Distribution of n-7 Fatty acid in *Brassicacea.*

Véronique J. Barthet

Canadian Grain Commission, Grain Research Laboratory, 1404-3030 Main St, Winnipeg, MB, Canada, R3C-3G8
Email: vbarthet@grainscanada.gc.ca

Abstract

Cis Vaccenic acid, a C18:1 (*n-7*) isomer of oleic acid (C18:1 (*n-9*)) has been found in several oilseeds. It is synthetized from palmitic acid (C16:0) via production C16:1 (*n-7*) by a Δ9 desaturase and elongation by an elongase giving C18:1 (*n-7*). While this fatty acid does not have a known antinutritional effect, there has been some interest in it, particularly in relation to its *trans* form *t*-vaccenic acid.

Brassica contained both (*n-9)* and (*n-7)* fatty acids for the C18:1, isomers of C20:1 and C22:1 fatty acids were also found. The amount of the C18:1(*n-7*) isomer varied from the seed sources.

Brassica specie relations have been established using electrophoretic studies of the proteins protein and chromosome pairing. This project assessed the use of *n-7* fatty acid isomers as common characteristic of several Brassica species and tried to establish if these fatty acids could be used as genetic markers.

Introduction

The cabbage or mustard family (*Brassicaceae*) includes over 3000 species, grouped in over 300 genres. They included weeds or domesticated plants grown as vegetables, ornamental flowers or for seeds. Middle East is the presumptive point of origin of the *Brassica* species but now they appear as cultivated plants or weeds in Europe, North and South America, and Australia. Seeds of the various *Brassica* species had very different relative fatty acid compositions; differences that have been amplified in the recent years by breeding to produce specialty oils. In our studies of the fatty acic composition of the oil from the weed, wild mustard or charlock (*Sinapis arvensis)*, (*n-9*) and (*n-7*) isomers for C18:1, C20:1and C22:1 fatty acids were identified. These fatty acids had been previously been identified in *B. napus* and *B. rapa (campestris)* (Applequist, 1969) and the (*n-7*) isomer of oleic acid was associated with structural lipids in *B. rapa* (Cv. Tobin) and *B. napus* (Cv. Westar) (Hu *et al*., 1994). However, there is little information on the distribution of (*n-7*) isomers of longer chain fatty acids in different *Brassica* species.

Materials and methods

Materials:

Samples: *Brassica* samples obtained from Mr. R.K. Gugel, curator of the Crucifer Node of the Plant Gene Resources of Canada included *Brassica carinata* (SRS1578, Dodolla, S67, PAK85490, SRS1460), *Brassica juncea* (Donskaja, Lethbridge 22A, Cutlass, Varuna, AC Vulcan, J197-102), *Brassica napus* (Argentine, AC Excel, Golden, Westar and Midas), *Brassica nigra* (SRS190, SRS586, SRS1170, SRS195), Brassica rapa (AC Parkland, Echo, Polish, R500, Torch), *Brassica tournefortii* (SRS349, PAK85655, SRS3036, SRS3038, SRS3043), *Camelina sativa* (SRS933), *Crambe abyssinica* (Prophet), *Eruca sativa* (PAK856392, PAK85886, PAK85889, PAK85873, PAK85896), *Raphanus sativus* (Nemex, Rauola, Zenit, IDC3098, SRS1078), *Sinapis alba* (Tilney, Ochre, Gisilba, Andante, AC Pennant) and *Sinapis arvensis* (SRS3100).

Reagents and Standards: Methanolic base was from Sigma (Sigma-Aldrich Canada Ltd., Ont. Canada). A gas chromatography reference standard, designed for this project was obtained from Nu Chek Prep Inc. (Elysian, MN, USA).

Fatty acid methyl esters: Samples (10 seeds) were used to prepared FAMEs using sodium methoxide as catalyser.

GC analysis: FAMES were analyzed using a Hewlett Packard 5890 gas chromatograph (Agilent Technologies, Mississauga, Ont., Canada) equipped with a flame ionization detector and a 7673A injector tower and a Agilent 6890N Network GC System with a 5973 inert Mass Selective Detector and equipped with a 7683B Autoinjector Module using the same temperature program. Methyl esters were separated on a Suplecowax 10 silica column (Sigma-Aldrich Canada Ltd, Mississauga, Ont., Canada) (60 m x 0.32 mm, 0.25 μm). Hydrogen was the carrier gas (2.5 mL/min), injection port temperature was 280°C and detector temperatures were kept at 300°C. The temperature program was as follows: the initial 190°C temperature was maintained for 3 min, a first gradient was made from 190 to 210°C at 2°C/min, then to 280°C at 20°C/min, the final temperature 280°C was held for 3 min for a total run time of 24 min.

Statistical analysis: The statistical analyses were performed using Origin® 6.0 (Microcal Software Inc., Northampton, MA, USA), InStat 3.05 (GraphPad Software Inc., San Diego, CA, USA) and SAS 9.1.3 (SAS Institute INC, Cary, NC, USA).

Results and discussion:

Cultivars of several species of Brassica were used in this study. Quintuplicate FAMEs were prepared for each sample; all were analyzed by GC (Figure 1) and one sample of each quintuplicate FAME was analyzed by GC-MS to allow a correct identification of the fatty acids. The cultivars had very different relative fatty acid compositions (Table 1); both isomers (*n-9*) and *(n-7)* were found for the C18:1 (oleic acid), the C20:1 (eicosenoic acid) and the C22:1 (erucic acid) fatty acids although in different proportions

Cis-vaccenic acid (11-*cis*-octadecenoic acid or C18:1(*n-7*)), an isomer of oleic acid (9-*cis*-octadecenoic acid or C18:1(*n-9*)), represented from 0.4 to 3.4% of the total relative fatty acid contents of the seeds. Some C20:1(*n-7*) and C22:1(*n-7*) isomers were also found. However, their levels were lower than C18:1(*n-7*) levels; they varied from below the limit of detection to 1.6% and below the limit of detection to 1.3% for C20:1 (*n-7*) and C22:1 (*n-7*), respectively. The percent of (*n-7*)/(*n-9*) varied depending on the species and sometimes the varieties. The ratios ranged from 2.3 to 17.9%, 0 to 34.2% and 0 to 3.9% for C18:1, C20:1 and C22:2, respectively.

The (*n-7*)/(*n-9*) ratios for C18:1, C20:1 and C22:1 were compared to establish if the (*n-7*) fatty acid isomers might a common characteristic of several *Brassica* species (Table 2**)**. Unfortunately, *S. arvensis*, *C. abyssinica* and *C. sativa* could not be used in these analyses since only one cultivar of each of these species was available for the study. The (*n-7*)/(*n-9*) ratios for C18:1 showed less variation than the (*n-7*)/(*n-9*) ratios for C20:1 and C22:1 within a species (Table 2); it was used to see if similarities or differences could be observed between the tested species. The C18:1 (*n-7*)/(*n-9*) ratios of *B. carinata, B. juncea* and *B nigra* were statistically different than the C18:1 (*n-7*)/(*n-9*) ratios for all the other tested species (Table 3). In contrast, *B. napus and B. tournefortii* were the species that had C18:1 *(n-7)/(n-9)* ratios statistically similar to the largest number of the tested Brassica species (Table 3). *B. napus* presented a C18:1 (*n-7*)/(*n-9*) ratio statistically similar to *B. rapa*, *E. sativa* and *B. tournefortii*. The C18:1 (*n-7)/(n-9)* ratio of *B. tournefortii* was similar to the one of *B. rapa*, *B. napus*, *R. sativus and S. alba*.

The evolution the *Brassicacae* followed the triangle of U theory, with *B. napus* (n = 19), an amphidiploid species, resulting from crosses between *B. campestris* (*rapa*) (n = 10) and *B. oleracea* (n = 9). *B. juncea*, another amphidiploid species, resulted from crosses between *B. campestris* (*rapa*) (n = 10) and *B. nigra* (n = 8) (U, 1935). Phylogenetic studies of *Brassica* could be contradictory. If the C18:1 *(n-7)/(n-9)* ratios were used to for the chemotaxonomy the tested Brassica, B. *napus, B. rapa, B. tournefortii* and *E. sativa* would be related and *B. tournefortii, R. sativus* and *S. alba* would be also related (Figure 2) It has been shown that *E. sativa* and *B. napus* belonged to the rapa/oleacea lineage along with *R. sativus* (Warwick and Black, 1991). *S. alba* and *B. tournefortii* with ratios similar would belong to the same group; these two species belonged to the nigra lineage (Warwick and Black, 1991). However they had ratios similar to *R. sativus* which was part of the rapa lineage (Warwick and Black, 1991). According to nuclear RFLP studies, *R. sativus* was closely related to *B. nigra* whereas chloroplast and mitochondria DNA restriction site analyses showed that *R. sativus* was closely related to *B. rapa/oleracea* (Yang *et al*., 1998).

Acknowledgments

Ms. Gemma Gibb, Mr. Alex Wishart assisted with FAME preparation. Mr. Ray Bacala carried out FAME analysis on GC and GC-MS. Mr. Don Gaba assisted with GC-MS analysis of the FAMES. Mr. Ken Howard assisted with the editing.

References

Appleqvist, L. (1969). Lipid in Cruciferae: IV. Fatty acid patterns in single seeds and seed populations of various cruciferae and in different tissues of *Brassica napus* L. Hereditas, 61(2), 9-44.

Hu, X., Daun, J.K. and Scarthe, R. (1994). Proportion of C18!n-9 fatty acids in canola seed coat surface and internal lipids. JAOCS, 71(2), 221-222.

U, N. (1935). Genome analysis in *Brassica* with special reference to the experimental formation o Brassica napus and peculiar mode of fertilization. Jpn Bot., 7, 389-452

Warwick, S.I. and Black, L.D. (1991). Molecular systematics of Brassica and allied genera (suntribe Brassicinae, Brassiceae) – chloroplast genome and cytodeme congruence. Theor., Appl. Genet., 82, 81-91.

Yang, Y.W., Tseng, P.F., Tai, P.Y. and Chang, C.J. (1998). Phylogenetic position of Raphanus in relation to Brassica species based on 5S rRNA spacer sequence data. *Bot. Bull. Acad. Sin*., 39, 153-160.

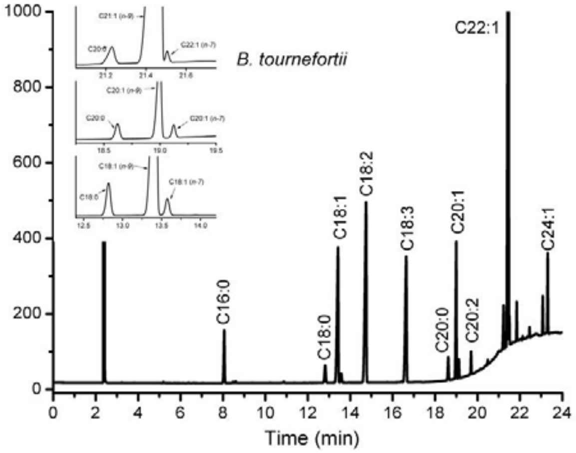

Figure 1: Gas chromatogram of fatty acid methyl esters from *B. tournefortii*. seed showing the presence of both *n*-9 and *n*-7 fatty acids.

Table 1: Relative fatty acid composition (average, n = 5) of the tested Brassica.

Species	Cultivar	Relative fatty acid composition (%)							Ratio (*n-7*/*n-9*) (%)		
		C16:1	C18:1		C20:1		C22:1				
			n-9	*n-7*	*n-9*	*n-7*	*n-9*	*n-7*	C18:1	C20:1	C22:1
B. carinata	Dodolla	0.10	7.50	0.89	5.81	1.01	40.42	0.74	11.93	17.42	1.84
	PAK85490	0.13	7.70	0.90	5.95	1.26	42.36	0.82	11.89	21.31	1.93
	S67	0.11	8.91	0.95	6.27	1.13	41.11	0.76	10.63	18.00	1.85
	SRS1460	0.14	7.36	0.88	6.77	1.18	40.60	0.76	12.08	17.50	1.86
	SRS1578	0.14	7.07	0.96	5.85	1.27	42.39	0.96	13.60	21.72	2.26
B. juncea	AC Vulcan	0.16	18.40	1.64	11.20	0.81	23.35	0.22	8.94	7.26	0.94
	Cutlass	0.15	16.98	1.60	11.20	0.87	24.97	0.24	9.47	7.78	0.94
	Donskaja	0.14	20.35	1.10	9.51	0.63	28.87	0.34	5.41	6.63	1.18
	J197-102	0.21	40.06	2.65	1.73	0.05	0.57	0.00	6.62	2.64	0.00
	Lethbridge 22A	0.19	20.53	1.61	11.30	0.78	22.28	0.26	7.82	6.94	1.18
	Varuna	0.17	9.63	0.76	4.76	1.29	45.86	1.01	7.89	27.25	2.21
B. napus	AC Excel	0.22	60.61	3.28	1.16	0.02	0.03	0.00	5.42	1.95	0.00
	Argentine	0.19	16.42	1.49	11.44	1.30	36.60	0.52	9.11	11.43	1.42
	Golden	0.16	14.84	1.10	9.92	1.40	43.03	0.65	7.46	14.17	1.51
	Midas	0.18	62.98	2.51	1.13	0.02	0.06	0.00	3.99	1.43	0.00
	Westar	0.19	60.80	3.12	1.38	0.01	0.03	0.00	5.13	0.62	0.00
B. nigra	SRS1170	0.27	9.73	1.47	6.61	1.13	34.80	0.91	15.18	17.21	2.61
	SRS190	0.26	9.47	1.46	6.96	1.14	33.63	0.82	15.43	16.45	2.44
	SRS195	0.26	10.23	1.64	7.82	1.23	27.85	0.67	16.14	15.94	1.94
	SRS586	0.24	6.94	1.16	5.39	1.14	38.54	1.01	16.74	21.29	2.62
B. rapa	AC Parkland	0.21	51.09	3.28	0.92	0.02	0.03	0.00	6.42	1.75	0.00
	Echo	0.14	28.80	1.80	10.40	0.61	24.85	0.33	6.24	5.90	1.33
	Polish	0.17	31.98	1.84	10.82	0.57	21.90	0.32	5.78	5.30	1.46
	R500	0.16	11.77	0.55	3.79	1.18	51.95	1.26	4.69	31.42	2.42
	Torch	0.17	58.00	2.56	1.73	0.04	0.72	0.00	4.41	2.11	-
B. tournefortii	PAK85655	0.06	7.94	0.45	5.01	0.66	49.99	0.46	5.63	13.21	0.92
	SRS3036	0.07	8.63	0.45	5.33	0.66	49.70	0.46	5.22	12.32	0.94
	SRS3038	0.07	8.52	0.44	5.48	0.63	49.70	0.53	5.16	11.58	1.07
	SRS3043	0.07	9.55	0.53	5.70	0.71	48.25	0.54	5.58	12.47	1.12
	SRS349	0.08	13.04	0.63	7.84	0.70	44.30	0.51	4.81	8.90	1.16
C. sativa	SRS933	0.07	13.98	0.77	14.13	0.45	3.12	0.03	5.54	3.18	1.04
C. abyssinica	Prophet	0.19	17.52	0.49	3.02	0.89	55.22	1.09	2.83	29.53	1.97
E.. sativa	PAK856392	0.31	12.48	0.94	6.53	1.26	45.52	0.92	7.49	19.40	2.01
	PAK85873	0.25	13.26	0.87	6.83	1.16	46.03	0.77	6.53	17.20	1.68
	PAK85886	0.24	13.89	0.89	8.09	1.01	43.40	0.69	6.38	12.54	1.60
	PAK85889	0.25	13.40	0.84	6.69	1.05	46.65	0.73	6.28	15.75	1.57
	PAK85896	0.25	13.49	0.91	7.98	1.13	44.77	0.70	6.77	14.12	1.57
R. sativus	IDC3098	0.24	32.53	1.55	8.02	0.41	16.09	0.17	4.79	5.07	1.04
	Nemex	0.14	34.93	1.29	7.26	0.19	9.61	0.05	3.71	2.58	0.51
	Rauola	0.17	33.69	1.48	8.84	0.30	12.95	0.07	4.39	3.38	0.52
	SRS1078	0.22	24.68	1.20	9.44	0.64	32.98	0.30	4.87	6.71	0.96
	Zenit	0.17	26.31	0.98	9.61	0.41	27.26	0.16	3.73	4.31	0.57
S. alba	AC Pennant	0.15	26.06	1.04	9.80	0.65	33.30	0.41	3.99	6.65	1.24
	Andante	0.20	24.65	1.30	9.88	0.74	30.49	0.50	5.29	7.47	1.64
	Gisilba	0.17	22.40	1.04	10.01	0.75	36.42	0.53	4.64	7.56	1.46
	Ochre	0.16	25.81	1.05	9.45	0.62	32.76	0.41	4.10	6.60	1.26
	Tilney	0.16	27.93	1.15	8.78	0.62	28.92	0.43	4.13	7.11	1.49
S. arvensis	SRS3100	0.16	31.15	1.88	10.92	0.17	7.47	0.07	6.05	1.55	0.95

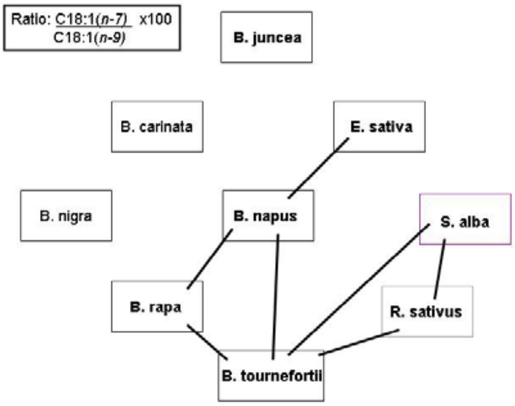

Figure 2: Chemotaxonomy of tested Brassicaceae according to the C18:1 (*n-7*)/(*n-9*) ratio.

Table 2: Ratio (*n-7*)/(*n-9*) for C18:1, C20:1 and C22:1 (as percent) for all tested *Brassica* species

Genus	Species	N	C18:1 Ratio (%)		C20:1 Ratio (%)		C22:1 Ratio (%)	
			Average	Stdev	Average	Stdev	Average	Stdev
Brassica	*carinata*	5	12.03	1.52	19.19	2.46	12.03	1.52
Brassica	*juncea*	6	7.95	1.55	9.46	7.65	7.95	1.55
Brassica	*napus*	5	6.22	1.89	5.92	5.85	6.22	1.89
Brassica	*nigra*	4	15.88	1.32	17.72	2.87	15.88	1.32
Brassica	*rapa*	5	5.51	0.87	9.30	11.53	5.51	0.87
Brassica	*tournefortii*	5	5.28	0.38	11.70	1.66	5.30	0.38
Eruca	*sativa*	5	6.69	0.66	15.80	3.0	6.69	0.67
Raphanus	*sativus*	5	4.30	0.60	4.41	1.48	4.30	0.60
Sinapis	*alba*	5	4.43	0.56	7.08	0.89	4.43	0.56
Camelina	*sativa*	1	5.54		3.18		1.04	
Cramble	*Abyssinica*	1	2.83		29.53		1.97	
Sinapis	*Arvensis*	1	6.05		1.55		0.95	

Table 3: Statistical analyse s of the ratio [(*n-7*)/(*n-9*)]x100 for C18:1, one-way analysis of variance.

	B. juncea	*B. napus*	*B. nigra*	*B. rapa*	*B. tournefortii*	*E. sativa*	*R. sativus*	*S. alba*
B. carinata	***	***	***	***	***	***	***	***
B. juncea		***	***	***	***	***	***	***
B. napus			***	NS	NS	NS	***	***
B. nigra				***	***	***	***	***
B. rapa					NS	*	*	*
B. tournefortii						**	NS	NS
E. sativa							***	***
R. sativus								NS

*** $P < 0.001$ ** $P < 0.01$ * $P < 0.05$ NS: $P > 0.05$