Biochemical and Cytochemical Transformations in Germinating Coconut (*Cocos nucifera* Linn.)

C. Balachandran and C. Arumughan*

Oil Seeds Research, Regional Research Laboratory, Council of Scientific & Industrial Research, Trivandrum - 695019, Kerala, India

ABSTRACT: The mechanism of mobilization and utilization of lipids by the developing seedling and the involvement of different tissues in this process was examined in germinating coconut. The endosperm (lipid reserve) and haustorium (spongy tissue developed during germination) were separated from germinating coconuts at intervals of two weeks up to week 22, and assayed for lipase, isocitrate lyase, malate synthase, and catalase, as well as for lipids and sugars. Except for lipase, activities of all the other enzymes were absent in the endosperm during the entire period of germination. However, these enzymes were found to be activated after the tenth week after germination and plateaued at week 16. Induction of these enzymes indicated the operation of the glyoxylate cycle in the haustorial tissue. Glyoxysomes also were observed in the haustorium. The haustorium was shown to absorb triacylglycerol and free fatty acids from endosperm for subsequent conversion to sugars in this tissue. Haustorium therefore is a biochemically active tissue and is the site of operation of the glyoxylate cycle in germinating coconut.

JAOCS 72, 1385–1391 (1995).

KEY WORDS: Catalase, coconut, free fatty acids, germination, glyoxylate cycle, glyoxysomes, haustorium, isocitrate lyase, lipase, malate synthase, sugars, triacylglycerols.

Coconut is the largest oil-bearing nut, with an average weight ranging from 100 to 1000 g for the dehusked nut (1,2). Anatomically, coconut is comprised of four distinct parts: the outer fibrous exocarp, the shell, the white solid endosperm, a central large water cavity filled with a liquid known as liquid endosperm, and a comparatively tiny embryo with a weight of 0.06–0.12 g (1), located in the germ pore of the solid endosperm. The solid endosperm is the lipid reserve and the liquid endosperm is the reservoir of sugars, and the growing embryo depends on these reserves as sources of energy during germination. For nurseries, the general field practice is to place well-matured coconuts in a horizontal position with two-thirds of the husk covered with soil, followed by periodic watering. Usually it takes about 22–24 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, namely the haustorium. The haustorium is a spongy tissue with extensive serrations on the periphery, and during germination it enlarges, keeping in close contact with the endosperm and finally filling the entire water cavity. This newly developed tissue is probably an adaptive feature to facilitate mobilization of nutrients by the embryo from a large area of reserves (3). Haustorium is characteristic within the palmae family. Coconut is unique in having an exceptionally large haustorium. Cooperative functions of various tissues, namely embryo, solid and liquid endosperms, and haustorium, are extremely important in bringing about the complex biochemical events that transform the embryo into a seedling.

Fat is converted into sugars through the glyoxylate cycle in germinating oil seeds (4–7). However, the site and mode of the biochemical events may vary depending on the anatomy of the seed. Among the palmae family, there are few reports on the operation of glyoxylate pathway in the germinating oil palm seed (8,9). Thus, little is known about the biochemical transformations, particularly the sites of biochemical transformations of lipids to sugars and the participation of various tissues in the germinating coconut. Balasubramanian *et al.* (10) reported that coconut depends only on sugar reserves during germination. Previously, we have reported the mobilization and transformation of nutrients and have presented evidence to establish the utilization of lipids by the germinating coconut (11). In this report, attempts have been made to quantify and localize key enzymes involved in the transformation of lipids to sugars, and also to show the role of various tissues in the germinating coconut. Cytochemical evidence supports the biochemical data from the progressive stages of germination.

EXPERIMENTAL PROCEDURES

Method of germination. Ten West Coast Tall variety trees of identical age and yield characteristics were selected for this study. At maturity (12 mon from flowering), the fruits (bunches) were harvested and the nuts with husk were dried under shade for 22 d (1). Nuts (150) of similar weight (733.9 ± 27.7 g) were used for sowing. The nuts with husk were placed in the horizontal plane so as to cover two-thirds of the nut with soil. Soil moisture was maintained by periodic watering. Germinated nuts were randomly sampled between

*To whom correspondence should be addressed.

10–22 wk after germination (WAG) at 2-wk intervals for analysis.

The coconuts were dehusked and broken into halves to expose the intact haustorium. The liquid endosperm (coconut water) was drained off and the haustorium was removed from the endosperm. The endosperm was removed from the shell. Endosperm remaining after removal of shell formed the endospermal region. The haustorium and endospermal regions from ten germinated nuts were pooled separately. Another set of ten nuts was used as the reference sample at "0"-h germination. The solid endosperm from all control nuts also was pooled. The pooled samples of the respective regions and the whole endosperm (reference sample) were disintegrated using a Waring blender, and representative samples were freeze-dried for analyses. Separate samples of fresh tissues of various regions were removed immediately from germinated nuts and preserved at 0°C for enzyme assays and cytochemical analyses.

Enzyme assays. The haustorial and endospermal tissues from coconuts at various stages of germination were chopped with a sharp knife and ground for three minutes in a prechilled mortar with ice-cold grinding medium containing 0.5 M sucrose, 0.15 M tricine buffer pH 7.5, 10 mM KCl, 1 mM $MgCl_2$, 1 mM ethylenediaminetetraacetic acid, and 10 mM dithiothreitol (12). The crude homogenate was filtered through four layers of cheese cloth. The creamy filtrate was centrifuged at 270 g for 10 min. The pellet and fatty layer were discarded. The remaining supernatant was centrifuged at 10,000 g for 30 min to obtain the crude particulate pellet. The pellet was then gently resuspended in grinding medium and used as crude particulate fraction for further enzyme assay studies. Catalase was determined according to the spectrophotometric method of Luck (13). Isocitrate lyase (ICL) and malate synthase (MS) were assayed by the method of Dixon and Kornberg (14).

Spectrophotometric measurements were made by using diode array spectrophotometer (Hewlett-Packard 8450; Hewlett-Packard, Palo Alto, CA). Lipase activity was measured by the titrimetric method of Bier (15). Freshly prepared polyvinyl alcohol (PVA)-emulsified coconut oil of 0.1 M was used for the assay. The assay system contained 10 mL of PVA-emulsified substrate, 5 mL of citrate–phosphate buffer, and 5 mL of the enzyme preparations. The mixture was shaken gently and incubated for 3 h at 37°C with constant shaking. At the end of the incubation, 25 mL of methanol/acetone solution (1:1, vol/vol) was added to stop the reaction and to break the emulsion. The production of free fatty acids (FFA) was measured by titration with 0.1 N NaOH. Control was done in the same way with enzyme extract inactivated by short boiling. One unit of lipase activity was defined as the amount of enzyme required to release 1 μm FFA per minute under specified experimental conditions. Protein was estimated by the method of Lowry *et al.* (16). Total enzyme activity was calculated as units/nut.

Cytochemical localization of glyoxysomes. Cytochemically, glyoxysomes were detected by localizing catalase according to the method of Vigil (17). The incubation medium was prepared by the method described by Frederick and Newcomb (18). The reaction medium contained 5 mL of 0.05 M 2-amino-2-methyl 1,3-propanediol (AMP) buffer, pH 10, 0.1 mL of 3% H_2O_2, and 10 mg 3,3′-diaminobenzidine tetrahydrochloride (DAB). The final pH of the medium was adjusted to 9 before use. Haustorial and endospermal tissues of coconut at different stages of germination (0–22 wk) were used for cytochemical preparations. Small pieces of experimental samples (1 mm^3) were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 for 6 h at 4°C. The tissues were washed thoroughly in cold cacodylate buffer for 2 h at 4°C. Fixed tissues were equilibrated in 0.1 M AMP buffer at pH 9 prior to incubation in the medium. The tissues were then incubated in DAB medium for 60 min at 37°C. After incubation, the tissues were rinsed once in chilled 0.1 M AMP buffer at pH 9, and several times in 0.1 M cacodylate buffer at pH 7.4. Incubated tissues were kept in 2% Osmium tetroxide in 0.1 M cacodylate buffer at pH 7.4 for 2 h at 4°C. Osmicated tissues were washed well in the same buffer and dehydrated in acetone series of 30, 50, 70, 90, and 100% for 10 min each. To increase the contrast of cellular structures during dehydration, the tissues were kept in 2% uranyl acetate in 70% acetone overnight. Dehydrated tissue pieces were embedded in the spurr's resin and polymerized for 24 h at 70°C. The embedded tissue blocks were sectioned by an ultratome (Nova; LKB, Bromma, Sweden). Ultrathin sections were mounted carefully in cleaned copper grids. The mounted grids were first stained for 15 min in 2% aqueous uranyl acetate. The grids were stained again in 4% lead citrate for 5 min, and were rinsed in double-distilled water and dried under light for 1 h. The dried specimen grids were viewed and photographed using a transmission electron microscope (Zeiss EM 109; TEM, Oberkochen, Germany). For control, the fixed tissue pieces were preincubated for 2 h in 0.05 M AMP buffer containing 0.02 M aminotriazole. After this treatment, the tissues were incubated in DAB medium containing 0.02 M aminotriazole for 60 min at 37°C, and the processing procedure for the experimental samples was followed.

Light microscopy. For localization of sugars, thin sections of haustorium were taken by freezing microtome and stained for sugars, following the PARS reaction (19). Sections were viewed and photographed under optical microscope (Nikon, Tokyo, Japan).

Triacylglycerols (TAG) and FFA. Total lipids were extracted from endospermal and haustorial tissues of germinating coconuts with chloroform/methanol (2:1, vol/vol) and purified according to the procedure of Folch *et al.* (20). The solvent fraction was evaporated under vacuum in a rotary evaporator to get the total lipids. TAG and FFA were separated by preparative thin-layer chromatography on 1-mm thick silica gel G (Merck, Bombay, India) adsorbent using a solvent system (21) of petroleum ether/diethyl ether/acetic acid (80:20:1, vol/vol/vol). Bands were detected by brief exposure to iodine vapor. The TAG and FFA bands were eluted from the gel with chloroform and quantitated by gravimetry, as well as by the oxidative dichromate method of Bragdon (22).

Analysis of sugars by high-performance liquid chromatography. Extraction of soluble sugars was carried out as per an Association of Official Analytical Chemists method (23) followed by a purification step using monobed mixed resign column (20 × 1 cm) of emberlite MB-1 (BDH Chemicals Ltd., Poole, England). The sugars were eluted with 100 mL of deionized water and were lyophilized. The residue was dissolved in 1 mL distilled water and filtered through a 0.5-μm celotate filter utilizing a Swinnex syringe filter (Millipore Corp., Milford, MA) before injection to liquid chromatograph. The volume of injection was 5 μL in all cases. A Shimadzu liquid chromatograph (Shimadzu Corp., Kyoto, Japan) equipped with C-R4A chromatopac data processor, LC-6AD solvent delivery system, RID-6A refractive index detector, and a Rheodyne injection module was used. The column was a 30 cm × 3.9 mm i.d. stainless-steel μ-Bondapak/Carbohydrate (Waters Associates, Milford, MA). The mobile phase consisted of a mixture of acetonitrile/water (850:150) at a flow rate of 2 mL/min. The solvent mixture was degassed for one minute under vacuum with shaking and was stirred continuously. Individual sugars were identified by using standards of ribose, rhamnose, arabinose, fructose, glucose, sucrose, maltose, and raffinose (Sigma, St. Louis, MO), and the peaks were quantitated by digital integration. Total soluble sugars (TS) and reducing sugars (RS) in the samples were estimated by the methods of Roe (24) and Somogyi (25), respectively.

RESULTS

Biochemical changes. The key enzymes involved in the conversion of TAG to sugars, such as lipase, ICL, and MS, were assayed in endosperm and haustorium obtained from different stages of germinating coconut. The enzyme catalase was also assayed from these tissues as a biochemical marker to localize glyoxysomes which contain the key enzymes of the glyoxylate cycle. The homogenates of the tissues were assayed for these enzymes following the procedure described previously, and the enzyme units were expressed on per nut basis. Figure 1 shows the lipase activities in endosperm and haustorium at various stages of germination. Endosperm lipase had a pH optimum in the acidic side, and the haustorium lipase exhibited its maximum activity in the alkaline side. Accordingly, the former is termed here as acidic lipase and the latter as alkaline lipase.

It may be further noted that the activity of lipase in the haustorium registered a rapid rise from 10 WAG, reaching a maximum value at 16 WAG and plateaued. The activities of ICL and MS were detected only in haustorium, whereas these enzymes were totally absent in the endosperm (Fig. 2). ICL and MS exhibited a steep rise in their activities at 14 WAG. Catalase enzyme also was found to be active only in haustorium; the endosperm had neglible activity (Fig. 3). The activity of catalase also exhibited a similar pattern as other enzymes. Total activity on per nut basis rose rapidly at 14 WAG; specific activities of the respective enzymes showed a high rate of increase from 10 to 16 WAG, and then declined.

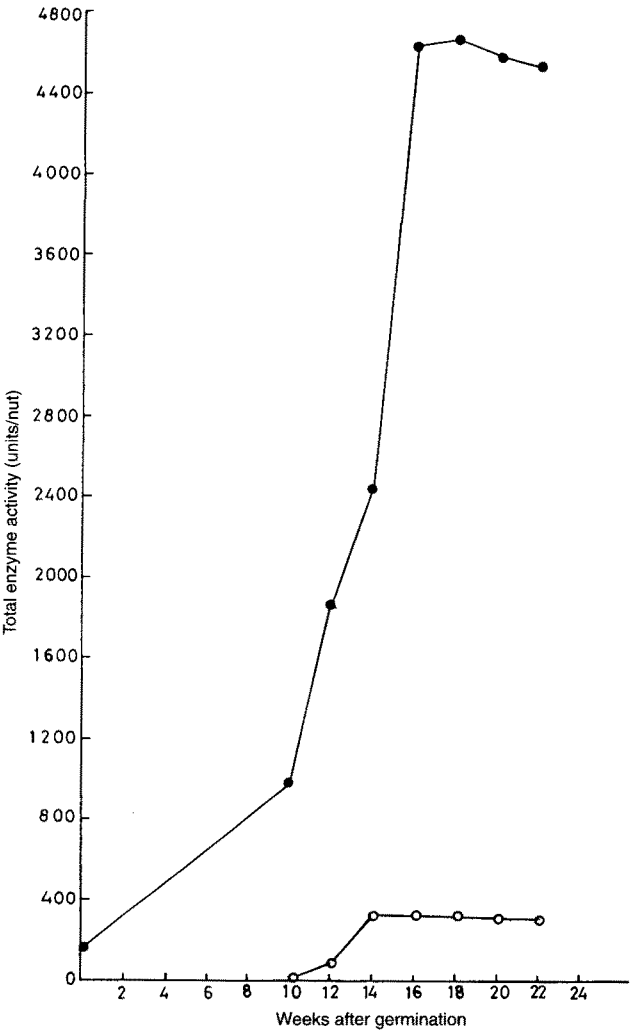

FIG. 1. Lipase activities in endosperm and haustorium of germinating coconut at different stages. The activity of the acid lipase was assayed at pH 5 and that of the alkaline lipase at pH 9. ●, Endosperm lipase; ○, haustorium lipase.

TAG and sugars from endosperm and haustorium were correlated with the enzyme activities during progressive stages of germination. In Figure 4, TAG remained almost constant until 12 WAG, and subsequently decreased rapidly until 22 WAG in the endosperm. Total depletion of TAG was 17.96 ± 1.25 g/nut. FFA content increased at a slow pace during the same period in the endosperm. The TAG level in the haustorium was insignificant until 10 WAG, but increased up to 22 WAG, with a total gain of 1.88 ± 0.05 g/nut. During the same period, FFA in the haustorium showed a similar rate of increase, but at a very slow rate (Fig. 5).

TS of the liquid and solid endosperms declined from 10 to 16 WAG while RS remained steady during the entire period of germination (Fig. 6). The TS of the endosperms at the beginning of germination, 12.23 ± 0.41 g/nut, declined to 1.89 ± 0.15 g/nut at 18 WAG. TS in haustorium increased, 8.4 ± 0.33 g/nut, from 10 to 22 WAG (Fig. 7). RS increased from 11.6% of TS at 10 WAG to about 36.8% at 22 WAG. Sucrose

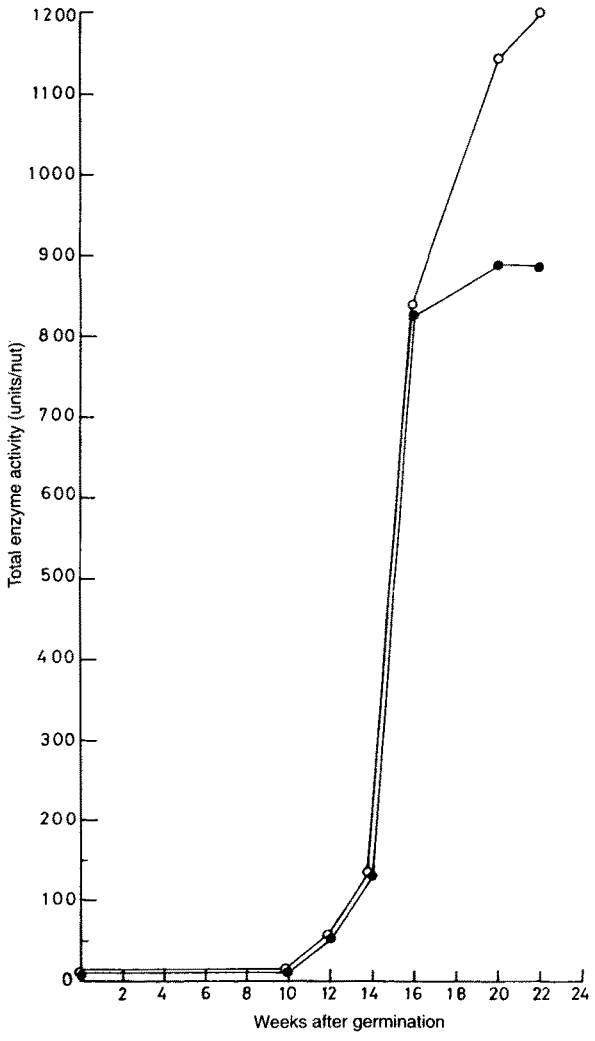

FIG. 2. Isocitrate lyase (ICL) and malate synthase (MS) activities in haustorium of germinating coconut at different stages. ○, MS activity; ●, ICL activity.

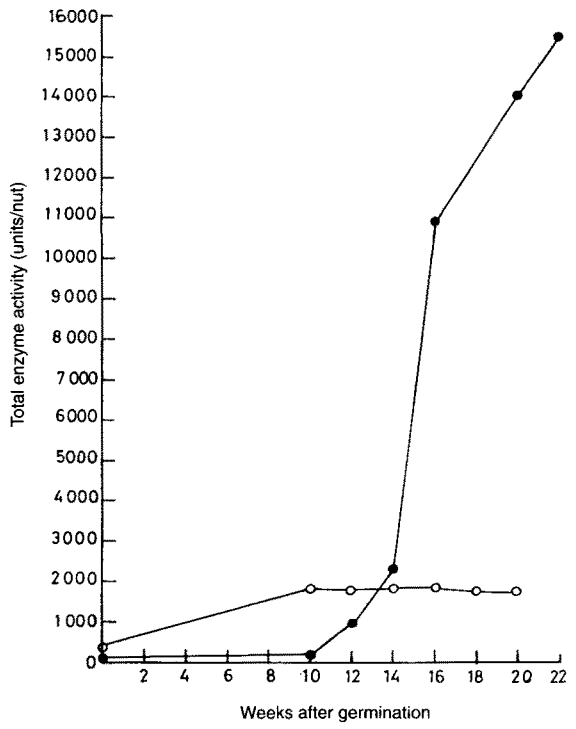

FIG. 3. Catalase activities in endosperm and haustorium of germinating coconut at different stages. ○, Endosperm; ●, haustorium.

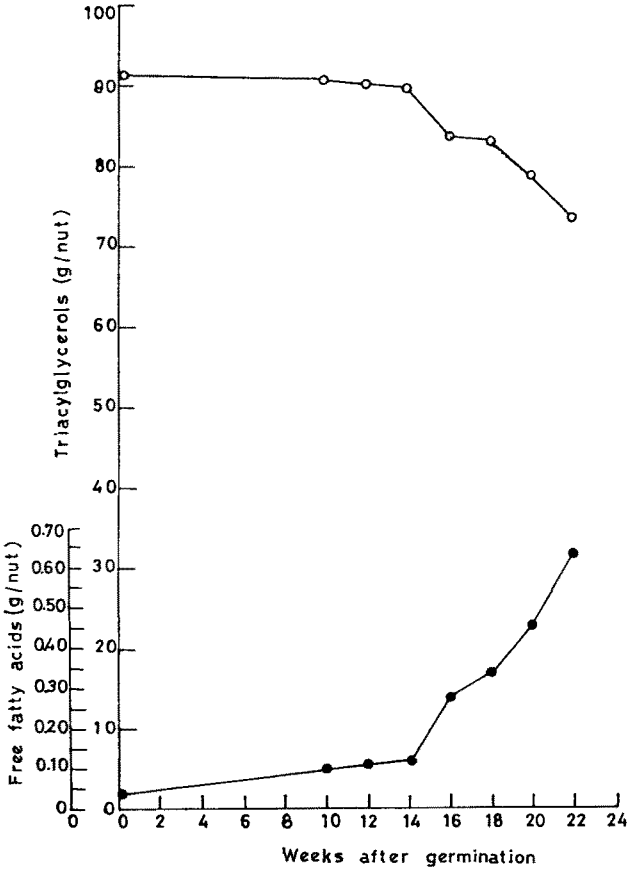

FIG. 4. Triacylglycerol (TAG) and free fatty acid (FFA) profiles in the endosperm of germinating coconut at different stages. ○, TAG; ●, FFA.

was the predominant sugar in solid endosperm (Table 1) at 10 WAG. Afterwards, sucrose concentration declined as glucose and fructose increased. At 22 WAG, the endosperm sugars comprised only 1.06% sucrose, whereas glucose and fructose predominated with 62 and 37%, respectively. A similar trend was observed for the individual sugars in haustorium (Table 2). However, the concentration of sucrose, glucose, and fructose remained fairly equal.

Cytochemical changes. The biochemical results were further corroborated with cytochemical evidence, particularly for the formation of glyoxysomes during germination. Both endospermal and haustorial tissues from different stages of germination of coconut were subjected to the cytochemical investigation following standard procedure using DAB staining followed by observation under TEM. Catalase was used as a marker for glyoxysomes. Glyoxysomes were stained as black bodies due to the specific staining technique for catalase, whereas in the control, they were observed as colorless entities (Fig. 8). As can be seen in Figure 9, the glyoxysomes

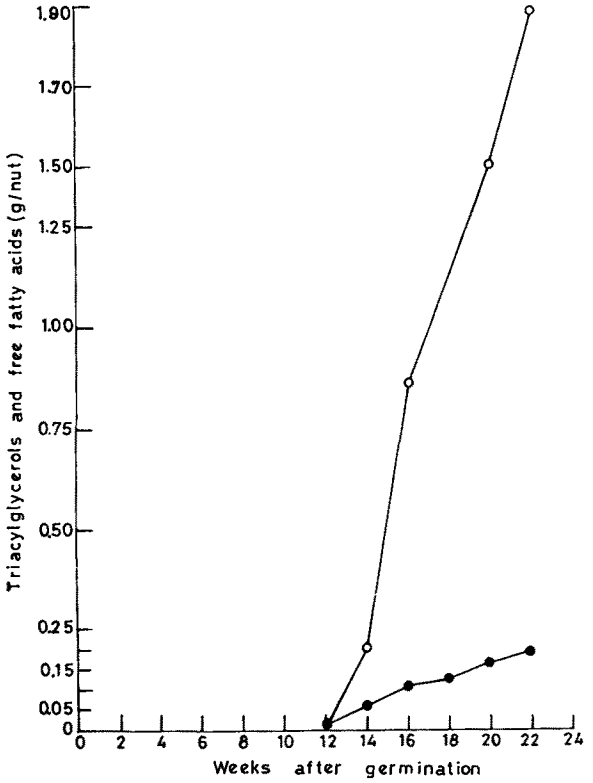

FIG. 5. TAG and FFA profiles in the haustorium of germinating coconut at different stages. ○, TAG; ●, FFA. Abbreviations as in Figure 4.

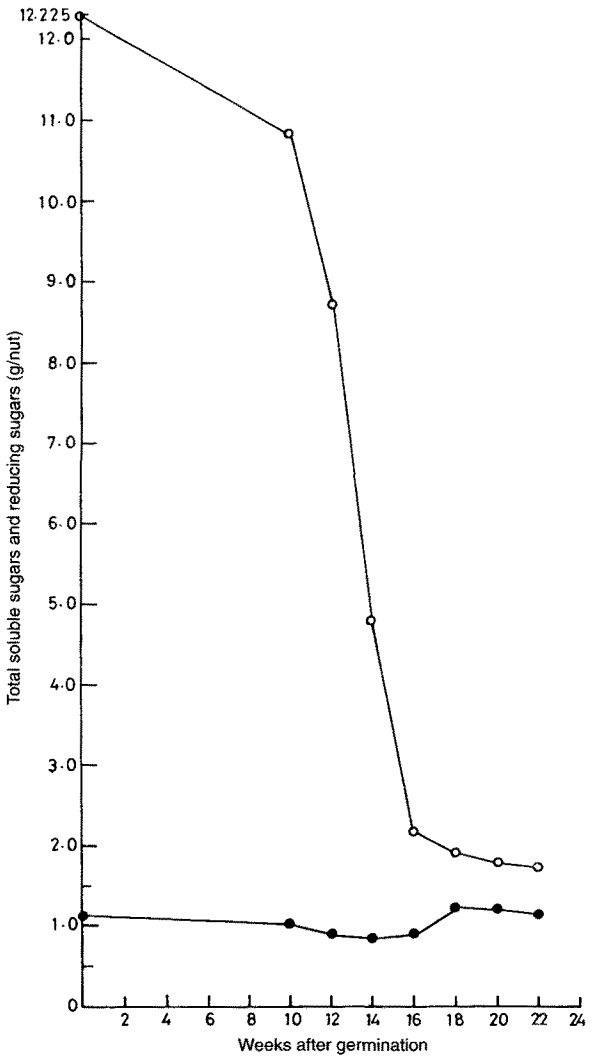

FIG. 6. Total soluble sugar (TS) and reducing sugar (RS) profiles in the endosperms (solid and liquid) of germinating coconut at different stages. ○, TS; ●, RS.

were detected in higher population around the fat globules (large black spherical bodies). Glyoxysomes were detected only in the haustorial tissues. The electron micrograph also showed a high population of mitochondria, indicating that the haustorial tissue was biochemically active. The population of glyoxysomes increased with time of germination and finally disappeared toward the end of this process. Presence of sugar crystals in the haustorial tissue also was observed using PARS reaction staining technique. The sugar crystals were found to be concentrated in the inner haustorium, which is a spongy structure formed by loose aggregations of cells (Fig. 10).

DISCUSSION

An average dehusked coconut contained 250 ± 10.5 g wet endosperm and 100 ± 10 mL liquid endosperm (coconut water). Solid endosperm contained 95.3 ± 5.3 g lipid and 10.5 ± 0.4 g sugar per nut; the liquid endosperm contained only sugars (1.88 ± 0.05 g/nut). Among the palmae family, coconut is the only seed that develops to be very large and has a well-developed haustorium. Various studies have shown that haustorium from germinating coconut is a biochemically active tissue (8,9,26–28). The present investigation established the metabolic role of haustorium during germination. Previously, we presented evidence for the translocation of lipids and sugars in germinating coconuts (11). The results of current experiments support these findings. Biochemical, histological, and cytochemical changes took place only after 10 WAG. ICL and MS are key enzymes in the glyoxylate cycle in germinating oil seeds (8,9,17,29,30). Although ICL and MS activities were not detected in endosperm throughout germination, acidic lipase (pH optimum 5) was active. Haustorial tissue recorded remarkably high activities for ICL and MS, and alkaline lipase exhibited low activity. The induction of these enzymes commenced at 10 WAG when the haustorial tissue was beginning to develop and increased until 20 WAG. Haustorium lipase had an optimum pH of 9; hence, it was termed as alkaline lipase. The effect of lipase action on TAG was not observed until 14 WAG in endosperm. However, there was a drastic depletion of sugar reserves from solid and liquid endosperms, 12.25 ± 0.41 g/nut to 2.71 ± 0.35 g/nut, respectively, at 16 WAG. After the sugar reserves were depleted, the TAG reserves were mobilized for further growth of the embryo at 16 WAG. Onset of lipase induction in the endospermal

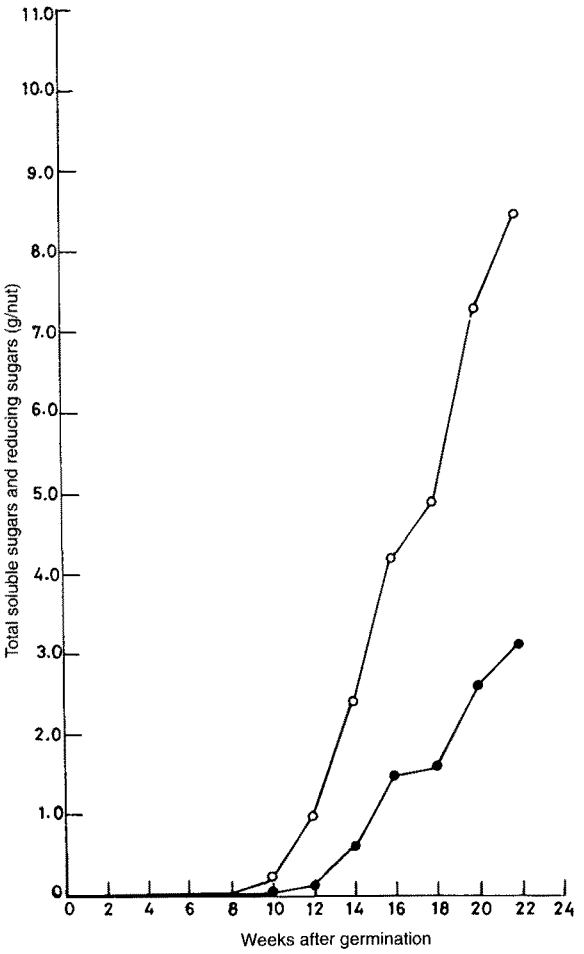

FIG. 7. TS and RS profiles in haustorium of germinating coconut at different stages. ○, TS; ●, RS. Abbreviations as in Figure 6.

tissue synchronized with the depletion of TAG in the endosperm. As can be seen in Figures 4–7, TAG content decreased progressively from 16 to 22 WAG in the endosperm, whereas TAG and TS increased in the haustorium during the same period. FFA increased in both endosperm and in haustorium. These results showed that both TAG and FFA were

TABLE 1
High-Performance Liquid Chromatography Profile of Sugars in the Endosperm from Three Different Stages of Germinating Coconut (% wt)[a]

	Weeks after germination		
Sugars	10	16	22
Rhamnose	0.20	0.14	trace[b]
Glucose	9.44	45.75	61.95
Fructose	1.14	35.01	36.98
Sucrose	89.22	19.10	1.06
Total soluble sugars (g/nut)	10.50	2.71	2.16
Reducing sugars (g/nut)	1.01	1.24	1.33

[a]Values are the means of three analyses.
[b]Trace amounts <0.01 g.

TABLE 2
High-Performance Liquid Chromatographic Profile of Sugars in the Haustorium from Three Different Stages of Germinating Coconut (% wt)[a]

	Weeks after germination		
Sugars	10	16	22
Rhamnose	trace[b]	0.14	0.04
Glucose	11.62	34.52	36.79
Fructose	7.57	19.22	28.57
Sucrose	80.81	46.12	34.60
Total soluble sugars (g/nut)	0.20	4.11	8.40
Reducing sugars (g/nut)	0.02	1.43	3.09

[a]Values are the means of three analyses.
[b]Trace amounts <0.01 g.

absorbed by haustorium and the TAG was further hydrolyzed by the haustorium lipase. Glyoxysomes were detected, using catalase as the marker, only in the haustorial tissue. It also was shown that the activity of catalase increased after 14 WAG, coinciding with activities of other enzymes in the haustorium. The increase in catalase activity indicated a rapid conversion of FFA to sugars by glyoxysomes. The presence of glyoxysomes was further confirmed by TEM. From these findings, it could be concluded that the TAG and FFA from the endosperm were mobilized and metabolized to soluble sugars for the growth of embryo. Haustorium, therefore, is a biochemically active tissue capable of synthesizing all the enzymes for fat metabolism in germinating coconut. Oo and Stampf (8,9), working with oil palm seed, could not detect lipase in the haustorium, but lipase was shown to be present in the shoot. Therefore, they hypothesized that the fat from the endosperm was transported to the shoot, where it was ly-

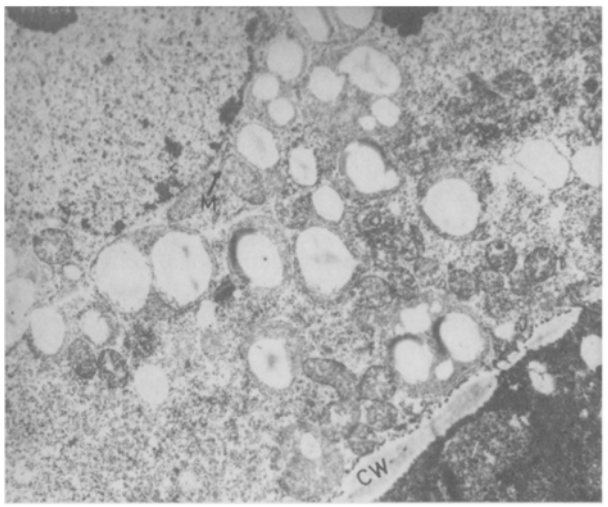

FIG. 8. Transmission electron micrograph as control for glyoxysomes. Aminotriazole was used to inhibit catalase enzyme and, consequently, the glyoxysomes were not stained with DAB, and are seen as white spherical bodies. Mitochondria also could be seen in abundance, indicating that haustorium is biochemically active. CW, cell wall; M, mitochondrion; magnification 8,160×.

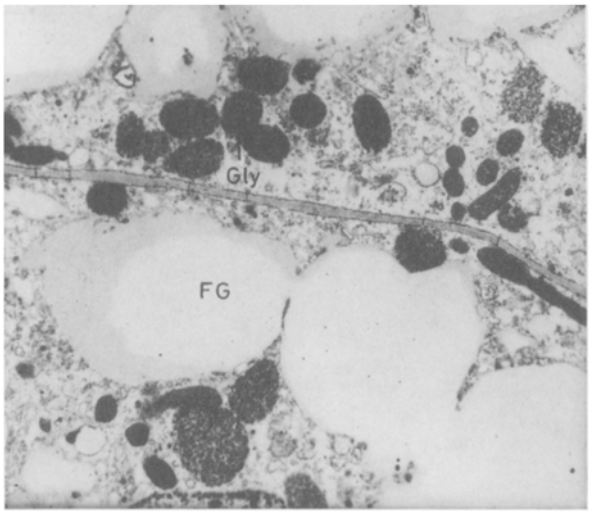

FIG. 9. Transmission electron micrograph of haustorial cell showing the glyoxysomes as black spherical bodies in close proximity with the fat globule. FG, fat globule; Gly, glyoxysomes; magnification, 8,160×.

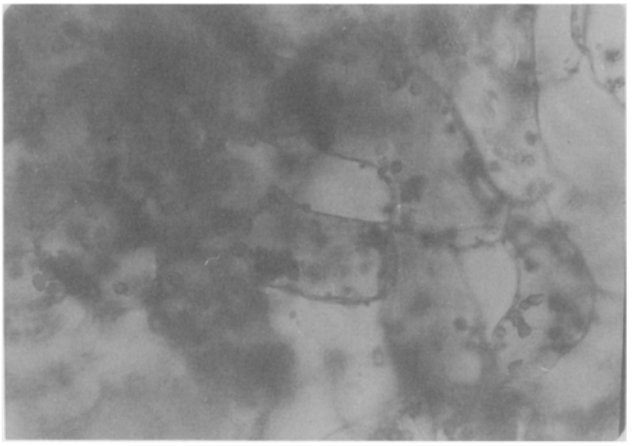

FIG. 10. Photomicrograph of haustorial tissue showing the sugar crystals stained as pink following PARS reaction. Magnification, 136×.

polized, and FFA were then transported back to haustorium for further degradation to sugars. Balasubramanian *et al.* (10) reported that coconut utilized only sugars during germination. Their study was confined to utilization of sugars only up to 16 WAG. As shown here, mobilization of fat reserve actually takes place after 16 WAG.

The present study has demonstrated that haustorium plays a key role in the mobilization of sugars and fat in the germinating coconut. The entire process of germination is therefore facilitated by cooperative and effective participation of endosperms (solid and liquid), haustorium and embryo, with the haustorium playing a key role as the site of the glyoxylate cycle.

ACKNOWLEDGMENT

The authors are grateful to Dr. A.D. Damodaran for providing facilities for conducting this investigation.

REFERENCES

1. Child, R., *Coconuts*, 2nd edn., Longman Group Limited, London, 1974.
2. Woodroof, J.G., *Coconuts: Production, Processing, Products*, 2nd edn., AVI Publishing Co., Inc., Westport, 1979.
3. Thampan, P.K., *Handbook on Coconut Palm*, Oxford and IBH Publishing Co., New Delhi, 1981.
4. Kornberg, H.L., and H. Beevers, *Biochim. Biophys. Acta 26*:531 (1957).
5. Canvin, D.T., and H. Beevers, *J. Biol. Chem. 236*:988 (1961).
6. Beevers, H., *Recent Advances in the Chemistry and Biochemistry of Plant Lipids*, edited by T. Gaillard and E.I. Mercer, Academic Press, New York, 1975.
7. Beevers, H., *Regulation of Developmental Processes in Plants*, edited by H.R. Schitte and Gross D. Fischer, Jena, Germany, 1978.
8. Oo, K.C., and P.K. Stumpf, *Plant Physiol. 73*:1028 (1983).
9. Oo, K.C., and P.K. Stumpf, *Ibid. 73*:1033 (1983).
10. Balasubramanian, K., T.M.S. Atukorala, S. Wijesundera and A.A. Hoover, *Ann. Bot. 37*:439 (1973).
11. Balachandran, C., and C. Arumughan, *J. Am. Oil Chem. Soc. 72*:647 (1995).
12. Beevers, H., and R.W. Breidenbach, *Methods in Enzymology, Part A: Biomembranes*, Academic Press, New York, 1975, p. 565.
13. Luck, H., *Method of Enzymatic Analysis*, Academic Press, New York, 1965.
14. Dixon, G.H., and H.L. Kornberg, *Biochem. J. 72*:3 (1959).
15. Bier, M., *Methods of Enzymology*, Academic Press, New York, 1955, p. 627.
16. Lowry, O.H., N.J. Roseburgh, A.L. Farr and B.J. Randall, *J. Biol. Chem. 193*:265 (1951).
17. Vigil, E.L., *J. Cell Biol. 46*:435 (1970).
18. Frederick, S.E., and E.H. Newcomb, *J. Cell Biol. 43*:343 (1969).
19. Hotchkiss, R.D., *Arch. Biochem. Biophys. 16*:131 (1948).
20. Folch, J., M. Lees and G.H. Sloane-Stanley, *J. Biol. Chem. 226*:497 (1957).
21. Mangold, H.K., *Thin Layer Chromatography*, edited by E. Stahl, Springer-Verlag, New York, 1969, p. 374.
22. Bragdon, J.H., *J. Biol. Chem. 190*:513 (1951).
23. *Official Methods of Analysis*, 14th edn., Association of Official Analytical Chemists, Viriginia, 1984.
24. Roe, J.H., *J. Biol. Chem. 212*:335 (1955).
25. Somogyi, N., *Ibid. 160*:61 (1945).
26. Khor, H.T., and K.C. Oo, *Phytochem. 23*:1579 (1984).
27. DeMason, D., *Ann. Bot. 52*:71 (1983).
28. DeMason, D., *Protoplasma 126*:159 (1985).
29. Breidenbach, R.W., and H. Beevers, *Biochem. Biophys. Res. Commun. 27*:462 (1967).
30. Huang, A.H.C., *Plant Physiol. 55*:870 (1975).

[Received January 3, 1995; accepted July 12, 1995]