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Effect of Growth Temperature on the Fatty Acid Composition of the Leaf Lipids in *Atriplex lentiformis* (Torr.) Wats.[1]

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ABSTRACT

Plants of *Atriplex lentiformis* had more saturated leaf lipids when grown at 43 C day/30 C night as compared to 23/18 C temperatures. In monogalactosyl diglyceride, the major change was the presence of hexadecatrienoic acid (16:3) at low but not high growth temperatures. In other lipids investigated, the major change was a decrease in linolenic acid (18:3) and increases in the more saturated fatty acids at high growth temperatures. Growth temperatures had little effect on the relative proportions of the galacto- and sulfolipids in the leaf. The increased lipid saturation is correlated with the greater thermostability of the photosynthetic apparatus at high growth temperatures in *A. lentiformis* but any cause and effect relationship is uncertain.

The photosynthetic apparatus of many plant species are capable of a physiological acclimation to prevailing growth temperatures resulting in improved photosynthetic performance. For *Atriplex lentiformis*, increased growth temperature results in a higher temperature optimum for photosynthesis and increased rates of CO_2 uptake at high temperatures due at least partially to a greater thermal stability of the photosynthetic apparatus (15). Experiments with both *in vivo* and *in vitro* measures of photosystem II have demonstrated a good correlation between an increased thermal stability of this system and that of photosynthetic CO_2 exchange. Other studies have demonstrated a similar effect of lipid solvents and high temperature on chloroplast photoreactions (12) suggesting that lipids may play a central role in the thermal stability of the photosynthetic apparatus. Membrane lipid composition may thus be an important factor in the changes in PSII thermal stability in *A. lentiformis*.

In higher plants, increases in unsaturation of fatty acids in response to chill hardening have been demonstrated for a number of species (4, 19) but there have been no reports of high growth temperature effects on fatty acid composition. In this study, the effects of high growth temperatures on the fatty acid composition of leaf lipids originating predominantly in the chloroplast are reported for *A. lentiformis*. The primary objective was to determine whether any correlation was apparent between changes in lipids and the marked increases in thermal stability of the photosynthetic apparatus reported previously (15).

MATERIALS AND METHODS

Plant Materials and Growth Conditions. *A. lentiformis* plants collected from a coastal habitat near Ventura, California and a desert habitat in Death Valley, California were grown in a growth chamber under either 23/18 C or 43/30 C day/night temperature regimes with a 16-hr photoperiod and a quantum flux density of 110 nE cm^{-2} sec^{-1}. Detailed descriptions of the growth conditions and collection sites are given elsewhere (14).

Extraction of Leaf Lipids. Ten g of mature leaves were harvested and heated in a steam bath for 3 min to inhibit any lipase activity (7). The lipids were then extracted by homogenization in a Waring Blendor with chloroform-methanol, 2:1. Nonlipid contaminants were removed according to the procedure of Folch *et al.* (5) and the extract was rotary-evaporated to dryness at room temperature. The extract was redissolved in chloroform containing 0.1 mg/ml butylated hydroxytoluene to prevent oxidation (6) and stored under N_2 in sealed vials at −20 C.

Separation of Polar Lipids. The crude extract was separated into: (*a*) pigment and neutral lipid, (*b*) glycolipid, and (*c*) phospholipid fractions with a modification of the silicic acid column chromatography procedures of Rouser *et al.* (18) and Kleinschmidt and McMahon (8). Aliquots containing 10 mg of lipid were applied to silicic acid columns (6 × 100 mm) and fractionated by successive elutions with 50 ml of 50:50 *n*-hexane-diethyl ether (neutral lipids and pigments), 75 ml of acetone (glycolipids), and 20 ml of methanol (phospholipids). Recovery from the column was quantitative.

The glycolipids and phospholipids were further separated by one-dimensional TLC on Silica Gel G plates with chloroform-methanol-acetic acid-water (170:30:20:7, v:v) as the solvent. The separated lipids were visualized with rhodamine 6G under UV light or with iodine vapors. The lipids MGDG[3] and DGDG were identified by comparison to spinach leaf lipid chromatograms, published R_F values (6), and by the presence of galactose (17). Identification of SL was made by comparison to chromatograms of a ^{35}S-labeled SL prepared from *Chlamydomonas reinhardtii*. For PG, identification was made by comparison to R_F values for a commercially available (Applied Sciences Labs) standard. The amounts of MGDG, DGDG, and SL were determined according to the procedures of Roughan and Batt (17).

Fatty Acid Composition. Lipids visualized with rhodamine 6G and corresponding to MGDG, DGDG, SL, and PG were removed from the chromatogram and methyl esters of the fatty acids were prepared according to the procedures of Metcalf *et al.* (11). The methyl esters were separated on a Perkin Elmer gas chromatograph equipped with a flame ionization detector. The column (180 × 0.3 cm) used in most analysis was packed with 10% ethylene-succinate-methylsilicone copolymer on Gas-chrom P (Applied Sciences Labs) and run at 175 C. Nitrogen (30 ml/min) was used as the carrier gas. Where required for identification of specific fatty acids, a 3% methyl silicone on Gas-chrom Q column (Applied Sciences Labs) was used in addition to the column described above. Fatty acid compositions were calculated from the peak areas as determined by electronic integration or cutting and weighing of the peaks from recorder traces.

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[3] Abbreviations: MGDG: monogalactosyl diglyceride; DGDG: digalactosyl diglyceride; SL: sulfolipid; PG: phosphatidyl glycerol.

Table I. Fatty acid composition of the glycolipids and phosphatidyl glycerol from leaves of high and low temperature grown Atriplex lentiformis plants.

Collection location	Growth Temp. C	Fatty acid composition, % of total 16:0	16:1	16:3	18:0	18:1	18:2	18:3
Monogalactosyl diglyceride								
Death Valley	23	1.2 ± 0.5[1]		13.8 ± 0.8			3.4 ± 0.6	81.4 ± 1.0
	43	5.3 ± 0.6	0.9 ± 0.5		1.2 ± 1.0	7.4 ± 0.9	7.7 ± 2.0	76.5 ± 5.1
Ventura	23	1.3 ± 0.3	T	13.7 ± 1.0	T		2.6 ± 0.5	82.1 ± 0.8
	43	4.5 ± 0.5	0.2 ± 0.2		T	6.2 ± 0.9	5.4 ± 0.5	83.3 ± 1.7
Digalactosyl diglyceride								
Death Valley	23	8.7 ± 2.2		2.1 ± 1.0		4.2 ± 1.2	4.6 ± 3.4	80.4 ± 1.5
	43	19.6 ± 2.8	T		1.9 ± 1.6	7.7 ± 3.3	8.1 ± 1.4	62.4 ± 5.1
Ventura	23	8.1 ± 0.9		2.1 ± 1.0	0.3 ± 0.1	4.2 ± 1.0	3.0 ± 0.3	82.1 ± 2.0
	43	16.6 ± 2.8			1.3 ± 0.9	5.0 ± 1.5	8.0 ± 1.5	68.8 ± 3.5
Sulfolipid								
Death Valley	23	30.3 ± 1.4			0.7 ± 0.3	5.1 ± 1.8	15.6 ± 2.7	48.0 ± 0.9
	43	35.7 ± 2.0	0.3 ± 0.2		1.6 ± 1.2	10.9 ± 1.3	16.3 ± 1.4	35.3 ± 2.3
Ventura	23	33.6 ± 0.6			3.2 ± 3.1	10.3 ± 0.8	11.5 ± 0.4	41.2 ± 4.0
	43	34.6 ± 4.0			3.0 ± 1.9	10.7 ± 0.8	19.5 ± 5.6	32.2 ± 3.8
Phosphatidyl glycerol								
Death Valley	23	24.9 ± 2.9	17.8 ± 1.4			11.7 ± 3.1	19.9 ± 2.0	25.4 ± 1.5
	43	37.5 ± 3.3	11.4 ± 4.5		3.2 ± 0.9	15.8 ± 4.4	16.7 ± 4.3	15.0 ± 1.2
Ventura	23	42.2 ± 1.3	9.3 ± 2.1			6.4 ± 1.8	17.0 ± 0.6	25.0 ± 1.5
	43	40.1 ± 2.7	10.5 ± 1.8		2.6 ± 1.6	13.9 ± 2.4	16.3 ± 1.2	16.4 ± 1.2

[1] Means and standard errors of 3 to 5 samples carried through all preparative procedures, T indicates trace amounts.

RESULTS

The fatty acid compositions (Table I) were generally similar to those reported previously for lipids from spinach and other higher plants (1, 6, 10). Growth temperature, however, had a consistent effect on the quantitative composition with greater amounts of more saturated lipids occurring at the higher growth temperatures. One qualitative difference was apparent in MGDG and DGDG where hexadecatrienoic acid (16:3) was present at the low but not at the high growth temperature regime. This highly unsaturated acid has been reported to be present in MGDG and to a lesser extent in DGDG from several species (1, 6) but is apparently absent in others (10). Growth temperature appears to be an important determinant of its presence or absence in *A. lentiformis*.

For lipids other than MGDG, the major effect of increased growth temperature on fatty acid composition was a decrease of 10 to 20% in linolenic acid (18:3) with resulting increases in the other more saturated fatty acids. However, because of the lower proportions of 18:3 in PG and SL the decrease was greatest in these lipids. For MGDG, growth temperature had little effect on 18:3 content so that the presence or absence of 16:3 was the major factor influencing the degree of saturation.

Changes in the amount of each lipid would also have a marked influence on the degree of saturation of the membrane because of the large differences in fatty acid composition. In the Death Valley plants, relative concentrations of MGDG, DGDG, and SL were the same in both growth regimes (Table II). For the Ventura plants, SL increased and MGDG decreased slightly in the high as compared to the low temperature regime but, this had only a slight effect on average saturation of the membrane lipids. Lipid concentrations of Chl basis were slightly higher in the high temperature-grown plants. Concentrations of PG were not determined but no differences in the weight of the total phospholipid fraction to Chl ratio or in the appearance of the PG spot on the thin layer chromatogram were present. Thus, it is unlikely that the results for PG would be significantly different.

Table II. Effect of growth temperature on concentrations of leaf lipids in Atriplex lentiformis.

Location	Growth temperature	Lipid MGDG	DGDG	SL
	C	µmol/mg Chl		
Death Valley	23/18	3.96(.65)	1.78(.29)	0.36(.06)
	43/30	5.31(.62)	2.67(.31)	0.59(.07)
Ventura	23/18	4.09(.71)	1.42(.25)	0.24(.04)
	43/30	4.76(.63)	1.91(.26)	0.87(.11)

[1] Mean of three determinations. Numbers in parentheses are the relative amounts.

DISCUSSION

Fatty acid compositions were determined on lipids extracted from leaves rather than from chloroplasts since this required much less tissue and leaves comparable to those used in the photosynthesis experiments (15) could be selected from the limited number of plants accommodated in the growth chamber, eliminating age effects (9). Rapid extraction of leaf lipids avoided possible losses due to oxidation or lipase action during chloroplast isolation procedures. Since large fractions (75–90%) of the MGDG, DGDG, SL, and PG in leaves are chloroplast in origin (13) it is unlikely that the differences observed could be explained by changes in the nonchloroplast fraction.

Growth of *A. lentiformis* at high temperatures results in moderate increases in saturation of chloroplast lipid fatty acids which correlates with the increased thermal stability of PSII. Although individual lipid classes differ somewhat in the degree of saturation, the changes seen here are comparable to differences present between a high temperature-adapted plant, *Tidestromia oblongifolia*, and two cool temperature-adapted *Atriplex* species (3). Greater lipid saturation might be expected to confer thermal stability to membranes because of higher melting temperatures. The simplest model for this would be increased temperatures of phase transitions. While phase transitions in chloroplasts have been reported over the range of 15 to 28 C in a chilling-sensitive plant (16) there is no evidence for any extending to 35 to 45 C where thermal inactivation of photosynthesis occurs in *A. lentiformis*. Furthermore, the high proportion of 18:3 even in the high temperature-grown plants would appear to rule them out at these temperatures. If lipids are at all important in high temperature stability of photosynthesis and not just an independent response to growth temperature, then clearly more complex mechanisms must be involved. The MGDG and DGDG have been postulated to be components of the fluid bilayer with SL and PG functioning as protein boundary lipids (2). The latter are much less saturated and show relatively greater changes in 18:3 in response to growth temperature. Possibly as suggested by Björkman *et al.* (3) these lipids are somehow specifically involved in the thermal stability of chloroplast membranes.

The essentially similar changes in fatty acid composition of plants from the coast and desert at first seemed surprising in view of the lower and much less variable temperatures in the former habitat. However, the coastal plants also appear to have a substantial capacity for adjustment of PSII thermal stability (Pearcy, unpublished data). It remains to be seen whether plants that have a low capacity for adjustment of the thermal stability of photosyn-

thesis also show small changes in membrane fatty acid composition.

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