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POLAR LIPID COMPOSITION OF LEAVES FROM NINE TYPICAL ALPINE SPECIES

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Abstract—The fatty acids and polar lipid compositions of leaves from nine alpine species were almost identical to that of plants growing in habitats with little seasonal variation in temperature. Furthermore each polar lipid had about the same fatty acid composition in all plant species studied. It is suggested that neither the relative proportions of different lipid classes nor the degree of saturation of individual classes are directly implicated in the adaptation of plant tissues to different climates.

INTRODUCTION

Temperature is prominent among the ecological variables that determine the natural distribution of higher plants [1, 2]. For example, many plants develop lesions (chilling injury) when exposed to temperatures at and below critical temperature [3, 4]. Since fluidity within the lipid bilayer is a central feature of the mosaic model of a biological membrane and maintaining this fluidity is essential for life processes [5], there have been several reports invoking changes in fatty acid unsaturation or lipid content in the cold acclimation process (for review see ref. [6]).

One of the important characteristics of leaves is their distinctive polar lipid composition. Unlike non-green tissues such as roots, they are dominated by galactolipids containing polyunsaturated fatty acids rather than phospholipids [7]. There have been many reports of the polar lipid content of a variety of leaves from different plants limited to habitats with little seasonal variations in temperature as well as analyses of the fatty acids associated with each of the major lipid classes [7]. In contrast, the polar lipid content of plants native to habitats characterized by great variations in temperature during growth is almost completely unknown. The present paper reports details of the lipid composition of leaves from various typical alpine species.

RESULTS

The polar lipid composition of leaves from nine alpine species is given in Table 1. The overall lipid compositions of these widely disparate species are remarkably similar. In all cases the uncharged lipids monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and phosphatidylcholine make up more than two-thirds of the polar lipids. The remaining fraction is mostly made up of anionic lipids principally sulphoquinovosyldiacylglycerol, phosphatidylglycerol and phosphatidylethanolamine. In addition, the amount of galactolipids exceeds that of phospholipids. If we consider that galacto-

Table 1. Polar lipid composition of leaves from nine alpine species*

Species	Altitude (m)	Total lipids (mg/g fr. wt)	% by weight MGDG	DGDG	SL	PG	PC	PE	PI	DPG
Elyna spicata	2100	9.6	34.0	34.3	4.6	6.0	12.7	5.4	2.2	0.9
Salix herbacea	2100	11.7	35.9	21.8	4.6	11.8	16.7	6.4	2.8	0.3
Salix retusa	2400	7.1	34.3	22.1	5.3	6.9	20.5	8.4	2.9	0.5
Salix reticulata	2400	6.3	33.5	20.4	5.7	7.8	20.6	7.8	3.2	1.0
Ranunculus glacialis	2700	3.9	37.8	22.8	5.6	11.7	14.0	6.0	2.1	0.3
Androsace helvetica	2800	3.5	26.7	21.3	5.0	4.9	27	9.2	4.6	1.2
Geum reptans	2700	5.9	30.5	22.5	3.7	9.1	20.2	9.8	3.6	0.6
Alchemilla pentaphyllea	2600	8.2	31.5	17.5	2.2	8.4	24.8	9.9	4.8	0.5
Saussurea depressa	2100	5.7	38.5	27.2	6.4	2.8	16	4.4	2.0	0.6
Saussurea depressa	2700	4.5	35.6	28.9	10.9	4.9	11.9	4.8	2.7	0.6

*Abbreviations: MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; SL, sulphoquinovosyldiacylglycerol; PC, phosphatidylcholine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; DPG, diphosphatidylglycerol or cardiolipin.

lipids, sulphoquinovosyldiacylglycerol and phosphatidylglycerol are concentrated in plastid membranes [8], the polar lipid composition in the leaves of alpine species reflects the predominance of chloroplastic membranes. Interestingly, the polar lipid composition of alpine species is almost identical to that of plants of more temperate habitats such as barley [9], sugar beet, maize and rye grass (for review see ref. [7]).

The relative proportions of the major fatty acids of the leaves from alpine species are compared (Table 2) to those of spinach ($C_{16:3}$ plant) and barley ($C_{18:3}$ plant), two typical higher plants. Again, the overall fatty acid compositions of these different species are remarkably similar. The saturated fatty acid palmitic and the unsaturated fatty acids [linoleic, linolenic and hexadecatrienoic ($C_{16:3}$ plant)], together account for almost all of the fatty acid content of the leaves. The most abundant fatty acid in all species was linolenic acid. It is noteworthy that in contrast to animal cells the fatty acid patterns of leaves are relatively simple (for review see ref. [7]).

The fatty acid composition of each polar lipid has been determined in leaves of alpine species and the data are presented in Table 3. Each polar lipid has about the same fatty acid composition in all plant species studied. The small differences observed are the decrease in the proportion of linolenic acid and the increase in the proportion of linoleic acid and *vice versa*. Phosphatidylinositol and phosphatidylglycerol contain higher amounts of palmitic acid than other phospholipids in higher plant leaves. All the galactolipids examined contain a high amount of polyunsaturated fatty acids and the proportion of palmitic acid increases when MGDG and DGDG from the same tissues are compared. Finally, the degree of unsaturation of individual lipid classes is almost identical in both the alpine species and in other plants such as barley [9] and spinach [7].

DISCUSSION

These results demonstrate that fatty acids from mature leaf tissue are remarkably constant from plant to plant (see also ref. [7]) and that the overall distribution of lipids reflects the high content of chloroplasts in the tissue. These results also demonstrate that the fatty acid patterns of the leaves of nine typical alpine species are almost identical with those from plants growing in habitats with little seasonal variations in temperature. Interestingly analysis of the leaves of 13 species of chill-sensitive and chill-resistant plants by Wilson and Crawford [10] showed no correlation between the degree of unsaturation of the fatty acids and resistance. In addition according to Vigh *et al.* [11] changes in linolenic acid levels alone cannot be responsible for adaptation of membrane fluidity of rye and wheat seedlings according to temperature. Likewise Patterson *et al.* [12] have also failed to find any correlation between the fatty acid distribution of polar lipid extracts of the leaves of different chilling-sensitive and chilling-resistant strains of passion fruit. These results indicate therefore that neither the relative proportions of different lipid classes nor the degree of saturation of individual classes are directly implicated in the acclimation of plant tissues to various climates. Finally the sum of C16:0 and C16:1^t from the phosphatidylglycerol of these alpine species ranges between 47 and 53% (Table 3), in the same proportions than that found by Murata *et al.* [13] in higher plants resistant to chilling.

EXPERIMENTAL

Plant material. Expanding leaves were taken from plants of various alpine species: *Elyna spicata* Schrad (Cyperaceae); *Ranunculus glacialis* L. (Ranunculaceae); *Androsace helvetica* (L.) All (Primulaceae); *Geum reptans* L. (Rosaceae); *Alchemilla pentaphyllea* L. (Rosaceae); *Saussurea depressa* (L.) DC. subsp. *depressa* (Gren) Nyman (Compositae); *Salix herbacea* L., (Salicaceae); *Salix reticulata* L.; *Salix retusa* L.

These studies were centred in the north and south slopes of the west part of the Cerces (French Alps) in a high contrasting alpine environment. Principal collecting stations were: (1) The Botanical Garden, Lautaret Pass, 2100 m; (2) intermediate station, 2400 m; (3) Galibier Pass, 2600–2800 m. The length of the growing season at these altitudes is restricted to July and August. The air temperature records averaged over several years are shown in Table 4.

Methods. Leaf aliquots (1–4 g) were fixed by boiling for 5 min in order to destroy phospholipases and lipolytic acylhydrolases [14]. Leaf lipids were then extracted according to Folch *et al.* [15]. In order to remove pigments from the chloroform extracts

Table 2. Fatty acid composition (% by weight) of total lipids from leaves of nine alpine species and of spinach (*Spinacia oleracea*) and barley (*Hordeum vulgare*)

Species	Altitude (m)	$C_{16:0}$	$C_{16:1^t}$	$C_{16:3}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Elyna spicata	2100	12.8	2.5		tr	1.2	7.8	75.7
Salix herbacea	2100	11.5	3.2		tr	1.6	12.0	71.8
Salix retusa	2400	13.8	3.4		0.3	1.3	13.3	67.9
Salix reticulata	2400	14.1	2.8		tr	1.7	16.9	64.4
Ranunculus glacialis	2700	12	4.8	12.9	tr	0.6	12.3	57.4
Androsace helvetica	2800	13.0	2.4		tr	5.3	29.6	49.7
Geum reptans	2700	11.0	4.1		1.3	2.6	8.6	72.4
Alchemilla pentaphyllea	2600	9.5	4.2		1	4.2	17.3	63.8
Saussurea depressa	2100	13.6	1.8		0.1	1.4	17.0	66.1
Saussurea depressa	2700	10	2.5		tr	1	11.2	75.3
Spinacia oleracea		12.9	2.6	9	tr	1	14.5	60
Hordeum vulgare		16	3		1.2	1.7	17.5	60.6

Table 3. Fatty acid composition (% by weight) of the polar lipids from leaves of nine alpine species*

Species		*Elyna spicata*	*Salix herbacea*	*Salix retusa*	*Salix reticulata*	*Ranunculus glacialis*	*Androsace helvetica*	*Geum reptans*	*Alchemilla pentaphyllea*	*Saussurea depressa*	*Saussurea depressa*
Altitude(m)		2100	2100	2400	2400	2700	2800	2700	2600	2100	2700
MGDG	$C_{16:0}$	1.4	1.4	1.2	1.4	1.2	1.4	0.9	0.7	1.1	1.2
	$C_{16:3}$	—	—	—	—	37.6	—	—		—	–
	$C_{18:1}$	0.2	0.1	tr	0.1	—	0.4	0.1	0.2	tr	tr
	$C_{18:2}$	1.5	1.6	1.1	1.2	1.8	9.4	0.9	1.8	3.0	1.4
	$C_{18:3}$	96.9	96.9	97.7	97.3	59.4	88.8	98.1	97.4	95.9	97.4
DGDG	$C_{16:0}$	11.3	9.5	9.1	0.5	13.3	7.9	7.1	4.6	8.0	10.8
	$C_{18:1}$	0.5	0.7	0.6	0.9	0.3	1.6	1	0.7	0.3	0.2
	$C_{18:2}$	2.6	1.5	0.9	1.7	3.3	8.2	0.9	1.3	2.2	2.3
	$C_{18:3}$	85.6	86.8	89.4	86.9	83.1	82.2	91.0	93.4	89.5	86.7
SL	$C_{16:0}$	32.9	33.3	32.3	32.4	34.3	24.9	33.9	26.3	25.9	31.9
	$C_{18:1}$	1.5	1.8	3.1	2.2	1.4	2.8	1.9	2.0	0.9	2.2
	$C_{18:2}$	2.6	3.5	2.5	2.0	1.3	24.0	3.9	6.0	10.8	4.0
	$C_{18:3}$	63.1	61.4	62.2	63.4	63.0	48.3	60.3	65.7	62.4	61.9
PG	$C_{16:0}$	14.5	24.6	24.6	26.6	15.0	24.2	15.0	18.3	25.7	13.4
	$C_{16:1^t}$	32.5	27.2	28.9	25.6	34.2	22.5	32.9	28.9	22.8	35.9
	$C_{18:1}$	4.0	7.7	3.7	4.2	1.7	3.2	1.6	2.7	7.2	5.9
	$C_{18:2}$	13.6	10.7	9.2	8.8	7.6	26.2	15.1	10.4	24.3	15.4
	$C_{18:3}$	34.5	29.7	33.6	34.8	41.5	23.8	35.4	39.7	20.0	29.4
PC	$C_{16:0}$	23	21.6	19.7	20.9	23.3	15.5	19.8	15.3	19.4	20.3
	$C_{18:1}$	1.4	0.7	3.3	1.5	3.4	13.6	5.5	11.0	2.1	2.0
	$C_{18:2}$	23.1	33.1	34.7	43.2	34.6	56.0	20.1	41.3	44.4	46.2
	$C_{18:3}$	52.5	44.6	42.3	34.4	38.7	14.9	54.6	32.4	34.1	31.5
PI	$C_{16:0}$	46.0	44.1	42.8	42.1	47.6	41.8	40.7	31.8	41.0	37.8
	$C_{18:1}$	1.2	0.8	2.1	1.0	1.5	4.3	4.7	8.8	2.4	1.4
	$C_{18:2}$	18.1	15.7	17.9	22.7	20.1	45.4	8.6	28.1	48.0	32.7
	$C_{18:3}$	34.7	39.4	34.5	34.2	30.8	8.5	46.0	31.3	8.6	28.1
PE	$C_{16:0}$	21.6	26.7	22.4	22.0	29.8	24.8	19.0	17.4	20.8	16.4
	$C_{18:1}$	0.4	0.3	2.1	0.8	0.7	3.8	4.5	6.7	1.3	1.4
	$C_{18:2}$	29.3	43.5	17.9	49.9	42.8	60.4	23.0	43.7	50.9	45.9
	$C_{18:3}$	48.7	29.5	37.2	27.4	26.7	11.0	53.5	32.2	27.0	36.4
DPG	$C_{16:0}$	15.2	5.1	11.3	12.5	7.2	5.3	28.1	9.0	23.3	18.8
	$C_{18:1}$	9.2	2.1	8.5	8.1	3.6	3.4	7.6	5.0	5.7	5.9
	$C_{18:2}$	24.5	42.6	38.1	40.8	39.3	65.8	14.0	13.3	40.0	36.6
	$C_{18:3}$	51.1	50.2	42.0	38.5	49.9	25.5	50.3	72.7	31.0	38.6

*See Table 1 for abbreviations used.

Table 4

	Mean daily temperatures (°) Botanical garden (2100 m)		Galibier Pass (2800 m)	
	maximum	minimum	maximum	minimum
June	12.8	1.8	8.5	−1.6
July	17.3	6.4	13.9	2
August	12.7	4.6	9.8	0.8

suitable aliquots (10–20 mg total lipid) were layered on top of a column containing 2 g silicic acid (Biorad; Bio-sil HA, Minus 325 mesh) equilibrated with $CHCl_3$. Pigments were rapidly eluted by washing the column with 200 ml $CHCl_3$. Polar lipids were eluted with 200 ml MeOH–$CHCl_3$ (9:1). Polar lipid extracts were evaporated to dryness under a stream of argon and the lipid residues were redissolved in 1 ml $CHCl_3$.

Individual glycolipids (galactolipids and sulpholipid) and phospholipids were separated from the bulk polar lipid extract by 2D-TLC (silica gel 60 precoated plates, Merck) using $CHCl_3$–MeOH–H_2O (65:25:4) in the first dimension and $CHCl_3$–Me_2CO–MeOH–HOAc–H_2O (10:4:2:2:1) in the second dimension [16]. Lipids for fatty acid analysis were located by spraying with anilinonaphthalene sulphonate (0.2% MeOH) and visualized under UV light (360 nm). Individual polar lipids were identified by their reaction with specific spray reagents and by comparing their R_f values with those of reference standards. Fatty acid methyl esters were made by transesterification of polar lipid fractions at 70° for 2 hr in MeOH–H_2SO_4–C_6H_6 (20:1:1). Methyl esters were extracted with hexane and chromatographed on an Intersmat gas chromatograph (IGC-131) equipped with a hydrogen flame ionization detector and an Intersmat integrator (ICR-1B). Separations were carried out at 150–175° (temp.

program: 0.2° min) using a column with 10% SP-222-PS (a highly polar polyester stationary phase, stabilized with phosphoric acid, Supelco, Inc.) on 100/120 mesh Supelcoport. Quantitative analysis of fatty acids and their parent lipids were made according to ref. [17].

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