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A Study of Phospholipids and Galactolipids in Pollen of Two Lines of *Brassica napus* L. (Rapeseed) with Different Ratios of Linoleic to Linolenic Acid[1]

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ABSTRACT

The phospholipids and galactolipids of the pollen-coat and internal domains of two lines of *Brassica napus*, Wesroona and IXLIN, with different linoleic/linolenic acid ratios (18:2/18:3) have been characterized by normal phase silica high performance liquid chromatography and gas liquid chromatography. The polar lipids of the pollen-coat are similar to leaf lipids in the high proportion of galactolipids (almost 50%) and the fatty acids; 18:3, palmitic (16:0) and hexadecatrienoic (16:3). In contrast, the pollen internal domain, although rich in 18:3, 18:2 and 16:0, is composed primarily of phosphatidyl-choline, -ethanolamine, and -inositol whose 18:2/18:3 ratio is correlated with that of the seed generation. The difference between the two divergent 18:2/18:3 ratio lines is most evident in the internal domain phospholipids. The 18:2/18:3 ratio of the galactolipids of both pollen domains is not significantly effected by the line genotype. The results are interpreted in terms of the previously described 'prokaryotic' and 'eukaryotic' plant desaturation pathways (PG Roughan, CR Slack [1982] Annu Rev Plant Physiol 33: 97–132). We propose that the eukaryotic pathway is the major desaturation pathway providing polyunsaturated fatty acids to the haploid-specified internal domain in which the IXLIN genotype modifies the activity of the *sn*-2 linoleoyl phosphatidylcholine desaturase/s of the endoplasmic reticulum. In the diploid-specified pollen-coat, our evidence suggests that a combination of the prokaryotic and eukaryotic pathways contribute polyunsaturated fatty acids.

Rapeseed pollen lipids can be separated by differential solvent extraction into pollen-coat and internal cytoplasmic domains, whose lipids have very different fatty acid compositions (10, 11). The neutral (storage) and polar (membrane) lipids of the pollen are rich in 18:3[2] and 16:0 which is characteristic of leaf lipids (25). However, when pollen and seed from traditional cultivars and those with high 18:2/18:3 ratios (IXLIN lines) were analyzed, a highly significant correlation was observed between the pollen internal domain and seed for the 18:2/18:3 ratio (11). Two different pathways for fatty acid desaturation have been described in plants; the prokaryotic '16:3' pathway, which confers the ability to desaturate 16:0 to 16:3 and 18:1 to 18:3 in association with MGDG, and the eukaryotic '18:3' pathway, which desaturates only 18:1 to 18:3 in association with PC and MGDG (9, 15, 24, 29).

The prokaryotic pathway is localized to plastids (9, 15, 24, 29) where MGDG is formed by acylation of glycerol-3-phosphate with strong preference being shown for 18:1 in the *sn*-1 position and 16:0 in the *sn*-2 position followed by glycosylation with UDP-galactose (13, 19). Desaturation then occurs at both positions to yield the characteristic *sn*-1 18:3/*sn*-2 16:3 MGDG species (3, 20, 21). Since C_{16} fatty acids are almost totally excluded from the *sn*-2 position of extraplastidial glycerolipids (24) and 16:3 is mainly associated with plastid-bound galactolipids (17, 30), there appears to be little export of the prokaryotic pathway products to the cytoplasm for storage lipid synthesis.

In the eukaryotic pathway the products of plastid fatty acid synthesis, mainly 18:1 and 16:0, are exported as CoA esters (2) to be incorporated into PC in the endoplasmic reticulum (9). At the endoplasmic reticulum, 18:1 PC is desaturated to 18:2 PC in both leaf and developing seed tissue, with the ability for desaturation at both the *sn*-1 and *sn*-2 positions (21, 27). 18:2 PC can then be desaturated at the *sn*-2 position to 18:3 PC, again common to both leaves and developing seed tissue (20, 21, 27). A further desaturation occurs after the transportation of PC to the plastid outer envelope by phospholipid transfer protein (23) where the DAG moiety is used to form MGDG (19), and *sn*-1 18:2 is subsequently desaturated to 18:3 (21). This final desaturation step has only been reported as significant in leaves (18, 23) with very little 18:2 transferred to MGDG in seed homogenates (26). This interpretation of the pathways for polyunsaturated fatty acid biosynthesis is consistent with recent findings (15, 29).

In rapeseed pollen, 16:3 is primarily located in the pollen-coat polar lipids which indicates that the internal lipids are most likely to be synthesized within the pollen grain with most desaturation to 18:3 occurring by the eukaryotic pathway. The 18:2/18:3 ratio correlations suggest that the eukaryotic pathway is also the major pathway in seed (11). In the

[1] We thank the Reserve Bank of Australia Rural Credits Development Fund for financial support.

[2] Abbreviations: 18:3, linolenic; 18:2, linoleic; 16:3, hexadecatrienoic; 16:0, palmitic; 18:1, oleic; PC, phosphatidylcholine; MGDG, monogalactosyldiacylglyceride; CL, cardiolipin; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PG, phosphatidylglycerol; SPH, sphingomyelin; DGDG, digalactosyldiacylglyceride; FAB-MS, fast atom bombardment mass spectrometry; FAME, fatty acid methyl esters; SQDG, sulfoquinovosyldiacylglyceride; 14:0, myristic; 16:1, palmitoleic; 16:2, hexadecadienoic; 18:0, stearic; MAG, monoacylglyceride; DAG, diacylglyceride; TAG, triacylglyceride.

pollen coat, which originates in the tapetum (16), the prokaryotic pathway probably plays a greater role (11).

Which pathway is the major desaturation pathway for 18:3 in pollen? Which region of the pathway is modified by the IXLIN genes? The purpose of this investigation is to suggest answers to these questions by HPLC separation of the polar lipids of the two pollen domains from two rapeseed lines differing in 18:2/18:3 ratio. Their fatty acids, particularly PC and MGDG which are integrally involved in 18:3 synthesis, will be examined by GLC.

MATERIALS AND METHODS

Plant Material

Twenty 4-inch pots of the rapeseed (*Brassica napus*) lines Wesroona (low 18:2/18:3 ratio) and IXLIN (line 81N61, high 18:2/18:3 ratio) were grown in a greenhouse at approximately 20°C with a 16 h d. Pollen was collected over a 7 week period and stored by the method of Evans *et al.* (10).

Lipid Extraction

The pollen-coat and internal lipid domains were extracted with acetone and chloroform/methanol (2:1), respectively, and separated into polar and neutral lipid classes by silicic acid column chromatography, by the method of Evans *et al.* (11). Polar lipids were passed through a Millipore (Teflon, 0.5 μm) filter with chloroform, dried with a stream of nitrogen and then taken up in isopropanol/hexane (3.5:3, 15.5 mg/mL). The filtrate was flushed with nitrogen and stored till use at −20°C. Neutral lipids were taken up in chloroform (1 mL) and stored under the same conditions.

HPLC Separation of Lipid Classes

The analysis was carried out using an ETP Kortec (Melbourne, Australia) HPLC consisting of two K 35D pumps controlled by a K 45 gradient computer and a Rheodyne injector (200 μL injection loop). Polar lipids were separated on a 4.0 × 300 mm column packed with silicic acid (5 μm Spherisorb, column efficiency 99,000 plates/m). Polar lipids (150–300 μg) were injected on to the column and eluted by modifying the procedures of Demandre *et al.* (7) and Christie (6). The elution was performed with a gradient program (Table I) of two solvent mixtures running from 100% solution A, isopropanol/hexane (3.5:3), to 100% solution B, isopropanol/hexane/water/ammonium acetate (8:5.3:1.9; 2.8 mM). The components used were HPLC grade isopropanol (May and Baker, Melbourne), nano grade hexane (Mallinckrodt, Melbourne), Milli Q water (Millipore, Melbourne) and AR grade ammonium acetate (Ajax, Melbourne). The solvent mixtures were degassed in an ultrasonic bath under vacuum. The flow rate was constant at 1 mL/min during the run. Polar lipids were detected at 205 nm using a model K 95 variable wavelength UV detector (ETP Kortec).

Lipid Class Identification

The classes eluted from the HPLC were manually collected for analysis and the solvents evaporated by a stream of nitrogen. Polar lipids were identified on silica gel TLC (Kieselgel 60 G254, Merck), mobile phase chloroform/methanol/water (65:25:4), by comparison with authentic standards purchased from Sigma (CL, PC, PE, PI, PG, phosphatidic acid, phosphatidylserine, and SPH) and Serva (MGDG and DGDG) and observation of characteristic color reactions after spraying with the specific reagents: Zinzadaze for phosphorous containing lipids, α-naphthol for galactolipids, Dragendorffs reagent for choline containing phospholipids, ninhydrin for amino containing phospholipids (5) and anthrone reagent for sulpholipids (14). The HPLC peak retention times for the standards and the eluted classes were also compared.

Table I. *Solvent Program for Solvents A and B at a Flow Rate of 1 mL/min*

Step	Time	B
		%
1	0	0
2	1	0
3	5	10
4	13	25
5	19	33
6	25	45
7	37	100
8	42	100
9	47	0
10	52	0

FAB-MS was used to confirm identification of PC and PE. HPLC collected samples (30–50 μg) were applied to a FAB tip with 1 μL of 0.1 M HCL. The solvent was slowly evaporated by a stream of warm air and the residue mixed with 2 μL of glycerol. FAB mass spectra were obtained with a Phrasor Fast Atom Capillaritron Source fitted to a Hewlett-Packard 5985A quadropole mass spectrometer as described elsewhere (12, 28). Xenon was used as the bombarding gas with an accelerating voltage of 8 KV and current of 50 mV. The ion source temperature was at 60°C. The FAB mass spectra of the phospholipid/glycerol mixture were computer averaged for a selected part of the scan range.

Fatty Acid Analysis

Fatty acids were analyzed as methyl esters by GLC and structural conformation obtained by GC-MS. FAMEs were prepared from the individual eluted fractions, with the inclusion of the internal standard heptadecanoate (17:0), using the micro procedure of Carreau and Dubacq (4). FAMEs of SPH were prepared by hydrolysis and transesterification in 1.5 mL of methanolic boron trifluoride (15%) in a sealed, Teflon-capped, glass tube at 110 to 120°C for 2 h. FAMEs were recovered with dichloromethane after which the solvent was evaporated with a stream of nitrogen. FAMEs were analyzed by a Packard-Becker 419 GLC equipped with a flame ionization detector and fitted with a 25 m RSL-950 fused-silica column (0.2 μm liquid phase coating, Alltech Deerfield, USA). The injection and detector ports were maintained at 240°C; the temperature of the oven was raised from 140°C at 5°C/min to a final temperature of 190°C; the carrier gas was helium. FAME samples were injected in 1.0 μL of dichloro-

methane and peak areas were calculated using a computing integrator. Peaks were identified by comparison with commercially available standard FAMEs and GC-MS (10). Lipid classes were quantitated by reference to the internal standard 17:0 and known stoichiometric ratios (5).

Statistical Analyses

LSD values and ANOVA (split plot design) were computed using the Genstat V program (Lawes Agricultural Trust, Rothamsted Experimental Station, UK).

RESULTS

HPLC Separation of Phospholipid and Galactolipid Classes

Polar lipid classes from the pollen-coat lipid domain (Fig. 1A) and the pollen internal cytoplasmic domain (Fig. 1B) separated within 43 min. Excellent class separation is obtained between CL,[3] MGDG, PG, PE, PC, and SPH, with several other unidentified minor phospholipids being separated. There is a minor contamination of PI by PE. DGDG and PI standards partially coelute. However, detectable amounts of PI and DGDG only appear in the internal and pollen-coat domains, respectively, so that this is not a problem in these analyses. SQDG is also partially contaminated by DGDG. SQDG was identified by its positive reaction with the α-naphthol and anthrone reagents, and its elution position (Fig. 1A) with reference to a published chromatogram with a similar HPLC system (7). MGDG coelutes with an unknown nonsaponifiable, UV absorbing compound. This compound can be separated from MGDG by two-dimensional TLC: first direction chloroform/methanol/7 M ammonium hydroxide (65:30:4); second direction chloroform/methanol/acetic acid/water (170:25:25:6, see ref. 5), and is possibly a pigment (see ref. 7). The injection front was collected and did not contain phospholipid or galactolipid. The small quantity of fatty acids that were associated with this region are of similar composition to the neutral lipids. Therefore, the solvent front contained a small amount of neutral lipid, air associated with injection and traces of highly UV absorbing chloroform which had not been removed during processing.

The two phospholipid standards, PC and PE dipalmitoyl, when analyzed by FAB-MS, show characteristic fragment ions in their mass spectra. The standard for PC dipalmitoyl gave diagnostic fragment ions at m/z 86, m/z 184, and m/z 224 which are characteristic of all PCs and distinguish them from other types of phospholipids (12, 28). These ions are due to the dehydrocholine ion, the choline phosphophosphate ion and an ion containing the carbon skeleton of glycerol attached to the choline phosphate chain, respectively. In addition to those ions, deacyl and deacyloxy-α-cleavages of the fatty acid esters are observed to give two doublet peaks at m/z 494/496 and m/z 478/480. A weak $[M + H]^+$ ion at m/z 734 is also observed which is consistent with the structure of PC dipalmitoyl. The same diagnostic fragment ions were observed for

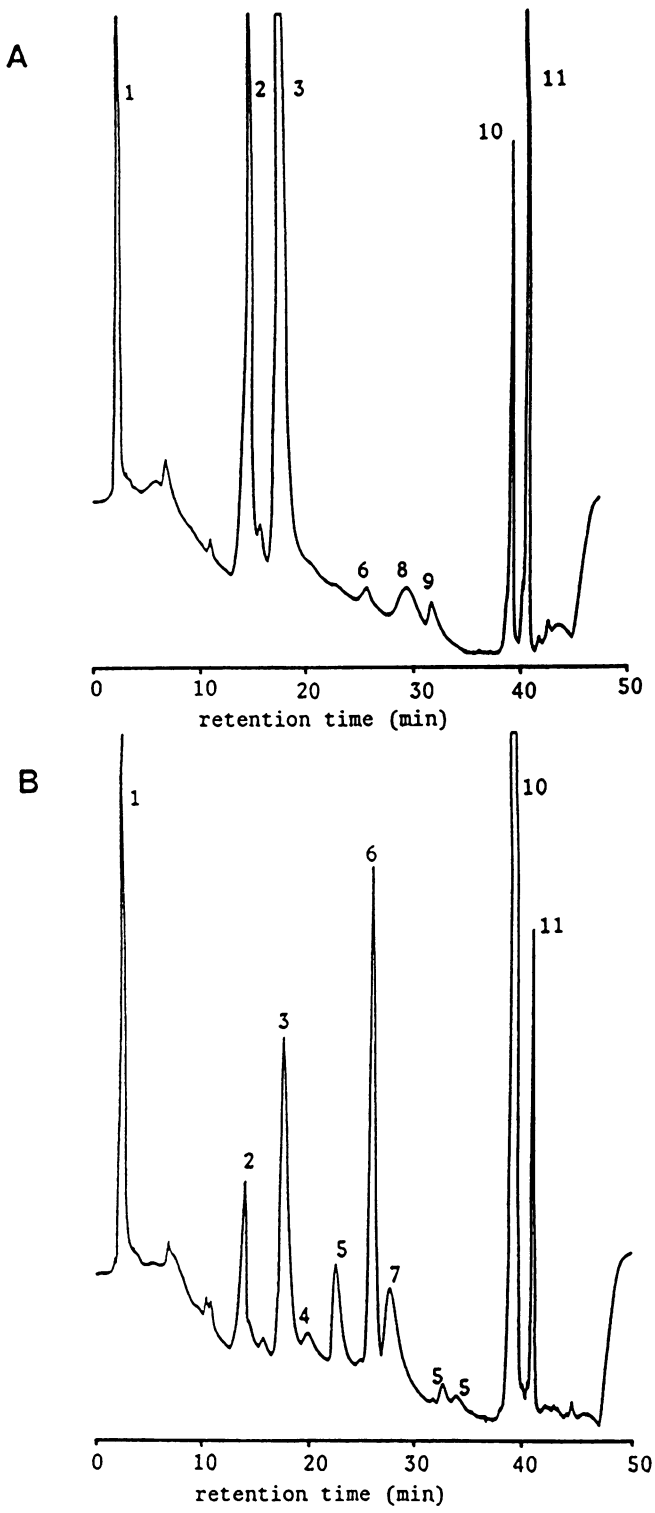

Figure 1. HPLC chromatograms of Wesroona pollen; A, pollen-coat; B, pollen internal. 1, injection front; 2, CL; 3, MGDG; 4, PG; 5, unknown phospholipids; 6, PE; 7, PI; 8, DGDG; 9, SQDG; 10, PC; 11, SPH.

[3] CL and DGDG were tentatively identified by co-chromatography and diagnostic color reactions after spraying with specific reagents on TLC plates.

samples of peak 10 (Fig. 1B), verifying it as PC, and is consistent with the assignments of Fenwick *et al.* (12) and Varenne *et al.* (28).

An $[M + H]^+$ ion could not be obtained for PE dipalmitoyl. The PE dipalmitoyl standards produced fragmentation ions at m/z 183 and m/z 239 presumably as a result of two deacyloxy-α-cleavages and generation of the $[C_{15}H_{31}CO]^+$ ion, respectively. Further ions at m/z 275 and m/z 313, presumably due to dealkylation of the $[M + H]^+$ ion and a deacyloxy-α-cleavage with the subsequent loss of the PE group are observed, respectively. Samples of peak 6 (Fig. 1B) produced the same diagnostic mass fragment ions as the standard reference compound. Different fragmentation patterns for PE dipalmitoyl have been reported by other authors (12, 22). Ohashi (22) observed fragment ions at m/z 142 and m/z 182 which were not observed by Fenwick *et al.* (12) or in these analyses of PE dipalmitoyl. Further FAB-MS analyses were not attempted due to consistent contamination of the source by the authentic standards and samples leading to inferior mass spectra.

Quantitation of Phospholipid and Galactolipid Classes

The polar lipids of the pollen-coat and internal domains differ considerably in lipid class composition although the genotypic source of the pollen did not have significant effect on the composition in either domain (Table II). Pollen-coat lipids accounted for 8.1% of the pollen dry weight, of which polar lipids comprised 15.7%. The major polar lipid classes were CL, DGDG, MGDG, of which galactolipids make up almost 50% of these lipids. Pollen internal lipids accounted for 18.0% of the pollen dry weight, of which polar lipids comprised 43.5%. In contrast to the pollen-coat, the major polar lipid classes are PC, PE, and PI with galactolipids comprising less than 2% of lipids. In addition variation between replicates is high in the pollen-coat polar lipids. This variation is also evident in the fatty acid compositions of the pollen-coat polar lipids.

Fatty Acid Composition of Phospholipids and Galactolipids

The expression of the low 18:3 IXLIN trait differed markedly in terms of the fatty acid compositions of the phospholipids and galactolipids (Table III). The prominent fatty acids of the phospholipids of the two domains are 16:0, 18:2, and 18:3 with the 18:2/18:3 ratio increasing significantly ($P < 0.05$) in the internal domain of the IXLIN line. In addition 16:3 is only a minor component of PC, PE and PI in either domain. In the pollen-coat phospholipids the 18:2/18:3 ratio does not differ significantly ($P = 0.05$) between lines. CL, however, is the exception since the increase in the 18:2/18:3 ratio is pronounced with 18:2 and 18:3 comprising greater than 75% of the fatty acids in both domains. In addition CL is the only phospholipid with a similar fatty acid composition in both domains.

In contrast the galactolipid 18:2/18:3 ratios are significantly ($P < 0.05$) less than those exhibited by IXLIN in both pollen-coat and internal phospholipids. In addition the 18:2/18:3 ratios for both lines analyzed are not greater than the ratios for internal PC, PE, or PI of Wesroona. The major galactolipid fatty acids are 16:0, 16:3, and 18:3 with 18:2 not exceeding 10.0% of fatty acids regardless of the line. Surprisingly, 16:3 was only detected in MGDG in one replicate of IXLIN of the samples analyzed. In this replicate the proportion of MGDG and 18:3 were also considerably higher. In the corresponding DGDG fraction of this replicate, the proportions of DGDG, 16:3, and 18:3 were reduced. This may suggest a substrate-product relationship for MGDG and DGDG in this case.

Substantial variation in fatty acid levels existed between replicates for both lines in phospholipids and galactolipids of the pollen-coat while the composition of the internal lipids remained stable. A further experiment (our unpublished data) confirmed the stability of the internal and dynamic nature of the pollen-coat fatty acid composition. An interaction between age of plant, temperature and possibly other unknown factors appears responsible for the observed variation.

Neutral Lipid Composition of Pollen

The neutral lipid class composition of the pollen domains (Fig. 2) appears to be similar. The main neutral lipid in both domain appears to be TAG with neither domain containing significant quantities of either DAG or free fatty acids. Also present is a small amount of unesterified cholesterol and MAG. Several unidentified 'lipid-like' compounds which do not comigrate with the standards are also observed.

Table II. *Normalized Percentage (mol) Phospholipid and Galactolipid Composition of the Pollen of Two B. napus lines*

Fraction	CL[a]	MGDG	PG	PE	PI	DGDG[a]	SQDG	PC	SPH	Unknown[b]
Pollen-coat										
Wesroona	21.5	17.7	ND[c]	6.5	ND	22.3	8.0	10.2	13.3	ND
IXLIN	17.5	24.0	ND	5.9	ND	25.6	6.4	7.3	12.9	ND
LSD (0.05)	(7.2)	(19.2)		(3.3)		(21.3)	(3.5)	(7.3)	(5.3)	
Internal										
Wesroona	1.7	1.6	2.3	31.5	9.0	ND	ND	45.6	3.1	5.0
IXLIN	2.6	1.5	2.6	30.8	9.1	ND	ND	45.7	3.1	4.7
LSD (0.05)	(0.8)	(0.5)	(0.8)	(3.5)	(1.2)			(2.0)	(1.5)	(2.7)

[a] Tentative identification based on co-chromatography and diagnostic color reactions. [b] Unknown phospholipids, assumed to be diacylglycerides. [c] Not detected.

Table III. *Normalized Percentage Fatty Acid Compositions of Representative Phospholipids and Galactolipids from Pollen of Two Lines of B. napus*

	Fraction/ Line[a]	16:0	16:1	16:3	18:0	18:1	18:2	18:3	Other[b]	18:2/18:3 Ratio
Phospholipids										
PC	Coat/H	30.0	0.9	1.3	5.8	9.5	13.1	30.4	9.2	0.48
	/L	30.0	2.9	2.3	4.6	7.0	17.6	33.5	2.1	0.53
	LSD (0.05)	(8.1)	(6.3)	(2.2)	(2.0)	(4.2)	(13.2)	(12.2)	(8.9)	(0.62)
	Internal/H	29.9	0.2	0.7	0.6	2.4	8.6	55.3	2.3	0.16
	/L	29.2	0.2	0.4	0.5	2.8	35.6	29.4	1.9	1.21
	LSD (0.05)	(0.9)	(0.2)	(0.0)	(0.3)	(0.1)	(0.6)	(0.7)	(1.5)	(0.01)
PE	Coat/H	32.6	1.2	2.1	9.2	13.6	8.0	25.2	8.1	0.34
	/L	25.2	1.7	5.2	10.6	9.6	12.7	27.0	7.8	0.48
	LSD (0.05)	(17.0)	(5.7)	(9.4)	(8.9)	(9.0)	(5.6)	(20.9)	(8.9)	(0.26)
	Internal/H	33.4	0.1	0.4	1.2	3.2	9.3	48.5	4.0	0.19
	/L	32.6	0.3	0.5	1.3	2.0	37.1	24.2	2.4	1.54
	LSD (0.05)	(1.4)	(0.4)	(0.8)	(0.7)	(1.7)	(2.0)	(1.9)	(3.7)	(0.03)
PI[c]	Internal/L	39.9	0.3	0.7	1.7	2.2	5.9	45.3	4.2	0.13
	/H	40.1	1.0	0.5	1.6	2.5	26.3	25.1	3.0	1.05
	LSD (0.05)	(0.8)	(1.6)	(0.1)	(0.6)	(2.7)	(2.2)	(2.7)	(3.3)	(0.04)
CL[d]	Coat/H	3.7	0.2	ND[e]	1.6	2.3	1.2	84.8	6.4	0.02
	/L	4.0	0.5	ND	1.9	1.8	51.5	35.4	4.8	1.45
	LSD (0.05)	(5.6)	(1.5)		(2.7)	(2.2)	(11.2)	(9.5)	(8.7)	(0.58)
	Internal/H	8.4	ND	ND	2.9	4.1	2.2	72.9	9.4	0.03
	/L	6.3	0.7	ND	2.3	2.8	52.3	31.2	4.2	1.67
	LSD (0.05)	(2.9)	(1.1)		(1.2)	(1.7)	(3.6)	(2.8)	(7.2)	(0.05)
Galactolipids										
MGDG	Coat/H	22.6	11.3	ND	8.0	14.0	2.6	23.5	17.9	0.11
	/L	16.1	6.3	12.5	3.3	8.4	4.1	35.3	13.4	0.18
	LSD (0.05)	(21.8)	(14.2)	(34.7)	(5.5)	(13.5)	(3.5)	(34.5)	(16.9)	(0.27)
	Internal/H	24.4	3.8	ND	7.8	16.6	3.5	23.3	20.5	0.16
	/L	22.3	0.9	ND	8.0	8.9	8.8	31.4	19.5	0.29
	LSD (0.05)	(9.0)	(7.9)		(3.0)	(4.1)	(2.0)	(12.5)	(20.2)	(0.12)
DGDG[bc]	Coat/H	11.0	2.7	28.8	4.8	6.6	1.4	37.7	7.0	0.04
	/L	11.7	2.8	20.3	6.8	4.0	4.2	35.8	14.5	0.12
	LSD (0.05)	(6.3)	(4.0)	(16.0)	(6.4)	(3.3)	(3.1)	(9.0)	(11.1)	(0.11)
SQDG[b]	Coat/H	16.4	1.0	23.2	6.2	10.1	1.8	30.7	10.2	0.06
	/L	13.1	1.6	28.1	5.2	6.0	3.1	37.1	6.1	0.09
	LSD (0.05)	(11.7)	(2.9)	(10.5)	(2.7)	(4.7)	(1.4)	(15.7)	(9.6)	(0.04)

[a] H, Wesroona; L, IXLIN. [b] Includes 12:0, 14:0, 16:2, 20:0, 20:1, 22:0, 22:1, 24:0, 24:1. [c] PI in coat, and DGDG and SQDG internal not detected. [d] Tentative identification based on cochromatography and diagnostic color reactions. [e] Not detected.

DISCUSSION

Marked differences exist between pollen-coat and internal domain lipid class and the fatty acid compositions of the polar lipids of *Brassica napus* pollen which may be interpreted in terms of the pathways of polyunsaturated fatty acid biosynthesis. The pollen-coat galactolipids are relatively rich in 16:3, a marker for the prokaryotic desaturation pathway, while the phospholipids and galactolipids of the internal domain possess only limited quantities of 16:3. The minor quantities of 16:3 present in the phospholipids and neutral lipids of rapeseed pollen (11) may signify that there is a limited export of prokaryotic fatty acids from plastids. The expression of the IXLIN trait is most evident in the 18:2/18:3 ratio of the internal phospholipids and in CL of the pollen-coat. In addition, studies where the activity of the plastid 16:2 to 18:2 desaturase is reduced by a temperature sensitive mutation (3, 20) or herbicide treatment (18, 21), there is an increase in the substrates, 16:2 and 18:2, in MGDG. Since 16:2 does not accumulate in detectable amounts in either domain, the 18:2/18:3 ratio of the galactolipids is not significantly affected by the IXLIN genotype and the fatty acid composition of the neutral and polar lipids of the internal domain are similar (11); the major pathway contributing fatty acids is most likely the eukaryotic pathway. Consequently it is proposed that the eukaryotic pathway is modified by the IXLIN genotype while the prokaryotic pathway is unaffected. Furthermore as the level of 16:3 is minor in the pollen internal neutral and polar lipids (11), desaturation by the prokaryotic pathway must be insubstantial in this lipid domain.

In the eukaryotic pathway 18:2 can be desaturated at *sn*-2 PC in the endoplasmic reticulum (20, 21, 27) and at *sn*-1

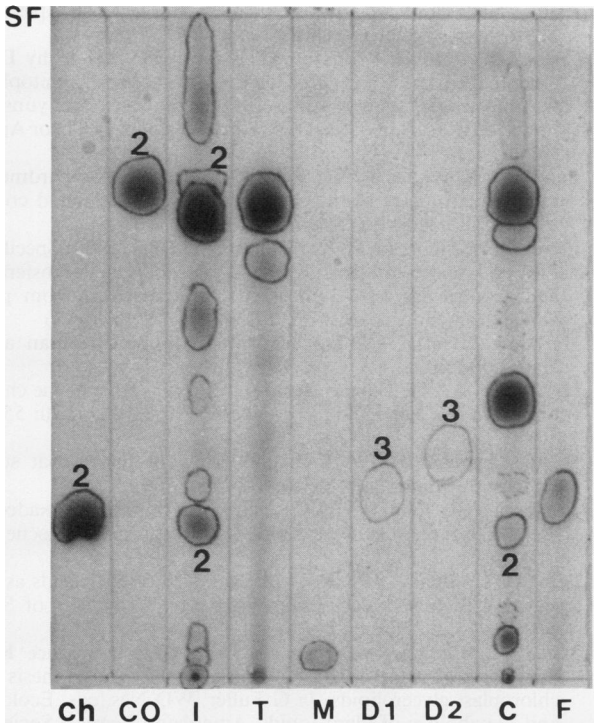

Figure 2. TLC of pollen neutral lipids and neutral lipid standards. TLC solvent phase, benzene/diethyl ether/ethylacetate/acetic acid (80:10:10:0.2); spray reagent, acidic ferric chloride (5); Ch, cholesterol; CO, cholesterol oleate; I, pollen internal neutral lipids; T, 18:1 TAG; M, *sn*-1 18:1 MAG; D1, *sn*-1,2 16:0 DAG; D2, *sn*-1,3 16:0 DAG; C, pollen-coat; F, 18:3; SF, solvent front. Stain purple with spray reagent is signified by 2. Spot detection with UV light on fluorescent F_{254} background is signified by 3.

MGDG after transfer of 18:2 to the plastid (21). Since the lipid class composition of both pollen domains is independent of the 18:2/18:3 ratio, it appears likely that the activity of one of these 18:2 desaturases is reduced in IXLIN. Therefore as the 18:2/18:3 of internal MGDG shows little change between lines, we propose that it is probably the activity of the *sn*-2 linoleoyl phosphatidylcholine desaturase/s of the endoplasmic reticulum which is modified by the IXLIN genotype.

The composition of lipids in the pollen-coat, excluding CL, are characteristic of leaf lipids due to the high proportion of galactolipids (25) and the concentration of 16:3 in them (17, 30). The 18:2/18:3 ratio and the levels of 16:3 of the galactolipids and phospholipids, although variable, did not show a significant relationship between the two lines of pollen analyzed. This implies that the prokaryotic pathway is the dominant desaturation pathway contributing polyunsaturated fatty acids to the pollen-coat polar lipids other than CL. CL of both pollen domains has a highly polyunsaturated fatty acid composition which exhibits the characteristic eukaryotic change for 18:2/18:3 ratio in IXLIN. This explains the strong correlation for 18:2 and 18:3 levels between the pollen-coat and seed polar lipids (11) which must be primarily due to the levels of 18:2 and 18:3 in CL. This indicates that the eukaryotic pathway also has a significant contribution to the levels of polyunsaturated fatty acids in the pollen-coat.

Composition of the Pollen Coat

The composition of the polar lipids and their fatty acids, in the pollen-coat, has a number of unusual features which have not been previously reported in other plant lipid extracts. CL and DGDG have been tentatively identified in this paper by cochromatography and diagnostic colour reactions on TLC. Their identity has not been confirmed by mass spectrometry, nor has CL been deacylated and its water soluble products separated by two dimensional TLC. For these reasons, caution must be applied in interpreting these results.

The ratio of CL to PC and PE in the pollen-coat is several times higher than that reported for the inner mitochondrial membrane of other plant species (1, 25). In the pollen-coat, the proportion of CL/PC is 2.1–2.4 and CL/PE is 3.0–3.3. In the inner mitochondrial membrane, the ratios of CL/PC and CL/PE have both been reported as between 0.3–0.7 (1, 25). One explanation is the low levels of PC and PE which are more characteristic of chloroplast membranes (25). A second explanation is that the acetone extraction of the pollen-coat may be responsible. Acetone is generally a poor solvent for phospholipids (5, 31). However the ability of acetone to extract phospholipid is improved by conditions where there are significant proportions of TAG and small amounts of water (5, 31). These conditions are present in this system. Extraction by chloroform/methanol (2:1) of intact pollen does not markedly improve lipid extraction (our unpublished data) and significant proportions of CL are found in the pollen-coat extract. Therefore the internal extract will at most be contaminated by insignificant amounts of pollen-coat phospholipids.

In both DGDG and SQDG, 16:3 accounted for 20 to 30 % of fatty acids. This is unusually high since levels of 16:3 in DGDG of other plant species have only been reported up to 9% (3, 17, 25, 30). One explanation is that some degradation of MGDG occurred during sample preparation. A second explanation is that a galactolipid:galactolipid galactosyltransferase could be responsible for the formation of the 16:3-containing DGDG during pollen-coat formation. As yet 16:3 has not been reported as being detected in SQDG although a portion of the 16:3 may be attributed to the partial contamination of this fraction by DGDG. Further, the large values for the LSD of the pollen-coat, suggest that the composition of lipids and fatty acids is sensitive to influence by the physiological state of the parent plant and its environment.

The pollen-coat of *Brassica* is reported to contain not only lipids but also cytoplasmic remnants including intact mitochondria and plastids (8). These findings are consistent with the present observations since the presence of galactolipids would appear to indicate plastid membranes (25), and CL the presence of mitochondrial membranes (1). Ultrastructural evidence supports the hypothesis that the pollen-coat is transferred from tapetum to the pollen grain wall late in pollen development (16). However, as pollen-coat neutral lipids do not appear to contain significant quantities of DAG or free fatty acids, there is no evidence to suggest that membrane lipid hydrolysis is involved in the deposition of pollen-coat lipids. The unusual features of the pollen-coat polar lipids and fatty acid composition are presumably the result of the proc-

esses involved in the formation of the pollen-coat which have yet to be described at a chemical level.

CONCLUSIONS

The polar lipid class and fatty acid compositions of the pollen-coat and internal domains of *Brassica* pollen are very different. By comparing two rapeseed lines with different 18:2/18:3 ratios we propose that the eukaryotic pathway is the major source of polyunsaturated fatty acids in the pollen internal domain. It is proposed that the IXLIN genotype contains genes which reduce the activity of the eukaryotic *sn*-2 PC desaturases in the endoplasmic reticulum. Since other modifications to 18:2 desaturase activity have interfered with the desaturation of 16:2 and 18:2 on MGDG (3, 18, 21), this system represents the first analysis of genes which modify the activity of the *sn*-2 linoleoyl phosphatidylcholine desaturase/s of the endoplasmic reticulum in the eukaryotic pathway.

In the pollen-coat it appears that a combination of the prokaryotic and eukaryotic pathways contribute polyunsaturated fatty acids. The pollen-coat is a product of the tapetum (16), hence specified by expression of the diploid genome. This report strongly supports the hypothesis that internal domain lipids are synthesized *de novo*, as a result of haploid expression, within each individual pollen grain (10, 11).

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