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Formation of Oil Bodies in Cultured Shoot Primordia of *Matricaria chamomilla*

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Matricaria chamomilla L., German chamomile, is an asteraceous herb, native to Europe. The essential oil from the flowers of these plants is known as German (or blue) chamomile oil and is used for folk medicine and as a bath additive, since it contains pharmacological secondary metabolites such as mono- and sesquiterpenoids, flavonoids, and coumarins[1-3]. In general, it is known that some aliphatic metabolites are contained in oil bodies, which may be a subcellular organelle for biosynthesis and/or a pool for the aliphatic metabolites. Although oil bodies in seeds (a sink organ) have been well investigated, little is known about the function of the oil body in multiplying cells (vegetative tissues). In connection with studies of the production of useful compounds with cultured cells[4-6], we investigated the formation of oil bodies in shoot primordia of *M. chamomilla.*

Accrding to the reported procedure[6], shoot primordia of *M. chamomilla* were subcultured in Murashige-Skoog's (MS) liquid media[7] supplemented with α-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP) at pH 5.8. Formation of oil bodies in the shoot primordia was examined with 16 kinds of the MS media supplemented with 0, 0.02, 0.2 and 2.0 mg/l of NAA in addition to 0, 0.02, 0.2 and 2.0 mg/l of BAP. Oil bodies were found to increase remarkably in the cultures with the MS media containing 0~0.2 mg/l of NAA in addition to 0.2 mg/l of BAP or 0.2~2.0 mg/l of NAA without BAP. However, the medium containing 0.02 mg/l of NAA and 0.2 mg/l of BAP was suitable for the formation of oil bodies, because the shoot primordia were morphologically homogeneous during subculture in the medium (**Fig. 1**). The size of the oil bodies in the cells was 0.3~1.5 μm in diameter; some small oil bodies (<0.3 μm) were occasionally observed in the outer layer of the shoot primordium, whereas middle-sized oil bodies (0.5~0.8 μm) were abundant in the inner meristematic region and its surrounding area.

Isolation of oil bodies was performed at 0~4°C. The shoot primordia (or seeds) were homogenized in 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 cheesecloth and the filtrate was centrifuged at 5,000×g for 15 min. to give a floating creamy surface layer. This layer was resuspended in the same buffer and the suspension was centrifuged again at

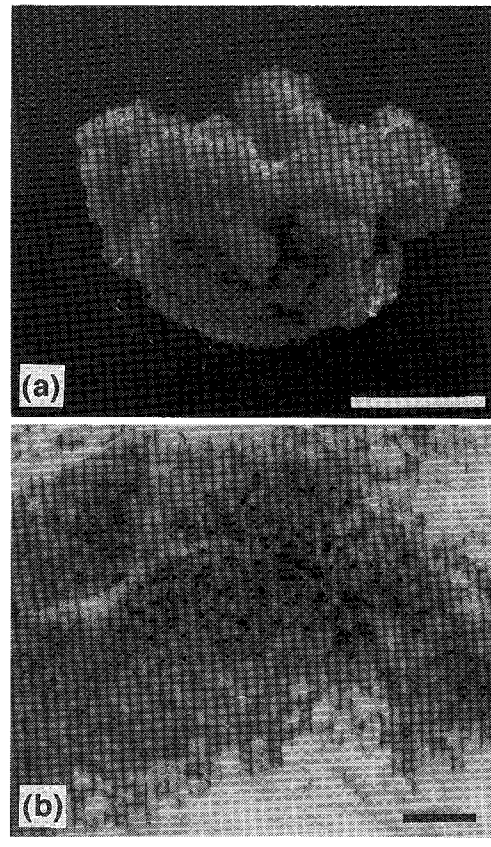

Fig. 1 Mass of shoot primordia of *Matricaria chamomilla*.
(a) Surface view, Bar=1 mm; (b) Cross section, Bar=50 μm, Oil bodies were stained black by sudan black.

30, 000×g for 30 min. The floating oil bodies were collected. This process was repeated more than three times, gradually increasing the centrifugal force to 100, 000×g, until the solution became clear and colorless. The size of the oil bodies obtained was about 0. 5~1. 2 μm in diameter.

Lipids of the oil body were extrated with $CHCl_3$-MeOH or isopropanol-CH_2Cl_2 according to the reported procedure[8,9]. The extract was subjected to thin-layer chromatography on boric acid-coated silica gel plates with $CHCl_3$-acetone (96:4 v/v) to separate mono-, di-, and triacylglycerols and free fatty acids. Phospholipids were subjected to 2-dimensional multistage separation on silica gel plate[10]. Individual glycerolipids separated were transmethylated with 5% HCl-MeOH[11] after addition of heptadecanoic acid as internal standard. The methyl esters formed were quantitatively analyzed by gas chromatography (GC) and GC-MS. Accumulation of secondary metabolites such as mono- and sesqui-terpenoids in the oil bodies could not be found in spite of careful analyses by thin-layer chromatography and GC. No remarkable difference in the lipid components between the shoot primordia and the seeds was observed, as shown in **Table 1**. Major fatty acid residue of acylglycerols and free fatty acids was linoleic acid. Triacylglycerol was the major component of the acylglycerols in both the shoot primordia and seeds. The content of triacylglycerol seems to be somewhat small, compared with the reported data (89~97% of triacylglycerol) for that of seeds such as linseed and safflower[12], crambe seed[13] and castor bean[14]. On the other hand, the content of phospholipid (0. 7%) was similar to those in the oil bodies of the seeds of *M. chamomilla* (**Table 1**), peanut cotyledon[15], and cotyledons of linseed and safflower[12]. The content is considered to be appropriate for the provision of a "half-unit membrane" for the organelle[16].

Table 1. Lipid components in the oil bodies of *M. chamomilla.*

Compd.*1	Content/wt %	
	Shoot primordia	Seeds
TG	78.7	75.7
1, 3-DG	2.8	11.9
1, 2-DG	5.1	0.9
2-MG	1.2	1.3
1-MG	3.3	1.1
FA	8.2	8.5
PL	0.7	0.6

*1 TG, DG, MG, FA, and PL denote triacylglycerol, diacylglycerol, monoacylglycerol, free fatty acid, and phospholipids, respectively.

Thus, the conditions for the formation of oil bodies in the cultured cells of *M. chamomilla* were determined and the lipid constituents of the oil bodies were elucidated. The cultured shoot primordia cells may be useful as a tool for further investigations, such as the function of oil body in the cells and the biosynthesis and metabolism of the constituents in the oil body.

Acknowledgments

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《和文要約》

カミツレ苗条原基におけるオイルボディーの生成

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カミツレ（*Matricaria chamomilla*）の苗条原基細胞におけるオイルボディーの生成とその構成脂質を調べた．α-ナフタレン酢酸（0.02 mg/*l*）と6-ベンジルアミノプリン（0.2 mg/*l*）を含むMS培地がオイルボディーの形成に最も適当であることがわかった．また，このオイルボディーはアシルグリセロールを主成分とし，遊離脂肪酸とリン脂質とから構成されていることがわかった．