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(*n*-7) and (*n*-9) *cis*-monounsaturated fatty acid contents of 12 *Brassica* species

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Abstract

cis-Vaccenic acid or *cis*-11-octadecenoic acid, a C18:1 (*n*-7) isomer of oleic acid (C18:1 (*n*-9)) has been found in several oilseeds. It is synthesized from palmitic acid (C16:0) via production of C16:1 (*n*-7) by a Δ9 desaturase and elongation by an elongase giving C18:1 (*n*-7). In this study, the fatty acid composition of 12 *Brassica* species was analyzed by GC-FID and confirmed by GC–MS. All species contained C18:1 (*n*-7), C20:1 (*n*-7) and C22:1 (*n*-7) fatty acid isomers, suggesting that C18:1 (*n*-7) was elongated. The levels of these fatty acids varied according to the species. C18:1(*n*-7)) represented from 0.4% to 3.3% of the total relative fatty acid contents of the seeds. The contents of C20:1(*n*-7) and C22:1(*n*-7) levels were lower than C18:1(*n*-7) contents; the relative fatty acid composition varied from 0.02% to 1.3% and from below the limit of detection to 1.3% for C20:1 (*n*-7) and C22:1 (*n*-7), respectively. The ratios of (*n*-7)/(*n*-9) ranged from 2.8% to 16.7%, 0.6% to 29.5% and 0% to 2.6% for C18:1, C20:1 and C22:2, respectively.

Using statistical similarities or differences of the C18:1 (*n*-7)/(*n*-9) ratios for chemotaxonomy, the surveyed species could be arranged into three groups. The first group would include *Brassica napus, B. rapa, and B. tournefortii* with *Eruca sativa* branching only related to *B. napus*. The second group would include *B. tournefortii, Raphanus sativus* and *Sinapis alba.* The last group would include *B. juncea*, *B. carinata* and *B. nigra* with no similarity/relationship between them and between the other species.

Results suggested that the level of C20:1 (*n*-7) influenced the levels of all monounsaturated fatty acids with chain length higher than 20 carbons. On the other hand, palmitoleic acid (C16:1) levels, C16:1 being the parent of all (*n*-7) fatty acids, had no statistically significant correlation with the content of any of the fatty acids of the (*n*-7) or (*n*-9) family.

Keywords: *Brassicaceae*; *Brassica carinata*; *B. juncea*; *B. napus*; *B. nigris*; *B. rapa*; *B. tournefortii*; *Camelina sativa*; *Crambe abyssinica*; *Eruca sativa*; *Raphinus sativa*; *Sinapis alba*; (*n*-7) Fatty acids; *cis*-Vaccenic acid; Chemotaxonomy

1. Introduction

The cabbage or mustard family (*Brassicaceae*) includes over 3000 species, grouped in over 300 genres. They include either weeds or domesticated plants grown as vegetables, ornamental flowers or those used for oilseeds. Eurasia and Middle-East (secondary centre) are the presumptive points of origin of the *Brassica* species (Weiss, 1983), now they appear as cultivated plants or weeds in Europe, North and South America, and Australia. Seeds of the various *Brassica* species have very different relative fatty acid compositions; differences that have been augmented in the recent years by breeding to produce specialty oils. In our studies of the fatty acid composition of wild mustard (charlock, *Sinapis arvensis*) seed, (*n*-9) and (*n*-7) isomers for C18:1, C20:1 and C22:1 fatty acids were identified (Daun et al., 2003). These fatty acids have previously been identified in *Brassica napus* and *B. rapa (campestris)* (Appleqvist, 1969). 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.

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This project investigated the fatty acid composition of the seeds of 12 *Brassica* species. The (*n*-7) fatty acid composition was assessed to establish if these fatty acids could be used as genetic markers for *Brassica* species.

2. Results and discussion

To avoid the formation of fatty acid artefacts, FAMEs were prepared at room temperature and one sample of each quintuplicate FAME was analyzed by GC–MS to allow for correct identification of the fatty acids. A typical chromatogram is presented in Fig. 1. The tested species had very different relative fatty acid compositions (Table 1). Both isomers (*n*-9) and (*n*-7) were found for C18:1, C20:1 and the C22:1 fatty acids although in different proportions.

cis-Vaccenic acid (11-*cis*-octadecenoic acid or C18:1 (*n*-7)) represented from 0.4% to 3.3% 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; the relative fatty acid composition varied from 0.02% to 1.4% and from below the limit of detection to 1.3% for C20:1 (*n*-7) and C22:1 (*n*-7), respectively (Table 1). The ratios of (*n*-7)/(*n*-9) varied according to the species and sometimes the varieties. The ratios ranged from 2.8% to 16.7%, 0.6% to 29.5% and 0% to 2.6% for C18:1, C20:1 and C22:2, respectively (Table 1).

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 be a common characteristic of several *Brassica* species (Table 2). Large variations were observed between the C18:1, C20:1 and C22:1 (*n*-7)/(*n*-9) ratios of the *B. napus* cultivars, resulting in the highest coefficient of variation for these ratios (Table 2). *S. arvensis*, *Crambe abyssinica* and *Camelina sativa* could not be used in these analyses since only one cultivar of each of these species was used in this 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). The data for the C18:1 (*n*-7)/(*n*-9) ratio 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 from 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 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*. If the C18:1 (*n*-7)/(*n*-9) ratios were used for chemotaxonomy, statistically similar C18:1 (*n*-7)/(*n*-9) ratios would be an indication of similarity between the tested *Brassica* species (Table 3). According to these results, the tested species could be arranged into three groups. The first group that would be related would include *B. napus, B. rapa, and B. tournefortii; Eruca sativa* would be a branch only related to *B. napus*. The second group would include *B. tournefortii, R. sativus* and *S. alba.* The last group would include *B. juncea*, *B. carinata* and *B. nigra* that showed no similarity/relationship between them and between the other species (Fig. 2). Phylogenetic studies of *Brassica* could be contradictory. The evolution the *Brassicacae* followed the Triangle of U theory (U, 1935), 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* ($n = 8$). Finally, crosses between *B. oleracea* ($n = 9$) and *B. nigra* ($n = 8$) led to *B. carinata* ($n = 17$), also an amphidiploid species (U, 1935). It has been shown that *E. sativa* and *B. napus* belonged to the rapa/oleracea lineage along with *R. sativus* whereas *S. alba* and *B. tournefortii* belonged to the nigra lineage (Warwick and Black, 1991). Nuclear RFLP studies suggested that *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). Later it was suggested that *Raphanus* was the result of hybridization between the *rapa*/*oleracea* and *nigra* lineages (Yang et al., 2002). In this experiment, *B. rapa, B. napus* and *E. sativa* were related, which agreed with Warwick and Black (1991) and the rapa/oleracea lineage whereas B. *tournefortii* was related to *S. alba*, agreeing with the nigra lineage. However, *B. tournefortii* was related to *R. sativus* and these two species belonged to a different lineage, but *B. nigra* was related to none of the species from the nigra lineage. The results suggested that *B. tournefortii* had more correlation with the tested *Brassica* species than *B. rapa* or *B. nigra*.

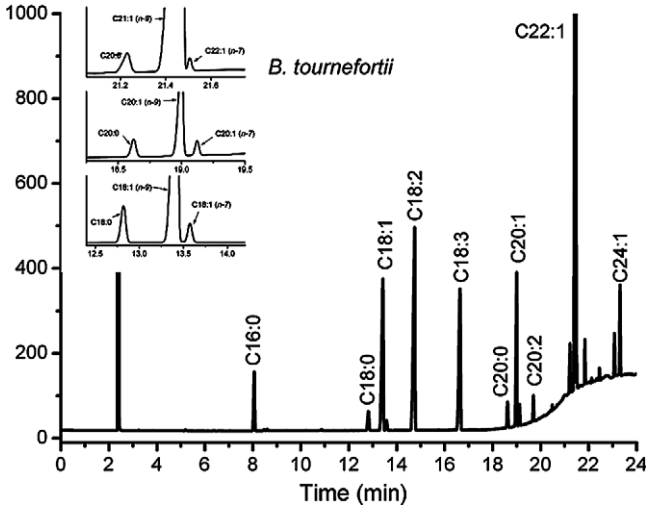

Fig. 1. Gas chromatogram of fatty acid methyl esters from *Brassica tournefortii*. seed showing the presence of both *n*-9 and *n*-7 fatty acids.

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
	Common brown	0.19	19.49	1.80	11.20	0.86	22.50	0.26	9.23	7.66	1.16
	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

In higher plants, *cis*-vaccenic acid is synthesized from palmitic acid (C16:0) via production of palmitoleic acid (C16:1 (*n*-7)) by a Δ9-desaturase and then elongated by an elongase giving C18:1 (*n*-7) (Southwell-Keely and Lynen, 1974; Mukherjee and Kiewitt, 1980; Shibahara et al., 1990). It was also found that *cis*-vaccenic acid could be isomerised into oleic acid with or without co-factors in kaki pulp (Shibahara et al., 1990). In *B. napus* (cv. Rapol and Tira) and *S. alba*, C18:1 (*n*-7) could be elongated to C20:1 (*n*-7), C22:1 (*n*-7) and C24:1 (*n*-7) (Mukherjee and

Table 2
Average of ratio (*n*-7)/(*n*-9) for C18:1, C20:1 and C22:1 (as percent) for each *Brassica* specie with *N* the number of varieties analyzed for each species

Genus	Species	*N*	C18:1 ratio (%)			C20:1 ratio (%)			C22:1 ratio (%)		
			Average	Stdev	CV (%)	Average	Stdev	CV (%)	Average	Stdev	CV (%)
Brassica	*carinata*	5	12.03	1.06	12.64	19.19	2.14	11.15	1.95	0.18	9.17
Brassica	*juncea*	6	7.86	1.51	19.16	11.15	9.01	80.79	1.34	0.50	37.24
Brassica	*napus*	5	6.22	2.04	32.81	5.92	6.37	107.67	0.58	0.80	137.03
Brassica	*nigra*	4	15.88	0.71	4.45	17.72	2.43	13.74	2.40	0.32	13.19
Brassica	*rapa*	5	5.51	0.91	16.55	9.30	12.51	134.55	1.04	1.04	99.82
Brassica	*tournefortii*	5	5.28	0.33	6.32	11.70	1.67	14.25	1.04	0.11	10.42
Eruca	*sativa*	5	6.69	0.48	7.20	15.80	2.66	16.86	1.69	0.19	11.19
Raphanus	*sativus*	5	4.30	0.56	12.95	4.41	1.59	36.11	0.72	0.25	36.11
Sinapis	*alba*	5	4.43	0.54	12.26	7.08	0.45	6.34	1.42	0.17	11.76
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 analyses of average of the C18:1 ratio (*n*-7)/(*n*-9) (as percent) for each species, 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

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

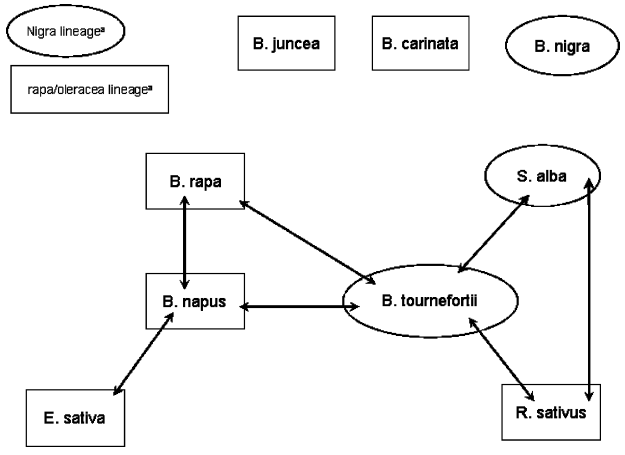

Fig. 2. Chemotaxonomy of tested *Brassicaceae* based on statistical difference between C18:1 (*n*-7)/(*n*-9) ratios. Statistically different C18:1 (*n*-7)/(*n*-9) ratios would be an indication of non-relationship between the species, statistically non-different C18:1(*n*-7)/(*n*-9) would be an indication of relationship between the tested *Brassica* species. Nigra lineage[a] and rapa/oleracea lineage[a] from Warwick and Black (1991).

Kiewitt, 1980). Linear correlations between the relative contents of each fatty acid were examined and the coefficient of correlation is reported in Table 4. The following was observed:

(a) a statistically significant inverse relationship between C18:0 and C22:1(*n*-9) contents;
(b) a statistically significant positive relationship between C18:1(*n*-9) and C18:1(*n*-7) contents and a statistically significant inverse relationship between C18:1(*n*-9) and both C20:1(*n*-7) and C22:1(*n*-9) contents;
(c) a statistically significant inverse relationship between C18:1(*n*-7) and C22:1(*n*-9)contents;
(d) a statistically significant positive relationship between C20:1(*n*-7) and C22:1(*n*-9), C22:1(*n*-7) contents and the ratios $[(n\text{-}7)/(n\text{-}9))] \times 100$ for the C20:1 and the C22:1;
(e) a statistically significant positive relationship between C22:0 and C24:0 contents;
(f) a statistically significant positive relationship between C22:1(*n*-9) and C22:1(*n*-7) contents and the ratio $[(n\text{-}7)/(n\text{-}9)] \times 100$ for the C20:1;
(g) a statistically significant positive relationship between C22:1 (*n*-7) content and the ratios $[(n\text{-}7)/(n\text{-}9)] \times 100$ for the C20:1 and the C22:1;
(h) a statistically significant positive relationship between the C20:1 [(*n*-7)/ (*n*-9)] and the C22:1 [(*n*-7)/(*n*-9)] ratios.

Table 4
Coefficient of correlation (R) for linear relationship between the relative content of each fatty acid and the ratio C18:1, C20:1 and C22:1 (n-7/n-9) ratio (%) for the tested *Brassica* seeds

	C16:1	C18:0	C18:1 (n-9)	C18:1 (n-7)	C18:2	C18:3	C20:0	C20:1 (n-9)	C20:1 (n-7)	C20:2	C22:0	C22:1 (n-9)	C22:1 (n-7)	C22:2	C24:0	C24:1	Ratio: (n-7)/(n-9) × 100		
																	C18:1	C20:1	C22:1
C16:0	0.4032***	0.5761***	0.3550***	0.2550***	0.0911	0.2377**	−0.0693	0.1068	0.3429***	−0.1396*	−0.4264***	−0.4662***	−0.4431***	−0.4161***	−0.2093**	−0.2366**	−0.1612*	−0.4361***	−0.3483***
C16:1		0.0245	0.1720*	0.3555***	−0.0458	−0.0707	−0.1847*	−0.1005	0.1775*	−0.2385*	−0.1559	0.1281*	0.1546*	−0.1649*	−0.2569***	−0.1086	0.2557***	0.1049	0.1903*
C18:0			0.6001***	0.5246***	0.5044***	0.3183***	0.2205**	0.0632	−0.6857***	−0.0600	−0.4077***	−0.7934***	−0.7486***	−0.5919***	−0.2421**	−0.5917***	−0.2874***	−0.6800***	−0.6382***
C18:1																			
n-9				0.8404***	0.3516***	−0.1789*	−0.4434***	−0.3567***	−0.8334***	−0.6395***	−0.5610***	−0.8290***	−0.7356***	−0.7455***	−0.5356***	−0.7237***	−0.4799***	−0.7051***	−0.7623***
C18:1																			
n-7					0.6155***	0.0000	−0.4009***	−0.3165***	−0.6064***	−0.3429***	−0.5933***	−0.8480***	−0.5173***	−0.5222***	−0.5561***	−0.6567***	−0.0141	−0.5952***	0.5911***
C18:2						0.3372***	−0.4987	−0.0995	−0.4055***	−0.2252**	−0.4350***	−0.6851***	−0.5278***	−0.1265*	−0.2713***	−0.3909***	0.2291**	−0.4136***	−0.4118***
C18:3							0.2760***	0.2812***	−0.0141	0.6464***	−0.2211**	−0.3198***	−0.1456*	0.1063	−0.5381	0.0316	0.3448***	-0.1100	0.0566
C20:0								0.2347**	0.2360**	0.5351***	0.5794***	0.2437**	0.1732*	0.2872***	0.5623***	0.2147**	0.1876*	0.1975*	0.2156**
C20:1																			
n-9									0.2500***	0.4246***	−0.2400**	0.0742	−0.1118	−0.1428*	0.2454**	0.2071**	0.0100	-0.2032*	0.1655*
C20:1																			
n-7										0.4637***	0.4022***	0.7774***	0.8446***	0.7145***	0.2787***	0.5920***	0.5937***	0.8149***	0.8380***
C20:2											0.1476*	0.1780*	0.2154**	0.5836***	0.2232**	0.3870***	0.6515***	0.2619***	0.3891***
C22:0												0.6979***	0.5875***	0.5524***	0.8362***	0.4376***	0.1221*	0.6194***	0.4147***
C22:1																			
n-9													0.8410***	0.6362***	0.5709***	0.6553***	0.1916***	0.7836***	0.7118***
C22:1																			
n-7														0.7331***	0.4336***	0.5745***	0.4651***	0.9449***	0.9159***
C22:2															0.6250***	0.6796*	0.7082***	0.7730***	0.6847***
C24:0																0.5614***	0.1924***	0.4716***	0.2980***
C24:1																	0.4116***	0.5100***	0.6102***
C18:1 ratio																		0.4474***	0.5846***
C20:1 ratio																			0.8109***

* Significant at $p < 0.05$.
** Significant at $p < 0.001$.
*** Significant at $p < 0.0001$.

It seems that the levels of C20:1(*n*-7) has a positive relationship with the content of all monounsaturated fatty acids with chain lengths higher than 20 carbons (Table 4). On the other hand, palmitoleic acid (C16:1) levels, C16:1 being the parent of all (*n*-7) fatty acids, had no significant relationship with the content of any of the fatty acids of the (*n*-7) or (*n*-9) family. The inverse correlation between oleic acid (C18:1-(*n*-9)) and erucic acid (C22:1 (*n*-9)) has been already observed in rapeseed (Loof and Appleqvist, 1964). It is known that part of the synthesis of the mono-unsaturated fatty acid occurs in the plastid via the catalytic action of a soluble Δ9-steraroyl-ACP desaturase (McKeon and Stumpf, 1982). Following hydrolysis of the acyl-ACP, catalyzed by acyl-ACP thioesterase, liberated fatty acids cross the plastidial envelope and become re-esterified as acyl-CoA (Pathak et al., 2004).. Further elongation and desaturation of acyl-CoA can occur in the endoplasmic reticulum (Cheesbrough, 1989). The acyl-transferase of the Kennedy pathway use acyl-CoAs to acylate the glycerol backbone in reaction leading to triacylglycerol (Harwood and Page, 1994). *sn*-1,3-Diaclyglycerol can be incorporated into phosphatidylcholine via the action of cholinephosphotransferase (Slack et al., 1985). Formation of polyunsaturated fatty acids is catalyzed by two specialized desaturase, which use phosphatidylcholine as a substrate and there are a number of possible mechanisms for incorporating the polyunsaturated acyl moieties into triacylglycerol (Browse and Somerville, 1991). In rapeseed oil, triacylglycerols contain C22:1 and C24:1 only in the *sn*1,3 positions (Fehling and Mukherjee, 1990), because (a) 1-acyl-*sn*-glycerol-3-phospahte acyltransferase discriminates against erucoyl-CoA and (b) lysophosphatidylcholine acyltransferase is inactive against this acyl-CoA (Bernerth and Frentzen, 1990). The biosynthesis of storage lipids seems to be controlled for 60% by the fatty acid synthesis in the plastid and for 40% by the Kennedy pathway in the endoplasmic reticulum (Ramil et al., 2002). The results (Table 4) suggested that the (*n*-7) fatty acids of C18:1, C20:1 and C22:1 were elongated as the more common (*n*-9) isomers were. The enzymatic system responsible for the (*n*-9) fatty acid synthesis could be responsible for the elongation and desaturation of the (*n*-7) fatty acids.

3. Experimental

3.1. Materials

3.1.1. Seed samples

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

3.1.2. Reagents and standards

Methanolic base was from Sigma (Sigma–Aldrich Canada Ltd., Ont. Canada). FAME standard CLC549 (NuChek Prep Inc., Elysian, MN, USA) was used as a gas chromatography reference standard.

3.1.3. Fatty acid methyl esters

Samples (10 seeds) were placed in a vial containing 2 ml of iso-octane. The seeds were homogenized for 60 s using an electric homogenizer, then 250 L of methanolic base was added and the sample mixed for 15 s. After incubating the mixture for 30 min at room temperature, 10 L of bromothymol blue (0.1%, wt/v in MeOH) was added. Then 150 L of HCl (1 M) and 500 L of Na_2CO_3 (0.15 M) were added consecutively, with mixing for 15 s after each solvent addition. Finally, deionized H_2O (N/ml) was added to the mixture without mixing and the vials were centrifuged for 7 min at 1500*g*. The top layer was transferred into a 100 μl GC vial insert for analysis.

3.1.4. GC analysis

The reference solution and samples were analyzed under the same operational conditions on a Hewlett–Packard 6890 gas chromatograph (Agilent Technologies, Mississauga, Ont., Canada) equipped with a flame ionization detector and a 7673A injector tower. Methyl esters were separated on a Supelcowax 10 silica column (Sigma–Aldrich Canada Ltd., Mississauga, Ont., Canada) (60 m × 0.32 mm, 0.25 m). Hydrogen was the carrier gas (2.5 mL/min), injection port temperature was 280 °C and detector temperature was kept at 300 °C. The temperature program was as follows: 190 °C initial temperature for 3 min, 2 °C/min ramp to 210 °C, 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. The split ratio was 25:1. Selected samples were also run on 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. The (*n*-9) fatty acids were identified by comparison to authentic standards (NuChek Prep Inc., USA). The (*n*-7) fatty acids were identified by their relative elution to the (*n*-9) standard based on the literature (Appleqvist, 1969) and by similarity of the MS spectrum to that of the (*n*-9) standard.

3.1.5. 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).

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