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LIPID CONSTITUENTS OF OIL BODIES IN THE CULTURED SHOOT PRIMORDIA OF *MATRICARIA CHAMOMILLA*

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Key Word Index—*Matricaria chamomilla*; Compositae, camomile; shoot primordia; oil body; lipid composition; triacylglycerol; fatty acids; steryl ester; sterols.

Abstract—The oil bodies in the cultured shoot primordia of *Matricaria chamomilla* comprised 80% triacylglycerol, 5% diacylglycerol, 6% monoacylglycerol and 9% free fatty acids. Free sterols and steryl esters were minor components of the oil bodies. The lipid components in the oil bodies from the cultured shoot primordia were similar to those from seeds, flowers and leaves in the intact plants. Linoleic acid (18:2) and linolenic acid (18:3) were major components of fatty acid residue in the triacylglycerols in the oil bodies from the shoot primordia, whereas the content of palmitic acid (16:0) and stearic acid (18:0) was lower than that in oil bodies from the seeds, flowers and leaves of *M. chamomilla*. The free sterol composition varied only slightly between the oil bodies from seed or shoot primordia, but a greater variation was observed in the composition of the steryl esters, in terms of both the sterol and fatty acid moieties. The seed oil bodies contained a higher proportion of precursor sterols than those of the shoot primordia. As in the acylglycerols, linoleic and linolenic acids were the major fatty acids in the esters from shoot primordia oil bodies, whereas the steryl esters of the seed oil bodies contained a much higher proportion of saturated fatty acids.

INTRODUCTION

Oil bodies in higher plants are believed to be a storage organelle for a pool of triacylglycerol, for example such as the oil bodies of seeds [1, 2]. However, the oil bodies are also present in multiplying cells of vegetative tissues. This fact suggests that the oil bodies in multiplying cells may have other physiological functions. Our knowledge on the function of the oil bodies in multiplying cells is poor, and little is known about the chemical composition of these oil bodies. Recently, we reported the formation of the oil bodies in multiplying cells of *Matricaria chamomilla* (German camomile) [3]. In continuation of these studies on the composition and function of the oil bodies in multiplying cells, we have now investigated the chemical composition of the oil bodies in the cultured shoot primordia of *M. chamomilla*.

RESULTS AND DISCUSSION

Shoot primordia of *M. chamomilla* were subcultured in Murashige-Skoog's (MS) liquid medium [4] containing α-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP). Light-microscopic observation of the cells showed that the size of the oil bodies in the cells was mainly in the range 0.3–1.5 μm in diameter and only relatively few smaller (< 0.3 μm) or larger (> 1.4 μm) size oil bodies were observed. The oil bodies were isolated by centrifugation and washed several times by buffer solution to yield mainly the intermediate sized oil bodies (0.5–1.1 μm).

Table 1 shows the lipid components of the oil bodies in the cultured shoot primordia, as well as those of the seeds, flowers and leaves of *M. chamomilla*. The oil bodies in the shoot primordia grown under the normal conditions comprised 90% of acylglycerols and 9% of free fatty acids as major components, with, in addition, minor amounts (< 1.0%) of phospholipids, steryl fatty acyl esters, free sterols and proteins. No remarkable difference of the lipid components was observed between the oil bodies in the cultured shoot primordia and the seeds. However, the relative amount of triacylglycerol in the oil bodies from the shoot primordia seemed to be somewhat lower than in the oil bodies obtained from the flowers and leaves of the intact plants. The amount of triacylglycerol in the oil bodies from the cultured shoot primordia grown under starvation conditions was similar to

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Table 1. Lipid components in the oil bodies from the shoot primordia, seeds, flowers and leaves of *M. chamomilla*

Compound	Relative content (wt%)				
	Shoot primordia				
	Normal*	Starvation†	Seeds	Flowers	Leaves
Triacylglycerols	80	93	76	91	92
1,3-Diacylglycerols	2	1	12	2	3
1,2-Diacylglycerols	3	1	1	1	3
2-Monoacylglycerols	2	tr.	1	1	tr.
1-Monoacylglycerols	4	1	1	1	tr.
Fatty acids	9	4	9	4	2

*Cultured under normal conditions for 6 days.
†Cultured in the starvation medium for 6 days.

Table 2. Relative contents of the lipids in the oil bodies from the shoot primordia of *M. chamomilla* by 3–14 days cultivation

Compound	Relative content (wt%)				
	3 days	6 days	9 days	12 days	14 days
Triacylglycerols	80	80	85	87	88
1,3-Diacylglycerols	2	2	2	2	2
1,2-Diacylglycerols	4	3	3	4	3
2-Monoacylglycerols	1	2	1	tr.	tr.
1-Monoacylglycerols	5	4	4	2	1
Fatty acids	8	9	5	5	6

that present in oil bodies from the flowers and leaves, as shown in Table 1. It is reported [5, 6] that some algae synthesized a large amount of triacylglycerol and accumulated it in discrete cytosolic droplets, the so called lipid bodies, when the algae was cultured on a starvation medium. In higher plants there may also be an increase in the relative amount of triacylglycerol when the plant is stressed under a nutrient limiting condition. The relative contents of the lipid components in the oil bodies from the cultured shoot primordia cultured for 3–14 day periods under normal conditions are shown in Table 2. The contents of triacylglycerol in the oil bodies increased slightly with the lapse of cultivation time.

The major fatty acid residues of the triacylglycerols in the oil bodies from the shoot primordia were linoleic acid (18:2) and linolenic acid (18:3), as shown in Table 3. There were obvious differences in the fatty acid compositions of the triacylglycerols from the oil bodies of the different tissues. Linolenic acid was scarcely present in the triacylglycerols from the seeds and flowers, whereas more than 20% of linolenic acid was observed in the triacylglycerols from the shoot primordia and leaves. The contents of palmitic acid (16:0) and stearic acid (18:0) in the triacylglycerols of the oil bodies from the shoot primordia were lower than those from the other tissues, especially the leaves. On the other hand, no significant difference was observed in the fatty acid compositions of the acylglycerols and free fatty acids from the oil bodies of the cultured shoot primordia after culture for 3 or 12 day periods (Table 4). However, the relative contents of palmitic acid (16:0) in mono- and diacylglycerols, and of free fatty acids in the oil bodies, were higher than found in triacylglycerol.

The amount of phospholipids (0.7%) in the oil bodies from the shoot primordia was similar to that present in the oil bodies of the seeds of *M. chamomilla*, peanut cotyledon [7] and cotyledons of the linseed and sunflower [8]. Such an amount of phospholipid may be considered adequate for the provision of a "half-unit membrane" for the organelle [9].

The oil bodies in the shoot primordia contained steryl fatty acyl esters and free sterols as only minor components. This contrasts with the oil bodies of maize scutella and a celery cell suspension culture which contain appreciably larger amounts of steryl ester and free sterol [10]. There was some evidence for the presence of steryl glycosides and acylsteryl glycosides in shoot primordia tissue (TLC R_f, GC, MS) but neither were detected in the oil bodies of this tissue, or in those of seeds.

The free sterol composition of oil bodies from camomile shoot primordia and seed are shown in Table 5. The major end-product plant sterols (campesterol, stigmasterol and sitosterol) constituted 91% of the total free sterols in both shoot primordia and seed oil bodies.

Table 3. Fatty acid compositions of triacylglycerols in the oil bodies from the shoot primordia, seeds, flowers and leaves of *M. chamomilla*

Origin	Relative composition (%)						
	14:0	16:0	16:1	18:0	18:1	18:2	18:3
Shoot primordia*	tr.	8	1	2	5	61	23
Seeds	tr.	13	tr.	15	1	69	tr.
Flowers	tr.	13	1	11	tr.	73	2
Leaves	2	34	2	9	4	15	34

*Cultured under normal conditions for 6 days.

Table 4. Fatty acid compositions of acylglycerols and free fatty acids in the oil bodies from the cultured shoot primordia of *M. chamomilla*

Compound	Culture period (days)	Relative composition (%)						
		14:0	16:0	16:1	18:0	18:1	18:2	18:3
Triacylglycerol	3	tr.	5	1	3	5	63	23
1,3-Diacylglycerol	3	3	18	tr.	10	6	49	14
1,2-Diacylglycerol	3	1	7	tr.	8	5	63	16
2-Monoacylglycerol	3	tr.	5	1	9	4	64	17
1-Monoacylglycerol	3	10	37	tr.	17	1	30	5
Fatty acids	3	2	16	tr.	10	10	48	14
Triacylglycerol	12	tr.	8	1	2	5	61	23
1,3-Diacylglycerol	12	2	14	tr.	5	8	53	18
1,2-Diacylglycerol	12	1	12	tr.	3	6	61	17
2-Monoacylglycerol	12	tr.	7	tr.	4	4	66	19
1-Monoacylglycerol	12	8	48	tr.	13	tr.	22	10
Fatty acids	12	1	18	tr.	4	13	48	15

Table 5. The free sterol compositions of the oil bodies from the shoot primordia and seeds of *M. chamomilla*

Sterol	Relative content (%)	
	Shoot primordia	Seeds
Campesterol	16	11
Stigmasterol	14	14
Sitosterol	61	66
Isofucosterol + C_{29} stanol	nd	9
Unknown	9	nd

Table 6. The compositions of the sterol and fatty acid moieties of the steryl esters of the oil bodies from the shoot primordia and seeds of *M. chamomilla*

Compd.	Relative content (%)	
	Shoot primordia	Seeds
Sterols		
Campesterol	19	11
Stigmasterol	3	3
Sitosterol	64	48
Others (see text)	14	38
Fatty acid moiety		
16:0	7	31
16:1	6	11
18:0	3	10
18:1	7	17
18:2	52	31
18:3	23	tr.

Isofucosterol and C_{29} stanol were also present in the seeds, whereas the shoot primordia contained an unidentified sterol. It is possible that the free sterols are involved, together with the phospholipids, in the bounding membrane of the oil body. The steryl esters could be present together with the triacylglycerols in the core of the oil body.

Table 6 shows the sterol and fatty acid compositions of the steryl esters isolated from the oil bodies of camomile shoot primordia and seeds. A considerable difference was observed between the sterol moieties of the steryl esters from oil bodies of shoot primordia and seeds. Principally, the shoot primordia had a higher proportion of the esters of the Δ^5-sterols, campesterol and sitosterol, whereas the seeds contained a greater variety of esterified sterols, including precursor sterols (isofucosterol, 24-methylenecholesterol, cycloartenol, 24-methylenecycloartanol, 24-ethylidenelophenol) as well as small amounts of α-amyrin and other triterpenols.

The fatty acid compositions of the steryl esters of the oil bodies from shoot primordia were similar to those of the triacylglycerols from the oil bodies, with linoleic and linolenic acids contributing the greater proportion (75%). However, linolenic acid was not detected in the steryl esters of seed oil bodies, and the proportion of more saturated fatty acids was much increased. This situation was also observed for the triacylglycerol fatty acids of the seeds (Table 4). Also notable was the greater amount of palmitoleic acid (16: 1) in the steryl esters of oil bodies from both shoot primordia and seeds than was found esterified to the triacylglycerol from these materials (Table 3).

The physiological functions of the oil bodies in the multiplying cells are uncertain and further investigations are necessary to gain a better understanding of their role(s) during tissue development, response to environmental stress and senescence. The cultured shoot primordia may be useful as a material for investigations of this nature concerning the function of oil bodies and the possible role they play in the regulation of the lipid metabolism in plant cells.

EXPERIMENTAL

Materials. Shoot primordia of *M. chamomilla* were subcultured according to the reported procedure [11] in test tubes (3 x 20 cm) containing MS medium (pH 5.8) [4] supplemented with 2.0 mg l^{-1} of α-naphthalene acetic acid, 2.0 mg l^{-1} of 6-benzylaminopurine and 30 g l^{-1} of sucrose at 25° under illumination (3000 lux). For the cultures under 'starvation', sucrose was omitted from the medium.

Isolation of oil bodies. Isolation of oil bodies was performed at 0–4°C for all operations. The cultured shoot promordia (50 g), seeds (0.5 g), flowers (50 g), and leaves (50 g) were separately homogenized in an equal weight of 50 mM Tris–HCl buffer (pH 7.5) containing 1 mM EDTA, 0.6 mM $MgCl_2$ and 0.5 M mannitol with a mortar for 10 min. The homogenate was filtered through three layers of cheese-cloth and the filtrate was centrifuged at 5000 g for 15 min to give a floating creamy surface layer. This layer was re-suspended in the same buffer and the suspension was centrifuged again at 3000 g for 30 min. The floating oil bodies were collected. This process was repeated more than three times, gradually increasing centrifugal force to 100 000 g, until the solution became clear and colourless.

Measurement of the lipid compositions. Lipids of the oil bodies were extracted with $CHCl_3$–MeOH or *i*-PrOH–CH_2Cl_2 by the method of Bligh and Dyer [12, 13]. After addition of a specified amount of heptadecanoic acid, 5α-cholestanol and cholesterol myristate as internal standards for quantitative analyses, the extract was subjected to TLC on silica gel with hexane–benzene (1 : 1) to separate four fractions containing steryl fatty acyl esters, mono-, di- and triacylglycerols, free sterols and phospholipids.

The glycerolipid fraction was further subjected to TLC on boric acid-coated silica gel plates with $CHCl_3$–Me_2CO (96:4) to separate triacylglycerols, 1,2- and 1,3-diacylglycerols, 1- and 2-monoacylglycerols and free fatty acids. The recovered compounds were transmethylated with 5% HCl–MeOH [14]. The fatty acyl methyl esters formed were quantitatively analysed by GC. GC was performed on an instrument equipped with an FID and a capillary column (0.25 mm x 30 m, OV-17) at 200°. GC-MS were taken on a mass spectrometer which was installed with an EI ion source (70 eV) and a gas chromatograph equipped with a capillary column (0.25 mm x 30 m, OV-17) at 200°.

Free sterols were analysed as their trimethylsilyl ethers by GC using a 25 m x 0.22 mm BP5 (0.25 μm capillary column, with the GC oven programmed from 50° to 120° at 50° min^{-1}, then to 280° at 6° min^{-1}, and held isothermally for 20 min. Intact steryl esters were analysed by GC on a 12 m x 0.22 mm BPI (0.1 μm) capillary column with the oven programmed from 50° to 150° at 50° min^{-1}, then to 340° at 4° min^{-1} and held isothermally for 10 min. The sterol moieties from the steryl esters were obtained following alkaline hydrolysis (8% KOH in 80% EtOH; 70°, 2 hr) and partition into hexane, and were analysed as described above for the free sterols. The fatty acid moieties were partitioned into hexane following acidification of the aq. phase, (2.5% H_2SO_4 in each MeOH; 70°; 1 hr), methylated and analysed by GC on a 25 m x 0.32 mm Carbowax (0.3 μm) capillary column with the oven temp. programmed from 50° to 230° at 10° min^{-1} and held isothermally for 20 min. GC-MS analyses were performed on a VG Quattro coupled to a HP5890 series II GC operated at 70 eV, 200 μA, source temp. 280°.

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