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Effects of Growth Temperatures on the Fatty Acid Composition of Isolated Chloroplasts From Two Species Differing in Heat Sensitivity

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THE EFFECTS OF GROWTH TEMPERATURES ON THE FATTY ACID COMPOSITION OF ISOLATED CHLOROPLASTS FROM TWO SPECIES DIFFERING IN HEAT SENSITIVITY

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ABSTRACT

Lipid analyses of chloroplasts isolated from wheat (*Triticum aestivum* L. cv. Arthur) and milo (*Sorghum bicolor* cv. Funk's hybrid 522) suggest no major heat effect on lipid class distribution. Assuming milo is more heat tolerant than wheat and that increased saturated/unsaturated fatty acid values increase thermal stability, changes in sulfoquinovosyldiglyceride (SL) appear to be more important than phosphatidylglycerol (PG) in conferring thermal stability to isolated chloroplasts.

INTRODUCTION

Although living organisms have the capacity to grow and maintain metabolic activity between −2 °C and 100 °C, individual organisms have much narrower temperature limits. The limits of temperature at which different organisms thrive may be related to membrane stability (Kleinschmidt and McMahon, 1970). Pearcy et al. (1977) cited evidence identifying photosynthesis as the most thermal sensitive process in cellular metabolism. Santarius (1975) compared the effects of heat on four different chloroplast stroma enzymes and found them to be inactivated at much higher temperatures than the photosynthetic process suggesting that chloroplast membranes are the most thermal sensitive component of the photosynthetic apparatus agreeing with Daniell et al. (1969).

The photosynthetic apparatus of many plant species is capable of physiological acclimation to changes in the prevailing temperatures. Pearcy (1978) suggested that thermally induced changes in chloroplast lipids are related to the greater thermal stability of the photosynthetic apparatus at higher growth temperatures.

High growth temperatures have consistantly been shown to induce increased saturated/unsaturated fatty acid ratios in lipids (Kleinschmidt and McMahon, 1970; Pearcy, 1978; Holton et al. 1964). The higher proportions of saturated fatty acids in lipids could increase thermal stability of membranes due to higher melting temperatures. Although all membrane lipids do not exist in the same fluidity state at any given temperature, the cells are able to function as long as enough of the lipids maintain the proper level of fluidity.

Shinitzky and Henkart (1979) suggested that although membrane fluidity can be affected by changes in lipid composition and saturated/unsaturated fatty acid ratios, lipid/protein ratios as well as changes in sterol levels can also be important factors. Chapman et al. (1983) suggested that the optimum fluidity levels in pea thylakoids is maintained by the lipid/protein ratios rather than changes in lipid composition.

Pearcy (1978) stated that if lipids are important in conferring thermal stability to photosynthesis, the mechanism is very complex. Pearcy (1978) further suggested that the theory postulated by Anderson (1975) identifying the galactolipids monogalactosyldiglyceride (MGDG) and digalactosyldiglyceride (DGDG) as components of the membrane fluid bilayer and sulfoquinovosyldiglyceride (SL) and phosphatidylglycerol (PG) as protein boundary lipids could be significant.

Chen et al. (1982) stated that although high temperature adaptations have been reported for many noncrop plants, very little information is available concerning adaptations in crop plants.

Comparisons of electrolyte leakage from heated chloroplasts isolated from *Sorghum bicolor* cv. Funks hybrid 522 (milo) and *Triticum aestivum* L. cv. Arthur (wheat) grown at 20 °C and 31 °C suggest that milo is more thermotolerant than wheat (unpublished data). The principal objective of this study was to compare milo and wheat grown at 20 °C and 31 °C to ascertain what, if any, correlation exists between chloroplast lipids and thermotolerance.

MATERIALS AND METHODS

Plant Materials and Growth Conditions.

Seeds of milo (*Sorghum bicolor* cv. Funks hybrid 522) and wheat (*Triticum aestivum* L. cv. Arthur) were grown in two Sherer model CEL 37-14 environmental chambers at constant day/night temperatures of 20 °C or 31 °C programmed for sixteen hours of light and eight hours of darkness with the light intensity increasing in the morning and decreasing in the evening to simulate normal environmental conditions. Each growth chamber contained eight 100-watt incandescent bulbs and sixteen mixed Sylvania soft-white and Gro-lux fluorescent tubes.

Isolation of Chloroplasts.

Chloroplasts were isolated from 5-10 grams of leaf tissue by modifications of the methods of Leech (1966) and MacKender and Leech (1974). The deveined leaves were cut into small pieces into a Waring blender containing 70 milliliters of ice-cold 0.3M sucrose in 67mM Sorensen phosphate buffer (pH 6.8) and homogenized by turning the blender on high for 5 seconds then off for 5 seconds. This on-off procedure was continued for one minute of grinding time.

The homogenate was filtered through one layer of nylon hose which was squeezed to remove as much crude filtrate as possible from the macerated leaf tissue. This removed most of the leaf fragments. The crude filtrate was filtered through eight layers of nylon hose three times (do not squeeze) to yield the crude chloroplast suspension.

The filtrate was centrifuged at 3000xg for 15 seconds at 4 °C in a Sorvall RC2-B superspeed refrigerated centrifuge using a Sorvall 50ml capacity swinging bucket head. The pellet was collected, resuspended in 10ml of cold buffer in 0.3M sucrose and layered on top of 10ml of

phosphate buffer in 0.4M sucrose (pH6.8) and centrifuged at 500xg for 12 minutes. The pellets consisted primarily of intact chloroplasts as determined under phase contrast microscopy using a Wild Heerbrugg M20 phase microscope under 400x magnification. Intact chloroplasts appear bright green with bright halos while ruptured chloroplasts appear gray and lack the halos.

Lipid Extraction and Isolation.

The chloroplast pellet was dried under N_2 and resuspended in 5ml of methanol. The chloroplasts in methanol suspension were heated under N_2 at 55 °C in a water bath to break the lipids away from the proteins in the membranes being careful not to let all of the methanol evaporate. The chloroplasts in methanol suspension were transferred to a 15ml glass vial and enough chloroform added to make a 2:1 chloroform-methanol suspension with a total volume of 15ml (MacKender and Leech, 1974). Vials were oven preheated at 35 °C. After 15 minutes, N_2 was again bubbled through the suspension and the cap was screwed down tightly. Vials were left in the oven overnight (about 15 hours).

The suspension was transferred to a test tube and fifteen ml of 2M KCl were added and mixed thoroughly by bubbling N_2 through the suspension. After standing a short period of time, the suspension separated into two layers with the chloroform and lipids on the bottom. The upper layer was aspirated and discarded; 2M KCl was again added and mixed thoroughly under N_2. The upper layer was aspirated and discarded. The chloroform suspension remaining was washed twice with 10ml of distilled water which was aspirated off and discarded.

The chloroform suspension was passed through a column of anhydrous Na_2SO_4 to remove all water, collected in a clean, weighed vial, and dried under N_2 in a water bath at 35 °C. The vial containing the lipids plus large quantities of pigments was reweighed. The weight of the contents was determined by subtracting the weight of the vial.

The lipid-pigment extract was stored in the freezer at −20 °C until analyzed.

Separation of Lipids by Thin Layer Chromatography.

Twenty-five grams of silica gel G (Acc. to Stahl) were mixed with 50ml of ethanol and water (1:1) to form a homogeneous slurry. Glass TLC plates (20cm x 20cm) were coated with the silica gel G slurry 250 microns thick using a Desaga Brinkman spreader. After drying, the plates were stored in a Brinkman storage cabinet over anhydrous $CaSo_4$ until used. (TLC plates were activated by heating 30 minutes at 110 °C just prior to use.)

Two dimensional thin layer chromatography was used to separate the lipid species according to the method of Schwertner and Biale (1973). The lipid extract was resuspended in 100 µl of chloroform. Twenty microliters of lipid extract were transferred to the TLC plate, dried under N_2 and placed in the developing tank containing chloroform-methanol-water (65:25:4). Following lipid separation, the plate was removed from solvent system 1 and dried under N_2. The front was measured and the silica gel scraped off to remove any impurities which could interfere with the separation of the second solvent system. The plate was rotated 90 °C and immersed in acetone-acetic acid-water (100:2:1) which effectively separated the galactolipids and sulfolipids from the phospholipids. The addition of acetic acid to the acetone helped prevent trailing, the loss of neutral lipid fatty acids, and contamination by other lipids (Schwertner and Biale, 1973).

Lipid spots were visualized with iodine and outlined using a clean scribe before the color faded. Measurements were made and RF values calculated for each solvent system. The different lipid species were identified using standards and RF values published by Schwertner and Biale (1973). The spots were scraped from the plates into clean, oil-free 15 ml vials. Nine lipid classes were separated: phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylcholine (PC), sulfoquinovosyldiglyceride (SL), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), digalactosyldiglyceride (DGDG), diphosphatidylglycerol (DPG), and monogalactosyldiglyceride (MGDG).

Transesterfication of Lipids.

Transesterfication of lipid fatty acids was accomplished by adding 0.5ml of 2,2-dimethoxypropane and 0.5ml of 5% HCl in lipopure methanol (Applied Science Laboratories). After bubbling N_2 through the solution, the vials were loosely capped and placed in an oven at 50 °C for 10 minutes. N_2 was again bubbled through the solution. The mouth of the vial was covered with teflon tape, the cap was screwed down tight, and the vials incubated overnight in the oven at 50 °C (12-15 hours).

Following transesterfication, an equal volume of deionized water was added followed by 3ml of n-hexane. The mixture was mixed by vigorously bubbling N_2 gas through the mixture. The upper hexane layer containing the fatty acid methyl esters was drawn off with a Pasteur pipette and dried by passing it through an anhydrous Na_2SO_4 column. The effluent was collected in a clean vial. This procedure was repeated three times to remove all fatty acid methyl esters from the aqueous layer. The Na_2SO_4 column was washed with 5ml of n-hexane which was also collected in the vial.

The n-hexane fatty acid mixture was evaporated under N_2 in a water bath heated at 45 °C. The fatty acid methyl esters were resuspended in 25 µl of carbon disulfide for injection onto the gas chromatograph. Carbon disulfide gives a smaller peak than hexane and a better base line with a hydrogen flame detector (Allen and Good, 1971).

Gas Chromatography and Quantitation of Lipids.

The concentrated fatty acid methyl esters were chromatographed on a Beckman gas liquid chromatograph with a matched pair of 6 foot x 2 mm ID glass columns containing 15% diethyleneglycol succinate (DEGS) on chromosorb W (HP) 100/120 mesh. Separation was accomplished with a column temperature of 175 °C (isothermic), detector temperature of 250 °C, and a helium flow rate of 40 ml/minute. Sample injections were made using the solvent-flush technique.

The retention time of each methyl ester was determined using standards obtained from Sigma Chemical Company and data reported by Allen et al. (1966). The retention time of each fatty acid was calculated relative to palmitic acid using three runs of each fatty acid methyl ester.

The lipid fatty acids were quantitated by multiplying the peak height by the peak width at one half peak height. Measured peak areas were converted to a molar quantity (mole adjusted peak area) by multiplying the peak area by a proportionality factor calculated by dividing the molecular weight of each fatty acid by the molecular weight of the internal standard, heptadecanoic acid. The fatty acid composition of each lipid was expressed as the mole percent of that lipid. The chloroplast lipids were quantitated by the method of Allen and Good (1971). These data were compiled from two samples of leaves from each species analyzed. Lipid analyses were repeated three times per sample.

RESULTS

The fatty acid composition of both wheat (Table 1) and milo (Table 2) chloroplast lipids showed higher ratios of saturated/unsaturated fatty acids grown at 31 °C in seven of the nine lipids analyzed (Tables 3 and 4).

Palmitic acid (16:0) was the major saturated fatty acid in both milo and wheat. Significant increases in palmitic acid (16:0) were determined for PG (+21.4%), PE (+20.3%) and DPG (+16.9%) in wheat while only SL (+14.4%) showed significant increase in milo chloroplasts. Stearic acid (18:0) did not appear to change significantly with growth temperature in either species studied. Although increasing at 31 °C in seven of nine wheat lipids analyzed, the increase was significant only in PG (+7.5%), the most abundant chloroplast phospholipid. Stearic acid (18:0) increased in only four of nine milo lipids analyzed. Minor increases did occur in MGDG (+1.6%) and DGDG (+2.7%), the two most abundant chloroplast lipids.

Monoenoic fatty acids (16:1, 18:1) were found in all lipids analyzed from both wheat and milo. However, the data does not suggest any significant temperature induced rearrangement of these acids.

Surprisingly, linoleic acid (18:2) was the most abundant fatty acid isolated from all wheat lipids analyzed at 20 °C. In milo, however, linoleic acid (18:2) was the second in abundance to palmitic (16:0) in PG, which was the most abundant phospholipid in both species. Decreases in

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Table 1. Fatty acid distribution in the various lipid species of wheat chloroplasts (mole percent).

	16:0		16:1		16:3		18:0		18:1		18:2		18:3		18:3		20:0		20:1		20:2		20:3	
	20°C	31°C	20°C	31°C	20°C	31°C	20°C	31°C	20°C	31°C	20°C	31°C	20°C	31°C	20°C	31°C	20°C	31°C	20°C	31°C	20°C	31°C	20°C	31°C
phosphatidyl-serine	12.8	26.7	2.6	4.1	4.1	4.7	8.8	0.9	10.8	8.9	27.0	18.5	3.0	7.1	13.8	14.7	—[a]	—	—	—	10.7	8.3	6.2	6.0
phosphatidyl-inositol	8.7	13.0	1.7	4.5	9.2	5.2	8.3	9.0	8.6	10.2	29.8	25.9	0.3	3.8	11.9	14.1	1.7	—	—	—	14.8	10.2	5.1	5.1
phosphatidyl-choline	16.7	13.8	3.9	2.3	6.4	10.9	6.6	9.9	11.7	11.9	29.1	19.1	1.7	10.1	14.2	9.0	t[b]	—	—	—	9.4	13.1	3.2	t
sulfoquinovosyl-diglyceride	13.8	8.9	5.6	3.5	7.4	8.0	8.2	8.6	7.3	7.7	23.7	27.5	3.8	4.1	11.3	9.7	1.3	t	—	—	12.4	13.1	3.2	6.8
phosphatidyl-glycerol	6.1	25.4	1.2	3.3	4.4	3.4	5.6	13.1	6.6	8.8	29.9	15.8	3.3	4.9	16.9	10.3	2.6	—	—	—	19.6	7.0	5.5	7.0
phosphatidyl-ethanolamine	3.8	34.1	.7	4.7	8.0	5.3	4.5	7.2	9.0	8.9	27.6	18.2	3.6	3.8	16.3	11.8	t	—	—	—	20.4	9.4	6.2	6.3
digalactosyl-diglyceride	4.9	16.6	2.0	3.4	7.5	5.3	10.3	6.8	8.6	9.4	27.5	31.4	4.7	1.2	13.5	12.7	t	t	—	—	14.2	10.1	7.2	2.9
diphosphatyl-glycerol	5.4	22.3	2.3	16.0	7.0	3.0	6.1	9.3	8.3	8.1	34.1	23.5	1.7	3.6	15.0	13.3	6.3	—	—	—	13.7	5.2	2.0	5.8
monogalactosyl-diglyceride	12.3	12.7	8.4	6.8	4.2	6.2	8.0	10.6	6.5	8.6	33.9	22.0	1.1	3.9	12.3	10.0	—	—	—	—	8.7	9.4	4.5	9.3

[a] dashes indicate non-detectable quantities

[b] t indicates trace amounts

Table 2. Fatty acid distribution in the various lipid species of milo chloroplats (mole percent).

	16:0		16:1		16:3		18:0		18:1		18:2		18:3		18:3		20:0		20:1		20:2		20:3	
	20°C	31°C	20°C	31°C	20°C	31°C	20°C	31°C	20°C	31°C	20°C	31°C	20°C	31°C	20°C	31°C	20°C	31°C	20°C	31°C	20°C	31°C	20°C	31°C
phosphatidyl-serine	14.4	19.6	1.0	6.0	5.9	7.4	6.3	12.5	8.9	4.7	28.2	18.8	4.3	3.5	13.9	12.3	t[a]	—[b]	—	—	11.7	10.6	5.3	4.7
phosphatidyl-inositol	14.8	16.1	3.5	8.3	8.6	4.6	9.2	8.9	6.7	6.7	25.1	24.6	2.6	2.1	13.1	11.4	t	t	t	—	11.4	10.4	5.0	6.8
phosphatidyl-choline	7.4	15.9	2.2	6.7	5.0	4.9	11.4	4.7	6.1	6.8	28.3	26.1	2.4	2.4	14.0	16.1	t	t	t	—	17.2	13.4	6.0	3.2
sulfoquinovosyl-diglyceride	9.6	24.0	6.8	3.2	6.6	7.3	6.6	4.5	10.7	7.9	27.9	16.5	4.1	4.1	14.1	10.2	t	t	—	—	11.9	16.6	8.2	5.2
phosphatidyl-glycerol	23.3	28.0	4.0	3.8	5.1	2.1	9.1	3.8	7.7	3.0	19.4	20.9	4.6	—	9.8	6.0	t	2.3	—	t	10.4	24.0	6.7	1.2
phosphatidyl-ethanolamine	15.0	15.9	4.7	6.4	5.8	2.2	9.4	6.2	7.3	6.5	20.0	28.4	4.7	1.0	9.2	10.0	1.5	—	—	—	12.2	19.7	10.2	3.6
digalactosyl-diglyceride	23.8	17.9	3.0	5.7	3.5	2.6	3.5	6.2	5.4	6.9	28.3	24.1	1.0	2.6	11.5	10.5	—	—	—	3.6	15.7	14.7	2.4	5.0
diphosphatyl-glycerol	19.2	27.9	3.9	5.3	8.3	3.0	6.5	9.6	7.5	9.2	22.1	15.4	3.2	3.4	11.7	11.8	t	—	—	—	12.1	7.2	5.4	5.2
monogalactosyl-diglyceride	13.0	15.9	4.4	6.0	5.0	4.4	6.6	8.2	9.3	8.3	27.2	28.8	2.5	—	13.8	14.8	—	t	—	t	12.6	12.5	5.6	3.5

[a] t indicates trace amounts

[b] dashes indicate non-detectable quantities

linoleic acid (18:2) occurred in all wheat chloroplast lipids analyzed except SL and DGDG. These increases are more significant on the basis of total lipids since DGDG is the second most abundant chloroplast lipid. Linoleic acid (18:2) decreased in seven of nine milo lipids at 31 °C. The most significant decrease occurred in SL (−11.3%). Significant increases were observed in milo PE, but this is a very minor chloroplast lipid.

Contrary to reports by Pearcy (1978), hexadecatrienoic acid (16:3) was found to be a component of all lipids analyzed in both wheat and milo. However, growth temperature induced alterations appear minor and unpredictable in all lipids analyzed from both wheat and milo.

Although linolenic acid (18:3) has been reported as the major chloroplast lipid fatty acid (Pearcy, 1978; Allen et al., 1966; MacKender and Leech, 1974), data reported here do not concur. Linolenic acid (18:3) was lower in concentration than linoleic (18:2) in all lipids analyzed in both wheat and milo. Contrary to previous reports, both α and linolenic acid (18:3) were found in all chloroplast lipids isolated from both milo and wheat.

Twenty carbon fatty acids were found in all chloroplast lipids analyzed from both plant species. Dienoic and trienoic species 11,14 eicosadienoic acid (20:2) and 11,14,17 eicosatrienoic acid (20:3) were the major twenty carbon fatty acids with trace amounts of eicosaenoic acid (20:0). Temperature induced alterations in these fatty acids were inconsistent.

Monoenoic fatty acids did not appear to be significant markers for temperature induced lipid alterations. Therefore, measurements of temperature induced changes in the degree of unsaturation of chloroplast lipids were based on dienoic and trienoic acids (Tables 3 and 4). Changes in levels of saturation of MGDG, DGDG, SL, and PG are of major importance. MGDG and DGDG are the most abundant chloroplast lipids while PG and SL are the two most abundant phospholipids (Table 5). PG (−41.8%), DGDG (−10.7%), and MGDG (−3.3%) from wheat chloroplast registered decreases in the sum of dienoic and trienoic fatty acids at 31 °C while SL (+7.6%) posted a slight increase. Milo, however, showed decreased levels of unsaturation of fatty acids in all four of these lipids. The largest drop in unsaturation occurred in SL (−12.2%) while the smallest drop occurred in PG (−1.8%) in sharp contrast to PG unsaturation at 31 °C from wheat.

The role of MGDG and DGDG as the major chloroplast lipids of both species makes any changes in their concentrations important to note. MGDG increased slightly in wheat at the higher growth temperature decreasing slightly in milo. DGDG, however, was altered in the opposite direction, decreasing slightly in wheat while increasing by the same amount in milo. The ratios of MGDG/DGDG (Table 5) at increasing growth temperature also registered inexplicable changes with growth temperature increasing in wheat at 31 °C while decreasing in milo.

CONCLUSIONS

Raju et al. (1976) suggested that the lipids of biomembranes may play a significant role in thermophily. Fatty acid analyses of lipids extracted from thermophilis fungi (Raju et al., 1976), higher plants (Pearcy, 1978), and thermophilic alga (Kleinschmidt and McMahon, 1970) suggest that saturated fatty acids predominate at higher temperatures.

Table 3. A comparison of saturated to unsaturated fatty acids, percent dienoic fatty acids, percent trienoic fatty acids, and the sums of percent dienoic and trienoic fatty acids in lipids isolated from wheat grown at 20°C or 31°C.

Lipid	(x 100) Sat/Unsat. 20°C	31°C	% Dienoic 20°C	31°C	% Trienoic 20°C	31°C	(Di + Tri) Polyunsat. 20°C	31°C
Phosphatidylserine	27.6	38.1	37.7	26.8	27.1	32.5	64.8	59.3
Phosphatidylinositol	23.0	28.2	44.6	36.1	26.5	28.2	71.1	64.3
Phosphatidylcholine	27.1	31.1	38.5	32.2	25.5	30.0	64.0	62.2
Sulfoquinovosyldiglyceride	30.4	21.2	35.9	42.6	27.7	28.6	63.6	71.2
Phosphatidylglycerol	14.0	54.7	49.5	23.2	30.7	15.2	80.2	38.4
Phosphatidylethanolamine	9.1	31.3	48.0	27.8	34.1	27.4	82.1	55.2
Digalactosyldiglyceride	17.4	30.5	41.7	41.8	32.9	22.1	74.6	63.9
Diphosphatidylglycerol	19.0	46.2	47.8	28.7	25.7	25.7	73.5	54.4
Monogalactosyldiglyceride	25.5	23.3	42.6	31.4	22.1	30.0	64.7	61.4

Table 4. A comparison of saturated to unsaturated fatty acids, percent dienoic fatty acids, percent trienoic fatty acids, and the sums of percent dienoic and trienoic fatty acids in lipids isolated from milo grown at 20°C and 31°C.

Lipid	(x 100) Sat/Unsat. 20°C	31°C	% Dienoic 20°C	31°C	% Trienoic 20°C	31°C	(Di + Tri) Polyunsat. 20°C	31°C
Phosphatidylserine	26.4	47.3	39.9	29.4	29.2	27.9	69.1	57.3
Phosphatidylinositol	32.0	33.0	37.0	35.0	22.3	25.1	59.3	60.1
Phosphatidylcholine	23.2	25.8	45.5	39.5	27.4	26.6	72.9	66.1
Sulfoquinovosyldiglyceride	19.3	39.9	39.8	33.1	32.9	27.4	72.7	60.5
Phosphatidylglycerol	47.4	51.7	29.8	44.9	26.2	9.3	56.0	54.2
Phosphatidylethanolamine	33.9	28.4	32.2	48.1	29.9	16.8	62.1	64.9
Digalactosyldiglyceride	36.1	31.8	44.0	38.8	20.4	20.7	64.4	59.5
Diphosphatidylglycerol	34.6	60.0	34.2	22.6	28.8	25.4	63.0	48.0
Monogalactosyldiglyceride	24.4	31.8	39.8	41.3	26.9	22.7	66.6	64.0

Bowler et al. (1973) postulated that the membrane is the primary lesion of heat death and that changes in the properties of membranes during thermal acclimation may be responsible for resistance to heat stress.

Data reported by Cossins (1976) on the effects of high temperature on crayfish indicated that thermal acclimation has no effect on the overall phospholipid class distribution or content agreeing with data presented here. Further analyses of data led Cossins (1976) to conclude that the primary event leading to the breakdown of membrane permeability may not be a breakdown of the bulk membrane lipid bilayer due to increased fluidity or phase change but rather may be the result of changes in the specific lipid halos of specific membrane bound enzymes thus inactivating these enzymes.

Farkas and Csengeri (1976) suggested that differences in the fatty acid synthesizing systems would facilitate the production of the necesesary chain lengths and saturated to unsaturated proportions at a particular temperature. They expressed confidence that this type of regulation is sensitive and rapid enough to ensure the proper functioning of cell membranes under changing conditions.

The well-known relationship between phase transition temperatures, carbon chain length, and double bonds explains why the same lipids species may have different temperatures for phase transition.

One might postulate that lipid fatty acid rearrangement confers thermal stability by increasing the phase transition temperature due to the higher melting temperatures of more saturated lipid fatty acid.

The fatty acid rearrangement associated with increased temperatures were similar to those reported in leaf senescence. Newman et al. (1973) found that senescence resulted in a decline in linolenic acid (18:3) with a concomitant relative increase in palmitic acid (16:0).

Tables 3 and 4 support the reported conclusions of Kleinschmidt and McMahon (1970), Holton et al. (1964), and others, indicating that plants adjust to higher growth temperatures by increasing the ratio of saturated to unsaturated fatty acids of their lipids. It is tempting to relate these changes to the relative thermal sensitivity of each plant species analyzed.

Wheat appears to adjust the saturated/unsaturated ratio primarily in PG, DPG, PE and DGDG in decreasing order, while milo adjusts primarily in DPG, SL, PS and MGDG. Since MGDG, DGDG, and PG are the most abundant chloroplast lipids, one could assume that changes in these lipids would be most important in conferring thermal stability. Bjorkman et al. (1976) included SL with PG as the major lipids involved in conferring thermal stability due to Anderson's (1975) theory identifying PG and SL as the membrane protein boundary lipids (halos) even though SL is a relatively minor chloroplast component (5-7%).

The difference in the ratio of saturated to unsaturated fatty acids

Table 5. The lipid composition of wheat and milo chloroplasts grown at 20 °C or 31 °C (mole percentage of total lipid).

Lipid	Wheat		Milo	
	20°C	31°C	20°C	31°C
Phosphatidylserine	1	1	1	1
Phosphatidylinositol	2	2	3	2
Phosphatidylcholine	6	5	6	7
Sulfoquinovosyldiglyceride	6	5	6	7
Phosphatidylglycerol	10	11	11	10
Phosphatidylethanolamine	1	1	1	1
Digalactosyldiglyceride	28	26	31	33
Diphosphatidylglycerol	1	1	1	1
Monogalactosyldiglyceride	41	44	40	38
Ratio of MGDG/DGDG	1.46	1.69	1.29	1.15

The results are the means of triplicate analyses.

from isolated wheat and milo chloroplasts grown at 20 °C and those grown at 31 °C showed the expected increases at higher temperatures. However, all lipid species did not alter their lipid fatty acid equally. Chloroplast PG isolated from wheat grown at 31 °C showed increased saturated/unsaturated ratio of more than 40%, while PG isolated from milo chloroplasts increased less than 4%. Analyses of SL fatty acid from wheat and milo showed increases of 20% in milo (31 °C) and an unexpected decrease of 9% in wheat (31 °C).

If, as the evidence suggests, milo is more thermal tolerant than wheat, then changes in saturated/unsaturated fatty acids of the two proposed membrane fluid bilayer lipids indicate that SL is more effective than PG in conferring thermal stability to these membranes. The rise in SL isolated from milo grown at 31 °C coupled with its significant drop in unsaturation could also be important. Furthermore, a comparison of temperature induced changes in dienoic and trienoic fatty acids of PG and SL in wheat and chloroplast lipids suggests that decreased levels of trienoic fatty acids in milo, although small, may be more imoprtant in conferring thermal stability than dienoic fatty acids.

Milo appears to be more heat tolerant because it seems to adjust to increased temperatures by decreasing the trienoic acid content of both SL and PG while dienoic fatty acids increase in both SL and PG. Wheat apparently adapts to higher temperatures by large decreases in the dienoic content of PG with slight increases in SL. The slight difference observed between the trienoic fatty acids of PG from wheat (−16%) and milo (−17%) suggests that chloroplast PG would have little effect on the apparent difference in thermal tolerance of these two plants. These observations place greater importance on the trienoic acid changes in wheat and milo chloroplast SL. Although these changes are small in comparison to PG (−6% in milo and +1% in wheat), they may be very important due to the significant decrease observed in milo SL.

The second possibility relating thermal tolerance to the stability of the membrane fluid bilayer is facilitated by adding the assumption that Anderson (1975) was correct in identifying galactolipids as the major lipids of the chloroplast membrane fluid bilayer.

The saturated/unsaturated fatty acids of MGDG show the expected increase at increased growth temperature in milo (+7%) while an unexpected decrease occurred in wheat (−2%). DGDG exhibited changes in saturated/unsaturated fatty acid ratios just opposite of those of MGDG with wheat increasing as expected (+12%) and milo decreasing (−4%). A comparison of temperature induced changes in wheat and milo galactolipids indicates a slight downshift in MGDG concentration in milo at 31 °C. This could be important considering its possible role in the membrane fluid bilayer and the most abundant membrane lipid.

The ratios of MGDG/DGDG further cloud the elucidation of the relationship of these lipids and membrane stability. Wheat showed increased MGDG/DGDG at increasing temperature while milo showed decreased ratios with increasing temperature (Table 5).

The possible functions of the other lipids are unknown. All except PC (5-7%) appear in very small quantities.

Result of these experiments suggest the presence of 20 carbon acyl groups associated with all chloroplast lipid classes analyzed. This data is contrary to previous fatty acid analyses of chloroplasts isolated from corn (Leese and Leech, 1976), *Atriplex lentiformis* (Pearcy, 1978), spinach (Allen et al., 1966;, and Allen and Good, 1971), barley (Newman et al., 1973), *Vicia faba* (MacKender and Leech, 1974), and others, where 18 carbon acids were the longest reported. Twenty carbon fatty acids have been reported in numerous lower plants including algae (Jamieson and Reid, 1976), fungi (Sawicki and Pisano, 1977), and others. Twenty carbon fatty acids have also been isolated from poikilothermic animals, e.g., crayfish (Cossins, 1976) and carp (Farkas and Csengeri, 1976). Reports of twenty carbon fatty acids in *Ginkgo biloba* leaves (Gellerman and Schlenk, 1972) was of special interest because of its position as a primitive gymnosperm.

Data presented in this study agree with results reported by Gawer et al. (1983) showing linoleic (18:2) as the major lipid fatty acid of tobacco cells grown at temperatures ranging from 12°-35 °C followed in decreasing order by palmitic acid (16:0) and linolenic acid (18:3) at most growth temperatures. Tremolieres and Lepage (1971) reported high linoleic acid (18:2) concentrations in leaves from dark grown pea seedlings.

Hitchcock and Nichols (1971) suggested that mineral deficiencies and low light intensity decrease the concentration of 18:3. The fatty acid distribution reported in this data may suggest cellular contamination of isolated chloroplasts.

These data seem to support the conclusion of Bjorkman (1975) conferring major significance to the protein boundary lipids SL and PG in conferring thermal stability to chloroplast membranes primarily by decreasing the trienoic acid composition of SL.

Gawer et al. (1983) reported that their data supported that of Tremolieres et al. (1982) suggesting that no single factor, such as temperature, can control the ability of a cell to adjust the degree of unsaturation of its cellular lipids. Genetic factors are also involved. The importance of genetic differences in temperature adaptation expressed in modifications of chloroplast lipid fatty acids was also reported by Horvath et al. (1983).

LITERATURE CITED

ALLEN, F., and P. GOOD. 1971. Acyl lipids in photosynthetic systems. J. Amer. Oil Chem. Soc. 49:21-28.

ALLEN, C. F., P. GOOD, H. F. DAVIS, P. CHISUM, and S. D. FOWLER. 1966. Methodology for the separation of plant lipids and application to spinach leaf and chloroplast lamellae. J. Am. Oil Chem. Soc. 43:223-231.

ANDERSON, J. M. 1975. The molecular organization of chloroplast thylakoids. Biochem. Biophys. Acta. 416:191-235.

BJORKMAN, O. 1975. Photosynthetic responses of plants from contrasting thermal environments. Thermal stability of the photosynthetic apparatus in intact leaves. Carnegie Inst. Wash. Yearbook 74:748-751.

BJORKMAN, O., J. BOYNTON, and J. BERRY. 1976. Comparison of the heat stability of photosynthesis, chloroplast membrane reactions, photosynthetic enzymes, and soluble proteins in leaves of heat adapted and cold adapted C-4 species. Carnegie Inst. Wash. Yearbook. 75:400-407.

BOWLER, K., C. DUNCAN, and R. GLADWELL. 1973. Cellular heat injury. Comp. Biochem. Physiol. 45:441-450.

CHAPMAN, D. J., J. DE-FELICE, and J. BARBER. 1983. Growth temperature effects on thylakoid membrane lipid and protein content of pea chloroplasts. Plant Physiol. 73:225-228.

CHEN, H. H., Z. Y. SHEN, and P. H. LI. 1982. Adaptability of crop plants to high temperature stress. Crop Sci. 22:719-725.

COSSINS, A. R. 1976. Changes in muscle lipid composition and resistance adaptation to temperature in fresh water crayfish, *Austropotamobius pallipes*. Lipids. 11:307-314.

DANIELL, J. E., W. E. CHAPELL, and H. B. COUCH. 1969. Effects of sublethal and lethal temperatures on plant cells. Plant Physiol. 44:1684-1689.

FARKAS, T., and I. CSENGERI. 1976. Biosynthesis of fatty acids by the carp, *Cyprinus carpio* L. in relation to environmental temperature. Lipids. 11:401-407.

GAWER, M., A. SANSONETTI, and P. MAZLIAK. 1983. Lipid composition of toacco cells cultivated at various temperatures. Phytochem. 22:855-859.

GELLERMAN, J. L., and H. SCHLENK. 1969. Preparations of 14 C-labelled fatty and anacardic acids from *Ginkgo biloba*. Lipids. 4:484-487.

HITCHCOCK, C., and B. W. NICHOLS. 1971. The lipid and fatty acid composition of specific tissues. In: Plant Lipid Biochemistry. Academic Press. New York. pp. 59-80.

HOLTON, R. W., H. H. BLECKER, and M. OHARE. 1964. Effects of growth temperature on the fatty acid composition of blue green algae. Phytochem. 3:595-602.

HORVATH, I., L. VIGH, P. R. VAN HASSETT, J. WOLTJES, and P. J. C. KUIPER. 1983. Lipid composition in leaves of cucumber genotypes as affected by different temperature regimes and grafting. Physiol. Plant. 57:532-536.

JAMIESON, G. R., and E. H. REID. 1976. The fatty acid composition of *Ulothrix aequalis*. Phytochem. 15:795-796.

KLEINSCHMIDT, M. G., and V. A. MCMAHON. 1970. Effects of growth temperature on the lipid composition of *Cyanidium caldarium*. I. Class separation of lipids. Plant Physiol. 46:286-289.

LEECH, R. M. 1966. Comparative biochemistry and comparative morphology of chloroplasts isolated by different methods. pp. 65-74. In Biochemistry of Chloroplasts. Vol. 1. T. W. Goodwin, Ed., New York.

LEESE, B., and R. M. LEECH. 1976. Sequential changes in the lipids of developing proplastids isolated from green maize leaves. Plant Physiol. 57:789-794.

MACKENDER, R. O., and R. M. LEECH. 1974. The galactolipid, phospholipid, and fatty acid composition of the chloroplast envelope membranes of *Vicia faba* L. Plant Physiol. 53:496-502.

NEWMAN, D. W., B. W. ROWELL, and K. BYRD. 1973. Lipid transformations in greening and senescing leaf tissue. Plant Physiol. 51:229-233.

PEARCY, R. W. 1978. Effects of growth temperature on the fatty acid composition of the leaf lipids in *Atriplex lentiformis* (Torr.) Wats. Plant Physiol. 61:484-486.

PEARCY, R. W., J. A. BERRY, and DAVID FORK. 1977. Effects of growth temperature on the thermal stability of the photosynthetic apparatus of *Atriplex lentiformis* (Torr.) Wats. Plant Physiol. 59:873-878.

RAJU, K. S., R. MAHESHWARI, and P. S. SASTRY. 1976. Lipids of some thermophilic fungi. Lipids. 11:741-746.

SANTARIUS, K. A. 1975. Sites of heat sensitivity in chloroplast and differential inactivation of cyclic and non-cyclic photophosphorylation by heating. J. Thermal. Biol. 1:101-107.

SAWICKI, E., and M. A. PISANO. 1977. The effects of temperature on the fatty acid and phospholipid composition of *Cephalosporium falciforme* and *Cephalosporium riliense*. Lipids. 12:125-126.

SCHWERTNER, H. A., and J. B. BIALE. 1972. Lipid composition of plant mitochondria and of chloroplasts. J. Lipid Res. 14:235-242.

SHINITZKY, M., and P. HENKART. 1979. Fluidity of cell membranes: current concepts and trends. International Rev. Cyt. 60:121-147.

TREMOLIERES, A., and M. LEPAGE. 1971. Changes in lipid composition during greening of etiolated pea seedlings. Plant Physiol. 47:329-334.

TREMOLIERES, A., J. P. DUBACQ, and D. DRAPIER. 1982. Unsaturated fatty acids in maturing seeds of sunflower and Rape: Regulation by temperature and light intensity. Phytochem. 21:41-45.

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