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THE FATTY ACID COMPOSITION OF SEEDS AND LEAVES OF *NICOTIANA* SPECIES

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Key Word Index—*Nicotiana*; Solanaceae; tobacco; seed; leaf; fatty acids; triacylglycerols; seed lipids.

Abstract—Fatty acid analyses of seeds in 62 *Nicotiana* species and leaves in 56 *Nicotiana* species are presented. The total fatty acid content on a dry wt basis ranged from 25 to 40% of seeds and from 2.1 to 4.4% of green leaves. Linolenate was the dominant fatty acid in the leaves of all species studied, comprising 50–63% of the total fatty acid content. In seeds of most species linoleate predominated, constituting 69–79% of the total fatty acid content. Fourteen of 21 species in the section *Suaveolentes* and one species in the section *Noctiflorae* had relatively high proportions (10–38%) of linolenate. In two linolenate-rich species studied, linolenate was the major fatty acid of triacylglycerols which predominated in the seed lipids.

INTRODUCTION

The fatty acid composition of the green leaves of higher plants follows a generally consistent pattern in which the major fatty acids are linolenate, linoleate and palmitate [1]. Tobacco leaves are also rich in these three fatty acids [2, 3]. On the other hand, seed lipids vary considerably in the fatty acid composition among plant species [1, 4]. Recent seed analyses suggest that fatty acids have taxonomic significance and evolutionary implications for higher plant classification and can be used to differentiate or relate taxa at several hierarchial levels [5–10]. Tobacco seed lipids are extremely rich in linoleate [2, 4, 11, 12]. However, such analyses are limited to only two commercial species, *Nicotiana tabacum* and *N. rustica*. The genus *Nicotiana* includes some 66 species. The present investigation provides the content and composition of leaf fatty acids in 56 species and seed fatty acids in 62 species.

RESULTS AND DISCUSSION

The content and distribution of fatty acids in green leaves of 56 *Nicotiana* species are summarized in Table 1. The classification essentially follows that of Goodspeed [13]; *N. kawakamii* [14] was later added to the *Tomentosae* section and *N. africana* [15] was tentatively grouped into the *Suaveolentes* section. The fatty acid contents on a dry wt basis varied from 2.12% (10. *N. tomentosa*) to 4.41% (46. *N. benthamiana* and 48. *N. cavicola*). Linolenate was the dominant fatty acid in all species studied, comprising 50–63% of the total fatty acid content. Palmitate and linoleate were the next major fatty acids constituting 13–18% and 5.0–18%, respectively. All species studied contained a considerable amount (2.6–7.7% of the total fatty acid content) of hexadecatrienoic acid in green leaves. The previous investigation [3] showed that green leaves of *N. tabacum* contained a considerable amount of hexadecatrienoic acid in monogalactosyldiacylglycerols and suggested that *N. tabacum* belongs to 16:3 plants [16]. The present results suggest that all *Nicotiana* species studied belong to 16:3 plants.

Table 1 also presents the content and distribution of seed fatty acids in 62 *Nicotiana* species. The fatty acid contents of all species studied were found to be relatively high, ranging from 25% (65. *N. fragrance*) to 40% (42. *N. spegazzini*) of seed dry wt. In 40 species (1–30, 32–45), linoleate was the dominant fatty acid comprising 69–79% of the total fatty acid content, followed by oleate (9.6–17%) and palmitate (6.7–10%) as the major fatty acids. Stearate and linolenate were the minor fatty acids in these species, constituting 1.4–4.7% and 0.5–5.5%, respectively. Different patterns from this of the fatty acid composition were observed in most species of the *Suaveolentes* section and *N. noctiflora* in the *Noctiflorae* section. Fourteen of 21 species in the *Suaveolentes* section had relatively higher proportions (10–38%) of linolenate than other species. This was accompanied by relatively lower proportions (58–29%) of linoleate. Moreover, in two species (52. *N. maritima* and 58. *N. rotundifolia*) the percentage of linolenate was higher than that of linoleate. *N. noctiflora* also had a relatively high proportion (23%) of linolenate and was the only exceptional species, apart from those in the *Suaveolentes* section.

It is reported that seed lipids of *N. tabacum* and *N. rustica* were preponderantly rich in triacylglycerols and that linoleate was the dominant fatty acid in triacylglycerols [4, 11, 12]. The present investigation showed that linolenate was one of the main fatty acids in seeds of several *Nicotiana* species. Further experiments were performed to see whether triacylglycerols were the dominant seed lipids in two linolenate-rich species and whether linolenate was the main fatty acid in triacylglycerols (Table 2). The total fatty acids of triacylglycerols in *N. noctiflora* and *N. rotundifolia* comprised 72 and 87%, respectively, of those of total lipids. Linolenate was found to be the major fatty acid in the triacylglycerols, constituting 17–30% of the total lipid fatty acids.

EXPERIMENTAL

Materials. Seeds of *Nicotiana* species were provided from Iwata Tobacco Experiment Station, The Japan Tobacco & Salt

Table 1. The content and distribution of fatty acids in *Nicotiana* species

Subgenus Section Species	Leaf								Seed					
	Content (mg/g dry wt)	Fatty acid composition (% total)							Content (mg/g dry wt)	Fatty acid composition (% total)				
		16:0	16:1	16:3	18:0	18:1	18:2	18:3		16:0	18:0	18:1	18:2	18:3
Rustica														
Paniculatae														
1. *glauca*	42.8	14.8	6.8	7.4	1.7	1.8	17.3	50.2	362	10.8	2.3	14.5	70.0	2.4
2. *paniculata*	35.3	17.1	7.0	6.0	2.7	2.8	8.0	56.4	316	10.2	2.7	15.6	70.3	1.2
3. *knightiana*	38.8	16.1	6.9	6.5	2.4	2.8	8.4	56.9	307	9.7	2.4	13.1	73.9	0.9
4. *solanifolia*	33.9	15.6	6.8	6.9	2.7	3.9	10.9	53.2	327	8.6	2.7	16.9	71.0	0.8
5. *benavidesii*	27.5	17.3	5.9	4.1	2.0	1.9	8.5	60.3	322	7.6	2.7	13.5	75.3	0.9
6. *cordifolia*	—	—	—	—	—	—	—	—	352	8.2	2.2	15.1	73.4	1.1
7. *raimondii*	35.0	15.0	7.0	5.8	2.4	2.1	10.2	57.5	352	9.5	2.0	15.3	72.1	1.1
Thyrsiflorae														
8. *thyrsiflora*	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Rusticae														
9. *rustica*	34.9	16.8	6.8	4.6	2.2	1.5	8.6	59.5	324	9.0	3.5	12.6	73.8	1.1
Tabacum														
Tomentosae														
10. *tomentosa*	21.2	17.2	4.9	3.8	3.6	2.0	10.7	57.8	284	7.2	2.0	11.2	79.1	0.5
11. *tomentosiformis*	40.9	14.9	4.4	5.0	2.8	1.9	10.7	60.3	308	7.1	1.9	14.1	76.3	0.6
12. *otophora*	36.4	14.8	2.7	5.2	2.6	1.3	10.0	63.4	304	10.6	1.7	12.5	74.6	0.6
13. *setchellii*	33.0	15.7	6.1	4.9	2.6	1.5	9.3	59.9	361	7.8	1.8	12.9	76.9	0.6
14. *glutinosa*	32.3	16.1	7.4	6.3	1.8	0.9	8.9	58.6	328	10.5	2.7	16.2	70.2	0.4
15. *kawakamii*	43.5	16.0	7.1	5.5	2.4	1.7	11.6	55.7	355	8.7	2.0	13.9	74.9	0.5
Genuinae														
16. *tabacum* (BY-4)	36.1	14.2	3.2	4.2	1.8	2.6	12.0	62.0	360	9.6	2.8	12.1	74.1	1.4
Petunioides														
Undulatae														
17. *undulata*	41.0	13.5	5.3	4.5	2.6	4.0	12.0	58.1	295	8.3	2.3	12.3	75.9	1.2
18. *arentsii*	41.9	14.3	6.0	4.1	2.2	2.7	11.8	58.9	287	9.0	2.1	11.7	76.2	1.0
19. *wigandioides*	29.6	15.8	6.6	3.8	2.0	2.4	12.7	56.7	304	7.9	2.8	10.9	76.9	1.5
Trigonophyllae														
20. *trigonophylla*	36.3	17.3	4.7	4.4	2.4	2.6	11.1	57.5	288	8.9	2.8	13.6	74.3	0.4
21. *sylvestris*	42.0	14.8	6.3	6.1	3.0	2.1	10.6	57.1	348	9.3	2.5	10.3	74.5	3.4
22. *langsdorffii*	35.2	15.2	6.4	5.6	2.8	0.8	10.1	59.1	293	8.1	2.8	11.6	75.7	1.8
23. *alata*	33.7	15.4	6.1	5.2	3.0	1.0	12.2	57.1	303	6.8	4.0	9.9	76.9	2.4
24. *forgetiana*	26.5	15.6	5.8	4.5	2.9	2.0	11.8	57.4	323	6.7	3.4	10.7	77.0	2.2
25. *bonariensis*	30.0	15.5	4.3	5.6	2.6	0.7	11.7	59.6	287	8.1	3.4	9.6	76.9	2.0
26. *longiflora*	28.7	17.2	4.4	6.3	3.3	1.6	13.4	53.8	359	8.5	3.0	11.4	75.0	2.1
27. *plumbaginifolia*	38.3	15.4	6.6	6.7	2.3	2.4	15.2	51.4	319	9.8	3.4	11.2	73.1	2.5
Repandae														
28. *repanda*	41.3	16.7	6.4	4.2	2.7	0.8	15.1	54.1	371	7.1	4.7	11.4	72.9	3.9
29. *stocktonii*	29.5	18.3	3.9	3.6	2.4	2.4	15.4	54.0	390	10.3	3.4	11.4	69.4	5.5
30. *nesophila*	—	—	—	—	—	—	—	—	305	10.0	4.6	10.3	70.2	4.9
Noctiflorae														
31. *noctiflora*	40.5	16.4	5.7	6.2	2.9	1.7	13.1	54.0	361	8.2	3.0	20.0	45.4	23.4
32. *petunioides*	—	—	—	—	—	—	—	—	396	8.6	2.6	17.4	70.3	1.1
33. *acaulis*	—	—	—	—	—	—	—	—	—	—	—	—	—	—
34. *ameghinoi*	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Acuminatae														
35. *acuminata*	34.8	15.2	6.8	4.2	2.5	1.1	13.5	56.7	353	7.7	1.4	13.3	76.7	0.9
36. *pauciflora*	30.1	14.0	4.6	3.1	2.2	1.3	17.1	57.7	366	8.5	1.5	14.3	74.7	1.0
37. *attenuata*	31.5	14.9	6.3	5.0	2.7	1.1	13.7	56.3	404	7.1	1.9	14.3	76.2	0.5
38. *longibracteata*	—	—	—	—	—	—	—	—	—	—	—	—	—	—
39. *miersii*	33.5	14.8	6.7	5.6	2.2	1.0	14.1	55.6	361	9.9	1.9	11.4	76.0	0.8
40. *corymbosa*	28.7	15.5	2.2	4.6	1.7	1.5	14.1	60.4	356	9.6	1.6	14.7	72.7	1.4
41. *linearis*	—	—	—	—	—	—	—	—	391	7.9	1.7	15.7	74.1	0.6
42. *spegazzini*	—	—	—	—	—	—	—	—	405	7.3	1.8	16.6	73.7	0.6
Bigelovianae														
43. *bigelovii*	35.1	15.4	6.0	4.8	1.8	2.0	11.4	58.6	381	8.2	1.7	12.6	76.9	0.6
44. *clevelandii*	33.1	17.5	7.3	5.8	3.0	1.2	13.0	52.2	349	9.9	3.1	12.3	73.8	0.9

Table 1. (*Contd.*)

Subgenus Section Species	Leaf Content (mg/g dry wt)	Leaf Fatty acid composition (% total) 16:0	16:1	16:3	18:0	18:1	18:2	18:3	Seed Content (mg/g dry wt)	Seed Fatty acid composition (% total) 16:0	18:0	18:1	18:2	18:3
Nudicaules														
45. *nudicaulis*	35.1	17.0	7.2	7.2	2.2	1.2	12.7	52.5	321	7.9	2.9	12.9	71.3	5.0
Suaveolentes														
46. *benthamiana*	44.1	16.4	6.8	4.9	2.8	2.8	5.7	60.6	393	12.2	2.6	17.5	66.1	1.6
47. *umbratica*	27.8	16.4	5.4	5.2	4.1	2.0	8.1	58.8	333	9.1	3.0	16.1	67.6	4.2
48. *cavicola*	44.1	15.8	5.8	7.7	3.8	1.3	9.0	56.6	316	8.9	2.8	19.2	58.4	10.7
49. *debneyi*	41.2	17.0	7.0	5.2	3.0	1.0	8.7	58.1	315	9.8	3.1	14.0	59.1	14.0
50. *gossei*	31.1	18.5	4.8	4.2	3.6	1.6	10.2	57.1	334	9.5	3.2	21.2	44.0	22.1
51. *amplexicaulis*	27.9	16.8	6.3	4.4	3.0	1.7	5.0	62.8	316	9.8	3.5	19.5	44.6	22.6
52. *maritima*	31.0	18.0	5.4	4.1	3.8	1.6	9.7	57.4	312	9.1	2.6	18.8	31.1	38.4
53. *velutina*	—	—	—	—	—	—	—	—	285	9.7	3.0	15.1	47.0	25.2
54. *hesperis*	35.7	16.7	5.8	4.3	3.2	1.7	12.2	56.1	287	10.5	2.5	17.0	51.5	18.5
55. *occidentalis*	29.9	17.6	5.3	4.7	2.9	1.7	12.2	55.6	339	11.0	2.3	16.9	67.4	2.4
56. *simulans*	27.3	17.9	5.7	4.0	3.8	1.4	10.9	56.3	317	10.9	3.1	17.8	65.1	3.1
57. *megalosiphon*	31.9	18.6	5.8	3.6	3.2	1.5	10.2	57.1	330	10.4	2.5	14.4	64.8	7.9
58. *rotundifolia*	42.1	16.3	6.3	5.4	2.4	1.6	10.3	57.7	352	9.6	2.2	22.8	29.2	36.2
59. *excelsior*	30.3	17.5	7.3	7.1	3.5	1.4	6.3	56.9	328	9.9	3.0	18.5	44.5	24.1
60. *suaveolens*	29.3	17.1	6.1	4.9	2.4	1.4	8.6	59.5	377	8.3	2.8	16.5	41.1	31.3
61. *ingulba*	31.3	18.2	6.3	4.3	3.3	1.3	7.7	58.9	268	11.4	2.5	13.9	62.7	9.5
62. *exigua*	22.3	15.7	6.7	7.7	1.9	1.7	11.4	54.9	320	9.4	3.1	15.8	45.0	26.7
63. *goodspeedii*	35.6	17.6	4.5	2.6	4.3	2.1	18.8	50.1	298	10.1	3.3	20.0	33.3	33.3
64. *rosulata*	29.3	17.9	5.5	4.6	3.4	1.3	7.3	60.0	291	10.4	3.2	20.3	39.7	26.4
65. *fragrance*	28.4	16.4	7.1	5.9	2.5	1.8	10.6	55.7	259	10.0	3.8	11.7	57.8	16.7
66. *africana*	29.4	17.5	7.0	5.1	2.1	3.7	11.1	53.5	285	7.1	2.7	20.9	64.6	4.7

Table 2. The fatty acid composition of lipid classes in seeds of *N. noctiflora* and *N. rotundifolia*

Species	Fatty acid	Fatty acid composition (% total) Triacylglycerols	Diacylglycerols	Sterol esters	Polar lipids
31. *N. noctiflora*	Total	72.5	8.2	7.4	11.9
	16:0	6.2	1.1	1.0	2.7
	18:0	2.0	0.8	0.3	0.8
	18:1	14.0	1.4	1.2	1.9
	18:2	32.8	3.4	3.6	4.7
	18:3	17.5	1.5	1.3	1.8
58. *N. rotundifolia*	Total	87.6	3.7	3.8	4.9
	16:0	8.1	0.7	1.1	2.5
	18:0	1.6	0.2	0.1	0.4
	18:1	18.3	1.0	0.5	0.9
	18:2	29.4	1.2	1.3	0.8
	18:3	30.2	0.6	0.8	0.3

Public Corporation. Seed samples were dried over Si gel in a desiccator. Tobacco plants were grown in a greenhouse at 28°. Middle leaves on the stalk were picked at 80 days after sowing, freeze-dried and powdered.

Fatty acid analysis. Leaf samples (10 mg dry wt) were incubated with 5% H_2SO_4 in MeOH at 40° overnight [17]. Seed samples were homogenized in a glass homogenizer with 5% H_2SO_4 in MeOH and incubated as above. The resultant fatty acid methyl esters were extracted with *n*-hexane and analysed by FID-GC using glass columns packed with 5% BDS on Chromosorb W for leaf samples [3] and with 5% DEGS on Gaschrom Q for seed samples [12]. The column temp. was maintained at 200° with a He flow rate of 50 ml/min.

Lipid analysis. Seed samples (50 mg dry wt) were homogenized × 3 with $CHCl_3$–MeOH (1:1) and the combined homogenates filtered. The filtrate was washed with H_2O, the $CHCl_3$ layer evaporated and the extract dried as described previously [17]. The total lipids obtained were weighed and dissolved in a small vol. of $CHCl_3$–MeOH (9:1). The total lipid soln was applied to a Si gel TLC. The plate was developed first with *n*-hexane–Et_2O

(49:1) and then re-developed with *n*-hexane–Et_2O–HOAc (50:50:1) [18]. Lipid areas were located under UV light after spraying with Rhodamine 6G soln. Each lipid area was scraped from the plate and individual lipid classes were quantitatively estimated as fatty acid methyl esters prepared by methanolysis as mentioned above.

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