# Triglyceride Deposition in Tissues of Germinating Coconut (*Cocos nucifera* Linn)

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**ABSTRACT:** Coconut is the largest oilseed. In the present investigation, various lipid classes, such as triacylglycerol, free fatty acid, diacylglycerol, and their fatty acid compositions were determined from three regions of germinating coconut at three stages of germination. In this process, triacylglycerol was hydrolyzed and resynthesized in the haustorium tissue. Cooperative participation of various tissues, such as endosperm, haustorium, and embryo, was therefore involved in the process of germination of coconut.

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**KEY WORDS:** Coconut, germination, haustorium, lipids, transformation, translocation, triacylglycerol.

Coconut is one of the major cash crops in the world, with a strong regional concentration in Asia and the Pacific accounting for 85% of the crop. Being the commercial source of lauric fat, coconut is valued for both industrial and food uses. It is the most extensively grown species of the Palmae family with unique anatomical features for its seed, which is popularly known as coconut. Coconut is the largest oil-bearing nut, with weights ranging from 100 to 1000 g for the dehusked nut (1,2). Anatomically, coconut is composed of four distinct parts: the outer fibrous exocarp or husk, the highly lignified endocarp or shell, the white solid endosperm, and a large central cavity filled with a liquid known as liquid endosperm. The most distinguishing characteristic of this seed is the large cavity, with its liquid endosperm that functions as a reservoir of nutrients for the germinating seed. Absence of dormancy is another characteristic feature of coconut (1).

For nurseries, the general field practice is to place well-matured coconuts with husk in a horizontal position and to cover them two-thirds with soil, followed by periodic watering. Usually, it takes about 22 to 24 wk for the sprout to come out of the husk. On germination, the embryo forces its way out through the germ pore, and the emergent embryo forms a button of tissue, which quickly develops into a plumule (shoot) and a radicle. At the same time, the basal part of the embryo enlarges to form a cotyledonary structure, named the haustorium. The haustorium is a spongy structure with extensive serrations on the periphery. During germination, the haustorium enlarges, keeps in close contact with the endosperm, and finally fills the entire water cavity in 20 to 24 wk. This coincides with the appearance of green tissue in the plumule but before opening into green leaves. During this period, the process of germination is entirely supported by the reserve nutrients of the liquid endosperm (sugars) and the solid endosperm (mainly triacylglycerols). The coconut seed is unique in having reserve nutrients in these two compartments. Further, the tiny embryo, embedded in one end of the oval endosperm, has to mobilize nutrients from a large area of liquid and solid endosperms. It is for this purpose that the basal part of the embryo enlarges into the haustorium to facilitate absorption of nutrients. The haustorium is characteristic of the Palmae family, and coconut is conspicuous by the presence of a very large haustorium. Cooperative functions of various tissues, namely embryo, endosperm, and haustorium, are extremely important to bring about the complex biochemical events that transform the embryo into a seedling.

Fat-bearing tissues depend largely on reserve fat as a source of energy during germination. This involves mobilization, translocation, and transformation of fat into soluble sugars for easy assimilation by the embryo. However, the mode of these events may vary, depending on the anatomy of the seeds, particularly in the case of coconut. Few authors have attempted to explain the functions of various tissues during germination of oil palm seed and date (3–8). Coconut has not been examined to understand how the storage lipids in the endosperm are utilized and the involvement of different tissues for mobilization, translocation, and transformation of the reserve lipids during germination. In this investigation, the authors have attempted to follow changes in lipid content among tissues during progressive stages of the germinating coconut.

## EXPERIMENTAL PROCEDURES

*Method of germination.* Ten West Coast Tall variety trees of identical age and yield characteristics were selected and their inflorescences were tagged. On maturity (12 mo from flowering), the bunches were harvested and the nuts with husk were dried under shade for 22 d by following the accepted practices under field conditions (1). Nuts (150) of identical weight (733.9 ± 27.7) were used for sowing. The nuts with husk were

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placed in the horizontal plain, and two-thirds of each nut was covered with soil. Soil moisture was maintained by periodical watering. Germinated nuts were randomly taken from 10 to 22 wk with 2-wk intervals for analysis.

The coconuts were dehusked and were carefully broken into two halves so as to avoid breakage of the haustorium. The liquid endosperm (coconut water) was drained off, and the haustorium was removed from the endosperm. The inner endosperm in contact with the haustorium was carefully scraped off to obtain the jelly-like mass containing free oil, termed here as "mucilage." The remaining endosperm after removal of mucilage formed the endospermal region. The various parts were weighed separately, and the respective regions from 10 germinated nuts were pooled. Another set of 10 nuts prior to sowing formed the reference sample at 0 germination. The solid endosperm from these nuts was also pooled. The pooled samples of the respective regions and the whole endosperm (reference sample) were disintegrated in a Waring blender, and representative samples were drawn and freeze-dried.

*Dry matter content.* Moisture and total dry matter were determined according to AOAC methods (9).

*Solvent extraction of lipids.* Total lipids (TL) were extracted from different parts of germinating coconuts with chloroform/methanol (2:1, vol/vol), and the filtrate was washed with 0.9% sodium chloride (10). The lower solvent fraction was evaporated under vacuum in a rotary evaporator to get the TL. The TL obtained were dissolved in a minimum quantity of chloroform and stored for further analyses.

*Separation of lipid classes.* About 20 mg of TL was spotted and separated into sterol ester, triacylglycerol (TG), free fatty acid (FFA), 1,3-diacylglycerol (1,3 DG), sterol, 1,2-diacylglycerol (1,2 DG), monoacylglycerol (MG), and polar-lipid (PL) classes by thin-layer chromatography on 1-mm thick silica-gel G adsorbent with hexane/diethyl ether/acetic acid (80:20:1, vol/vol/vol) solvent system (11). Plates were prewashed and equilibrated prior to development as in standard chromatographic procedures. Lipid bands were detected by exposure to iodine vapor. Sterol ester and sterol fractions were eluted from the gel with chloroform/methanol (4:1, vol/vol); TG, DG, MG, and FFA fractions with chloroform; and the PL band with chloroform/methanol/water (5:5:1, vol/vol/vol). Individual lipid classes were quantitated by the oxidative dichromate method of Bragdon (12).

*Determination of fatty acid composition.* Methyl esters of the fatty acids were prepared by saponification with alcoholic potassium hydroxide, followed by esterification with alcoholic sulfuric acid reagent according to the IUPAC procedure (13). A Hewlett-Packard 5840A gas chromatograph equipped with a flame-ionization detector (FID) (Hewlett-Packard, Palo Alto, CA) was used for gas–liquid chromatographic (GLC) analysis of the methyl esters. Methyl esters were analyzed on an HP-FFAP (cross-linked FFAP) 30 m × 0.5 nm column of 1.0 μm, film thickness (Hewlett-Packard). The injection and detector temperatures were maintained at 250 and 300°C, respectively. Flow rate of the carrier gas (nitrogen) was 20 mL/min. The column temperature was programmed from 100 to 180°C at the rate of 5°C/min. Methyl esters were identified by using authentic standards (Sigma Chemical Co., St. Louis, MO), and the peaks were quantitated by digital integration. Fatty acid levels are reported as relative proportions of the total composition.

## RESULTS

Figure 1 shows longitudinal cross-sections of the germinating coconut at 0, 10, 16, and 22 wk. The parts identified for the analysis of lipids were endosperm, mucilage (degraded inner endosperm), and haustorium. The morphological changes of the coconut are obvious in the photograph. The most conspicuous change was the enlargement of the basal part of the embryo into the spongy tissue called haustorium. The haustorium is in close contact with the inner endosperm. The entire water cavity was filled with this new tissue by the 22nd wk of germination. Another visible change noticed was the degradation of the inner part of the endosperm that was in contact with the haustorium. Degraded inner endosperm (mucilage) began to appear after the 16th wk. The mucilage was semisolid in nature and contained oil. TL were extracted from endosperm and haustorium at 10, 16, and 22 wk. Changes in the lipid profile of the endosperm are presented in Table 1. There were no significant changes from 0 to 10 wk, and therefore these data are not shown. The dry matter content of the endosperm was reduced from 131.12 ± 2.52 g/nut at 10 wk to 93 ± 7.42 g/nut at 22 wk. The reduction was partly due to the separation of the endosperm as mucilage after 16 wk. Although mucilage was 10.55 ± 0.65 g/nut, the actual reduction of the endosperm dry matter was 27.57 ± 1.85 g/nut. Among the lipid fractions, remarkable reduction was observed for TG, which accounted for 17.96 ± 1.25 g/nut. Among the minor constituents, the partial glycerides and FFA of the endosperm registered an increase of four to five times from the 10th to the 22nd wk. However, their combined concentration

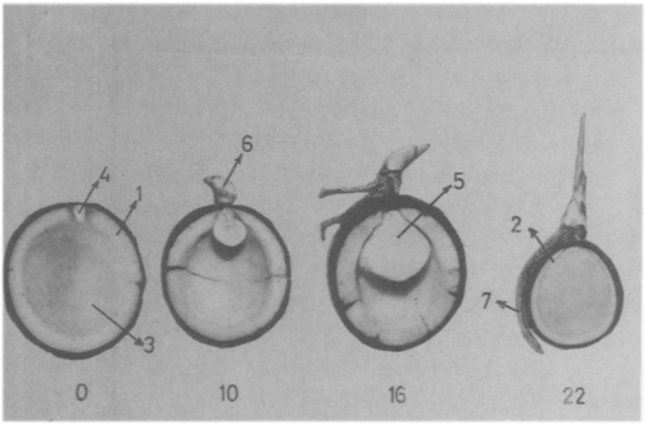


**FIG. 1.** Photograph showing longitudinal cross-sections of germinating coconut during different stages 0, 0 wk; 10, 10th wk; 16, 16th wk; 22, 22nd wk. Regions: 1, endosperm; 2, mucilage; 3, liquid endosperm (coconut water); 4, embryo; 5, haustorium; 6, shoot; 7, root.

**TABLE 1**
**Lipid Composition of Endosperm of Germinating Coconut at Different Stages[a]**

| Stage of germination (wk) | Lipid class (wt in g/nut) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total lipid | Sterol ester | Triacylglycerol | Free fatty acid | 1,3-Diacylglycerol | 1,2(2,3)-Diacylglycerol | Monoacylglycerol | Sterol | Polar lipids |
| 10 | 95.28 | trace[b] | 91.14 | 0.10 | 0.19 | 0.33 | trace | 0.20 | 3.24 |
| 16 | 86.68 | trace | 81.33 | 0.28 | 0.47 | 1.06 | 0.15 | 0.20 | 3.16 |
| 22 | 73.06 | trace | 67.03 | 0.44 | 0.77 | 1.57 | 0.25 | 0.18 | 2.78 |

[a]Values are the means of three analyses. [b]Trace amounts: <0.05 g.

**TABLE 2**
**Lipid Composition of Mucilage of Germinating Coconut at Different Stages[a]**

| Stage of germination (wk) | Lipid class (wt in g/nut) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total lipid | Sterol ester | Triacylglycerol | Free fatty acid | 1,3-Diacylglycerol | 1,2(2,3)-Diacylglycerol | Monoacylglycerol | Sterol | Polar lipids |
| 16 | 2.75 | trace[b] | 2.51 | trace | trace | 0.10 | trace | trace | 0.10 |
| 22 | 7.15 | trace | 6.15 | 0.19 | 0.13 | 0.43 | trace | trace | 0.19 |

[a]Values are the means of three analyses. [b]Trace amounts: <0.05 g.

was not appreciable. The PL and sterols showed no significant difference in the endosperm during this period.

Table 2 shows the lipid pattern of mucilage obtained at the 16th and 22nd wk. Quantitatively, the dry matter and TG showed appreciable increases. The results for the lipid fractions of the haustorium are presented in Table 3. The haustorium registered a rapid rate of accumulation of dry matter and all lipid components. From the nil level at the start of germination, the dry matter content increased to 16.75 ± 0.75 g/nut, and the TL increased from 0.1 to 3.35 g/nut during the same period. The TG content also showed a pattern similar to TL. FFA and 1,2(2,3)-DG, though low in concentration, also showed significant increases.

Fatty acid compositions of TL and TG from endosperm, mucilage, and haustorium at 10, 16, and 22 wk are shown in Tables 4–6. The fatty acid compositions of the dormant seed and of germinated seed at 10 wk were not different; therefore, the fatty acid composition of the dormant seed was not shown. Table 4 shows that the proportion of 12:0 declined significantly from the 10th to 22nd wk. A similar trend was observed for the TG fraction of endosperm. The fatty acid composition of mucilage, shown in Table 5, followed the pat-

**TABLE 3**
**Lipid Composition of Haustorium of Germinating Coconut at Different Stages[a]**

| Stage of germination (wk) | Lipid class (wt in g/nut) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total lipid | Sterol ester | Triacylglycerol | Free fatty acid | 1,3-Diacylglycerol | 1,2(2,3)-Diacylglycerol | Monoacylglycerol | Sterol | Polar lipids |
| 10 | 0.10 | trace[b] | trace | trace | trace | trace | trace | trace | trace |
| 16 | 1.42 | 0.05 | 0.86 | 0.10 | 0.07 | 0.13 | trace | trace | 0.18 |
| 22 | 3.35 | 0.14 | 1.88 | 0.19 | 0.17 | 0.37 | 0.10 | 0.10 | 0.40 |

[a]Values are the means of three analyses. [b]Trace amounts: <0.05 g.

**TABLE 4**
**Fatty Acid Profile of Lipid Classes of Endosperm from Germinating Coconut at Different Stages[a]**

| Stage of germination (wk) | Lipid class | Fatty acid (wt%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 6:0 | 8:0 | 10:0 | 12:0 | 14:0 | 16:0 | 18:0 | 18:1 | 18:2 |
| 10 | Triacylglycerol | 0.30 | 7.22 | 5.50 | 44.05 | 19.07 | 12.24 | 3.33 | 7.27 | 1.02 |
| 16 | | 0.33 | 6.90 | 5.49 | 35.05 | 24.25 | 13.08 | 4.39 | 9.12 | 1.39 |
| 22 | | 0.30 | 6.73 | 5.63 | 27.57 | 22.70 | 15.98 | 5.50 | 13.67 | 1.90 |
| 10 | Total lipids | 0.48 | 7.93 | 5.42 | 45.78 | 19.88 | 8.28 | 4.88 | 4.95 | 2.40 |
| 16 | | 0.38 | 7.26 | 5.16 | 42.27 | 19.77 | 9.07 | 6.40 | 5.74 | 3.95 |
| 22 | | 0.13 | 3.90 | 2.71 | 36.65 | 21.56 | 14.45 | 6.64 | 9.60 | 4.36 |

[a]Values are the means of three analyses.

**TABLE 5**
**Fatty Acid Profile of Lipid Classes of Mucilage from Germinating Coconut at Different Stages[a]**

| Stage of germination (wk) | Lipid class | Fatty acid (wt%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 6:0 | 8:0 | 10:0 | 12:0 | 14:0 | 16:0 | 18:0 | 18:1 | 18:2 |
| 16 | Triacylglycerol | 0.70 | 7.70 | 6.75 | 43.64 | 19.51 | 9.92 | 4.02 | 4.35 | 3.41 |
| 22 | | 0.23 | 4.24 | 3.52 | 31.55 | 23.76 | 16.09 | 5.61 | 11.22 | 3.78 |
| 16 | Total lipids | 0.43 | 6.60 | 6.15 | 43.18 | 21.23 | 12.53 | 5.39 | 3.99 | 0.50 |
| 22 | | 0.50 | 5.75 | 5.02 | 37.14 | 22.32 | 15.04 | 7.03 | 5.73 | 1.47 |

[a]Values are the means of three analyses.

**TABLE 6**
**Fatty Acid Profile of Lipid Classes of Haustorium from Germinating Coconut at Different Stages[a]**

| Stage of germination (wk) | Lipid class | Fatty acid (wt%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 6:0 | 8:0 | 10:0 | 12:0 | 14:0 | 16:0 | 18:0 | 18:1 | 18:2 |
| 10 | Triacylglycerol | 0.25 | 2.09 | 2.95 | 30.03 | 21.16 | 18.21 | 7.75 | 10.55 | 7.01 |
| 16 | | 0.27 | 2.13 | 2.61 | 30.15 | 24.12 | 15.15 | 8.93 | 9.43 | 7.21 |
| 22 | | 0.15 | 2.73 | 2.41 | 32.74 | 21.30 | 13.25 | 8.05 | 11.12 | 8.25 |
| 10 | Total lipids | 0.24 | 3.20 | 3.02 | 35.88 | 17.40 | 17.27 | 8.09 | 11.90 | 3.00 |
| 16 | | 0.28 | 2.54 | 2.70 | 27.50 | 23.02 | 15.85 | 9.73 | 11.28 | 7.10 |
| 22 | | 0.18 | 2.76 | 2.26 | 25.01 | 19.83 | 20.52 | 7.20 | 12.38 | 9.86 |

[a]Values are the means of three analyses.

tern of endosperm. However, the FFA fraction of mucilage was much smaller when compared to that of endosperm. The fatty acid profile of the TL and TG of haustorium exhibited remarkable deviation from the dormant endosperm (Table 6). TL and TG had low levels of low-molecular weight fatty acids 6:0 to 12:0 with higher amounts of fatty acids 14:0 to 18:1. The fatty acid profile of the lipids of haustorium were closely similar at different stages of germination.

## DISCUSSION

During germination, it is assumed that the lipids move from the outer endosperm to the haustorium through the inner endosperm. These three parts were separated from the germinating coconut to verify this process. Results showed that the lipid mobilization started after the 10th wk of germination. From the anatomical features of the coconut, it may be concluded that formation of haustorium during germination is an adaptive and transient feature to facilitate the transfer of nutrients from the reserve site to the embryo. The large haustorium is unique even within the Palmae family. Fat released from inner endosperm cells may be absorbed by the haustorium through the peripheral serrations embedded in the inner endosperm. There was a net loss of lipid from endosperm (15.08 ± 1.25 g/nut) from the 10th to the 22nd wk, with the major loss occurring after 16 wk. The other tissues studied here, such as mucilage and, particularly haustorium, showed an increase in the lipid content, as germination proceeded. The lipids from the endosperm, therefore, could have been leached to the inner region, which on later stages transformed into mucilagenous mass. Lipids from this mass were further absorbed by the haustorium and stored in the outer haustorium for subsequent conversion into sugars. Formation of sugars from lipids in germinating oilseeds through the glyoxylate pathway has already been reported (14–17). Utilization of individual fatty acids by germinating oilseeds has not been fully understood. This is more relevant in the case of coconut, which contains numerous fatty acids, with predominance of short-chain fatty acids (6:0 to 12:0) unlike other oilseeds. The fatty acid composition of the haustorium lipids presented here, as compared to those of mucilage and endosperm, reveals some pattern of preferential utilization of certain fatty acids. Table 6 shows that TL and TG had lower levels of fatty acids 6:0 to 12:0.

Oo and Stumpf (3,4) tried to follow the translocation of lipids in the germinating oil palm seed. They explained that the movement of lipids is from endosperm through haustorium to the shoot; the lipase present in the shoot, as reported by these authors, releases the FFA there, and the FFA are further transported back to the haustorium for subsequent conversion to sugars. They further reported the presence of requisite enzymes of the glyoxylate pathway in haustorium itself, which makes the haustorium of germinating palm seed not only an absorptive tissue but also a biochemically active one. Balasubramaniam and co-workers (18) noticed that germinating coconut utilized only sugars present in the liquid endosperm, with the lipids having no role to play. These authors studied germination of coconut only up to 120 d (17 wk). Our previous studies (Balachandran, C., and C. Arumughan, unpublished data) also indicated that germinating coconut depends mostly on free sugars of liquid and solid endosperms during the early stages (up to 16 wk). Failure to notice utilization of lipids by germinating coconut by Balasubramaniam and co-workers is due to the fact that the major consumption

of lipid takes place only after 16 wk as revealed by the present investigation. Thus, in the germinating coconut, the movement of lipid takes place from endosperm to haustorium, where it is converted to sugars to provide the energy for the growing seedling.

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