Cocoa Bean Cell and Embryo Culture

MING-CHE WEN, BRUCE GERMAN and **JOHN E. KINSELLA,** Institute of Food Science, Cornell University, Ithaca, NY 14853

ABSTRACT

Callus culture of cocoa bean was initiated from immature cotyledons on agar medium. By dispersing these callus cells, a liquid suspension culture was established. The lipid composition of cocoa suspension culture was investigated and compared with those of cocoa beans of different maturities. Factors affecting fatty acid and triglyceride synthesis in cocoa suspension cultures also were studied. In parallel studies, asexual embryos were proliferated from immature zygotic embryos cultured in semisolid or liquid medium. The factors affecting the lipid composition in cultured embryos are discussed.

INTRODUCTION

Cocoa butter, the natural fat in the cocoa bean, usually is obtained by hydraulic expressing from milled mature cocoa beans which grow in pods. The unique properties of cocoa butter, quick-melting at 33 C, brittle fracture below 20 C and contraction on solidification make it an indispensable ingredient in manufacturing chocolate-type confectionary products. The sharp melting property of cocoa butter is especially important for confectionary manufacturers (1,2). This property directly reflects the fatty acid composition and stereospecific arrangement of fatty acids on triglycerides which form homogenous crystals with a sharp melting point at the mouth-temperature.

Cocoa butter is the most expensive edible fat in the world. According to the Public Ledger (3), the price of cocoa butter was 2,400 British Pounds per ton, which was about twice that of the next most expensive oil, olive oil, and about 10 times that of soybean oil. In 1982, FAO statistical data showed the U.S, the largest importer of cocoa butter in the world, imported 37,326 tons, about twice the amount imported in 1972 (4). However, the total value in 1982 was about seven times that of 1972. Also, in 1977, according to the U.S. Department of Commerce (5), the amount of money spent by the US confectionery industry on ingredients was highest for cocoa butter, which also showed the highest unit price among all ingredients. America must import all its cocoa beans and cocoa butter. The supply of cocoa beans is unstable because of climatic, economic and political reasons which in turn cause the price to rise to yet higher levels.

The high price and erratic supply have forced manufacturers of chocolate products to examine ways to supplement cocoa butter with other types of fats without altering final product quality. However, there are problems for these replacement fats such as bad melting properties, high price or legal arguments in many countries that these fats may not be used in chocolates described as such (2). Because of their high cost, fluctuating supply and problems of replacement, we explored the possibility of using plant cell and embryo culture to produce cocoa butter-type products.

In the case of the cocoa plant, the cocoa butter is deposited solely in mature embryos. The challenge hence is to induce cells or tissues to produce and accumulate cocoa butter triglycerides outside these physiological constraints. Since both cells and asexual embryos derived from zygotic embryos carry the same genes as the intact plant (totipotency), it may be possible to induce the expression of desirable genes in cultured cells leading to cocoa butter synthesis. One approach would be to modify the conditions surrounding single cells in culture which would cause these cells to alter their lipid metabolism grossly to accumulate triglycerides. Another approach would be to proliferate and differentiate cultured embryos to a physiological stage comparable to mature zygotic embryos and allow these to accumulate fat in culture. Growing these cells or asexual embryos in liquid medium analogously to growing industrial microorganisms using submerged fermentation technique would offer advantages over conventional field cropping (6,7). It would give a steady, year-round supply without restriction by climate; production would be free of contamination from pests or microorganisms, and genetics or metabolism of cultured cells or embryos could be modified or regulated to obtain maximum yield. With this reasoning, we started a long term investigation of the possibility of production of cocoa butter by plant cell and tissue culture.

Cell Culture

Different stages of maturity of cocoa zygotic embryos were tested to determine the optimum stage for the initiation of calli (dedifferentiated cell masses) (8) on Murashige and Skoog agar medium (9) supplemented with 2.26 μM 2,4-D and 0.465 μM kinetin (10). It was found that cocoa callus culture could be initiated consistently from embryos harvested 120 to 130 days after pollination. This is 60 days prior to maturity. Upon initiation, the calli were maintained at an active growing state by subculturing them every 4 weeks. From left to right, Figure 1 shows callus one day, one month and 2 months after reculture. By dispersing these callus cells in liquid medium of the same composition, a liquid suspension culture was started. The suspension cultures exhibited an approximate doubling time of 3 days with fresh weight being increased 20 times from 300 mg to 6 grams in two weeks (10).

Lipid compositions of these cells were analyzed in order to compare them with those of cocoa beans (11). As shown in Table I, immature cocoa beans (110 days after pollination) contained 11% total lipids which increased to a level of 54% in ripe beans. This represents a drastic change in lipid content during maturation. The same result also has been observed by Lehrian and Keeney (12) and Wright et al. (13). Also, the lipid contents of cocoa calli or suspension cells were lower than those of cocoa beans. Lower total lipids in cell culture and higher total lipids in beans also were observed in oil seeds *Glycine soja* (14) and *Pinus elliotti* (15).

The lipid classes of different samples also were quite different (Table I). The total lipids of immature beans contained 80% triglycerides, 10% phospholipids and small

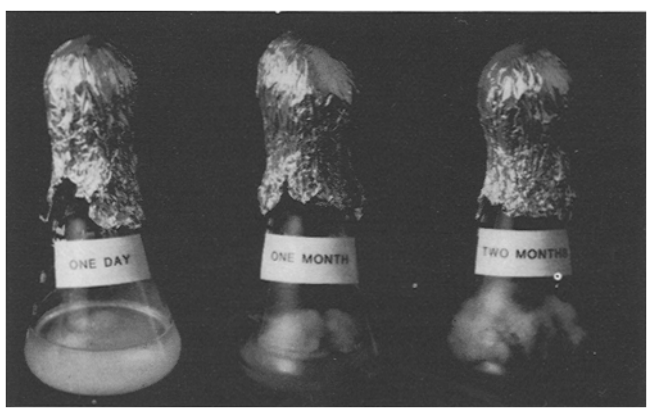

FIG. 1. Cocoa calli, from left to right one day, one month and 2 months after subculture.

TABLE I

Lipid Composition of Different Cocoa Bean Preparations

	Immature cocoa beans[a] %	Ripe cocoa beans %	Cocoa calli %	Cocoa suspensions %
Total lipids[b]	11.0 ± 0.5	54.0 ± 1.0	5.3 ± 0.2	6.5 ± 1.2
Neutral lipids				
triglycerides[c]	80.0 ± 2.1	96.8 ± 0.4	13.2 ± 0.6	12.6 ± 1.0
Diglycerides[c]	2.6 ± 0.3	0.6 ± 0.1	0.6 ± 0.2	0.2 ± 0.0
Free fatty acids[c]	4.2 ± 0.3	0.5 ± 0.1	0.5 ± 0.2	0.6 ± 0.2
Sterols[c]	1.2 ± 0.2	0.2 ± 0.0	8.6 ± 0.7	9.2 ± 1.0
Phospholipids[c]	10.0 ± 0.5	0.5 ± 0.2	26.7 ± 0.4	22.0 ± 2.5
Glycolipids[c]	3.0 ± 0.3	0.5 ± 0.1	46.6 ± 2.2	56.0 ± 2.2
16:0[d]	31.1 ± 1.3	23.9 ± 1.3	29.3 ± 1.4	28.3 ± 1.3
16:1[d]	–	–	0.8 ± 0.1	1.1 ± 0.3
18:0[d]	5.8 ± 0.4	38.2 ± 1.2	5.2 ± 0.3	2.4 ± 0.2
18:1[d]	7.6 ± 0.3	33.9 ± 1.3	22.3 ± 1.2	11.5 ± 0.7
18:2[d]	45.4 ± 2.6	3.2 ± 0.2	34.4 ± 1.6	48.6 ± 1.6
18:3[d]	3.6 ± 0.2	–	5.2 ± 0.4	7.7 ± 0.8

[a]110 days after pollination.
[b]% of dry matter.
[c]% of total lipids.
[d]F.A. composition of total lipids (% *w/w*).

amounts of other lipid classes. During maturation, triglycerides increased to 97% while other lipid classes decreased to less than 1% (19). These results confirmed the earlier findings by Lehrian and Keeney (12). However, in calli or suspension cells glycolipid was the major lipid component and phospholipid second. Unlike those from beans, lipids from cell cultures contained only small portions of triglycerides amounting to just 13% of the total lipids. This suggested that the lipids synthesized in cell culture were used mainly for construction of membranes during active growth, while the lipids accumulated during the maturation of zygotic beans were predominantly storage lipids.

The major fatty acids in the ripe cocoa beans were found to be stearic, oleic and palmitic acids (Table I). However, unsaturated fatty acids including linoleic and oleic acids were dominant in cell cultures. It also is interesting to note that cocoa suspension culture contained a pattern of fatty acids similar to immature beans, with 50% linoleic acid and about 30% palmitic acid.

Studies subsequently were directed to determining the change of total lipid content and fatty acid composition (Table II) during growth of suspension cells (11). There was no change observed in these lipid components. Apparently, cells actively growing in the exponential or stationary phase do not alter their storage lipid metabolism significantly. Therefore, these cultured cells are not suitable for producing cocoa butter under the conditions used in this study. Questions were raised on the low lipid content and different lipid pattern of these cultured cells. Was it because they were dedifferentiated (8) when compared with the differentiated bean cells, or because they needed a special environment such as indicated by Radwan and Mangold (16) that cultural conditions influence the content and composition of lipids in cultured cells?

The direction of our investigation focused on optimizing those culturing conditions which might enhance differentiation or alter lipid composition to obtain cell cultures that synthesize triglycerides similar in composition and structure to cocoa butter. The approaches taken included altering specific factors such as plant growth hormones; adding exogenous substrates (eg. stearic acid, coconut water, sucrose and osmoticum), and varying the carbon to nitrogen ratio. The immediate objective of our research was to understand the factors which might affect lipid synthesis in suspension cells. Ultimately we wished to determine if the lipid content and composition could be changed, first,

TABLE II

Total Lipids and Fatty Acid Composition of Total Lipids Extracted from Cocoa Cell Suspensions at Different Stages of Growth

Days in culture	6	10	14
Total lipids (% Of dry matter)	7.7 ± 0.7	5.7 ± 0.5	6.5 ± 1.2
Fatty acids in total lipids (% *w/w*)			
16:0	24.6 ± 0.6	25.2 ± 1.0	28.3 ± 1.3
16:1	1.2 ± 0.2	1.2 ± 0.1	1.1 ± 0.3
18:0	2.4 ± 0.3	2.6 ± 0.3	2.4 ± 0.2
18:1	15.8 ± 1.2	11.2 ± 0.8	11.5 ± 0.7
18:2	43.8 ± 1.0	47.3 ± 1.2	48.6 ± 1.2
18:3	10.5 ± 0.7	10.9 ± 0.8	7.7 ± 0.8
20:0	1.7 ± 0.3	1.4 ± 0.2	–

by increasing the content of triglycerides, and second, by increasing stearic and oleic acid and decreasing linoleic acid in triglycerides by varying culturing conditions.

Plant growth hormones are known to exert regulatory effects on growth and development of plant cells and tissues in culture. Omission of hormones enhanced embryogenesis of many species including carrot, coriander, date palm, orange and parsley (17). Hormones also have been shown to exert effects on the fatty acid composition of soybean suspension cultures. That is, the addition of kinetin and gibberellic acid was found to cause a higher proportion of linoleic acid and a lower proportion of linolenic acid relative to control cells (18).

In our laboratory, different combinations of 2,4-D and kinetin were tested for their effects on the lipid composition of cultured cells (19). Although the response to 2,4-D or kinetin was not significant in either total lipids or triglycerides at a level of $p=0.05$, there was a significant effect as the result of the interaction between 2,4-D and kinetin. The optimum concentration of 2,4-D and kinetin was found to be 2.26 μM and 0.465 μM, respectively. Our later work on the effect of growth hormones showed that complete elimination of all hormones normally used in cell culture systems to enhance growth had an apparently beneficial effect on the fatty acid pattern. At the same time, however, a combination of 2.26 μM 2,4-D, 5.0 μM gibberellic acid (GA3) and 5.0 μM zeatin (another type of cytokinin used to replace kinetin) further reduced the linoleic

TABLE III

Effect of Plant Growth Hormone on Fatty Acid Composition of Total Lipids Extracted from Cocoa Cell Suspensions

Treatment	2.26 μM 2,4-D +0.50 μM kinetin (Control)	Omission of Hormone	2.26 μM 2,4-D +5.0 μM GA3 +5.0 μM Zeatin
Fatty acids in total lipids (% *w/w*)			
16:0	34.9 ± 0.2	26.4 ± 0.3	26.4 ± 0.2
16:1	1.1 ± 0.1	2.0 ± 0.1	1.6 ± 0.1
18:0	1.8 ± 0.1	1.3 ± 0.1	1.4 ± 0.1
18:1	10.1 ± 1.1	23.2 ± 0	26.5 ± 0.2
18:2	47.0 ± 1.1	41.8 ± 0.1	37.2 ± 0.3
18:3	5.2 ± 0.3	5.4 ± 0.2	6.8 ± 0.1

acid and palmitic acid and increased the oleic acid in the suspension cells. Oleic acid was increased, but stearic acid remained low. Although plant growth hormones seemed to exert significant effects on the lipid and fatty acid compositions of cells in culture (Table III), more experiments are needed before their mechanism of action can be fully understood and lipid metabolism manipulated.

It was thought it might be feasible to enrich the stearic acid in cultured cells simply by exogenous addition (11), since previous results showed exogenous fatty acids could rapidly be incorporated into lipids (20). The results (11) showed that stearic acid exogenously added enhanced the triglyceride content about two times. However, after investigating the fatty acid composition of the triglycerides, no significant change was found.

Coconut water has been used widely in plant tissue culture. In particular it has been shown to enhance cacao *in vitro* embryogenesis (21,22). Since the embryo is the site of triglyceride synthesis, coconut water might be expected to have some effect on triglyceride production. Sucrose is the carbon source for fatty acids and glycerol which form triglycerides. In order to regulate the lipid content of cultured cells, 10% coconut water and/or 5% more sucrose were added to the suspension culture (11,19). Results are shown in Figure 2. Coconut water alone increased the triglyceride content from 11% to 19%. A concurrent decrease of linoleic acid from 37% to 19% and increase of oleic acid from 10% to 33% was observed. Sucrose alone did not exert any drastic change on triglyceride nor its fatty acid composition in cells. However, the combination of coconut water and sucrose seemed to enhance the above effects.

The component(s) of coconut water responsible for the above changes was investigated further. According to Radwan and Mangold (16), 100ml of coconut water contained up to 35.8 mg of lipid including hydrocarbons, fatty acids, sterols and steryl glycolipid. Analyzing the total fatty acid content of the coconut water used in our laboratory, we found it contained very little lipid (Table IV). Also, its fatty acid composition did not reflect the drastic change discussed above. Another factor contributed by coconut water is osmolarity. However, growing cocoa suspension cells in the standard medium supplied with different concentrations of osmoticum (polyethylene glycol) did not cause any change in lipid composition of these suspension cells.

It has been found that the carbon to nitrogen ratio plays a very important role in the oil accumulation of many oleaginous microorganisms (23). This lipid accumulation follows a two-stage pattern. During the first stage, there is active growth but slow lipid accumulation. After nitrogen is depleted, the lipid content in cells starts to increase significantly. Thus we were interested in determining the effect of nitrogen depletion on the lipid production in our sus-

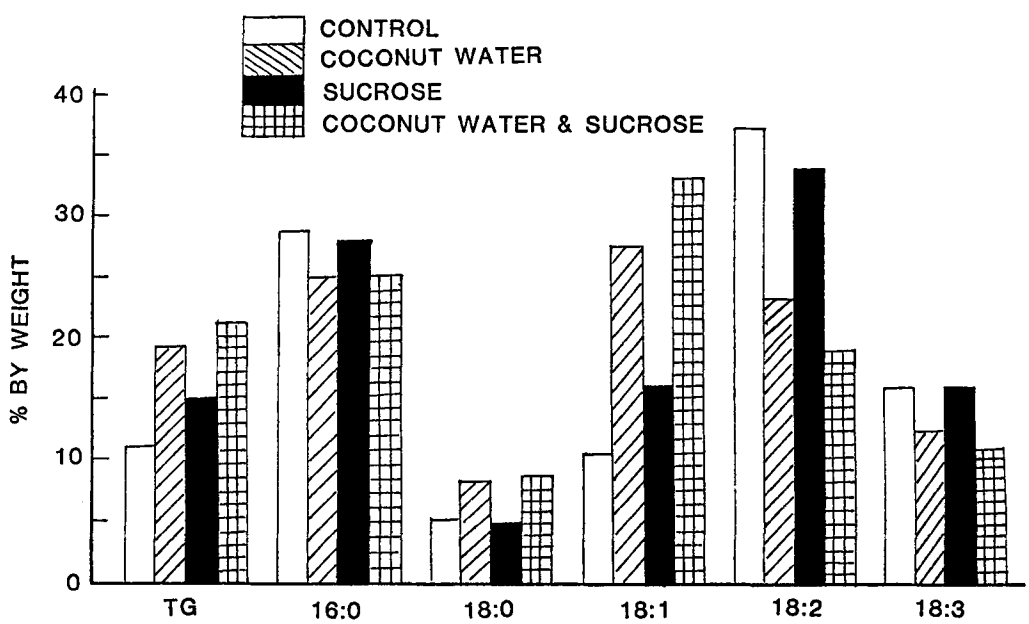

FIG. 2. Effects of coconut water and/or sucrose on the content and fatty acid composition of triglycerides of cocoa cell suspension cultures. Results are expressed as % by weight of triglycerides in total lipids and % (*w/w*) fatty acids in triglycerides (10,19).

TABLE IV

Fatty Acid Composition of Deproteinized Coconut Water

Total Fatty Acid (μg/5ml)	1.67 ± 0.15
Fatty Acid (% *w/w*)	
16:0	38.2 ± 3.6
16:1	2.7 ± 0.4
18:0	28.9 ± 0.6
18:1	24.6 ± 3.5
18:2	5.7 ± 0.1

pension cells. We tried different carbon to nitrogen ratios from 15:1 to 480:1. A ratio of 15:1 was used routinely in our system. In all cases the carbon source was maintained constant using 3% sucrose. We observed that the highest growth of suspension cells was at a carbon to nitrogen ratio of 30:1. When the ratio was higher than 30:1, the growth started to decrease. However, over the range tested we saw no effect of the carbon to nitrogen ratio on the production of triglycerides by suspension cells.

It seems that triglycerides in cocoa suspension culture may be increased modestly by the addition of coconut water, stearic acid or a combination of coconut water and sucrose. Linoleic acid content in triglycerides could be decreased by the addition of coconut water or stearic acid, or by a combination of sucrose and coconut water. Oleic acid was increased by the addition of coconut water, omission of plant growth hormone and addition of gibberelic acid and zeatin. We currently are continuing our work to enhance the triglyceride content and fatty acid composition of suspension cells to simulate that found in cocoa butter.

Embryo Culture

Because cocoa butter is naturally produced in cocoa embryos, it might be possible to propagate cocoa zygotic embryos *in vitro*, via asexual embryogenesis to grow these asexual embryos to full maturity and harvest the cocoa butter. In vitro embryogenesis first was reported by Esan in 1977 (24). He observed the budding of adventive embryos from all parts of immature cocoa zygotic embryos cultured *in vitro*. These adventive embryos seemed to be epidermal in origin. Later, Pence et al (20,21) found that the same phenomenon also occurred in zygotic embryos cultured in Murashige and Skoog medium (9) supplied with 0.1% casein hydrolysate, 10% coconut water and 1.5 mg/l of NAA.

The asexual embryos originated in many ways (21,22). In the budding process, the asexual embryos emerged from specific epidermal cells of cotyledon and hypocotyl origin. In the nonbudding process, portions of the cotyledon swelled, folded and formed asexual embryos with axes and cotyledons. Later, Esan (25) also reported the initiation of asexual embryos from embryogenic callus. Pence et al. (21) observed that *in vitro* embryogenesis occurred more frequently in liquid media than in agar media. They also found tissue explants initiating embryos could be transferred to basal Murashige and Skoog medium without auxin and coconut water and still continued to initiate asexual embryos during a year of reculturing in this medium.

In our laboratory, asexual embryos could be initiated from immature zygotic embryos cultured in Pence et al. medium (21) with 8μM 2,4-D or NAA. The growth of these asexual embryos eventually was arrested. After analyzing the fatty acid composition of these asexual embryos, it was found that the composition was similar to that of immature zygotic embryos.

The results were similar to those reported by Pence et al. (27). Factors shown to affect the lipid composition in cocoa asexual embryos include sucrose concentration (26,27) and temperature (28). By gradually increasing the sucrose concentration in the medium from 3% to 27%, it was possible to alter the fatty acid composition in the asexual embryos so it was similar to that of mature zygotic embryos. The total fatty acid (dry weight basis) and total fatty acid to total lipid ratio also were increased by the same treatment. The temperature factor was studied by Wright et al. (28). In this study, asexual embryos were grown in the same medium (26,27) at different temperatures ranging from 10 to 35 C. It was shown that embryos grown at 26 C exhibited the fatty acid composition and fatty acid to total lipid ratio most similar to that of cocoa butter. Clearly, the synthesis of storage lipid was enhanced by these two factors. However, although the lipid content was increased, it was still far from that of the mature zygotic embryos. It seems more factors might be involved in the synthesis and accumulation of storage lipids.

Plant cell and tissue culture does seem to offer the distinct possibility of producing cocoa butter *in vitro* (29). However, more basic research is needed before industrial application is possible. This includes understanding the lipid metabolism of cells and asexual embryos *in vitro* and zygotic embryos *in vivo*, studying factors affecting the above mechanism and applying these methods to trigger the genetic information in cells or embryos to synthesize cocoa butter.

REFERENCES

1. Johnston, G.M., JAOCS 49:462 (1972).
2. Minifie, B.W., in Chocolate, Cocoa and Confectionery: Science and Technology, 2nd ed., Avi. Pub. Co., Westport, Connecticut, 1980, pp. 67-88.
3. Public Ledger, January 31, 1981.
4. FAO Trade Year Book 36:191 (1983).
5. US Dept. of Commerce, Confectionery Manufacturers Sales and Distribution, Industry and Trade Administration (1978-1981).

TABLE V

Fatty Acid Composition (% *w/w*) of Zygotic Embryos and Asexual Embryoids

	Immature zygotic embryos[a]	Mature zygotic embryos	Asexual embryoids
16:0	31.1 ± 1.3	23.9 ± 1.3	27.5 ± 3.9
16:1	–	–	1.9 ± 0.8
18:0	5.8 ± 0.4	38.2 ± 1.2	12.3 ± 2.6
18:1	7.6 ± 0.3	33.9 ± 1.3	9.1 ± 2.9
18:2	45.4 ± 2.6	3.2 ± 0.2	38.7 ± 1.1
18:3	3.6 ± 0.2	–	10.5 ± 2.5

[a]110 days after pollination.

6. Crocomo, O.J., in Plant Tissue Culture Methods and Applications in Agriculture, edited by T.A. Thorpe, Academic Press, New York, New York, 1981, pp. 359-372.
7. Zenk, M.H., in Frontiers of Plant Tissue Culture, edited by T.A. Thorpe, International Association for Plant Tissue Culture, 1978, pp. 1-13.
8. Street, H.E., in Plant Tissue and Cell Culture, 2nd ed., edited by H.E. Street, 1977, pp. 504.
9. Murashige, T., and F. Skoog, Physiol. Plant. 15:431 (1962).
10. Tsai, C.H., and J.E. Kinsella, Ann. Bot. 48:549 (1981).
11. Tsai, C.H., M.C. Wen and J.E. Kinsella, J. Food Sci. 43:768 (1982).
12. Lehrian, D.W., and P.G. Keeney, JAOCS 57:61 (1980).
13. Wright, D.C.; W.D. Park; N.R. Leopold; P.M. Hasegawa and J. Janick, JAOCS 59:475 (1982).
14. Shin, B.S., H.K. Mangold and E.J. Staba, in Les Cultures de Tissus de Plantes, Colloq. No. 193, CNRS, Paris, 1971, pp. 51.
15. Laseter, J.L.; G.C. Lawler; C.H. Walkinshaw and J.D. Weete, Phytochemistry 12:817 (1973).
16. Radwan, S.S., and H.K. Mangold, Adv. Lipid Res. 14:171 (1976).
17. Evans, D.A., W.R. Sharp and C.E. Flick, in Plant Tissue Culture Methods and Applications in Agriculture, edited by T.A. Thorpe, Academic Press, New York, New York, 1981, pp. 45-113.
18. Stearns, E.M. Jr., and W.T. Morton, Phytochemistry 14:619 (1975).
19. Tsai, C.H., Lipids in Callus and Suspension Cultures of Cocoa Beans (*Theobroma cacao* L.), Ph.D. Thesis, Cornell University, (1981).
20. Tsai, C.H., and J.E. Kinsella, Lipids 17:848 (1982).
21. Pence, V.C., P.M. Hasegawa and J. Janick, J. Amer. Soc. Hort. Sci. 104:145 (1979).
22. Pence, V.C., P.M. Hasegawa and J. Janick, Z. Pflanzenphysiol. 98:1 (1980).
23. Ratledge, C., Prog. Ind. Microbiol. 16:119 (1982).
24. Esan, E.B., Proceedings V. International Cocoa Res. Conf. Ibadan, Nigeria, 1977, pp. 116-125.
25. Esan, E.B., Proceedings VIII. International Cocoa Res. Conf., Lagos, 1980, pp. 245-252.
26. Janick, J., D.C. Wright and P.M. Hasegawa, J. Amer. Soc. Hort. Sci. 107:919 (1982).
27. Pence, V.C., P.M. Hasegawa and J. Janick, Physiol. Plant. 53:378 (1981).
28. Wright, D.C., J. Janick and P.M. Hasegawa, Lipids 18:863 (1983).
29. Kinsella, J.E., and C.H. Tsai, Cocoa Bean Culture, U.S. Patent No. 4,306,022. Issued 12/15/81.

[Received June 14, 1984]

☘Prospects and Problems in the Large Scale Production of Metabolites from Plant Cell Tissue Cultures

M.L. SHULER*, J.W. PYNE and **G.A. HALLSBY,** School of Chemical Engineering, Cornell University, Ithaca, New York 14853

ABSTRACT

A wide range of plant products can be made directly by using plant cell tissue cultures. However, the economic production of even high-value products from such cultures has not been conclusively demonstrated. One problem is that rapid growth and high product yields often appear to be mutually exclusive with plant cell tissue cultures. Some level of cellular differentiation often is required for the expression of genes associated with product formation; only unorganized cells grow rapidly. Another problem results from the tendency of plant cells to form aggregates, which leads to a mixture of cell types in culture. The biological response is a function not only of the chemical environment but also the physical (e.g. hydrodynamic) environment which makes scale-up of suspension processes difficult. In addition, cell lysis due to high liquid shear is a significant design constraint. Some of these problems can be circumvented by using multi-stage continuous culture devices or with immobilized cell reactors. Emphasis will be on membrane entrapped cultures.

INTRODUCTION

The purpose of this paper is to provide an overview of the potential role of plant cell tissue culture in the commercial production of oils and fats. These products are of particular importance in the food and fragrance industries. Compounds of potential interest as oils or to the flavor and fragrance industry include jasmine, rose oil, chamomile, capsicum and cocoa butter. The emphasis in this paper is on the "factory" production of non-growth associated chemicals made by plants. The techniques described in this paper are applicable only to high value products.

Problems

The use of cell culture for chemical production has long been considered an attractive solution to the problems of extracting chemicals from the whole plant (1-4). The use of tissue culture insures a continuous supply of homogenous material which is independent of disease, weather, or politics; offers a significant opportunity to rapidly and greatly improve yields through selection of high-yielding variants, and can lead to the formation of novel products, primarily through the biotransformation of chemical analogs of natural substrates. The last feature is of particular interest in the pharmaceutical industry, but modified oils could prove to be of significant interest.

Although the "factory" production of chemicals from plant cells has been considered for 3 decades, it is only within the last year that a plant cell tissue culture process has been commercialized successfully. The Japanese corporation, Mitsui Petrochemical Industries Ltd., has begun producing shikonin from suspension cultures of *Lithospermum erythrorhizon.* (5) Shikonin can be used as a dye or pharmaceutical. Shikonin produced by Mitsui apparently will be used in lipstick.

Since the potential for the "factory" use of tissue cultures has been recognized for a long period and only one commercial process exists, it is clear that significant problems impede commercialization of tissue culture systems. Slow growth rates (doubling times are typically 20 to 100 h.) make experimentation tedious, require large bioreactors since the corresponding volumetric productivity is low, and result in systems very sensitive to contamination. Frustrating efforts further is the genetic instability often encountered with high yielding strains. The shear sensitivity of the cells constrains bioreactor selection. The major problem, however, is the low yields of non-growth associated products from most cultures. The latter problem can be attacked through selection procedures to isolate high-yielding variants and the selection of bioreactors which control the environment in a manner which leads to more complete gene expression. Both approaches are semi-empirical because of the paucity of knowledge about basic plant biochemistry. Nonetheless, some encouraging progress has been made on the selection of variants (6). Even with

*To whom correspondence should be addressed.