The use of acetyl-CoA carboxylase activity and changes in wall composition as measures of embryogenesis in tissue cultures of oil palm (*Elaeis guineensis*)

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(Received 14 May 1982/Accepted 15 July 1982)

With some lines of oil-palm tissue cultures embryogenesis occurs spontaneously within the callus grown on a medium containing 2.5 mg of 3-naphthylacetic acid/litre. One of the initial biochemical events that occurs just before the embryoid can be seen is the accumulation of fat droplets within the cells. This accumulation of lipid is correlated with an increase in acetyl-CoA carboxylase activity. The carboxylase is thus probably a rate-limiting step in fatty acid synthesis in these cells and can be used as a quantitative marker of somatic embryogenesis within the tissue. During the development of the embryoid tissue there is an increase in cell division and the differentiation of vascular cells with secondary thickened walls. These stages of the differentiation may be monitored by measuring the ratio of pectin synthesis (polygalacturonic acid formation) to hemicellulose synthesis (xylan formation).

Many plant species can be grown and maintained as disorganized calluses on a nutritive medium and induced to differentiate by changing the environmental conditions. The differentiation may take different forms, e.g., root or shoot formation, somatic embryogenesis (sometimes resulting in the regeneration of plantlets) or simply the formation of nodules containing xylem and phloem. The stimulus that induces the differentiation is usually a change in the balance of growth substances in the medium. The ratio of auxin to cytokinin has been proposed as the determining factor in the formation of roots or shoots in callus tissue (Skoog & Miller, 1957).

The importance of plant-growth factors for the formation of vascular nodules has also been well documented (Torrey, 1953; Wetmore & Sorokin, 1955; Wetmore & Rier, 1963; Jeffs & Northcote, 1966; Wright & Northcote, 1972; Haddon & Northcote, 1975).

Somatic embryogenesis was first observed in carrot cultures (Reinert, 1958; Steward *et al.*, 1958) and has subsequently been achieved in many plant species including *Antirrhinum* (Sangwan & Harada, 1975), *Petunia* (Rao *et al.*, 1973) and *Nigella* (Raman & Greyson, 1974). In these species embryogenesis was induced by transfer of the cultures from medium containing auxin and a reduced nitrogen source to medium lacking, or with a very low

concentration of, auxin. Many plant species do not respond to this transition from high to low auxin concentrations and other factors are probably important in inducing embryogenesis. Cytokinins promote embryogenesis in some species, e.g. *Apium* (Al Abta & Collin, 1978), and in *Daucus* embryogenesis only occurs below a critical level of dissolved O₂ (Kessel *et al.*, 1977). Activated charcoal has also been used to promote embryogenesis, e.g., *Daucus* and *Allium* (Fridborg *et al.*, 1978) and *Hedera* (Banks, 1979).

Recently, interest has centred on the possibility of propagating the oil palm (*Elaeis guineensis*) by tissue-culture methods. The oil palm is an important commercial crop, used in the manufacture of margarine and soap. The palm cannot be propagated vegetatively as it possesses a single terminal meristem, inflorescences develop from each axillary bud and it does not regenerate new tissues after wounding. Tissue culture is a possible solution to the problem of propagating individual, high-yielding, F₁ hybrid palms. Successful cultures of palm have been established from crown meristem and root tissue of seedlings (Jones, 1974a). The growth of the cultures is very slow compared with other plant species; doubling times of 40 days are usual. In some cultures, initiated from regions near the embryonic axis of germinated seedlings, white globular embryoids have developed spontaneously. These become elongated and form a thick primary root at

Abbreviation used: NAA, 3-naphthylacetic acid.

the end proximal to the parent callus, and in some cases a shoot at the distal end. Successful plantlet regeneration from clonal embryoids has been achieved (Corley *et al.*, 1978).

Although somatic embryogenesis has been observed in the oil palm, it has not been reproducibly brought about by controlled changes in the growth medium or other environmental conditions. Reynolds & Murashige (1979) have reported an increased rate of embryogenesis by the inclusion of activated charcoal in the medium but this method has not been successful with some cultures used in this laboratory. Greater embryoid yields would be possible if a suitable induction medium for the differentiation could be found.

The work presented in this paper has been concerned with the investigation of biochemical changes that occur during embryogenesis in tissue cultures of palm, with the aim of identifying a suitable parameter for use as a biochemical marker of embryogenesis. This parameter could be used to enable a more rapid screening of various media in the search for the optimal conditions for differentiation.

Changes in the composition of the plant cell wall have been monitored in several systems as an index of differentiation to vascular tissue (Northcote, 1982). The cell wall composition during embryogenesis in oil palm has been investigated in this work. Oil-palm embryoids produced *in vitro* contain deposits of lipid and storage protein (Jones, 1974b) and in these respects resemble the structure of the embryo in the mature seed. If the deposition of triacylglycerol is a unique feature of palm embryoids and is not present in callus, then the level of triacylglycerol and the activities of enzymes associated with triacylglycerol synthesis may be used as markers of differentiation.

We have shown that embryogenesis in oil-palm tissue culture is characterized by the deposition of lipid, which can be correlated with changes in the activity of acetyl-CoA carboxylase. This enzyme catalyses the ATP-dependent carboxylation of acetyl-CoA to malonyl-CoA, the first committed step in the synthesis of fatty acids. The cell wall of the palm has been analysed and changes in cell-wall composition were observed during embryogenesis to detect possible periods of cell division and differentiation of xylem tissue.

Materials and methods

Tissue cultures of oil palm lines JB.20.1C and JH were provided by Unilever Research, Sharnbrook, Bedford, U.K. Murashige and Skoog medium was obtained from Flow Laboratories, Irvine, Ayrshire, Scotland, U.K. Agar Noble and casamino acids were from Difco Laboratories, Detroit, MI, U.S.A.

$\text{NaH}^{14}\text{CO}_3$ (sp. radioactivity 2.05 GBq/mmol) was purchased from Amersham International, Amersham, Bucks., U.K. ATP, avidin and CoA were obtained from Sigma Chemical Co., Kingston upon Thames, Surrey, U.K. All chemicals used were of A.R. quality wherever possible.

Acetyl-CoA was synthesized by acetylation of CoA with acetic anhydride (Simon & Shemin, 1953) and its concentration was determined by the method of Ramsey & Tubbs (1975).

Growth of tissue cultures

Media and instruments were sterilized by autoclaving at 120°C and 103 kPa for 20 min. All manipulations were carried out on a sterile laminar-air-flow bench. Undifferentiated callus cultures of oil palm were maintained on half-strength Murashige and Skoog medium (Murashige & Skoog, 1962) with the addition of 1 g of casamino acids/litre, 2.5% sucrose and 2.5 mg of 3-naphthylacetic acid/litre. The medium was solidified by the addition of 1% agar Noble. The cultures were maintained at 25°C and illuminated at a light intensity of 100 lx, with an 18 h photo period. They were routinely subcultured every 8 to 10 weeks. Cultures of line JH differentiated spontaneously under these conditions to produce small hard globular embryoids. Line JB.20.1C showed very little spontaneous embryogenesis under the same conditions.

Preparation of enzyme extracts

All procedures were carried out at 4°C. The tissue (approx. 2 g for time courses, 20 g for preparation of enzyme extract for optimization) was homogenized in 100 mM-Tris/HCl, pH 7.5, containing 20% glycerol, 0.2 mM-phenylmethanesulphonyl fluoride and 15 mM-mercaptoethanol (5 ml of buffer/g of tissue). The tissue was first ground in a pestle and mortar with acid-washed fine sand, then homogenized in an MSE overhead homogenizer at full speed for 2 min. Triton X-100 was added to a final concentration of 0.5% and the homogenate was stirred for 10 min, followed by centrifugation at 20 000 g for 10 min in a Sorvall RC2-B centrifuge. The supernatant was taken to 80% saturation with saturated $(\text{NH}_4)_2\text{SO}_4$ solution and stirred for 1 h. The precipitated protein was pelleted at 20 000 g for 10 min and redissolved in a small volume of homogenization medium.

Assay of acetyl-CoA carboxylase (EC 6.4.1.2)

This was assayed by measuring the fixation of radioactivity from $\text{NaH}^{14}\text{CO}_3$ into malonyl-CoA as described by Egin-Buhler *et al.* (1980). The optimum conditions for the enzyme assay were determined. The standard incubation mixture contained, in a total volume of 90 μl , 10 μmol of Tris/HCl, pH 8.7, 1.4 μmol of KHCO_3 , 0.1 μmol of $\text{NaH}^{14}\text{CO}_3$,

(205 kBq), 0.04 μmol of acetyl-CoA, 0.5 μmol of ATP, 1.0 μmol of MgCl_2 . Up to 80 μg of protein in a volume of 100 μl was used per assay. All assays were performed in duplicate for two different enzyme concentrations. The duplicate values did not deviate by more than 10% from the mean value. Controls without acetyl-CoA were included for each sample. After incubation at 30°C for 10 min, glacial acetic acid (20 μl) was added to terminate the reactions and remove $\text{H}^{14}\text{CO}_3^-$. After centrifugation at 12000 g for 2 min in a Beckman microfuge to remove precipitated protein, samples (100 μl) were dried on to Whatman GF-C discs. The discs were immersed in aqueous scintillation fluid (1.5 litres of toluene, 0.75 litre of Triton X-100 and 6 g of 2,5 diphenyloxazole) and radioactivity was counted with a Searle Mark III liquid-scintillation counter.

Lipid extraction

Tissue (approx. 2 g) was homogenized in propan-2-ol at 80°C (20 ml/g of tissue) to inhibit lipase activity. After filtration the residue was extracted overnight at 4°C with chloroform/methanol (3:2, v/v) (20 ml/g of tissue) and then refiltered. The propanol extract was rotary-evaporated to dryness, taken up in chloroform/methanol (3:2, v/v) and recombined with the first chloroform/methanol extract. The extracted lipids were purified by washing by the method of Folch *et al.* (1957). The purified extracts were rotary-evaporated to dryness, taken up in a small volume of chloroform and stored at -20°C.

Estimation of lipid

Lipid was measured by the method of Van Handel & Zilversmit (1957) with tripalmitoylglycerol (0–100 μg) as standard.

Fractionation of the cell wall

The cell-wall material from young palm stems was fractionated to determine the sugar composition of pectin, hemicellulose and cellulose fractions. Stems (cut into small pieces, 15 g fresh weight) were plunged into boiling ethanol and refluxed in ethanol for 1 h, followed by refluxing in benzene for a further hour to remove soluble phenolics. The residue was dried, suspended in water (2 ml/g of residue) and delignified by using sodium chlorite (0.3 g/g dry wt. of tissue) and glacial acetic acid (1 drop/g dry wt. of tissue). The suspension was incubated at 60°C for 5 h with further additions of sodium chlorite and acetic acid each hour. After delignification the suspension was filtered and the residue boiled with 2% sodium hexametaphosphate, pH 3.7, for 2 h. The supernatant from this extraction was dialysed overnight against running water and for 1 h against glass-distilled water. Polysaccharide was precipitated from the non-diffusible material in 85%

ethanol. This fraction was designated the pectin fraction. The residue remaining after sodium hexametaphosphate extraction was refluxed with 3% NaOH for 2–3 h under N_2 , then filtered. The filtrate was dialysed and polysaccharide was precipitated (first hemicellulose fraction). The residue was refluxed with 5% NaOH containing 3% sodium borate for 3 h. The extracted polysaccharide was dialysed and precipitated (second hemicellulose fraction). All fractions were incubated with α -amylase (Olaitin & Northcote, 1962) to remove starch before dialysis and precipitation. The residue remaining after all extractions was washed with water. All fractions were freeze-dried.

For the analyses of the wall material from callus and embryoid tissue the cells were extracted with boiling ethanol under reflux and dried.

Analysis of wall fractions

Neutral sugars were determined by g.l.c. of alditol acetate derivatives with inositol as an internal standard. The inositol was added immediately before hydrolysis of the polysaccharide (Wright & Northcote, 1975). A Pye series 104 model 24 chromatograph fitted with a flame ionization detector and a column of 3% SP 2340 on 100/120 Supelcoport (Supelcoport Inc., Bellefonte, PA, U.S.A.) was used. The carrier gas was argon at 30 ml/min and the oven temperature was 235°C.

Uronic acids were determined by the method of Blumenkrantz & Asboe-Hansen (1973) with galacturonic acid (0–20 μg) as standard.

Protein determination

Protein was determined by the method of Lowry *et al.* (1951), after precipitation with trichloroacetic acid, with bovine serum albumin as standard.

Determination of cell number

The number of cells per g fresh wt. of tissue was determined as described by Henshaw *et al.* (1966). A known weight of tissue (approx. 200 mg) was added to 5 ml of 15% chromium trioxide, incubated at 70°C for 5 min, then shaken vigorously. The samples were diluted until between 5 and 10 cells were present per field and counted by using a calibrated slide.

Electron microscopy

Callus and embryoid tissue was fixed overnight in 4% glutaraldehyde in 0.1 M-phosphate buffer, pH 7.2, rinsed in phosphate buffer (30 min), and post-fixed in 1% OsO_4 (2 h). The fixed material was dehydrated through an ethanol series over 2 days, then left overnight in absolute ethanol. The tissue was incubated in increasing concentrations of Spurr's resin (25–100%) for 5 days, and finally was cured in fresh resin at 60°C. Thin sections were cut on a

Sorvall Porter-Blum MT-2 ultramicrotome using a glass knife and mounted on carbon-coated grids. The sections were stained with uranyl acetate (30 min) and alkaline lead hydroxide (5–10 min). The grids were examined by using an A.E.I. EM6B microscope.

Results

Fractionation of the cell wall of palm stems

The sugar analyses of the various fractions are shown in Table 1.

The pectin fraction contained a high percentage of uronic acids with only small amounts of other sugars, mainly glucose, xylose and arabinose. Paper electrophoresis of the hydrolysate of the pectin fraction in pyridine/acetate buffer, pH 3.5 (Wright & Northcote, 1975), showed that the uronic acid was galacturonic acid. Two hemicellulose fractions were isolated; the first, soluble in 3% NaOH, consisted mostly of xylose, glucose and arabinose with a low percentage of uronic acids. The second hemicellulose fraction (soluble in 5% NaOH plus 3% sodium borate) was composed predominantly of xylose and glucose.

The residue remaining after hemicellulose extractions was characteristic of cellulose, with a very high percentage of glucose.

In the oil palm the most suitable marker sugar of pectin deposition would therefore be uronic acid and xylose could be used as a marker of hemicellulose deposition during secondary wall formation.

Composition of the cell walls of callus and embryoid tissue

The sugar compositions of ethanol-extracted callus and young embryoid material were compared (Table 2). The callus tissue was characterized by a high percentage of arabinose and xylose and a low

percentage of uronic acid. The embryoids had a higher percentage of uronic acids and a lower percentage of xylose. A distinguishing feature of young oil palm embryoids was therefore probably an increased rate of cell division correlating with an increased deposition of primary wall material (pectin), characterized by a high percentage of galacturonic acid.

An unusual feature of oil palm callus tissue was the relatively high percentage of fucose (10.2%), which was not observed in the embryoid wall. The sugar composition of the ethanol-extracted callus material, apart from the presence of fucose, was very similar to that of other monocotyledons in tissue culture (Burke *et al.*, 1974).

Optimization of acetyl-CoA carboxylase assay

The optimum conditions for assay *in vitro* of acetyl-CoA carboxylase activity were determined to ensure that the enzyme activity was measured under

Table 2. *Sugar composition of ethanol-extracted material from oil palm callus and young embryoids*

Callus and embryoid material were each extracted in boiling ethanol and dried. The neutral sugars were determined by g.l.c. of alditol acetate derivatives and the uronic acids by *m*-hydroxybiphenyl uronic acids.

Sugar	Composition (%)	
	Callus	Embryoids
Rhamnose	0.49	1.9
Fucose	10.2	2.0
Arabinose	20.5	18.1
Xylose	25.7	10.1
Mannose	3.1	3.2
Galactose	11.0	8.2
Glucose	19.4	25.4
Uronic acids	8.5	29.3

Table 1. *Sugar composition of wall fractions isolated from oil palm stems*

Stems were extracted in organic solvents, delignified and fractionated into pectin (material soluble in 2% sodium hexa-metaphosphate) and two hemicellulose fractions, one extracted with 3% NaOH and the other by further extraction with 5% NaOH containing 3% sodium borate. The neutral sugars were determined by g.l.c. of the alditol acetate derivatives and the uronic acids by reaction with *m*-hydroxybiphenyl.

Sugar	Composition (%)			
	Pectin fraction	First hemicellulose fraction	Second hemicellulose fraction	Residue (cellulose)
Rhamnose	1.08	1.03	1.03	0.34
Fucose	1.19	0.89	1.23	0.79
Arabinose	5.56	18.17	4.14	1.46
Xylose	6.75	38.0	45.56	14.75
Mannose	3.49	0.89	2.04	4.39
Galactose	2.78	6.50	2.86	0.68
Glucose	12.3	23.93	27.78	76.01
Uronic acid	63.3	10.19	13.30	Not determined

Fig. 1. The optimum conditions of the acetyl-CoA carboxylase assay

(a) HCO₃⁻ concentration; (b) acetyl-CoA concentration; (c) Mg²⁺ concentration; (d) ATP concentration; (e) pH (Δ, phosphate buffer; ●, Tris/HCl buffer; ▲, glycine buffer); (f) protein concentration; (g) time; (h) presence of avidin.

non-limiting conditions for the amount of substrate. The enzyme activity was measured in an extract of culture line JH which showed some differentiation to embryos. For each optimization the other variables were maintained at their optimal values. The results are shown in Fig. 1. The assay system saturated at approx. 7 mM-HCO_3^- (Fig. 1a), $0.05\text{ mM-acetyl-CoA}$ (Fig. 1b), 3 mM-Mg^{2+} (Fig. 1c) and 2.0 mM-ATP (Fig. 1d). The optimum pH for the assay was pH 8.7 in Tris buffer (Fig. 1e). Under optimum conditions the assay was linear up to $80\text{ }\mu\text{g}$ of protein (Fig. 1f) and for at least 10 min (Fig. 1g). The assay was shown to be sensitive to avidin (Fig. 1h) (97% inhibition of HCO_3^- incorporation at $5.3\text{ }\mu\text{M-avidin}$).

The product of the reaction was identified as malonyl-CoA by t.l.c. (Huang, 1970). The reaction was terminated with conc. HCl, brought to 3 M-KOH and incubated for 10 min at 30°C to hydrolyse the thioester and then neutralized with HCl. Hydrolysed and unhydrolysed samples were chromatographed on Polygram silica-gel t.l.c. plates, in water-saturated diethyl ether/formic acid (7:1, v/v). Unlabelled malonic acid standards were detected using 0.04% Bromothymol Blue in 0.01 M-NaOH . For radioactive samples, the t.l.c. plate was cut into 1 cm strips and the radioactivity was measured. In unhydrolysed samples the radioactive material remained at the origin (characteristic of CoA esters), whereas in hydrolysed samples the radioactivity co-chromatographed with authentic malonic acid.

Biochemical changes occurring during differentiation of oil palm callus

(a) *Changes in the levels of lipid and acetyl-CoA carboxylase activity in callus (line JH).* On medium containing 2.5 mg of NAA/litre, callus (line JH) undergoes spontaneous differentiation to produce numerous embryos.

Electron micrographs of callus and embryoid tissue revealed several differences between the two tissues. The callus (Plate 1a) consisted of thin-walled cells containing small vacuoles and starch grains. The embryoid cells (Plate 1b) were characterized by the presence of protein-storage bodies and numerous lipid droplets that were absent from callus cells.

The deposition of lipid over the period of differentiation and the activity of acetyl-CoA carboxylase were measured (Figs. 2 and 3). Since the amount of protein and the number of cells in the culture both vary throughout the time course (Fig. 4) the results can be obtained either as specific activities of the enzyme (Fig. 2) or in relation to a fixed number of cells (Fig. 3). Nevertheless, both types of measurement show the same variation for the enzyme activity over the growth period. Embryoids became visible in the cultures from day 39 and

Fig. 2. Lipid content and activity of acetyl-CoA carboxylase in tissue cultures of oil palm (line JH) measured per kg of protein

Differentiation of the cultures to form embryos was evident (arrow) 39 days after subculture during the 100 day period of growth on medium containing 2.5 mg of NAA/litre. Total lipid ($\blacktriangle$) was measured in chloroform/methanol extracts of callus and the activity of acetyl-CoA carboxylase ($\circ$) was assayed in the $20\,000\text{ g}$ supernatant of cell homogenates by measuring the acetyl-CoA-dependent fixation of $\text{NaH}^{14}\text{CO}_3$ into malonyl-CoA.

Fig. 3. Lipid content ($\blacktriangle$) and activity of acetyl-CoA carboxylase ($\circ$) in tissue cultures of oil palm (line JH) measured per 10^6 cells

For details see the legend to Fig. 2. Embryoids were visible in the cultures from day 39 (arrow).

increased in number throughout the time course. Two peaks of acetyl-CoA carboxylase activity were observed; the activity rose to a maximum at day 31, then fell, rising again to another peak at day 59. The amount of lipid deposited correlated with the changes in enzyme activity; the amount of lipid per 10^6 cells increased 2-fold during the period of the first increase in enzyme activity and after a decrease subsequently rose a further 2-fold to a plateau at day

EXPLANATION OF PLATE 1

Electron micrographs of oil palm callus (a; $\times 24\,500$) and embryoids (b; $\times 9400$)

Large numbers of lipid droplets are present within the embryoid cells (arrow), but are absent from callus tissue.

Fig. 4. Changes in cell number/g fresh weight ($\blacktriangle$), protein content/g fresh weight ($\triangle$) and protein content/cell number ($\bullet$) during differentiation of tissue cultures of oil palm (line JH)

Cell number was measured after maceration of cell clumps in 15% chromium trioxide. Protein was assayed by the method of Lowry *et al.* (1951). Embryoids were visible in the cultures from day 39.

79. The level of lipid fell considerably between the two peaks of enzyme activity when estimated per 10^6 cells but the drop was less pronounced when measured per mg of protein. This drop in stored lipid probably represents utilization of the lipid reserves by the cells, although when considered in terms of the number of cells it can be partially explained by an increase in cell number of approx. 23%, which occurred between days 31 and 46 (Fig. 4). A second rise in cell number per g fresh wt. (17.8%) occurred between days 59 and 86. The level of soluble protein in the tissue also showed two periods of rapid increase correlating with these increases in cell number.

(b) *Acetyl-CoA carboxylase activity and lipid deposition in callus line JB.20.1C.* Callus (line JB.20.1C) shows very little spontaneous differentiation on medium containing 2.5 mg of NAA/litre. The activity of acetyl-CoA carboxylase and amount of lipid were measured over a period of about 11 weeks. The results are shown in Fig. 5. No embryoids were visible on the cultures during this period. The specific activity of acetyl-CoA carboxylase showed some changes over this period but was constantly low compared with the activity in cells of line JH [maximum observed activity in line JB.20.1C

Fig. 5. Lipid content ($\blacktriangle$) and activity of acetyl-CoA carboxylase ($\triangle$) in tissue cultures of oil palm (line JB.20.1C)

No embryogenesis was observed in this cell line during the 80 day time course on medium containing 2.5 mg of NAA/litre.

was $1.17 \text{ nkat} \cdot (\text{kg of protein})^{-1}$ compared with a maximum activity in line JH of $6.18 \text{ nkat} \cdot (\text{kg of protein})^{-1}$. In addition the level of lipid was low, although some increase was evident over the time course.

Fig. 6. Xylose (●), uronic acid (○) content and xylose/uronic acid ratio (△) of cell-wall material of cultures of line JH during growth on medium containing 2.5 mg of NAA/litre

Sugar composition was determined in the ethanol-insoluble material by g.l.c. of alditol acetate derivatives (neutral sugar) and after reaction with *m*-hydroxybiphenyl (uronic acids). Embryoids were visible in the cultures from day 39 (arrow).

(c) *Levels of uronic acids and xylose in the cell wall during differentiation of line JH.* Ethanol-extracted material was analysed for xylose and uronic acids during the time course of embryogenesis of line JH cells (Fig. 6). The amount of uronic acid per mg of total carbohydrate in ethanol-insoluble material showed a rise over a period, day 17–46, with possibly a second but slower rise after day 67. This rise in uronic acid content correlated with the increases in cell number per fresh weight and the results suggest a phase of increased cell division during the time course, with a corresponding increase in primary wall (pectin) deposition.

The ratio xylose/uronic acid composition of the wall increased from day 46 during the time when vascular tissue was apparent in the embryos.

Discussion

In an actively expanding cell a primary wall is laid down, consisting of a meshwork of cellulose fibrils in a matrix composed mostly of pectin and some hemicellulose. When the cell differentiates during xylem formation, the pattern of deposition of wall components changes (Northcote, 1982), pectin deposition ceases and a secondary wall composed of a higher proportion of hemicellulose and cellulose is laid down. Lignification of the cell may also occur.

In studies on xylem differentiation in dicotyledonous plants the ratio of xylose (a marker sugar of hemicellulose, xylan, deposition) to arabinose (occurring in the pectin) has been used as an index of the state of differentiation of the tissue (Jeffs & Northcote, 1966; Dalessandro & Northcote, 1977). The polysaccharide characteristic of hemicellulose in monocotyledonous species is glucuronoarabinoxylan (Timell, 1965), although some glucoxylan can occur in primary walls (Burke *et al.*, 1974). The glucoxylan will only represent a small fraction of the total xylan extracted from a secondary thickened wall. In the palm, xylose was shown to be an effective marker of hemicellulose and the most suitable indicator of pectin deposition was the level of uronic acids. Our results have shown an increased deposition of pectin in the embryoid cell walls, and this could be correlated with a rise in cell number during embryogenesis. Light microscopy revealed numerous meristematic areas, consisting of clusters of small, highly nucleated cells, in embryoid tissue. These clusters of meristematic cells elongated to produce secondary thickened vascular strands at later stages in the development of the embryo. Differentiation in the palm callus can therefore be regarded as, first, the formation of embryoid regions in which organised cell division can occur, followed by development of xylem and phloem in vascular tissue.

Oil palm embryos *in vivo* and the embryoids that are developed in tissue cultures lay down deposits of triacylglycerol in oleosomes (Jones, 1974b). This storage lipid is mobilized and utilized during the subsequent germination of the embryo. Electron microscopy has shown that storage lipid is a specific product of differentiated embryoid cells and thus it is probably a more specific and reliable marker of embryogenesis than changes in wall composition. Measurement of the activity of acetyl-CoA carboxylase, which catalyses the first committed step in fatty acid biosynthesis, may therefore be a more sensitive indicator of the differentiation state of the tissue.

An increase in the activity of acetyl-CoA carboxylase has been correlated with increased rates of fatty acid synthesis in animals (Majerus *et al.*, 1968; Majerus & Kilburn, 1969; Geelen *et al.*, 1978; Lane *et al.*, 1979), micro-organisms (Mishina *et al.*, 1976) and plants. Simcox *et al.* (1979) showed that a marked rise in the activity of the enzyme in developing castor bean seeds occurred between 20 and 30 days after anthesis, shortly before the period of rapid oil synthesis in the seeds. The enzyme activity declined as oil synthesis slowed and the seed matured. In contrast, however, Nikolau *et al.* (1981) found no correlation between enzyme activity *in vitro* and the rate of fatty acid biosynthesis at different developmental stages in maize leaves, indicating that the enzyme was not limiting fatty acid synthesis.

We have shown that during embryogenesis in tissue cultures of oil palm (line JH) there was an increase in the activity of acetyl-CoA carboxylase, which occurred slightly before the increase in the lipid content of the cells. Two waves of lipid deposition were observed during the time course (100 days), associated with two peaks of acetyl-CoA carboxylase activity. Tissue cultures of line JB.20.1C, which showed very little somatic embryogenesis and had a much lower lipid content, had a much lower acetyl-CoA carboxylase activity throughout a similar time course (although some variation in activity did occur).

These results can be used to construct a general pattern for the course of embryogenesis in the palm. In the tissue culture, cells at certain positions in the callus are stimulated to differentiate to embryoids, the stimulus for this differentiation is presumably the levels of particular growth factors or nutrients at that point in the callus. The stimulated cells differentiate and synthesize specific products characteristic of embryoid cells. One of these is triacylglycerol and the synthesis of the fatty acids is preceded by a rise in the activity of acetyl-CoA carboxylase, which is a limiting enzyme step in the synthesis. After these changes, an increase in cell number per g fresh wt. of tissue occurs, plus an increase in the percentage of primary wall material

deposited in the tissue, and it is these later changes that are indicated by the changes in cell-wall composition. This rise in rate of cell division occurs as embryoids become visible in the cultures and may be associated with the appearance of meristematic regions which are observed in young embryoids by light microscopy. The gradients of hormones and nutrients in the callus are constantly changing as the tissue grows and utilizes the substances available to it. After the initial stimulation of some cells to differentiate, other cells in other areas of the callus may be similarly stimulated to undergo embryogenesis with corresponding increases in acetyl-CoA carboxylase activity, triacylglycerol deposition and rate of cell division.

The reasons for the lack of spontaneous embryogenesis in line JB.20.1C cultures are unknown. The two culture lines behave differently under identical conditions, showing that there are genetic differences between the two that affect their potential to differentiate in tissue culture. Jones (1974a) has suggested that the ability of palm tissue culture to produce embryoids is influenced more by the origin of the callus rather than by the hormonal and nutritional composition of the medium.

Our experiments have indicated a correlation between acetyl-CoA carboxylase activity and the rate of triacylglycerol deposition during embryogenesis in oil palm. The triacylglycerol is a specific product of embryoid cells and thus it seems feasible to utilize measurements of acetyl-CoA carboxylase activity as a marker of the differentiation state of palm tissue cultures. If the differentiation of callus to embryoids could be controlled by means of specific induction media, improved yields of somatic embryoids and subsequent plantlets could be achieved. The use of acetyl-CoA carboxylase activity as a marker of somatic embryogenesis may enable media to be screened more rapidly to determine their potential to induce differentiation.

We thank the S.E.R.C. for a C.A.S.E. award with Unilever, during the tenure of which this work was carried out. We thank Dr. Laurie Jones for supplying the two lines of palm tissue cultures and Dr. T. Slabas and Dr. C. Lloyd, together with Dr. L. Jones, for many helpful discussions.

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