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CHANGES IN FATTY ACID COMPOSITION OF LIPID CLASSES IN DEVELOPING MUSTARD SEED

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Key Word Index—*Sinapis alba*; Cruciferae; mustard seed; lipid biosynthesis; fatty acid composition; triacylglycerols; gadoleic acid; erucic acid.

Abstract—Maturation of mustard (*Sinapis alba*) seed proceeds with a sharp decrease in the amounts of palmitic and linoleic acids in the total lipids up to 6 weeks after flowering (WAF). Concomitantly, the concentration of oleic acid increases, reaching a plateau at 4 WAF, which is followed by chain elongation of oleic acid to gadoleic and erucic acids. Compositional changes in constituent fatty acids of individual lipid classes indicate that the very long-chain monounsaturated fatty acids (C_{20} and C_{22}), as opposed to common long-chain fatty acids (C_{16} and C_{18}), are metabolized to triacylglycerols mainly by esterification to preformed diacylglycerols and monoacylglycerols, rather than via esterification to glycerol-3-phosphate or lysophosphatidic acids.

INTRODUCTION

Triacylglycerols of cruciferous seeds, such as rapeseed, constitute a major source of edible fat [1]. Compositional changes in lipids and their constituent fatty acids during maturation of cruciferous seeds have been investigated extensively in rape (*Brassica napus*) [2, 3] and crambé (*Crambé abyssinica*) [2–4], and to some extent in mustard (*Sinapis alba*) [5]. Very long-chain monounsaturated fatty acids, e.g. gadoleic and erucic acids, are known to be the major constituents of triacylglycerols of most cruciferous seeds. Since the introduction and widespread cultivation of rape and turnip rape varieties that yield seeds which are virtually devoid of gadoleic and erucic acids [6], attention has been focused on alternative crops, such as crambé or mustard, as potential sources of very long-chain monounsaturated fatty acids which are of interest as raw materials for the chemical industry [7, 8].

The biosynthesis of triacylglycerols in developing mustard seed has been investigated recently in our laboratory using radioactive precursors [9]. We now report the compositional changes in fatty acids of all the major lipid classes of mustard seed during maturation in support of the biosynthetic pathways observed earlier by radiolabelling studies.

RESULTS AND DISCUSSION

Lipid content and fatty acid composition of total lipids of mustard seeds at various stages of maturation are given in Table 1. Lipids of mustard seed contain, at various stages of maturation, different proportions of common fatty acids, i.e. palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3), and very long-chain monounsaturated fatty acids, i.e. gadoleic (20:1), erucic (22:1) and nervonic (24:1), in addition to minor proportions of very long-chain saturated fatty acids, i.e. arachidic (20:0), behenic (22:0) and lignoceric (24:0) (Table 1). The figures given in Table 1 for oleic and gadoleic acids also include minor proportions of their ($n-7$) isomers, i.e. ($n-7$) 18:1 and ($n-7$) 20:1.

The patterns of lipid accumulation and compositional changes of fatty acids in mustard seed with progressive

Table 1. Changes in lipid content and fatty acid composition of developing mustard seed

Weeks after flowering	Lipid content (% fr. wt)	Fatty acid* composition (wt %)											
		16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	24:0	24:1
2	0.6	21.0	1.2	3.6	4.5	49.5	14.6	tr	0.1	0.4	0.7	tr	0.2
3	0.5	17.5	1.5	3.1	9.3	48.5	15.1	0.8	0.5	0.3	0.5	0.3	0.4
4	2.0	10.3	1.7	2.8	25.5	30.0	13.2	0.8	4.9	0.3	6.3	0.2	0.9
5	4.4	7.8	1.2	2.1	27.8	22.2	11.2	0.8	8.7	0.3	13.4	0.2	1.2
6	8.3	4.6	0.5	1.1	25.7	13.8	11.8	0.5	10.5	0.3	28.6	0.1	1.6
Mature seed	24.9	2.9	0.4	0.8	21.3	11.6	11.0	0.7	9.8	0.3	38.1	tr	2.1

*Fatty acids are designated by the number of carbon atoms: the number of double bonds; in addition to the fatty acids listed minor proportions of 14:0, 20:2 and 22:2 were also detected. tr = Trace.

maturation (Table 1) broadly resemble those observed in developing seeds of rape [2, 3] and crambé [3, 4], and are in good agreement with earlier data on mustard seed [5]. Thus in both mustard (Table 1) and crambé [4], seed maturation and lipid accumulation proceed with a steep decline in the amounts of 16:0 and 18:2. However, the decrease with progressive maturation in the concentration of 18:3—a characteristic fatty acid of photosynthetic tissues—is far less in mustard (Table 1) than in crambé [4].

Seed maturation in mustard was also accompanied by a steep increase in the quantity of 18:1 up to 4 weeks after flowering (WAF), after which time the concentration of 18:1 remained essentially unchanged (Table 1). Subsequent accumulation of 20:1, 22:1 and 24:1 (Table 1) was consistent with the pathways observed in other higher plants, where 18:1, synthesized *de novo*, has been found to be elongated to very long-chain monounsaturated fatty acids [10–13].

In an earlier study on developing mustard seed, compositional changes in fatty acids had been reported only for neutral lipids [5]. We report here the compositional changes of all the major classes of neutral lipids, phospholipids and glycolipids of developing mustard seed at 4, 5 and 6 WAF. Table 2 records the compositional changes in unesterified fatty acids (FA), monoacylglycerols (MG), diacylglycerols (DG) and triacylglycerols (TG). The compositional changes of the major phospholipids, i.e. phosphatidylcholines (PC), phosphatidic acids (PA) and phosphatidylinositols (PI), and those of the major glycolipid, monogalactosyldiacylglycerols (MGDG), are given in Table 3. Other minor lipids detected included phosphatidylethanolamines, phosphatidylglycerols and diagalactosyldiacylglycerols. The stage of maturation chosen (4 until 6 WAF) represents the phase of seed development when extensive synthesis of very long-chain monounsaturated fatty acids and their conversion into TG occur (Table 1 and ref. [5]). TG was found to constitute 53, 65 and 78 %, respectively, of the total lipids at 4, 5 and 6 WAF.

It is generally accepted that in higher plants the common long-chain fatty acids are metabolized to TG and phospholipids via the glycerol-3-phosphate pathway [14–17] in a similar manner as in animal tissues. Radiolabelling studies in crambé [18], and more recently in nasturtium [12] and mustard [9], have provided evidence that the glycerol-3-phosphate pathway is operative also in the biosynthesis of TG containing very long-chain monounsaturated and saturated fatty acids. These findings are consistent with the data reported here.

The amounts of saturated fatty acids (16:0 and 18:0) in TG were quite low and relatively constant at all three stages of seed maturation, whereas in all other lipid classes considerable fluctuations in the quantities of both acids

Table 2. Fatty acid composition (wt %) of neutral lipids* in mustard seeds at various stages of development

Fatty acid†	FA			MG			DG			TG		
	(4 WAF)	(5 WAF)	(6 WAF)	(4 WAF)	(5 WAF)	(6 WAF)	(4 WAF)	(5 WAF)	(6 WAF)	(4 WAF)	(5 WAF)	(6 WAF)
16:0	18	32	7	12	36	22	15	24	8	7	6	4
18:0	7	13	3	7	11	10	4	14	2	3	2	2
18:1	35	28	29	10	25	45	22	31	38	31	32	31
18:2	26	11	16	55	14	9	39	4	18	22	19	13
18:3	5	2	8	tr	2	2	13	tr	10	10	10	9
20:1	4	2	8	3	3	2	3	4	9	9	10	10
22:1	3	2	22	3	1	3	2	5	12	11	17	26

*FA, Unesterified fatty acids; MG, monoacylglycerols; DG, diacylglycerols; TG, triacylglycerols.
†See footnote, Table 1.
WAF = Weeks after flowering.

Table 3. Fatty acid composition (wt %) of major phospholipids* and galactolipids* in mustard seeds at various stages of development

Fatty acid†	PC			PA			PI			MGDG		
	(4 WAF)	(5 WAF)	(6 WAF)	(4 WAF)	(5 WAF)	(6 WAF)	(4 WAF)	(5 WAF)	(6 WAF)	(4 WAF)	(5 WAF)	(6 WAF)
16:0	32	21	19	14	20	16	30	24	19	17	10	27
18:0	24	7	5	10	4	10	10	6	9	8	3	8
18:1	17	18	32	12	14	46	16	23	38	13	12	27
18:2	11	26	23	40	42	14	30	32	16	30	22	16
18:3	tr	22	19	2	14	4	7	11	5	12	39	8
20:1	2	1	1	1	2	2	1	1	1	1	1	3
22:1	1	4	2	3	< 1	1	3	< 1	< 1	5	1	1

*PC, Phosphatidylcholines; PA, phosphatidic acids; PI, phosphatidylinositols; MGDG, monogalactosyldiacylglycerols.
†See footnote, Table 1.
WAF = Weeks after flowering.

were observed (Tables 2 and 3). Similar findings have been reported for developing rapeseed and crambé seed [3, 4], indicating the dynamic turnover of 16:0 and 18:0 in the aforesaid lipids.

The concentration of 18:1 in the total lipids changed little between 4 and 6 WAF (Table 1), and concomitantly that of 18:1 in TG was also essentially constant (Table 2). In all other lipid classes, with the exception of FA, the concentration of 18:1 consistently increased with progressive seed maturation (Tables 2 and 3).

Among the polyunsaturated fatty acids, the proportion of 18:2 in the total lipids was found to decrease with progressive seed maturation (Table 1) and this was reflected by a decline in the amounts of 18:2 in all the lipid classes with the exception of PC (Tables 2 and 3). The concentration of 18:3 altered little between 4 and 6 WAF (Table 1), and during this phase of seed development the concentration of 18:3 in TG also remained constant (Table 2). In all other lipid classes substantial fluctuations in the proportions of 18:3 were observed (Tables 2 and 3).

Large proportions of the common fatty acids, i.e. 16:0, 18:0, 18:1, 18:2 and 18:3, were found in PA at all three stages of development of mustard seed (Table 3), indicating extensive esterification of these fatty acids to glycerol-3-phosphate and/or lysophosphatidic acids. All these acids, with the exception of 18:3, were also abundant in MG (Table 2), which is likely to have been derived, in part, from lysophosphatidic acids by the action of phosphohydrolases [19]. Apparently, most of the common long-chain fatty acids enter the glycerol-3-phosphate pathway by esterification to glycerol-3-phosphate and/or lysophosphatidic acids.

The occurrence of insignificant proportions of very long-chain monounsaturated fatty acids (20:1 and 22:1) in PA and MG (Tables 2 and 3) suggests that only small quantities of 20:1 and 22:1 are esterified to either glycerol-3-phosphate or lysophosphatidic acids. This conclusion is further substantiated by the low concentrations of 20:1 and 22:1 in PC and PI (Table 3), which are known to be derived from PA. Distinctly higher amounts of 20:1 and 22:1 in DG at both 5 and 6 WAF than in MG or PA (Tables 2 and 3) are indicative of the pathways by which MG, derived from lysophosphatidic acids [19], are converted to DG by esterification with 20:1 and 22:1.

The quantities of long-chain monounsaturated fatty acids (20:1 and 22:1) between 4 and 6 WAF were distinctly higher in TG and DG than in all other lipid classes, with the exception of FA at 6 WAF (Tables 2 and 3). Increase in the proportion of 20:1 and 22:1 in the total lipids with progressive seed maturation (Table 1) was paralleled by increments of both 20:1 and 22:1 in DG and that of 22:1 in TG (Table 2). Moreover, the amounts of 20:1 and 22:1 in TG were consistently higher than in DG (Table 2). These findings indicate that a major pool of long-chain monounsaturtated fatty acids is esterified to preformed DG to yield TG containing 20:1 and 22:1. The occurrence of substantial proportions of 20:1 and 22:1 in FA at 6 WAF (Table 2) is indicative of a fairly large pool of these acids, which, after activation to acyl-CoAs, are esterified to DG by acyl-CoA:diacylglycerol acyltransferase. This enzyme, hitherto found to be involved in the biosynthesis of TG containing common fatty acids [20, 21], also seems to accept very long-chain monounsaturated acyl-CoAs and DG containing 20:1 and 22:1 as substrates.

In summary, the data presented suggest that in contrast to the common fatty acids, which are metabolized to TG mainly via esterification to glycerol-3-phosphate and/or lysophosphatidic acids, the long-chain monounsaturated fatty acids are converted to TG predominantly by esterification to preformed DG, which in turn are partly derived from MG by esterification with 20:1 and 22:1. Thus, in mustard seed the direct esterification of long-chain monounsaturated fatty acids to either glycerol-3-phosphate or lysophosphatidic acids seems to play a minor role in the biosynthesis of TG. These findings, which have been complemented by radio-labelling studies [9], seem to be linked with the preference of acyl-CoA:glycerol-3-phosphate acyltransferase for the common acyl-CoAs [16, 22], rather than for long-chain acyl-CoAs [23].

EXPERIMENTAL

Seeds of white mustard, *S. alba* L., cv Albatros, were provided by Norddeutsche Pflanzenzucht, Hans-Georg Lembke KG, D-2331 Hohenlieth, Federal Republic of Germany. The plants were field-grown and insect-pollinated during the months of May until August, and the developing seeds were collected at definite periods after flowering.

Lipids were extracted from the fresh seeds and separated from non-lipid contaminants according to established procedures [24]. Fractionation of lipids by TLC on silica gel H was carried out with hexane–Et_2O–HOAc (70:30:1) for the separation of neutral lipid classes, and with $CHCl_3$–Me_2CO–MeOH–HOAc–H_2O (10:4:2:2:1) for the separation of the various phospholipid and glycolipid classes. Lipid fractions were located under UV after spraying with anilinonaphthalenesulphonate (0.2% in MeOH) and identified by co-chromatography with authentic standards. The identity of phospholipid and glycolipid fractions was also ascertained by TLC on $(NH_4)_2SO_4$ impregnated silica gel G using Me_2CO–C_6H_6–H_2O (91:30:8) [25].

Procedures used for the transmethylation of total lipids and lipid fractions, purification of methyl esters and their quantitative analysis by FID–GC were the same as those described previously [13]. The figure given in Tables 1–3 are mean values of two separate GC analyses.

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