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HYDROXY AND NORMAL FATTY ACID DISTRIBUTION IN STIGMAS OF *NICOTIANA* AND OTHER PLANTS

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Key Word Index—*Nicotiana*; Solanaceae; stigma; fatty acid composition; ω-hydroxy fatty acid; lipid; triacylglycerol; diacylglycerol.

Abstract—The ω-hydroxy and normal fatty acid distribution in stigmas of 51 *Nicotiana* species and 25 other plant species is presented. All *Nicotiana* species studied had high proportions of ω-hydroxy fatty acids (C18:1-ω-OH and C18:2-ω-OH), constituting from 22 to 83% of the total fatty acid content. In most *Nicotiana* species, the percentage of C18:1-ω-OH was higher than that of C18:2-ω-OH and the percentage of C18:1 was higher than that of C18:2. The reverse pattern of this fatty acid composition was observed in the self-incompatible *Nicotiana* species. Many plant species with wet type stigma and a relatively few species with dry type stigma contained considerable amounts of C18:1-ω-OH and C18:2-ω-OH. The ω-hydroxy fatty acids were contained not only in the triacylglycerol and diacylglycerol fractions but also in the polar fraction of stigma lipids.

INTRODUCTION

The stigma is the receptive part for pollen. *Nicotiana tabacum* has a wet type stigma where fluid secretions occur during the receptive period. We have previously isolated and identified the triacylglycerol (TG) and diacylglycerol (DG) types of multiacylglycerol from stigma of *N. tabacum* [1, 2]. These multiacylglycerols have ω-hydroxy fatty acids (C18:1-ω-OH and C18:2-ω-OH) and normal fatty acids in their molecules. The ω-hydroxy fatty acids are found only in the stigma of *N. tabacum* [3]. Although multiacylglycerols have been detected in other plant sources, i.e. ergot oil [4] and kamala oil [5], the existence of ω-hydroxy fatty acids such as C18:1-ω-OH and C18:2-ω-OH in these multiacylglycerols has not been reported. ω-Hydroxy fatty acids so far reported in other plant species are those found as constituent fatty acids in polymerized cuticular and suberin layers [6]. This study examined the ω-hydroxy and normal fatty acid composition in the stigma of 51 *Nicotiana* species and 25 other plant species. Also studied was the distribution of ω-hydroxy and normal fatty acids in stigma lipid classes of three plant species.

RESULTS AND DISCUSSION

The genus *Nicotiana* includes some 66 species and the classification essentially follows that of Goodspeed [7, 8]. The ω-hydroxy and normal fatty acid distribution in stigmas of 51 *Nicotiana* species is summarized in Table 1. The stigmas of all species studied contain high proportions of ω-hydroxy fatty acids (C18:1-ω-OH + C18:2-ω-OH), ranging from 22% (*N. solanifolia*) to 83% (*N. goodspeedii*) of the total fatty acid content. The major normal fatty acids are oleate (C18:1) and linoleate (C18:2). In most species, the percentage of C18:1-ω-OH is higher than that of C18:2-ω-OH and the percentage of C18:1 is higher than that of C18:2. Thus, the amount of an ω-hydroxy fatty acid in stigma of *N. tabacum* seems to

Table 1. The ω-hydroxy and normal fatty acid distribution in stigmas of *Nicotiana* species

Section / Species	Fatty acid composition (% total)						
	16:0	18:0	18:1	18:2	18:3	18:1-OH	18:2-OH
Paniculatae							
glauca	2	+	36	8	2	42	10
paniculata	2	1	46	10	3	30	8
solanifolia	2	1	56	16	3	14	8
benavidesii	3	1	18	6	2	49	21
raimondii	2	1	27	6	2	49	13
Rusticae							
rustica	3	1	27	24	5	19	21

Table 1. *Continued*

Section / Species	Fatty acid composition (% total)						
	16:0	18:0	18:1	18:2	18:3	18:1-OH	18:2-OH
Tomentosae							
tomentosiformis	4	2	20	7	3	52	12
otophora	3	1	41	5	4	41	5
setchellii	2	+	51	13	6	20	8
glutinosa	5	2	16	9	9	45	14
kawakamii	4	1	35	11	5	33	11
Genuinae							
tabacum	3	1	26	9	3	45	13
Undulatae							
undulata	3	1	23	7	3	53	10
arentsii	1	+	26	15	2	36	20
wigandioides	2	1	24	19	2	26	26
Trigonophyllae							
trigonophylla	1	+	42	2	4	49	2
Alatae							
sylvestris	3	+	42	12	2	32	9
langsdorffii	3	+	19	37	6	10	25
alata	3	1	17	35	6	13	25
forgetiana	1	+	18	26	2	23	30
bonariensis	1	+	15	20	2	28	34
plumbaginifolia	3	1	10	9	3	51	23
Repandae							
repanda	4	1	6	5	5	72	7
stocktonii	5	+	11	5	8	64	7
nesophila	4	1	7	5	5	64	14
Acuminatae							
acuminata	1	+	13	6	3	46	31
pauciflora	1	+	17	6	2	50	24
attenuata	2	+	22	8	4	50	14
corymbosa	3	+	14	7	5	49	22
spegazzini	2	+	12	8	3	51	24
Bigelovianae							
bigelovii	1	+	26	5	2	54	12
clevelandii	6	1	20	11	10	42	10
Nudicaulis							
nudicaulis	3	1	25	12	5	39	15
Suaveolentes							
umbratica	1	+	13	2	2	72	10
cavicola	3	1	21	5	6	54	10
debneyi	4	1	22	10	5	43	15
gossei	1	+	16	2	2	71	8
amplexicaulis	3	+	29	5	7	49	7
maritima	4	+	20	4	5	59	8
velutina	3	+	32	5	6	49	6
hesperis	7	1	14	7	11	46	14
occidentalis	4	1	23	6	8	49	10
simulans	2	1	9	2	4	74	8
megalosiphon	3	+	21	3	4	64	5
excelsior	1	+	28	4	2	58	7
suaveolens	1	+	20	4	2	63	10
ingulba	2	+	11	2	4	74	7
exigua	5	1	18	4	7	57	8
goodspeedii	2	+	10	2	3	76	7
rosulata	3	+	13	3	4	68	9
fragrance	4	1	18	6	4	58	9

be reflected by the amount of the corresponding normal fatty acid. It is reported that ω-hydroxylation of fatty acids to form the ω-hydroxy fatty acids found in cuticular and suberin layers is catalysed by microsomal preparatins from pea seedlings [9] and germinating *Vicia faba* [10]. Therefore, it is of interest to study the biosynthetic pathways of ω-hydroxy fatty acid formation in tobacco stigma. A different pattern from that of most species is seen in the fatty acid composition of four species in the *Alatae* section, i.e. *N. langsdorffii*, *N. alata*, *N. forgetiana* and *N. bonariensis*. In these species, the proportion of C18:2-ω-OH is significantly higher than that of C18:1-ω-OH, which corresponds with a higher proportion of C18:2 than C18:1. *N. alata*, *N. forgetiana* and *N. bonariensis* are self incompatible species and *N. langsdorffii* is a species with some self incompatible races [7]. Most other *Nicotiana* species examined in the present study are self compatible ones. The results show that four of the self incompatible species have a different hydroxy and normal fatty acid pattern to the other self compatible species. However, it is uncertain if this is concerned with self incompatible systems.

The ω-hydroxy and normal fatty acid distribution in stigmas of 15 plant species with wet type stigma are shown in Table 2. The classification of stigma types, wet or dry, essentially followed that of Heslop-Harrison and Shivanna [11]. All four species studied in the *Solanaceae* family, i.e. *Solanum melongena*, *Salpiglossis sinuata*, *Petunia hybride* and *Lycoperisicon esculentum*, were found to have high proportions of ω-hydroxy fatty acids (C18:1-ω-OH + C18:2-ω-OH) comprizing 61-96% of the total fatty acid content. *Rhododendron obtusum*, *R. indicum* and *Lilium auratum* also had high proportions (48–77%) of ω-hydroxy fatty acids. Significant proportions of ω-hydroxy fatty acids are present in *Convallaria majalis*, *Prunus yedoensis*, *P. persica* and *Citrus natsudaidai*. In these wet type species mentioned above, the proportion of C18:1-ω-OH is higher than that of C18:2-ω-OH, cf most *Nicotiana* species. However, the proportion of normal fatty acids such as C18:1 and C18:2 is not correlated with that of the corresponding ω-hydroxy fatty acids. That is, the percentage of C18:2 is often higher than that of C18:1. The other four species with wet type stigma contain only small, if any, amounts of ω-hydroxy fatty acids.

Of the 10 plant species studied with dry type stigma (Table 3), only three species, i.e. *Antirrhium majas*, *Pelargonium inquinans* and *Forsythia suspensa*, have relatively high proportions (15–33%) of ω-hydroxy fatty acids. The other seven species contain little or no ω-hydroxy fatty acids. The present results show that relatively many plant species with wet type stigma and a relatively few species with dry type stigma contain considerable amounts of C18:1-ω-OH and C18:2-ω-OH fatty acids in their stigma.

We have previously reported that ω-hydroxy fatty acids exist in both TG and DG fractions of *N. tabacum* stigma lipids as the constituent fatty acids of TG and DG types, respectively, of multiacylglycerol [3]. Therefore, the content and composition of the constituent fatty acids in stigma lipid fractions of three plant species were analysed and compared (Table 4). Stigma lipids of *N. tabacum* contain a large amount of TG (28.2%), followed by considerable amounts of DG and polar fractions. All three lipid fractions have relatively high proportions of ω-hydroxy fatty acids (C18:1-ω-OH major and C18:2-ω-OH next). On the other hand, in *R. indicum* and *L. auratum* the polar fraction is the dominant lipid class and contains C18:1-ω-OH as the major fatty acid. The present results showed that ω-hydroxy fatty acids are contained not only in the TG and DG fractions but also in the polar fraction of plant stigma lipids. This suggests that some ω-hydroxy acid-containing lipids other than TG and DG types of multiacylglycerol [1, 2] may exist in plant stigma.

EXPERIMENTAL

Materials. Tobacco plants (51 species in the genus *Nicotiana*) were grown in soil in 110 cm² pots in a greenhouse at 28°. At the

Table 2. The ω-hydroxy and normal fatty acid distribution in stigmas of plants with wet type stigma

	Fatty acid composition (% total)						
Species	16:0	18:0	18:1	18:2	18:3	18:1-OH	18:2-OH
Solanum melongena	3	1	3	5	2	77	9
Salpiglossis sinuata	10	1	12	5	4	64	4
Petunia hybrida	1	+	1	1	1	72	24
Lycopersicon esculentum	5	3	9	16	6	45	16
Rhododendron obtusum	5	2	6	9	3	70	5
Rhododendron indicum	4	1	6	6	6	76	1
Lilium auratum	13	1	13	14	11	48	+
Convallaria majalis	21	2	6	45	13	9	4
Prunus mume	24	7	4	27	35	1	2
Prunus yedoensis	54	12	4	10	9	10	1
Prunus persica	21	4	16	28	16	14	1
Viola mandshuria	22	2	3	52	17	3	1
Viola × *Wittrockiana*	26	2	3	54	14	+	1
Orchis sp.	24	1	11	49	10	4	1
Citrus natsudaidai	11	3	24	22	29	8	3

Table 3. The ω-hydroxy and normal fatty acid distribution in stigmas of plants with dry type stigma

Species	Fatty acid composition (% total)						
	16:0	18:0	18:1	18:2	18:3	18:1-OH	18:2-OH
Antirrhium majus	17	4	6	21	19	30	3
Pelargonium inquinans	20	1	10	31	21	16	1
Forsythia suspensa	17	+	12	30	26	9	6
Hibiscus syriacus	30	4	6	45	15	0	0
Hibiscus mutabilis	32	7	6	29	24	2	0
Crinum asiaticum	30	2	22	37	9	0	0
Narcissus tazetta	23	2	4	51	15	4	1
Erythrina indica	34	17	9	25	15	0	0
Pharbitis nil	48	7	2	3	40	0	0
Mirabilis jalapa	27	2	8	34	29	0	0

Table 4. The content and composition of fatty acids in stigma lipid fractions

Species	Lipid fraction	Fatty acid content (μg/mg lipid)	Fatty acid composition (% total)						
			16:0	18:0	18:1	18:2	18:3	18:1-OH	18:2-OH
Nicotiana tabacum	TG	282	1	+	34	6	1	42	16
	DG	139	1	+	27	4	+	50	18
	Polar	127	5	1	19	11	8	42	14
Rhododendron indicum	TG	5	35	12	21	21	11	0	0
	DG	16	21	8	22	6	1	42	0
	Polar	612	4	1	6	6	4	78	1
Lilium auratum	TG	18	20	4	18	16	9	33	0
	DG	37	10	2	10	12	7	59	0
	Polar	166	16	1	8	13	12	50	+

onset of flowering, 10 stigmata were obtained from the flowers of each species and freeze-dried. The stigmata of other plant species were collected in the field and freeze-dried.

Fatty acid analysis. The ω-hydroxy fatty acids (C18:1-ω-OH and C18:2-ω-OH) were identified by GC-MS as described previously [1]. Stigma samples were homogenized in a glass homogenizer with 5% H_2SO_4 in MeOH and incubated at 40° overnight [8]. The resultant normal and hydroxy fatty acid methyl esters were extracted with Et_2O, after adding a known amount of methyl heptadecanoate as an int. standard. The normal fatty acid methyl esters were analysed by FID-GC at 200° using glass columns packed with 5% BDS on Chromosorb W(AW-DMCS). The ω-hydroxy fatty acid methyl esters were analysed at 230° after trimethylsilylation with BSTFA on a fused silica capillary column (0.3 mm × 50 m) of OV-1. The standard ω-hydroxy fatty acid methyl esters were prepared as described previously [1].

Lipid extraction and lipid separation. Stigma samples (50–100 mg dry wt) were homogenized (×3) with $CHCl_3$–MeOH (1:1) and the combined homogenates filtered. The filtrate was washed with 5% NaCl soln, the $CHCl_3$ layer evaporated and the extract dried. The total lipid obtained was weighed and dissolved in a small vol. of $CHCl_3$–MeOH (9:1). The soln was applied to a silica gel TLC plate [3, 6] and the lipid classes separated by developing first with hexane–Et_2O (49:1) followed by hexane–Et_2O–HOAc (50:50:1) [12]. Lipid spots (TG, DG and polar fractions) were located under UV light after spraying with Rhodamine 6G soln, and scraped off. Individual lipid fractions were methylated and the fatty acid methyl esters were analysed by GC as mentioned above.

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