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Δ^6-Unsaturated fatty acids in species and tissues of the Primulaceae

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Abstract

The Δ^6-unsaturated fatty acids γ-linolenic acid (GLA; $18{:}3\Delta^{6,9,12}$) and octadecatetraenoic acid (OTA; $18{:}4\Delta^{6,9,12,15}$) were present in seed lipids of the tribe Primuleae, but not in other tribes of the Primulaceae. Within the genus *Primula* both fatty acids were present in seed lipids from 22 species (from 12 sections), with combined levels increasing from 1.1 to 27.4%. High levels of Δ^6-unsaturated fatty acids were also present in leaves of ten species (from nine sections), but with lower levels generally being present in root lipids. In general, the levels of octadecatetraenoic acid were higher than that of γ-linolenic acid. The results indicate that Δ^6-unsaturated fatty acids could be used as taxonomic markers within the genus *Primula*.

Keywords: *Primula* spp; Primulaceae; Seeds; Leaves; Roots; Δ^6-Unsaturated fatty acids

1. Introduction

The Δ^6-unsaturated fatty acids γ-linolenic acid (GLA; $18{:}3\Delta^{6,9,12}$) and octadecatetraenoic acid (OTA; $18{:}4\Delta^{6,9,12,15}$, also called stearidonic acid) are synthesised from linoleic acid ($18{:}2\Delta^{9,12}$) and α-linolenic acid (ALA; $18{:}3\Delta^{9,12,15}$), respectively, catalysed by a Δ^6 fatty acid desaturase. GLA has a limited distribution, being reported in seed oils of some 160 species from ten botanically diverse plant families, being particularly abundant in species of the Boraginaceae, Onagraceae, Saxifragaceae and Scrophulariaceae (Ucciani, 1995). The Boraginaceae and Onagraceae include *Borago officinalis* and *Oenothera biennis*, respectively, which are used as commercial sources of GLA-containing oils. OTA has been less widely studied, but has been reported to occur, together with GLA, in seed oils of the Boraginaceae, Primulaceae and Saxifragaceae (Aitzetmüller & Werner, 1991; Kleiman, Earle, Wolff, & 1964; Miller, Earle, Wolff, & Barclay, 1967; Traitler, Winter, Richli, & Ingenbleek, 1984; Wolf, Kleiman, & England, 1983). The only current commercial plant source of OTA is the seed oil of *Ribes nigrum* (Traitler et al., 1984). The occurrence of GLA and OTA in vegetative tissues has not been widely studied, although these fatty acids have been reported in total leaf lipids of the Boraginaceae and Caryophyllaceae (Hansen, Stoessel, & Rossi, 1991; Jamieson & Reid, 1969, 1971; Sayanova, Shewry, & Napier, 1999; Sewòn & Tyystjärvi, 1993).

The Primulaceae comprises some 1000 species and 22 genera which are grouped into five tribes (Table 1). Previous studies of seed oils have been restricted to the genus *Primula*, showing 11–14% OTA in three species of the section *Sikkimensis* (*P. alpicola*, *P. sikkimensis*, *P. florindae*) but only a low level (0.1%) in one species of the section *Petiolares* (*P. pulverulenta*). In all species lower levels of GLA (0.2 to 4.3 mol%) were present (Aitzetmüller & Werner, 1991). These results indicate that the occurrence and proportions of GLA and OTA could be taxonomic markers for differentiation of sections of the genus *Primula*. In view of this and the economic importance of GLA and OTA, we have made a detailed study of the distribution of these fatty acids in seed oils and vegetative tissues of the Primulaceae, focusing on sections of the genus *Primula*.

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Table 1
Tribes and genera of the Primulaceae according to Heywood, 1978. Number of species are given in parentheses

	Tribes				
	(1) Primuleae	(2) Cyclamineae	(3) Lysimachieae	(4) Samoleae	(5) Corideae
Genera	*Primula* (~500) *Soldanella* (~11) *Dodecatheon* (~50) *Androsace* (~100) *Hottonia* (2) *Dionysia* (~44)	*Cyclamen* (15)	*Lysimachia* (~200) *Anagallis* (~28) *Glaux* (1) *Trientalis* (4)	*Samolus* (15)	*Coris* (2)

2. Results and discussion

2.1. Identification of Δ^6-unsaturated fatty acids in seed lipids of species of the tribe Primuleae

Preliminary analyses of seeds of species from all five tribes of the Primulaceae showed no evidence for GLA or OTA in the Tribes Cyclamineae (*Cyclamen neapolitanum* L.), Lysimachieae (*Lysimachia vulgaris* L., *L. lichiangensis* L., *L. minorciensis* L., *Anagallis blue* L., *A. arvensis* L., *Trientalis europaea* L.), Samoleae (*Samolus valerandi* L.) and Corideae (*Coris monspeliensis* L.) and no further studies of these were therefore carried out. In contrast, both OTA and GLA were widely distributed in seed lipids of species of the tribe Primuleae, including species of *Soldanella*, *Dodecatheon* (Table 2) and *Primula* (Table 3). However, Δ^6-unsaturated fatty acids were not ubiquitous in this tribe, seeds of *Androsace villosa* L., *A. chameajasme* L., *A. alpina* L. and *Douglasia vitaliana* L. all lacked GLA or OTA with the latter two species containing high levels of C16:1 fatty acid (Table 2).

The genus *Primula* comprises some 500 species, including many ornamentals, which are classified into 37 sections (Richards, 1993). We analysed seed oils from 22 species from 12 sections (Table 3). The proportions of OTA and GLA varied widely, allowing the species to be divided into five broad groups as defined in Table 3. In most cases species in the same section were placed in the same group. The exception was the section *Oreophlomis*, with *P. rosea* Royle placed in group IV and *P. luteola* Ruprecht in group V. With the exception of group I, all species contained higher amounts of OTA than GLA. Furthermore, the levels of OTA in the group II species (17.5 and 22.5%) were similar to the highest levels so far reported for plant oils (Tsevegsüren & Aitzetmüller, 1996). Statistical analysis showed no correlation between the amounts of OTA and GLA but a strong negative correlation between the proportion of 18:3 and GLA ($r = -0.683$, $p < 0.001$) was observed. The latter was not surprising since 18:3 and GLA compete for the same substrate.

Table 2
Fatty acid compositions of seed lipids of some species of the tribe Primuleae

Genus/species	16:0	16:1[a]	18:0	18:1	18:2	GLA	18:3	OTA
Soldanella alpina L.	15.7	5.2	0.4	16.8	29.7	15.8	2.2	1.7
S. pusilla L.	16.0	8.0	0.7	17.2	34.1	13.2	2.1	1.4
Dodecatheon meadia L.	11.1	1.7	0.5	25.5	27.0	4.5	19.9	11.9
D. *tetrandrum* L.	10.5	1.9	0.6	20.0	26.8	2.5	27.9	12.2
Androsace alpina L.	10.0	46.9	0.1	20.6	18.2	–	2.8	–
A. vitaliana L.	8.7	54.0	0.1	16.3	13.0	–	2.1	–

[a] Position of double bond not determined.

2.2. Δ^6-unsaturated fatty acids of vegetative tissues from Primula species

The fatty acid compositions of vegetative tissues of ten selected species from nine sections of *Primula* were also determined (Table 4). High levels were present in leaves, the combined proportions of GLA + OTA ranging from 15.8 to 34.7% (mean 29.5), with OTA being the major component in all cases. The levels of both fatty acids were generally lower in roots (0.8 to 16.1%, mean 6.0) than in leaves or seeds (mean 13.1) but OTA was again the major component with the exception *of P. macrophylla* D. Don, which contained only low levels (0.4%) of both fatty acids.

Statistical analysis showed that there was no relationship between the levels of OTA in the three tissues or between the levels of GLA in leaves and seeds or in leaves and roots. A correlation between the levels of GLA in seeds and roots was observed ($r = 0.896$, $p < 001$) but this was determined solely by high levels of this fatty acid in the two tissues of the two group I species (*P. cortisoides* L. and *P. malacoides* Franchet). It is concluded, therefore, that the levels of OTA, GLA and OTA + GLA vary independently in the three tissues. The reasons for the variation in composition between the different species and tissues are not known, but the latter could reflect their different contents of acyl lipids, with predominantly storage triacylglycerols in seeds, membrane phospholipids in roots and a mixture of membrane phospholipids and chloro-

Table 3
Fatty acid compositions of seed lipids of *Primula* species

Section	Species	16:0	18:0	18:1	18:2	GLA	18:3	OTA	GLA + OTA
Group I: high GLA, medium OTA									
Monocarpicae	*M. malacoides* Franchet	14.1	0.8	4.6	33.8	15.7	13.8	11.7	27.4
Cortusoides	*C. cortusoides* L.	11.1	0.8	7.3	33.0	16.6	20.1	4.5	21.1
Group II: low GLA, high OTA									
Aleuritia	*A. scotica* W.J. Hooker	7.7	0.4	10.3	26.9	2.2	29.0	22.5	24.5
Aleuritia	*A. farinosa* L.	9.1	0.4	7.3	29.9	1.8	29.2	17.5	19.3
Group III: low GLA, medium OTA									
Crystallophlomis	*C. macrophylla* D. Don	7.4	1.0	11.1	38.1	3.8	25.5	7.4	11.2
Obconicolisteri	*O. obconica* Hance	11.6	1.3	5.5	51.5	4.8	20.1	4.5	9.3
Group IV: very low GLA, medium OTA									
Auricula	*A. auricula* L.	14.1	0.4	6.9	26.5	0.8	35.3	8.9	9.7
Auricula	*A. glaucescens* Moretti	12.4	0.4	9.8	42.1	2.3	22.0	5.1	7.4
Auricula	*A. integrifolia* Scopoli	9.4	0.6	9.2	38.6	1.6	32.2	5.6	7.2
Auricula	*A. viscosa* Allioni	16.5	0.9	10.0	34.0	0.6	32.5	3.7	4.3
Auricula	*A. pedemontana* Gaudin	19.9	1.3	8.1	31.8	0.4	31.5	3.2	3.8
Primula	*P. juliae* Kusnetsow	10.6	0.6	12.3	22.1	0.7	35.5	11.5	12.2
Primula	*P. veris* L.	18.8	0.8	7.6	27.8	0.7	33.5	8.9	9.6
Primula	*P. vulgaris* Hudson	13.1	0.9	23.1	28.9	0.9	25.5	8.5	9.4
Primula	*P. elatior* Hill	16.0	1.1	10.0	26.5	0.7	26.9	8.5	9.2
Petiolares	*P. petiolaris* Wallich	10.0	0.5	8.0	39.1	2.2	33.0	4.3	6.5
Muscarioides	*M. vialii* Franchet	12.2	0.9	15.1	31.1	0.2	32.1	6.1	6.3
Oreophlomis	*O. rosea* Royle	12.0	0.6	15.5	33.7	0.6	30.0	5.3	5.9
Group V: traces of GLA, low OTA									
Denticulata	*D. denticulata* J.E. Smith	10.0	0.5	11.4	32.2	0.1	39.7	2.9	3.0
Oreophlomis	*O. luteola* Ruprecht	12.0	0.4	6.8	30.6	0.1	49.1	1.5	1.6
Proliferae	*P japonica* Gray	9.7	1.0	8.0	37.6	0.2	38.2	1.1	1.3
Proliferae	*P. beesiana* Forrest	13.4	1.0	6.8	33.4	0.1	44.5	1.0	1.1

plast glycerolipids in leaves. Further studies of individual lipid classes would be required to confirm this.

In conclusion, the analyses reported here indicate that the distribution and proportions of Δ^6-unsaturated fatty acids do indeed have potential value as taxonomic markers in the Primulaceae and the genus

Table 4
Fatty acid compositions of leaf and root lipids from *Primula* species

Section	Species		16:0	18:0	18:1	18:2	GLA	18:3	OTA	GLA + OTA
Monocarpicae	*M. malacoides*[a]	leaf	21.7	–	–	13.0	10.9	33.4	21.1	32.0
		root	48.1	0.4	1.6	37.7	1.4	9.2	1.5	2.9
Cortusoides	*C. cortusoides*	leaf	11.5	0.4	1.2	13.3	4.0	47.3	18.8	22.8
		root	23.6	–	1.4	42.1	1.9	23.3	6.4	8.3
Aleuritia	*A. scotica*	leaf	20.7	–	–	17.8	6.7	35.3	15.6	22.3
		root	45.7	–	3.5	38.6	0.2	14.6	4.0	4.2
Aleuritia	*A, farinosa*	leaf	16.0	–	–	16.2	5.4	43.0	29.3	24.7
		root	31.9	–	0.5	41.7	0.1	22.9	4.4	4.4
Crystallophlomis	*C. macrophylla*	leaf	19.6	–	3.0	14.8	5.7	38.0	19.7	25.4
		root	31.5	–	0.6	42.1	0.4	22.2	0.35	0.7
Auricula	*A. glaucescens*	leaf	12.3	–	4.3	13.4	6.0	37.8	9.8	15.8
		root	31.5	–	0.6	42.0	0.3	20.2	4.7	5.0
Primula	*P. vulgaris*	leaf	18.8	0.8	5.3	16.6	0.6	36.0	22.0.	22.6
		root	37.4	–	1.5	36.4	0.8	13.3	10.2	11.0
Muscarioides	*M. vialii*	leaf	16.2	–	1.4	14.4	1.5	36.8	25.6	27.1
		root	32.8	0.2	0.8	47.1	0.1	14.0	4.0	4.1
Denticulata	*D. denticulata*	leaf	13.8	–	1.2	12.8	5.9	31.5	27.7	33.2
		root	39.2	0.4	0.7	42.6	0.2	13.3	3.3	3.5
Oreophlomis	*O. luteola*	leaf	14.7	0.5	2.3	14.5	1.9	40.0	20.0	21.9
		root	21.0	0.4	1.0	37.5	0.6	24.5	15.5	15.5

[a] Botanical authorities are as in Table 3.

Primula. Furthermore, the high proportions of OTA in some species indicate the potential for commercial exploitation as a source of this important fatty acid.

3. Experimental

3.1. Plant material

Seeds of *Coris monspeliensis* L. were obtained from Istituto ed Orto Botanico 'Hanbury', University of Geneva, Italy. Seeds of *Samolus valerandi* L. were obtained from Jardin Botanique de Dijon, France. All other seeds were obtained from Chiltern Seeds, Cumbria.

3.2. Fatty acid analysis

The levels of individual fatty acids were determined by gas chromatography of methyl esters of total tissue extracts (Sayanova et al., 1999) and expressed as percentages of the total fatty acids.

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