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Fatty-acid composition of polar lipids in fruit and leaf chloroplasts of "16:3"- and "18:3"-plant species

B.D. Whitaker

U.S. Department of Agriculture, Agricultural Research Service, Horticultural Crops Quality Laboratory, Beltsville Agricultural Research Center-West, Beltsville, MD 20705, USA

Abstract. The fatty-acid composition of polar lipids from fruit and leaf chloroplasts was compared in five Solanaceous and two cucurbit species. The acylated fatty acids in monogalactosyl diglycerides (MGDG) from leaf chloroplasts of all five Solanaceous species included substantial amounts of $\Delta^{7,10,13}$-hexadecatrienoic acid (16:3). In contrast, the MGDG from fruit chloroplasts of the Solanaceae contained very little of this plastid-specific polyunsaturate, and instead included a proportionately greater percentage of linoleic acid (18:2). In MGDG from leaf chloroplasts of two cucurbits, α-linolenic acid (18:3) constituted 94–95% of the acylated fatty acids. Fruit-chloroplast galactolipids of the cucurbits had a greater abundance of 18:2, and hence a higher 18:2/18:3 ratio, than found in the corresponding leaf lipids. Among the phosphoglycerides, the unusual fatty acid Δ3-*trans*-hexadecenoate (*trans*-16:1) constituted from 15 to 24% of the acylated fatty acids in phosphatidyl glycerol (PG) from leaf chloroplasts (all species). In sharp contrast, *trans*-16:1 was virtually absent in PG from fruit chloroplasts of both Solanaceous and cucurbit species, and was replaced by a proportionate increase in the content of palmitate (16:0). The observed differences in the polar lipid fatty-acid composition of fruit and leaf chloroplasts are discussed in terms of the relative activity of several intrachloroplastic enzymes involved in lipid synthesis and fatty-acyl desaturation.

Key words: Chloroplast (lipids) – Fruit (lipids) – Galactolipid – Phospholipid – Leaf (lipids) – Lipid (desaturation).

Abbreviations: MGDG = monogalactosyl diglyceride; DGDG = digalactosyl diglyceride; PC = phosphatidyl choline; PE = phosphatidyl ethanolamine; PG = phosphatidyl glycerol

Introduction

During work undertaken to investigate changes in membrane-lipid composition and metabolism associated with ripening and chilling injury in the tomato fruit, I noted striking differences in the fatty-acid composition of specific polar lipids from chloroplasts of tomato fruit as compared to those from tomato leaves. These findings prompted a survey of several other Solanaceous species. Among the Solanaceae, the consistent differences in chloroplast-lipid fatty-acid composition of fruits and leaves involved C-16 unsaturated fatty acids at the 2-position of galactolipids and phosphatidyl glycerol. In the interest of comparison, analyses of fruit and leaf chloroplast lipids from two species of Cucurbitaceae were also performed, as members of this family do not synthesize galactolipids with hexadecatrienoic acid (16:3) at C-2 (Roughan 1970), but do synthesize PG with Δ3-*trans*-hexadecenoic acid (*trans*-16:1) at C-2 (Murata 1983). The results of these combined studies, reported here, are taken to indicate that the level or activity of one or more enzymes involved in polar-lipid synthesis and-or fatty-acyl desaturation are reduced in chloroplasts from fruit tissues.

Materials and methods[1]

Fruits and leaves of *Capsicum annuum* L. (bell pepper), *Lycopersicon esculentum* Mill. (tomato) and *Cucurbita pepo* L. (zucchini squash) were harvested from plants grown locally in field plots. Fruits and leaves of *Cucumis sativus* L. (cucumber) were taken from plants grown in a local greenhouse. Fruits and

[1] Use of a company or product name by the U.S. Department of Agriculture does not imply approval or recommendation of the product to the exclusion of others which may also be suitable

leaves of the Solanaceous weed species *Solanum dulcamara* L. (woody night shade), *Solanum carolinense* L. (horse nettle) and *Physalis subglabrata* Mackenz. et Bush (ground cherry) were gathered from plants found in fields in the Beltsville area. Harvested fruits were selected for uniform size and maturity. The Solanaceous fruits ranged from late immature to mature green (based on size and seed development); the cucurbit fruits were young, ranging from 15 to 20 cm in length, with a dark-green epidermis. For all species, young, fully expanded leaves were selected.

A crude chloroplast fraction was prepared from leaves of all species prior to lipid extraction. Midribs were first excised from leaves or leaflets; the larger cucurbit leaves were then cut into smaller pieces. The leaf laminae (approx. 3–5 g FW) were immersed and allowed to equilibrate in 120 ml of cold (4° C) Tricine-sorbitol buffer (0.1 M N-tris(hydroxymethyl) methyl-glycine-KOH, pH 7.8, containing 0.33 M D-sorbitol) (Sigma Chemical Co., St. Louis, Mo., USA) prior to homogenization in a Waring blender at low speed for 5 s. The homogenate was filtered through one layer of Miracloth (Calbiochem, San Diego, Cal., USA) and the filtrate centrifuged at 200 *g* for 45 s. The supernatant was distributed in 30-ml Corex glass (Corning Glass Works, Corning, N.Y., USA) centrifuge tubes and centrifuged at 5000 *g* for 5 min. The supernatant was discarded, and the crude chloroplast pellet extracted immediately with hot *iso*propanol followed by chloroform-methanol (2:1, v/v).

Essentially the same procedure was used in the isolation of fruit chloroplasts, with the following exceptions: 1) pericarp tissue from pepper and tomato fruit (80–100 g FW) was diced, immersed in cold Tricine-sorbitol buffer, and gently homogenized with a mortar and pestle prior to filtration; and 2) skins of cucumber and zucchini fruit were peeled in thin strips with a potato peeler to a depth of 2–3 mm. The strips (8–10 g FW) were cut into small pieces, equilibrated in Tricine-sorbitol buffer, and homogenized in culture tubes, 25 mm diameter, 150 mm long, with two 5-s bursts of a Kinematica polytron (Brinkmann Instruments, Westbury, N.Y., USA) tissue homogenizer. Berries of the three Solanaceous weed species were too small for isolation of a crude chloroplast fraction and hence lipids were extracted from whole or quartered fruit by boiling in *iso*propanol followed by grinding with a mortar and pestle in chloroform-methanol (2:1, v/v).

All solvents used in the lipid work-ups were high-performance liquid chromatography (HPLC) grade. Combined *iso*propanol and chloroform-methanol lipid extracts from chloroplasts or fruit tissue were washed by the procedure of Folch et al. (1957) in sealed tubes under N_2. Following centrifugation, the chloroform phase, which contained the total lipids, was dried under a stream of N_2 and redissolved in 2 ml chloroform. Total lipids were separated into neutral, glyco- and phospholipid fractions by silicic-acid column chromatography on 100–200 mesh Bio-Sil A (Bio-Rad Laboratories, Richmond, Ca., USA). The glycolipid and phospholipid fractions were further separated by thin-layer chromatography (TLC) on 20·20 cm^2 glass plates precoated with a 0.25-mm thickness of silica gel 60 (EM Reagents, Darmstadt, FRG) in solvent systems consisting of acetone-acetic acid-water (100:2:1, by vol.) and chloroform-methanol-acetic acid-water (85:15:10:3.5, by vol.), respectively. Individual galactolipids and phospholipds were identified by co-chromatography with authentic standards (Sigma Chemical Co., St. Louis, Mo., USA and Supelco, Bellefonte, Pa., USA), and by detection with spray reagents specific for hexose sugars (Christie 1973) or phosphate (Dittmer and Lester 1964). After drying under N_2, the TLC plates were sprayed with distilled water. Individual lipid bands were then scraped and eluted in chloroform-methanol (2:1, v/v) followed by a Folch wash (Folch et al. 1957). For transesterification, the individual polar-lipid fractions were dried under N_2 in screw-cap culture tubes, then redissolved in a few drops of chloroform followed by addition of 0.5 ml 0.6 N KOH in dry methanol. The tubes were flushed with N_2, tightly sealed, and placed on a rotary shaker for 2 h at room temperature. Following additions of 0.5 ml distilled water and 50 µl 6 N HCl, fatty-acyl methyl esters (FAMEs) were recovered by extraction with 2 ml hexane (Christie 1973). Analysis of the FAMEs was performed by gas-liquid chromatography (GLC) using a Hewlett-Packard (Palo Alto, Cal., USA) model 5710A gas chromatograph in tandem with an HP 3380A integrator. The FAMEs were separated on a glass coil column (1.8 m in length, 2 mm inner diameter, 6.4 mm outer diameter) packed with 10% SP2330 on chromosorb 100/120 (Supelco). Oven, injector and detector temperatures were, in this order, 190°, 250° and 300° C, with an N_2 flow rate of 30 $ml \cdot min^{-1}$. Individual FAMEs were identified by comparison of retention times with those of authentic standards (Supelco). This tentative identification of the major polar lipid fatty acids was corroborated by further analysis of the FAMEs by gas chromatography-mass spectrometry (GC-MS) using a Hewlett-Packard (Palo Alto, Cal., USA) model 5800 capillary GC, an HP 5790A mass-selective detector, and an HP 9825B data terminal.

Results

Galactolipid fatty acids in chloroplasts from the Solanaceae. Table 1 presents the fatty-acid composition of mono- and digalactosyl diglycerides (MGDG and DGDG, respectively) from fruit and leaf chloroplasts of the five Solanaceous species studied. The fatty-acid profiles of MGDG from fruits and leaves of the five Solanaceae are similar, particularly in the percentage of the predominant fatty acid 18:3 ($\Delta^{9,12,15}$). However, there is a striking difference in the proportion of 16:3 ($\Delta^{7,10,13}$) in MGDG from plastids of the two plant organs. While this hexadecatrienoic acid constituted between 8.5% and 19.2% of the total fatty acids in MGDG from Solanaceous leaves, it was undetectable in the MGDG from fruits of three species, and constituted a maximum of 5.5% in MGDG from fruit of *Solanum dulcamara*. The reduced percentage of 16:3 in MGDG of chloroplasts from Solanaceous fruits was largely offset by an increase in the level of 18:2 ($\Delta^{9,12}$). A small amount of 16:3 (approx. 1–2%) was also detected in the digalactosyl diglycerides from leaf chloroplasts of each Solanaceous species, while this polyunsaturate was not detectable in the corresponding DGDG of fruit plastids. Other differences in the fatty-acid profiles of DGDG from fruit versus leaf chloroplasts included decreases in the percentages of 16:0 and 18:3, offset by increases in the proportions of 18:0, 18:1 and 18:2.

Phospholipid fatty acids in chloroplasts from the Solanaceae. Table 2 presents the fatty-acid composi-

Table 1. Galactolipid fatty acids in fruit and leaf chloroplasts from five Solanaceous species. Values are expressed as the percent of total fatty acids in the individual galactolipids

Fatty acids	*Capsicum annuum*		*Lycopersicon esculentum*		*Solanum dulcamara*		*Solanum carolinense*		*Physalis subglabrata*	
	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit[a]	Leaf	Fruit[a]	Leaf	Fruit[a]
MGDG										
16:0	2.8	3.4	4.0	6.7	2.2	2.8	1.9	3.1	1.9	3.0
16:1	0.5	0.1	0.1	0.1	0.3	–	0.5	0.1	0.2	1.2
16:2	1.5	–	0.2	–	0.8	0.2	1.7	–	1.0	0.2
16:3	11.4	0.1	19.2	2.3	18.6	5.5	13.5	–	8.5	–
18:0	0.2	1.1	0.5	1.0	–	0.1	0.1	2.2	0.1	0.3
18:1	0.1	0.2	0.1	0.4	–	1.0	0.3	4.0	0.4	12.4
18:2	8.9	20.5	2.4	12.9	3.3	8.9	7.4	14.5	3.4	10.3
18:3	74.6	74.6	73.5	76.6	74.8	81.5	74.5	76.1	84.5	72.5
DGDG										
16:0	15.0	15.4	19.6	16.8	20.5	15.1	15.8	8.6	15.9	10.5
16:1	–	–	0.1	0.1	0.4	0.1	0.6	0.1	0.2	1.2
16:2	0.6	–	0.1	–	0.1	–	0.8	–	0.4	–
16:3	1.4	–	1.6	–	2.1	–	2.0	–	1.0	–
18:0	2.0	8.2	2.1	3.1	2.0	2.8	2.4	10.4	1.1	1.9
18:1	0.6	0.9	0.4	1.4	0.5	4.0	0.9	4.0	1.3	10.9
18:2	8.8	22.8	6.7	17.4	7.7	14.2	9.7	17.2	4.7	12.4
18:3	71.6	52.7	69.4	61.2	66.7	63.8	67.89	59.7	75.4	63.0

[a] From lipid extracts of whole fruit

Table 2. Phospholipid fatty acids in fruit and leaf chloroplasts from five Solanaceous species. Values are expressed as the percent of total fatty acids in the individual phospholipids

Fatty acids	*Capsicum annuum*		*Lycopersicon esculentum*		*Solanum dulcamara*		*Solanum carolinense*		*Physalis subglabrata*	
	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit[a]	Leaf	Fruit[a]	Leaf	Fruit[a]
PG										
16:0	19.6	53.0	16.8	47.9	14.6	44.3	34.8	38.7	23.3	44.4
t-16:1(3)	17.3	–	15.9	–	15.0	0.6	16.7	–	21.6	0.3
c-16:1(3)?	2.6	–	2.7	–	3.6	–	1.3	–	0.8	–
18:0	1.2	5.2	0.3	2.8	1.6	1.4	2.2	5.5	0.1	0.1
18:1	16.8	9.2	7.9	6.0	9.9	9.5	5.8	7.5	11.9	25.2
18:2	29.3	25.9	35.5	25.8	41.5	26.4	26.2	33.1	27.8	17.6
18:3	13.2	6.8	20.9	17.5	13.8	17.8	13.0	15.2	14.5	12.4
PC										
16:0	23.1	23.6	31.4	27.9	16.0	15.7	20.6	16.2	30.4	12.8
16:1	–	–	0.2	0.1	0.1	0.1	0.1	0.2	–	1.3
18:0	6.2	9.2	0.1	2.1	3.0	2.9	7.8	9.7	0.8	1.7
18:1	10.3	2.2	1.7	4.0	10.7	10.7	9.4	12.0	9.8	29.0
18:2	45.6	56.0	48.4	56.3	38.5	38.6	43.3	50.8	22.6	28.8
18:3	14.8	9.0	18.2	9.6	31.7	32.0	18.8	11.0	36.4	26.4
PE[b]										
16:0	20.7	20.3	28.8	26.3	19.9	17.8	12.7	18.0	15.9	16.5
16:1	–	–	0.1	0.1	0.1	0.1	0.1	0.2	0.2	1.2
18:0	4.3	7.3	0.2	2.2	1.0	1.9	6.8	9.0	1.0	1.3
18:1	2.4	1.3	0.4	2.8	4.8	6.5	1.7	7.0	9.3	18.8
18:2	60.3	65.7	54.7	60.3	45.4	43.9	58.8	55.1	44.1	33.0
18:3	12.3	5.4	15.8	8.3	28.8	29.8	19.9	10.7	28.5	29.2

[a] From lipid extracts of whole fruit
[b] Predominantly an extrachloroplastic phospholipid

Table 3. Galactolipid fatty acids in fruit and leaf chloroplasts from two cucurbit species. Values are expressed as the percent of total fatty acids in the individual galactolipids

Fatty acids	*Cucurbita pepo*		*Cucumis sativus*	
	Leaf	Fruit	Leaf	Fruit
MGDG				
16:0	2.9	2.0	1.0	1.6
16:1	0.4	0.1	0.4	0.3
18:0	0.5	0.3	0.1	0.3
18:1	0.2	0.3	0.8	1.2
18:2	2.1	7.0	2.5	20.4
18:3	93.9	90.3	95.2	76.2
DGDG				
16:0	15.7	11.1	9.5	9.1
16:1	–	–	0.5	0.2
18:0	2.7	2.7	0.9	1.5
18:1	0.6	1.0	1.2	1.6
18:2	1.8	7.8	2.3	12.8
18:3	79.3	77.4	85.6	74.8

Table 4. Phospholipid fatty acids in fruit and leaf chloroplasts from two cucurbit species. Values are expressed as the percent of total fatty acids in the individual phospholipids

Fatty acids	*Cucurbita pepo*		*Cucumis sativus*	
	Leaf	Fruit	Leaf	Fruit
PG				
16:0	43.3	62.8	30.0	47.4
t-16:1(3)	23.7	–	19.4	–
c-16:1(3) ?	1.5	–	1.5	–
18:0	5.7	4.0	12.0	11.3
18:1	8.8	10.5	19.0	19.0
18:2	4.6	8.8	5.0	12.7
18:3	12.4	13.9	13.1	9.6
PC				
16:0	33.8	23.4	27.2	36.9
16:1	0.2	–	0.1	–
18:0	4.0	2.6	6.9	3.6
18:1	1.9	1.5	1.1	5.0
18:2	7.8	24.4	15.8	36.7
18:3	52.3	48.1	48.9	17.8
PE[a]				
16:0	44.5	33.6	30.0	32.7
16:1	0.1	–	0.1	0.1
18:0	0.8	0.5	3.1	1.2
18:1	1.6	0.5	1.2	2.4
18:2	10.5	28.5	21.4	47.5
18:3	42.5	36.9	44.2	16.1

[a] Predominantly an extrachloroplastic phospholipid

tion of phosphatidyl glycerol (PG), phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) from fruits and leaves of the Solanaceae studied. As PE represents, at most, a very small percentage of chloroplast membrane phospholipid (Kirk and Tilney-Bassett 1978, p. 56), it presumably was derived from other cell membranes. In crude chloroplast preparations from leaves it accounted for $\leq 10\%$ of the total phospholipid, while in lipid extracts of fruit chloroplasts or whole fruits the percentage of PE was ≥ 25 (Table 5). Of the three phosphoglycerides analyzed, the most dramatic difference was observed in PG of fruit versus leaf chloroplasts. The chloroplast-specific fatty acid Δ 3-*trans*-hexadecenoate constituted from 15.0 to 21.6% of the total in PG from leaves of the five Solanaceous species, but was virtually absent ($\leq 0.6\%$) in the corresponding fruit PGs. The absence of *trans*-16:1 in fruit PG was more than compensated for by an increased percentage of 16:0. Other comparative differences in the proportions of the C-18 fatty acids were noted in PG, but these were not consistent among the five species. In both PC and PE, the extent of fatty-acid unsaturation was consistently less in fruit compared to leaf phosphoglycerides with the exception of *S. dulcamara*, in which the fatty-acid profiles of fruit and leaf PC and PE were remarkably similar. This was largely the result of differing proportions of the C-18 fatty acids. In particular, the ratio 18:2/18:3 was consistently higher in fruit compared to leaf PC and PE.

Galactolipid fatty acids in chloroplasts from the Cucurbitaceae. The fatty-acid compositions of galactolipids from fruit and leaf chloroplasts of two species of Cucurbitaceae are shown in Table 3. As anticipated, neither fruit nor leaf galactolipids of the cucurbits included the fatty acid 16:3 ($\Delta^{7,10,13}$). Fatty-acid profiles of both MGDG and DGDG from fruit versus leaf chloroplasts of *Cucurbita pepo* were quite similar, with a slightly higher 18:2/18:3 ratio, and a slightly lower percentage of 16:0, in the fruit galactolipids. In *cucumis sativus*, the increased ratio of 18:2/18:3 in fruit- compared to leaf-plastid galactolipids was far more dramatic, with no significant difference in the level of 16:0.

Phospholipid fatty acids in chloroplasts from the Cucurbitaceae. Fatty-acid compositions of the phosphoglycerides PG, PC, and PE from fruits and leaves of the cucurbits are shown in Table 4. As observed in the Solanaceae, *trans*-16:1 (Δ^3) constituted a major portion of the acylated fatty acids in PG from leaf chloroplasts (19.4–23.7%), but was totally absent in PG from fruit chloroplasts. The lack of 16:1 in cucurbit fruit-plastid PG was

Table 5. Polar-lipid content of crude chloroplast preparations from leaves and fruits of tomato and bell pepper. Individual polar lipids were separated by thin-layer chromatography (see *Materials and methods* for details). Galactolipids and phospholipids were quantified by the spectrophotometric assays of Roughan and Batt (1968) and Ames (1966), respectively. Total chlorophyll (Chl) and the Chl a/b ratio were measured spectrophotometrically by the method of Inskeep and Bloom (1985). Values represent the mean ± 1 SD.

Polar lipid (μmol·(mg Chl)$^{-1}$)	*Lycopersicon esculentum*		*Capsicum annuum*	
	Leaf ($n=4$)	Fruit ($n=5$)	Leaf ($n=4$)	Fruit ($n=3$)
MGDG	2.40±0.24	3.54±0.42	2.06±0.19	2.93±0.31
DGDG	1.23±0.10	2.74±0.33	0.96±0.08	2.14±0.24
PC	0.32±0.04	2.83±0.34	0.28±0.05	1.28±0.18
PG	0.29±0.03	0.48±0.12	0.26±0.02	0.28±0.07
PE	0.08±0.02	2.68±0.41	0.05±0.03	0.69±0.10
Lipid ratios (molar)				
Chl a/b	3.11±0.14	2.82±0.11	3.10±0.05	2.52±0.17
MGDG/DGDG	1.95±0.15	1.29±0.11	2.14±0.07	1.37±0.10
MGDG/PG	8.28±0.73	7.38±0.81	7.92±0.65	10.46±0.98

offset by a proportionate increase in the percentage of 16:0. An increase in the ratio 18:2/18:3 was also noted in fruit compared with leaf PG of *C. pepo* and *C. sativus*. Differences in the fatty-acid profiles of both PC and PE from fruits versus leaves of the two cucurbits were similar to, but more dramatic than, those observed in the galactolipids. Hence, in *C. pepo*, a large increase in 18:2 was balanced by decreases in 16:0 and 18:3 in fruit- compared with leaf-PC and PE. In *C. sativus*, the 18:2/18:3 ratio was much higher in both PC and PE of fruits versus leaves.

Proportions of polar lipids in chloroplasts from the Solanaceae. Table 5 presents data on the relative amounts of the major galactolipids (MGDG, DGDG) and phospholipids (PC, PG, PE) in the crude chloroplast preparations from leaves and fruits of tomato and bell pepper. Based on total chlorophyll, fruit chloroplasts contained roughly 70% more galactolipid than the corresponding leaf chloroplasts. In addition, both the MGDG/DGDG and chlorophyll a/b ratios were appreciably lower in fruit compared with leaf chloroplasts. The amount of PE (and to a lesser extent, PC) per milligram of chlorophyll was much greater in the crude chloroplast preparations from fruits compared with those from leaves of the Solanaceae, indicating the probability that the fruit-plastid preparations were heavily contaminated with other organelles and cell membranes. On a chlorophyll basis, the level of PG was approx. 65% higher in fruit versus leaf chloroplasts of tomato, but roughly equal in fruit and leaf chloroplasts of pepper. Conversely, the proportion of PG relative to MGDG was roughly the same in fruit and leaf plastids of tomato, but significantly lower in fruit versus leaf plastids of pepper.

Discussion

It is generally accepted that two pathways are involved in synthesis of the diacylglycerol precursors of chloroplast-membrane galactolipids (Heinz and Roughan 1982; Williams et al. 1983). In the extrachloroplastic ("eukaryotic") pathway, molecular species of phosphatidylcholine produced in the microsomes, composed mainly of 18:2 and 18:3 at both the C-1 and C-2 positions, serve as precursors of MGDG synthesis in the chloroplast (Williams et al. 1983; Roughan 1975). In the second, intrachloroplastic ("prokaryotic") pathway, galactolipid synthesis begins with the assembly of predominantly 1-oleoyl-2-palmitoyl-phosphatitidic acid (Sauer and Heise 1982). This PA is cleaved by a phosphatase, yielding diacylglycerol which is subsequently galactosylated to form MGDG (Frentzen et al. 1982). The galactolipid products of the extrachloroplastic pathway are already highly unsaturated, requiring only one final desaturation step to yield the predominant dilinolenoyl MGDG species (Williams et al. 1983). In contrast, MGDG produced by the intrachloroplastic pathway requires five additional desaturation steps to yield the 18:3/16:3 species (Siebertz et al. 1980). It is the relative contribution of these two pathways to the MGDG pool which establishes the fatty-acid composition of MGDG in a given plant species (Roughan 1975). Thus, if solely the extrachloroplastic pathway is utilized the plant is

classified as an "18:3" species, whereas if the intrachloroplastic pathway contributes significantly the classification is "16:3" species. Among the angiosperms, a wide variation in the percentage of 16:3 in MGDG has been observed (Jamieson and Reid 1971), and this variation is thought to reflect the relative activity of enzymes involved in the two separate biosynthetic pathways (Roughan 1975).

Given the above information, the data from this study indicate that the contribution of the intrachloroplastic pathway to galactolipid biosynthesis in chloroplasts from fruit of the Solanaceae, a "16:3" family, is greatly diminished. The simplest explanation for a reduction in galactolipid synthesis via the intrachloroplastic pathway in Solanaceous fruits would be a decrease in the synthesis of the "prokaryotic" PA precursor of MGDG. If this were the case, one might also expect decreases in the levels of PG and sulfolipid (SQDG), as a recent study has shown that all of the PG and much of the SQDG in leaf chloroplasts of tobacco and spinach (both 16:3 species) is produced via the prokaryotic pathway (Bishop et al. 1985). Unfortunately, the data in Table 5 on the relative level of PG in leaf and fruit chloroplasts of tomato and bell pepper must be regarded as inconclusive in light of the obvious contamination of the crude fruit-plastid preparations with other cell membranes. However, the data on the levels of MGDG, DGDG and chlorophyll support the conclusion that the ratio of envelope to thylakoid membranes is much greater in fruit compared with leaf chloroplasts, and hence the amount of chlorophyll, MGDG and PG per plastid should be less in fruits versus leaves. To obtain a satisfactory answer to the question of the relative abundance of "prokaryotic" polar lipids in fruit and leaf chloroplasts of Solanaceous species, it will be necessary to work with highly purified preparations of intact plastids and-or thylakoids.

It has also been shown in this study that PG from fruit chloroplasts of several 16:3- and 18:3-plant species essentially lacks the unusual fatty acid Δ^3-*trans*-hexadecenoate, which is a major constituent in the corresponding leaf chloroplast PGs. The sum of 16:0 + *trans*-16:1 in PG isolated from leaf chloroplasts of Solanaceous and cucurbit species, calculated from the data in Tables 2 and 4, is somewhat low in comparison with previously reported values (Murata et al. 1982; Roughan 1985). This is probably a consequence of low-level contamination with DGDG, some of which eluted with the phospholipid fraction during silicic-acid column chromatography and had an Rf close to that of PG in the thin-layer-chromatography system used. Despite this, it is clear from the data that fruit plastids of the Solanaceous and cucurbit species studied performed very little post-synthetic desaturation of 16:0 at C-2 of PG, leading to the conclusion that the desaturase enzyme involved is either inactive or present at much reduced levels.

Addendum: After this paper had been submitted and was under review a related, independent study by J.R. Kenrick and D.G. Bishop, "Phosphatidylglycerol and sulphoquinovosyldiacylglycerol in leaves and fruits of chilling-sensitive plants", was published in Phytochemistry (vol. 25, No. 6, pp. 1293–1295, 1986), reporting on analyses of the fatty-acid composition of PG from leaves and fruits of five chilling-sensitive plants. Among these were two Solanaceous species (tomato and bell pepper) and one cucurbit species (cucumber) also used in my investigation. The methodology used by Kenrick and Bishop differed from mine in the use of leaf and fruit tissue rather than crude chloroplast preparations, and also in the column fractionation and derivatization of the lipids. Methods shared by the two studies included use of leaves and fruits from the same plant and use of the same tissues from Solanaceous and cucurbit fruits (pericarp including the epidermis from tomato and bell pepper; thin epidermal strips from cucumber). Kenrick and Bishop's results on the level of *trans*-16:1 in PG from leaves and fruits of these species were closely parallel with mine, i.e., they found that while *trans*-16:1 constituted a major portion of the fatty acids in PG from leaf tissue, it was virtually absent ($\leq 2\%$) in PG from green fruit tissue. They concluded (as I have) that the reduced level of *trans*-16:1(3) in fruits may be the consequence of a deficiency of the enzyme which performs the post-synthetic desaturation of 16:0 to *trans*-16:1(3) at C-2 of PG.

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