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LIPID COMPOSITION OF TOBACCO CELLS CULTIVATED AT VARIOUS TEMPERATURES

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Key Word Index—*Nicotiana tabacum*; Solanaceae; cell suspensions; biosynthesis; fatty acids; lipid classes; desaturation; temperature.

Abstract—Tobacco cell suspensions were grown under controlled conditions to determine whether temperature affected the fatty acid pattern of the cellular lipids. At any temperature ranging between 17° and 35°, the total fatty acid content and the levels of fatty acids or individual lipids varied during the growth period, The optimum temperature for lipid biosynthesis and polyunsaturated fatty acid accumulation was between 20° and 26°. Increase in the level of polyunsaturated fatty acids was associated with lower temperatures during the active cell division period.

INTRODUCTION

It is claimed that low temperatures induce an increase in the polyunsaturated fatty acid content of plant cells [1–3], as a direct result of which optimum membrane fluidity is maintained [3]. Plant cell cultures are particularly useful for the study of the effect of temperature on the cellular lipid content, and previous studies [4] on cell suspensions from *Glycine max*, *Catharanthus roseus* and *Nicotiana tabacum* have shown that the lipid composition of cells is altered when the cultures are grown at 25° or 15° with increased levels of linoleic and linolenic acids being observed at 15°. With sycamore cell suspensions, it has been shown [5] that low temperatures have no effect on the fatty acid composition of cells; in marked contrast, the formation of unsaturated fatty acid is tightly controlled by the oxygen concentration of the liquid medium. We have found [6] that the total lipid content of these cells is three-fold higher in cells grown at 12° than at 25°. It was thus of interest to reinvestigate the effect of temperature on plant cell cultures using a wider range of temperatures. For this purpose, we have used a line of tobacco cells which can grow in liquid medium over the temperature range 17 33°, without a noticeable increase of dead cells.

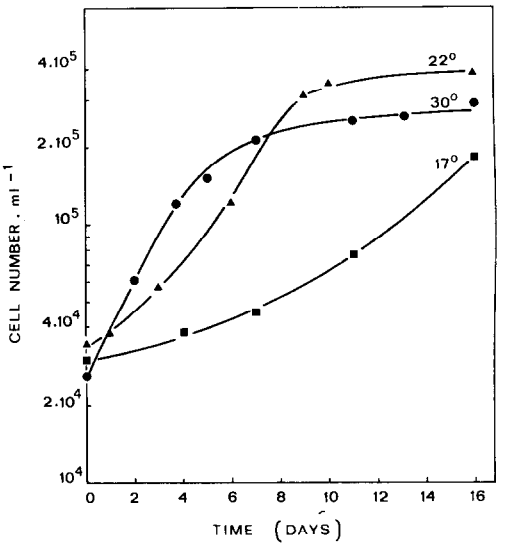

Fig. 1. Growth curves of tobacco cell suspensions maintained in batch cultures at different temperatures.

RESULTS AND DISCUSSION

Effects of temperature on cell suspension growth

The growth of tobacco cell suspensions as measured by changes in cell number as a function of time was investigated at various culture temperatures. It was found that numerous parameters were affected by temperature (Fig. 1): doubling time, duration of the exponential phase and maximum cell yield at the stationary phase. As summarized in Table 1, cell division was stopped at 13° and promoted at higher temperatures; the shortest doubling time was observed at 30° and the maximum cell yield at 26°.

In this cell line, the proportion of dead cells was particularly low and stable (3–5%) during one growth

Table 1. Growth parameters of tobacco cell suspensions cultivated at different temperatures

	Temperature (°)						
	13	17	20	22	26	30	35
Doubling time (hr)	00	166	144	72	56	48	50
Maximum cell yield ($\times 10^{-3}$/ml)	40	200	200	350	390	300	200

cycle at temperatures ranging from 17 to 30°, but increased up to 15–30% at the extremes of the temperature range tested, i.e. 13° and 35°.

The growth of tobacco cell suspensions occurred in the same range of temperatures (17–35°) as that described for *Haplopappus* [7] or *Catharanthus roseus* [8], but was inhibited by temperatures (12° or 14°) which allowed the growth of sycamore cells [9].

The cell line utilized in our experiments required cytokinin for its growth and the addition of this hormone to the liquid medium promoted chlorophyll synthesis. During one growth cycle at 26°, the chloroplasts underwent stepwise morphological modifications [10], so that the suspension initiated with 14-day-old green cells was composed of bleaching cells during the early log phase (4–5 days) and then greening ones with well differentiated chloroplasts during the stationary phase.

Variations of the lipid composition of tobacco cells during a growth cycle at 26°

The total fatty acids content per cell varied as a function of time (Fig. 2). Fatty acid biosynthesis was initiated during the first hours of subculture and was maximal during the exponential phase leading to a five-fold increase in the cellular concentration of fatty acids. The cellular fatty acid content then dropped from 500 to 100 $\mu g/10^6$ cells during the last divisions and remained stable throughout the stationary phase.

Such quantitative variations in the lipid content of cells had been observed in growing sycamore cells [6]; however, the changes were less marked. The rapid increase in the cell content of fatty acids during the exponential phase was so large that it cannot be related solely to membrane development during cell division. Thus, the accumulation of lipid reserves must accompany the first cell divisions in the period 2–7 days. While the cell division rate remained stable throughout the exponential phase, the fatty acid content of the cells decreased before the end of this phase, indicating a modified lipid metabolism: presumably most cellular lipid reserves would be utilized in the period 7–11 days. It must be emphasized, however, that no change in the proportions of neutral lipids, the usual components of lipid reserves, were observed during the exponential phase (Table 2). Therefore, some membrane destruction may well take place during the last part of the exponential growth phase.

Changes in the fatty acid composition (Fig. 3) were discrete and mainly affected linoleic and linolenic acids. Palmitic acid represented 22–25% of total fatty acid weight and remained fairly constant, myristic and palmitoleic acids appeared as traces, and stearic acid accounted for less than 5%, like oleic acid. However, this last acid represented up to 13% of total fatty acids at the onset of the subculture. Linoleic acid was present in greatest proportion in the middle of the log phase (*ca* 58%) whereas linolenic acid declined slightly in the early log phase and then increased gradually with the greening of cells. Separation of the various lipid classes (Table 2)

Table 2. Changes in the lipid composition of tobacco cells, cultivated at 26°, during one growth cycle

Age (days)	Composition (%/lipid wt)			
	NL	PL	MGDG	DGDG
2	10	75	6	9
4	11	74	7	8
7	10	78	4	8
14	8	73	7	12
39	12	64	11	13

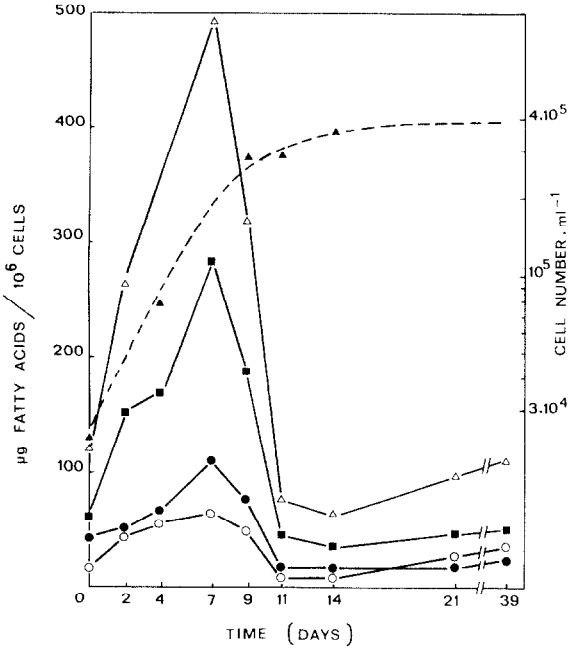

Fig. 2. Changes in cellular fatty acid content during the growth of tobacco cell suspensions cultivated at 26°. (▲) Cell number; (△) total fatty acid content; (●) $C_{16:0}$; (■) $C_{18:2}$; (○) $C_{18:3}$.

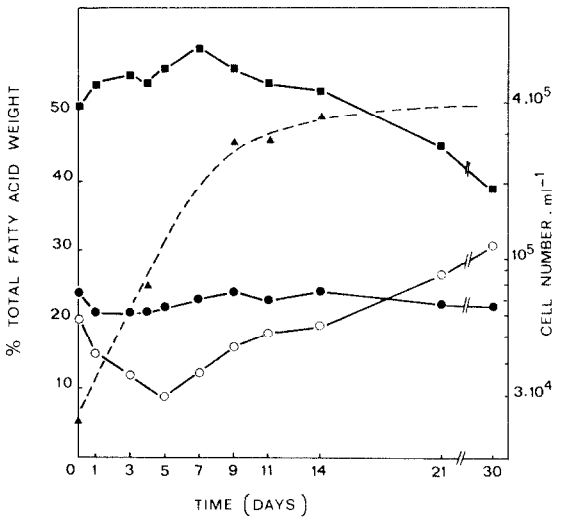

Fig. 3. Variations in fatty acid composition of the lipids of tobacco cell suspensions cultivated at 26°. (●) $C_{16:0}$; (■) $C_{18:2}$; (○) $C_{18:3}$; (▲) cell number. Plotted values are the means of four separate analyses.

showed that polar lipids [phospholipids (PL) and galactolipids (MGDG and DGDG)] were largely predominant over neutral lipids (NL). Phospholipids were the major components accounting for 70% of the total lipid weight and were mainly represented by phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI). Few changes were detected in the relative proportions of the various molecular types during the growth period except for some increase in the percentages of PC, during the exponential phase, and of MGDG and DGDG, during the stationary phase. This last increase may be related to the development of chloroplasts in non-dividing cells. It should be noted that the phospholipid content decreased significantly at the end of the exponential phase. The predominant fatty acids were, respectively, linoleic acid for phospholipids (55–65% of the total fatty acid) and linolenic acid (30–45% of total fatty acid) for galactolipids.

Changes affecting lipids in cells grown at different temperatures

In the range of chosen temperatures (17–35°) the concentration of fatty acids per cell increased immediately after incubation of cells in fresh medium, for periods varying with culture temperature, then decreased just before the last cell divisions. The optimal temperature for fatty acid biosynthesis seemed to be 20°; lower or higher temperatures resulted in a decrease of the amount of total fatty acids in the cells (Fig. 4). At the same cell density, this amount was three-fold higher in cells grown at 20° than in cells cultivated at 17° or 35°. The fatty acid composition of cells in active division varied with culture temperature. Thus, the percentages of polyunsaturated fatty acids were highest at the lowest temperatures (Table 3).

At the stationary phase, the cellular fatty acid content was influenced by growth temperature (Table 4). A high temperature caused a decrease in the total fatty acid amount per cell. However, the temperatures (22–35°) which promoted the best greening of cells induced an increase in the relative amount of linolenic acid accompanied by a decline of linoleic acid. Lower temperatures inhibited the greening of cells and maintained linolenic acid at a low level.

Chloroplast development in tobacco cells cultivated at higher temperatures was not accompanied by great changes in the galactolipid percentages: the MGDG percentage hardly changed while the DGDG percentage increased from 7–8% to 10–12%.

Table 5 shows that temperature related changes of the lipid classes detected in dividing or non-dividing cells were small, except for an increase in the MGDG and DGDG percentages in greening cells at temperatures higher than 20°.

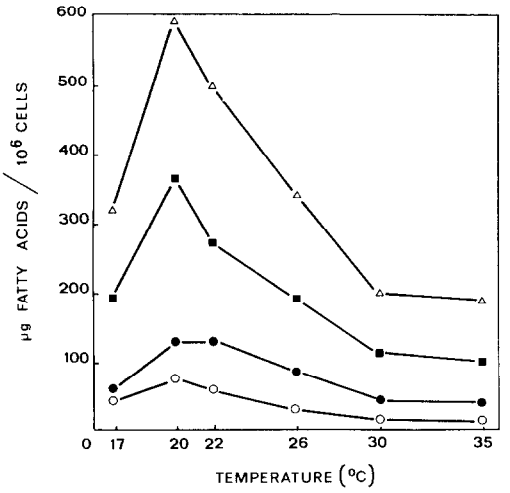

Fig. 4. Changes in fatty acid content of tobacco cell suspensions in active division (10^5 cell/ml) as a function of temperature. (△) Total fatty acid content; (●) $C_{16:0}$; (■) $C_{18:2}$; (○) $C_{18:3}$.

Comparison with sycamore cell suspensions

Previous work with sycamore cells [6] had shown that their lipid content was highest at low temperatures (12°) and that the amounts of polyunsaturated fatty acids were three-fold higher in cells grown at 12° compared with cells grown at 25°. A comparison of the lipid metabolism in sycamore and tobacco cell suspensions revealed marked differences between the two cell lines. Tobacco cells had a higher fatty acid content (500 µg fatty acids/10^6 cells) than sycamore cells (200 µg fatty acids/10^6 cells) which reflected the larger cell size in the tobacco suspension. Furthermore, at low temperatures, no lipid accumulation was observed in tobacco cell suspensions during the first days of subculture, which may be associated with the fact that cell division could not occur at temperatures lower than 17°, whereas during the stationary phase the fatty

Table 3. Fatty acid composition of dividing tobacco cell suspensions (10^5 cells/ml) cultivated at various temperatures

Culture temperature (°)	Age of culture (days)	Fatty acids: Amount (µg/10^6 cells)	Composition (wt %) $C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
17	14	320	19	3	3	61	14
20	12	597	22	2	2	61	13
22	6	500	26	4	4	54	12
26	4	336	25	2	5	57	11
30	3.5	200	23	5	5	57	9
35	4	180	24	5	7	55	9

Table 4. Fatty acid composition of total lipids of tobacco cell suspensions, at the stationary phase of growth, cultivated at various temperatures

Culture temperature (°)	Age of culture (days)	Fatty acids: Amount ($\mu g/10^6$ cells)	Composition (wt %) $C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
13	14	291	21	4	3	59	13
17	18	350	18	3	2	63	14
20	18	305	20	3	2	63	12
22	17	160	20	4	2	46	28
26	16	101	19	4	2	52	23
30	15	100	19	5	1	57	18
35	14	127	19	6	2	53	20

Table 5. Lipid composition of tobacco cells cultivated at different temperatures

	Composition (% total lipid wt)									
	17°		20°		22°		26°		30°	
Lipid species	Exp. (6)	Stat. (18)	Exp. (7)	Stat. (24)	Exp. (5)	Stat. (10)	Exp. (6)	Stat. (14)	Exp. (7)	Stat. (12)
NL	7	10	8	11	10	7	12	8	10	11
PL	80	76	79	77	74	78	74	73	79	72
MGDG	6	7	5	4	7	5	6	7	4	7
DGDG	7	7	8	8	9	10	8	12	7	11

Exp., exponential phase; Stat., stationary phase. Figures in parentheses indicate the number of days for which cells were cultured.

acid content increased between 13° and 20°. Optimal temperatures for cell growth, lipid biosynthesis and polyunsaturated fatty acid accumulation in tobacco cells, were found at 20–26°. The variations in lipid contents, related to changes in culture temperatures, interfered with the ability of tobacco cells to elaborate chloroplasts.

Thus, the responses of tobacco cells to changes in growth temperatures were different from those generally reported for higher plant tissues [1] or blue-green algae [11]. Lower temperatures neither promoted active polyunsaturated fatty acid accumulation nor resulted in great modifications in the proportions of the various types of lipids. A similar lack of a prominent increase in the polyunsaturated fatty acid percentage at lower temperatures had already been noticed for sycamore cells [5]. These data confirm previous suggestions [12] that a single physical factor, such as temperature, cannot totally control the fatty acid desaturation ability of plant cells; genetic factors are also probably involved.

EXPERIMENTAL

Batch culture. Cells of a cytokinin-requiring tobacco strain (clone 193, originally cloned by Tandeau de Marsac and Jouanneau [13]) were subcultured on Jouanneau and Peaud Lenoel liquid medium [14], supplemented with 2×10^{-7} M 2,4-D and 6×10^{-6} M benzyladenine as growth regulators, and 2×10^{-4} M sucrose. Cell suspensions were maintained at 26° ± 1° under continuous fluorescent light (2000 lx) in 800-ml round bottomed bottles shaken at 100 rpm.

The O_2 concn of the liquid medium of cell suspensions cultivated at various temps. (17–35°) was 95–97% of the O_2 satn.

Determination of cell number and viability. Samples of cell suspension were macerated in an equal vol. of 10% Cr_2O_3 soln, for 24 hr. After mechanical agitation cells were counted by means of a Nageotte cell. Viability of cells was detected by a dying test using erythrosine B [15].

Lipid extraction and analysis. After filtration of an aliquot of the suspension, 200–1000 mg of harvested cells were fixed in boiling H_2O and the lipids extracted in a mixture of MeOH and $CHCl_3$ according to ref. [16]. Different lipid classes were separated by TLC, following Lepage [17]. The component fatty acids were saponified and methylated [18] then analysed in a Girdel GC equipped with a 50 m capillary glass column packed with P.E.G. (column temp. 185°; He 0.4 bar; FID 220°).

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