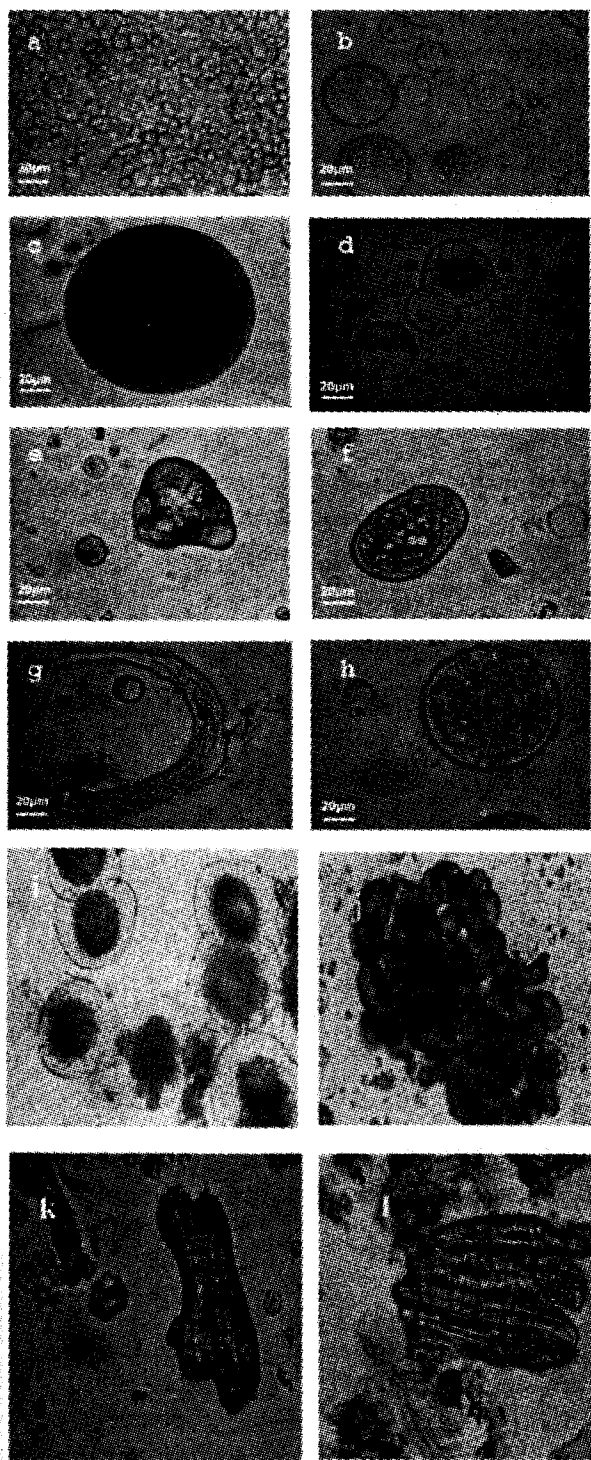


Eleven month old matured endosperm mutant coconut showing abnormal development of the endothelial veins

Fig. 1. Rare endosperm mutant of coconut

There were spherical syncytial nuclei of varying sizes in the other nuts (Fig. 2b). No aberrations were observed at this stage and the syncytial nuclei were normal. The number of syncytial nuclei of various sizes was counted in a single field of vision of the microscope. The nuts differed in the syncytial nuclei development and the nuts were in successive stages of development (Table 1).

In six-month-old coconuts, one of them had a normal development of nut. There was solid endosperm deposition on the endothelium. The other



a) Numerous 1-2  $\mu\text{m}$  granular bodies aggregating to form the spherical syncytial nuclei.  
 b) Spherical normal syncytial nuclei in six month old coconut  
 c) A highly active and intensely black syncytial nucleus in endosperm mutant coconut  
 d) The abnormal condensation of the nuclear content inside the syncytial nuclei  
 e) Few abnormal granular aggregate in endosperm mutant coconut  
 f) An elongate syncytial nucleus in abnormal endosperm  
 g) A long mucilaginous particle in the liquid endosperm of abnormal coconut  
 h) An abnormal condensation of syncytial nucleus  
 i) Mutant syncytial nuclei with dense centre j) Abnormal globular bodies k) and l) A few elongate structures

Fig. 2. Syncytial nuclei development in the endosperm mutant coconut

three nuts did not show any deposition but were comparatively larger in size. The normal nut had less than 1000 syncytial nuclei/ml but numerous 1  $\mu\text{m}$  free bodies could be observed. In one of the abnormal nuts, few spherical syncytial nuclei and numerous 1  $\mu\text{m}$  bodies were seen aggregating. The other two abnormal nuts had spherical syncytial nuclei and absence of 1  $\mu\text{m}$  bodies (Table 2). The large sized syncytial nuclei in these two nuts had turned intensely brown (Fig. 2c) and some of the nuclei were seen condensing (Fig. 2d). Other structures such as granular aggregate (Fig. 2e), an elongate syncytial nucleus (Fig. 2f), a long mucilaginous particle (Fig. 2g) and abnormally condensing syncytial nucleus were found (Fig. 2h). Out of the two nuts studied in seven month old mutant coconuts, both had normal development of the jelly endosperm. In one of the nuts there were no syncytial nuclei while in the other there was  $2 \times 10^3$  nuclei/ml. The size of the nuclei varied from 20-40  $\mu\text{m}$  in size (Table 3). Three coconuts at 8 months of development were observed; out of which one was normal while the other two were mutants. (Table 4). The endothelium had turned dark with prominent 'varicose' veins. The normal coconut had no syncytial nuclei while the mutant coconut had  $1 \times 10^4$  nuclei/ml. In the other mutant coconut, nuclei with dense centre were found (Fig. 2i). Some globular bodies were also seen (Fig. 2j). A few elongate structures (Fig. 2k and 2l) were also observed. The coconut water is very sweet in this mutant nut at 8 months old and it turns to black in colour. This may be possibly due to degradation of syncytial nuclei or oxidation of the dissolved testa coat. In mutant coconuts, no embryo formation and development was taking place because of lack of support for embryo growth.

The three 5-month-old nuts were in different stages of development (Table 1). At 5<sup>th</sup> month, particle aggregation seems to be the mode of syncytial formation. Similar results were obtained in our previous studies (Devakumar and Prabakaran, in press) where, a single nut was studied at a time. More than  $5 \times 10^4$  granular particles were observed in the 1<sup>st</sup> nut, while granular particles were found to be synthesizing membrane around them, multiplying inside to form matured nuclei ranging in size from 10-50  $\mu\text{m}$  in diameter.

In normal nuts, towards the end of 6<sup>th</sup> month, the syncytial nuclei deposited on the endothelial layer and soft jelly endosperm begins to grow (Table 2). Millions of minute particles which aggregate and deposit during the kernel maturation were observed.

**Table 1. Syncytial nuclear characteristics in 5 month old coconut fruits from abnormal coconut yielding palm**

5 <sup>th</sup> Month	I Nut	II Nut	III Nut
<b>Shape of nuclei</b>	Very few large spherical syncytial nuclei, few granular aggregates observed	Spherical	Spherical
<b>Size</b>	>40µm - 1% 30-40µm - 0% 20-30µm - 5% 10-20 µm - 44% <10 µm - 55 % granular not spherical	>40µm - 40% 30-40µm - 30% 20-30µm - 20 % 10-20 µm - 5% <10 µm - 5 %	>40µm - 2% 30-40 µm - 5% 20-30 µm - 8% 10-20 µm - 25% <10 µm - 60 % 90% of nuclei completely filled
<b>No of Nuclei / ml</b>	>5 ×10 <sup>4</sup> particles	1×10 <sup>4</sup> nuclei	2×10 <sup>4</sup> nuclei
<b>Activity</b>	Very Small sized activity could not be observed	Highly active	Highly active
<b>Endothelium</b>	Varicose No visible syncytial nuclei deposition	Varicose No visible syncytial nuclei deposition	Varicose.No visible syncytial nuclei deposition.
<b>Taste</b>	Salty	Salty	Salty

**Table 2. Syncytial nuclei characteristics in 6 month old coconut fruits from abnormal coconut yielding palm**

6 <sup>th</sup> month	I Nut	II Nut	III Nut	IV Nut
<b>Nuclei shape</b>	Spherical	Spherical Many 0.1µm bodies could be seen aggregating	Spherical Millions of 0.1µm bodies Few elliptical well developed nuclei	Spherical to Oval nuclei 50-100µm nuclei are dense
<b>Size</b>	>90µm - 2% 50-60µm - 5% 40-50µm - 20% 10-20µm - 30% <10µm - 43 %	>90µm - Nil 50-60 µm - Nil 40-50µm - 10% 10-20 µm - 60% <10 µm - 30%	>90µm - Nil 50-60 µm - Nil 40-50µm - 1% 10-20 µm - 95% <10 µm - 4%	>90µm - 5% 50-60µm - 20% 40-50µm - 15% 10-20µm - 40% <10µm - 20 %
<b>No. of Nuclei/ml</b>	3.4×10 <sup>4</sup>	0.5×10 <sup>4</sup>	1000	2.5×10 <sup>4</sup>
<b>Syncytial nuclei Activity (Internal movements)</b>	Highly active Few large nuclei have turned intensely brown Some are condensing	Less active	Millions of 0.1µm bodies Too small to judge the activity	Highly active Few large nuclei have turned intensely brown Some are condensing
<b>Taste</b>	Slight salty	Slight salt	Sweet	Salty
<b>Endothelium</b>	Varicose No visible syncytial nuclei deposition	Varicose. No visible syncytial nuclei deposition	Vein seen but not prominent 1-2 mm thick sweet endosperm deposition observed	Varicose veined

In mutant nuts, there was no visible soft jelly endosperm but nuclei of previous stages could be seen floating in coconut water and the abnormal endothelial veins could be observed on the endothelium. In one of the mutant coconut, the millions of particles could be observed which indicate that the contents of the liquid endosperm during the 6<sup>th</sup> month are normal.

The syncytial nuclei studies on the endosperm mutant coconuts show that the syncytial nuclei development is normal until the fifth month of fruit development. The deposition and normal development of solid endosperm was hampered. The observation of large, dark spherical syncytial nuclei suggests that the syncytial nuclei is defective in condensation i.e. the syncytial nuclei does not mature and become a

compact structure to organize into a sheet for settling on the endothelium. Conversely, the endothelium may not provide adherence for the deposition of the syncytial nuclei. The "varicosed" appearance of the endothelial veins in mutant coconut indicates that they may play a role in providing nourishment for the development and hardening of the kernel. As a result the kernel development and maturation may not depend entirely on the liquid endosperm alone. In our study, both the coconuts at 7<sup>th</sup> month of development happened to be normal coconuts.

This mutant coconut is different from macapuno and kopyor coconuts where abnormality arises during the cellularization and hardening of the deposited syncytial nuclei. The defective syncytial nuclei

deposited on the lower endothelial surface fail to proliferate and grow along the endothelial wall. The defective syncytial nuclei may have arisen as a result of deficiency in endogenous factors such as hormones or nuclear factors essential for syncytial nuclei maturation. Further the nut water also undergoes changes in amino acid content such as the sudden increase of alanine and  $\alpha$ -amino butyric acid during the 6<sup>th</sup> month of coconut development. (Baptist, 1956). Since, amitosis was observed to be the mode of syncytial nuclei proliferation in coconuts, any chromosomal abnormalities in this mutant coconut could not be ascertained. Abraham and Mathew (1963) reported that after the syncytial nuclei deposit and cellularize, the endosperm cells close to the endothelium were triploid (3n) followed by hexaploid nuclei (6n=96) and (12n=192). Further studies on this interesting material should provide clues to the appearance of chromosomes in the syncytial nuclei, its transition between the amitosis to mitotic mode of development.

In Arabidopsis and other crops, mutant forms have played a major role in understanding the gene regulation during the endosperm development. A mutant library and database has been set up to help exchange of such material among researchers. ([http://](http://www.arabidopsis.org/)

[www.arabidopsis.org/](http://www.arabidopsis.org/)). In a similar fashion, mutant types of coconut can also be maintained as germplasm collections in separate locations to prevent their pollen being transmitted to normal palms. In this study, the presence of both normal nuts and mutant nuts in a single bunch and the absence of mutant nuts in the neighbouring palms indicate that a single recessive gene is involved in this mutant formation. The study of reproductive biology of these mutant coconut trees may explain the genetic inheritance behind these mutants.

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**Table 3. Syncytial nuclei characteristics in 7 month old coconut fruits from abnormal coconut yielding palm**

7 Month	I Nut	II Nut
Nuclei shape	No spherical Nuclei. $1 \times 10^4$ $1 \mu\text{m}$ spherical bodies. Aggregations of such bodies could be observed.	Spherical to elongate nuclei
Size	Nil	$2 \times 10^3$ nuclei
No. of Nuclei/ml	Nil	$> 40 \mu\text{m}$ - 6% $20-40 \mu\text{m}$ - 94%
Syncytial nuclei Activity (Internal movements)	No activity observed	Moderately active
Taste	Very sweet	Very sweet
Endothelium	2-3 mm thick jelly endosperm formed Normal development of nut	2-3 mm thick jelly endosperm formed Normal development of nut

**Table 4. Syncytial nuclei characteristics in 8 month old coconut fruits from abnormal coconut yielding palm**

8 Month	I Nut	II Nut	III Nut
Nuclei shape	Spherical.	No free Nuclei.	Many spherical bodies with dense centre, globular bodies and syncytial nuclei were found.
Size	20-30 $\mu\text{m}$	—————	—————
No. of Nuclei/ml	$1 \times 10^4$	—————	—————
Syncytial nuclei Activity (Internal movements)	No activity observed	—————	—————
Taste	Not tasted	Not tasted	Not tasted
Endothelium	Endothelium turned Black in colour. Water normal	Normal nut 8 mm thick kernel	Endothelium turned Black in colour Water slight brownish

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