J Am Oil Chem Soc (2008) 85:159–168
DOI 10.1007/s11746-007-1181-y

ORIGINAL PAPER

Genotype and Growing Environment Effects on the Tocopherols and Fatty Acids of *Brassica napus* and *B. juncea*

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Received: 23 August 2007 / Revised: 14 November 2007 / Accepted: 27 November 2007 / Published online: 15 December 2007

Abstract The effects of genotype and growing environment on the tocopherols and fatty acids (FA) of experimental *Brassica juncea* and *B. napus* breeding lines were investigated. For both species, with the exception of a few genotypes, the concentration ratio of γ-tocopherols to α-tocopherol was practically constant. The genotype influenced the tocopherol concentration in *B. napus*, and to a lesser degree, *B. juncea*. The environment also had a similar effect, and a positive correlation existed between the daily maximum temperature and the α-tocopherol concentration in *B. napus*. Genotype effects on the FA composition were significant for the conventional but not for Clearfield or triazine tolerant traits of *B. napus*. The genotype had no effect on the FA of the *B. juncea* genotypes. In contrast, the growing environment had a significant influence on the FA composition of both species with apparent influence from temperature and rainfall. For both species, the concentration of γ-tocopherol as well as total tocopherols was inversely related to the 18:3 concentration, which could have resulted from opposite and independent effects of temperature on the two variables. No relationship existed between the concentrations of tocopherol and the remaining unsaturated FA 18:1 and 18:2. The positional distribution of unsaturated FA within the oil triacylglycerol was a function of their total concentration.

Keywords *Brassica juncea* · *Brassica napus* · Canola oil · FA composition · FA positional distribution · Genotype · Growing environment · Mustard seed oil · Tocopherols

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Introduction

Tocopherols have long been known as one of the most potent classes of fat-soluble antioxidants occurring in nature. More recently, beneficial biological roles for natural tocopherols, which are independent of their antioxidant/radical scavenging abilities have also been suggested, although a definitive function in human health and disease prevention has not been established [1]. Vegetable oils are the most abundant source of naturally occurring tocopherols. Therefore, oilseed crops such as canola and soybean represent the best target for biotechnological cost-efficient production of tocopherols for use as dietary supplements and food additives [2].

Considerations of both the positive and negative effects of vegetable oils on human health have led to growing interest in modifying the fatty acid (FA) composition of natural vegetable oils, by either conventional breeding techniques or by genetic modification. One major focus has been on the development of high-oleic and low-linolenic varieties of canola and soybean oils, which would also make them more resistant to oxidative rancidity. Such modifications would also augment their suitability as *trans* fat alternatives. Efforts are also being made to develop

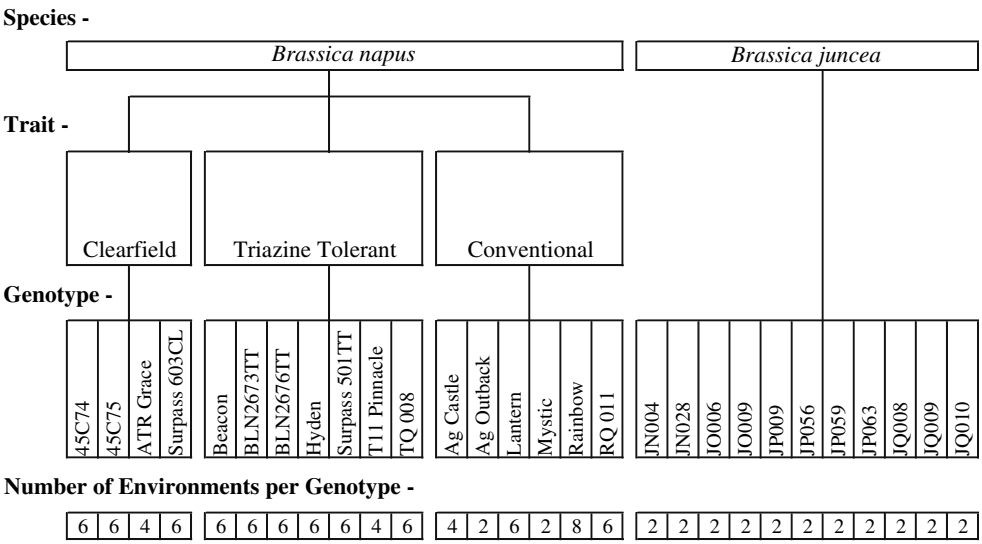

Fig. 1 The genotype and growing environments of the *B. napus* and *B. juncea* seed samples

vegetable oils with increased levels of the biologically important long-chain polyunsaturated FA such as docosahexaenoic acid (DHA) [3]. The effects of such FA modification on the minor components in oil such as the tocopherols are not well understood.

Previous studies have shown that the tocopherol content in canola oil is influenced by the genotype [4–6] as well as by the growing environment [4, 5]. Such genotype and environmental effects have been reported for soybean [7–11].

Canola (*Brassica napus*) is the main oilseed crop grown in Australia and the area under canola cultivation is projected to increase rapidly. A second *Brassica* species, *B. juncea* has been released for the low rainfall cropping areas of Australia with greater tolerance to heat and water stress, as well as enhanced resistance to the fungal disease blackleg, than *B. napus*. In this study, a large collection of experimental *B. juncea* and *B. napus* genotypes were investigated for effects of genotype and environment on the oil tocopherol content, FA composition, and FA positional distribution.

Experimental Procedures

Seed Samples

Seed samples from advanced breeding lines or released cultivars of *B. juncea* and *B. napus* genotypes were obtained from the Victorian Department of Primary Industries, Horsham, VIC, Australia. These seeds were collected from experimental plants grown in 2002 in locations within one or more of the following Australian States: New South Wales, South Australia, and Western Australia. All lines were sown and harvested at similar time points within the season. Samples consisted of ninety *B. napus* samples in total, representing three trait types (conventional, triazine tolerant and Clearfield). The *B. napus* samples comprised six conventional genotypes grown in two to eight environments ($n = 28$), seven triazine-tolerant genotypes grown in four to six environments ($n = 40$) and four Clearfield genotypes grown in four to six environments ($n = 22$). The *B. juncea* samples consisted of eleven genotypes grown in two environments ($n = 22$) (Fig. 1). Rainfall and monthly maximum temperature data for the 2002 season (April through to November) are presented in Tables 1 and 2, respectively.

Oil Extraction and Fatty Acid Analysis

Extractions were performed using petroleum ether (b.p. 40–60 °C). Briefly, the seed samples (5–10 g) were crushed and transferred into a thimble topped with cotton wool. The thimble was placed into the extraction apparatus and solvent added (200 mL). Samples were refluxed for four hours at a rate of three drops per second. The samples were emptied from the thimble and re-crushed, to be re-extracted for a further 2 h (×2). The solvent containing the extracted oil was filtered onto a pre-weighed flask and evaporated to dryness. Fatty acid methyl esters (FAME) were prepared by transesterification with methanolic sodium methoxide [12] and analyzed immediately by gas chromatography (GC) using a Perkin Elmer Model Autosystem XL instrument equipped with a SP-2560 fused silica capillary column (100 m, 0.25 i.d., 0.20 μm film). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min.. The oven temperature was programmed from 100 to 130 °C at a rate of 3 °C/min and then to 170 °C at a rate of 5 °C/min, and finally to 200 °C at the rate of 10 °C/min, and held at the final temperature for a further 30 min. The

Table 1 Average monthly rainfall from April to November in 2002 (n = 11)

Environment	Average monthly rainfall (mm)								
	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Total
New South Wales									
Moree	20.9	31.4	29.9	43.7	31.6	32.7	56.8	82.6	329.6
Tamworth	24.4	28.9	37.3	42.0	40.5	42.4	61.7	71.4	348.6
Wagga Wagga	42.5	53.6	50.2	55.4	52.9	50.8	60.0	43.5	408.9
Western Australia									
Katanning	30.2	60.5	77.2	76.0	63.6	46.7	35.6	21.5	411.3
Esperance	57.6	16.0	38	46.4	40.0	45.2	32.4	53.2	328.8
Wongon Hills	22.4	9.4	38.8	34.2	29.8	21.4	15.4	1.6	173.0
Mingenew	25.8	0.0	34.4	68.0	52.0	40.6	20.4	3.6	244.8
Merredin	43.4	6.6	20.2	22.8	23.8	15.2	4.6	5.8	142.4
South Australia									
Straun	19.2	40.0	58.8	118	60.8	51.8	33.6	40.4	422.6
Bordertown	7.5	34.0	64.5	38.0	40.5	47.0	20.5	59.0	311.0
Turretfield	35.9	53.4	56.4	58.8	57.7	53.7	44.5	28.6	389.0
Average	30.0	30.3	46.0	54.8	44.8	40.7	35.0	37.4	319.1
Std. Dev.	13.9	20.5	16.9	25.9	13.4	12.6	19.1	27.8	95.6

injector and flame ionization detector (FID) temperatures were held at 220 and 240 °C, respectively. Samples of FAME (1.0 μL) were injected using an autosampler at a split ratio of 50:1. Peaks were identified by comparison of their retention times with those of an authentic standard mixture containing common saturated and unsaturated FA (37 fatty acid methyl ester standard mix, Sigma, Sydney, Australia). Peaks were integrated using Turbochrom Workstation software (version 6.1.1, PE Nelson). Each extract was analyzed in triplicate.

Table 2 Average monthly maximum temperature from April to November in 2002 (n = 11)

Environment	Average maximum temperature °C							
	April	May	June	July	Aug.	Sept.	Oct.	Nov.
New South Wales								
Moree	26.8	22.1	19.1	18.0	20.3	24.5	27.2	29.8
Tamworth	24.9	20.4	17.1	15.9	18.3	21.7	24.7	27.5
Wagga Wagga	22.4	17.2	13.7	12.6	14.4	17.4	21.1	25.4
Western Australia								
Katanning	22.8	18.4	15.5	14.5	15.4	17.7	20.7	25.1
Esperance	22.3	22.0	17.7	17.2	17.4	18.6	22.3	24.6
Wongon Hills	24.5	22.4	17.4	16.1	16.4	19.2	23.8	29.3
Mingenew	26.4	31.8	19.2	17.7	17.1	20.0	24.3	30.7
Merredin	24.6	22.8	17.5	16.8	16.9	19.9	24.7	29.5
South Australia								
Straun	21.2	17.5	14.5	13.9	14.9	16.7	19.5	22.5
Bordertown	22.4	18.3	15.5	15.0	16.1	18.5	21.4	24.6
Turretfield	23.0	18.6	15.5	14.7	15.7	18.1	21.6	25.4
Average	23.8	21.0	16.6	15.7	16.6	19.3	22.8	26.8
Std. Dev.	1.8	4.1	1.8	1.7	1.7	2.2	2.3	2.7

Positional Distribution of Fatty Acids

The oil (0.06 g) was dissolved in 1-butanol (400 μL) and added to a test tube containing immobilized Lipozyme IM (250 mg) obtained from *Mucor miehei* (activity = 30 U/g). The empty oil vial was washed with a further aliquot of 1-butanol (200 μL) and combined with the enzyme mix. The mixture was agitated at room temperature for a total of 4 min, and extracted with *n*-pentane (3 × 7 mL). The combined extract was washed with saturated sodium chloride (3 × 5 mL), followed by a single distilled water wash, and dried over anhydrous sodium sulfate.

The FA composition at the *s*n-2 position was calculated using the formula:

$$\begin{aligned} &s\mathrm{n}-2\,\mathrm{composition}(\%\mathrm{mol}) \\ &\quad = 3\times[\mathrm{total\,composition}(\%\mathrm{mol})]-2 \\ &\qquad \times[s\mathrm{n}-1(3)\mathrm{composition}(\%\mathrm{mol})]. \end{aligned}$$

Tocopherol Analysis

Freshly extracted oils were dissolved in trimethyl pentane (10%, w/v) and analyzed for tocopherols by HPLC on a silica column (150 × 4.6 mm, 3 μm). The column was eluted isocratically with a mixture of trimethyl pentane and *iso*-propyl alcohol (99.5:0.5, v/v) at a flow rate of 0.8 mL/min. Peaks were detected with a diode array detector set at

a wavelength of 292 nm. The α-, β-, and γ-tocopherols were quantified by reference to an external calibration with their authentic standards. The α- and γ-tocopherols standards were purchased from Sigma, Sydney, Australia whereas the β-tocopherol was a gift from The Victorian Department of Primary industries, Werribee, VIC, Australia. Each extract was analyzed in triplicate.

Statistical Analysis

Analysis was conducted using GenStat software. Variables were FA composition (16:0, 18:0, 18:1, 18:2, 18:3) and tocopherol content (α-, β- and γ- and total tocopherol). Analysis of Variance (ANOVA) algorithm was used for *B. juncea* genotypes, while a restricted maximum likelihood (REML) multi-variate model was used for *B. napus* data due to inequalities in sampling. The *B. juncea* and *B. napus* genotypes had significantly different FA profiles. Therefore, each species was analyzed separately to prevent incorrect identification of significant genotype effects due to differences between the species.

Overall statistical significance and general trends were determined at the 0.05 level (95% confidence intervals) for all variables through *p* values generated from the GenStat program. Significance between individual genotypes and environments for each variable was determined using least significant difference (LSD) values.

Results and Discussion

Tocopherols

In both species, α- and γ-tocopherols were the main components while relatively small amounts of β-tocopherol were also present. Although δ-tocopherol has been previously reported in rapeseed oil [13–16], it was not found in our study. Tocotrienols and plastochromanol-8 were also absent. Most previous studies have failed to find β-tocopherol in canola or rapeseed oil [17]. However, Ferrari et al. [14] found low levels of β-tocopherol in solvent-extracted crude rapeseed oil and refined rapeseed oil, but not in pressed crude rapeseed oil. β-Tocopherol concentrations of 0–140 μg/g have been reported in canola oil [16]. β-Tocopherol is an isomeric form of γ-tocopherol, which is the most abundant tocopherol in canola oil. The two isomers are well separated on normal-phase (silica) columns but inadequately separated on reversed-phase columns [16], and the failure to detect β-tocopherol in canola oil in some studies may have been due to inadequate separation of the two isomers by the HPLC method used.

The first and second most abundant tocopherols in the oils examined were γ-tocopherol (mean of 350 μg/g, range of 163–924 μg/g) and α-tocopherol (mean of 186 μg/g, range of 76–336 μg/g), respectively. Averaged across all samples ($n = 90$, irrespective of genotypes and growing environment) the *B. napus* oils contained 358 μg/g of γ-tocopherol (range of 241–924 μg/g) and 181 μg/g of α-tocopherol (range of 108–336 μg/g). The corresponding values for the *B. juncea* oils ($n = 22$) were 379 μg/g (range of 163–777 μg/g) and 200 μg/g (range of 76–335 μg/g). The concentration of β-tocopherol was relatively low in both species with averages of 25 μg/g (range of 11–64 μg/g) for *B. napus* and 16 μg/g (range of 4–57 μg/g) for *B. juncea.*

Although previous studies by Marwede et al. [4] identified highly significant genotype x growing environment interactions as a major source of variation in the tocopherol content of *B. napus* samples, no statistically significant genotype × environment interactions existed for the *B. napus* genotypes examined in this study. This enabled genotype effects to be evaluated independently of the environment and vice versa. The total tocopherol concentration in the *B. napus* oils varied widely between genotypes within the conventional samples (Table 3) with RQ011 and Lantern having the highest (820 μg/g) and lowest (389 μg/g) values, respectively, relative to all other *B. napus* samples examined within this study. Relative quantities of the tocopherols were in the range of 55–65% for γ-tocopherol and 30–40% for α-tocopherol. The ratio of γ- to α-tocopherol was fairly constant, approximately 3:2 in favour of γ-tocopherol. This relationship has been noted previously [5, 6]. Exceptions were RQ011, 45C74, and Surpass 501TT genotypes for which the γ- to α-ratio surpassed normal values. In *B. napus*, the content of β-tocopherol was generally within 3–8% of the total; however, T11 Pinnacle and Ag Castle contained amounts over 10%. The total, as well as the individual tocopherol levels were significantly ($p < 0.01$) affected by the genotype.

The concentrations of γ-, α- and β-tocopherol within the *B. juncea* genotypes were similar to that of *B. napus* with values of 55–65, 30–40 and 2–7%, respectively. Nevertheless, unlike *B. napus*, where the levels of tocopherols were significantly influenced by the genotype, genotype effects were less apparent in *B. juncea*.

Marwede et al. [4] showed both strong environmental and genotype effects on tocopherol content in rapeseed oil. In contrast, our results showed that genotype has a much greater influence than the environment. Table 4 shows the effects of environment on the tocopherols of *B. napus* and *B. juncea* oils. For *B. napus*, significant ($p = 0.001$) environmental effects were observed for α- and β-tocopherols but not for γ-tocopherol. A significant ($p = 0.004$) environmental effect was observed within the *B. juncea*

Table 3 The effect of genotype on the tocopherol composition of *B. napus* and *B. juncea* seed oils

	Tocopherol Content (μg/g of oil)			
	α-Tocopherol	β-Tocopherol	γ-Tocopherol	Total
B. napus Clearfield				
45C74	168 (37)^{a}	13 (6)^{d}	468 (65)^{klm}	649 (66)^{pqrs}
45C75	230 (84)^{bc}	29 (24)^{defg}	330 (64)^{jkl}	589 (90)^{nopqr}
ATR Grace	158 (85)^{ab}	28 (25)^{def}	302 (47)^{jkl}	488 (78)^{nopq}
Surpass 603 CL	201 (103)^{ab}	29 (17)^{defg}	402 (179)^{jkl}	632 (264)^{pqrs}
B. napus triazine tolerant				
Beacon	176 (45)^{ab}	23 (15)^{def}	292 (120)^{jk}	490 (148)^{nopq}
BLN2673TT	199 (71)^{ab}	41 (20)^{gh}	291 (76)^{jk}	531 (124)^{nopq}
BLN2676TT	158 (36)^{ab}	16 (12)^{de}	241 (54)^{j}	415 (69)^{no}
Hyden	207 (47)^{ab}	22 (13)^{def}	312 (41)^{jkl}	541 (40)^{nopq}
Surpass 501 TT	166 (52)^{ab}	19 (3)^{de}	512 (51)^{lm}	697 (77)^{qrs}
T11 Pinnacle	240 (16)^{bc}	64 (9)^{i}	290 (11)^{jk}	594 (14)^{nopqrs}
TQ 008	161 (137)^{ab}	36 (137)^{efg}	418 (233)^{jkl}	615 (361)^{opqrs}
B. napus conventional				
Ag Castle	145 (58)^{ab}	49 (18)^{hi}	284 (21)^{jk}	480 (45)^{nop}
Ag Outback	222 (131)^{abc}	20 (3)^{de}	303 (112)^{jkl}	636 (337)^{pqrs}
Lantern	121 (76)^{a}	16 (8)^{de}	251 (77)^{j}	389 (150)^{n}
Mystic	314 (95)^{c}	36 (3)^{efg}	425 (23)^{jklm}	775 (115)^{rs}
Rainbow	150 (44)^{abc}	16 (10)^{de}	246 (60)^{j}	412 (89)^{no}
RQ011	162 (61)^{ab}	24 (8)^{def}	634 (170)^{m}	820 (219)^{s}
LSD	105	20	214	212
p	0.011	0.001	0.001	0.001
B. juncea				
JN004	271 (180)^{t}	31 (29)^{v}	518 (16)^{za}	820 (59)^{cd}
JN028	244 (188)^{t}	21 (2)^{uv}	485 (97)^{yza}	750 (280)^{cd}
JO006	213 (94)^{t}	15 (1)^{uv}	366 (95)^{xyz}	594 (188)^{bcd}
JO009	208 (102)^{t}	9 (9)^{uv}	323 (156)^{wxyz}	539 (267)^{bcd}
JP009	76 (2)^{t}	18 (13)^{uv}	163 (35)^{w}	256 (50)^{b}
JP056	335 (279)^{t}	14 (1)^{uv}	579 (10)^{a}	928 (268)^{d}
JP059	203 (86)^{t}	13 (2)^{uv}	287 (64)^{wxy}	503 (152)^{bc}
JP063	203 (86)^{t}	4 (1)^{u}	283 (5)^{wx}	470 (67)^{bc}
JQ008	183 (74)^{t}	10 (4)^{uv}	351 (75)^{wxyz}	538 (176)^{bcd}
JQ009	194 (63)^{t}	26 (6)^{uv}	453 (138)^{xyza}	673 (203)^{bcd}
JQ010	195 (139)^{t}	14 (11)^{uv}	305 (97)^{wxy}	514 (225)^{bcd}
LSD	223	25	201	419
p	0.226	0.0194	0.001	0.155

For each genotype, average values based on analysis of 90 samples ($n = 90$) of *B. napus* representing 17 genotypes, and 22 samples ($n = 22$) of *B. juncea* representing 11 genotypes, grown in different locations in 2002, are shown. The standard deviation is shown in parenthesis. Values in columns, for the same species, followed by different letters are significantly different ($p < 0.05$)

samples for α-tocopherol content but not for others. Rainfall (Table 1) and temperature (Table 2) were two key environmental variables. It was found that the daily maximum temperature, and not rainfall, had a significant positive correlation with α-tocopherol level in *B. napus* ($R^2 = 0.38$). Previous studies have shown that biosynthesis of tocopherol in oilseeds, including rapeseed [18] and soybean [8] is promoted by increased temperature. Temperature or rainfall effects on tocopherols in *B. juncea* could not be determined via linear extrapolation due to the limited number of environments included in the study for this species ($n = 2$).

Fatty Acid Composition

Table 5 shows the FA composition of the *B. napus* and *B. juncea* oils. The *B. napus* oils had FA compositions typical of canola oil with 18:1, 18:2, and 18:3 levels of approximately 60, 20, and 10%, respectively. The *B. juncea* oils

Table 4 The effect of growing environment on the tocopherol composition of *B. napus* and *B. juncea* oils

	Tocopherol Content (μg/g of oil)			
	α-Tocopherol	β-Tocopherol	γ-Tocopherol	Total
B. napus				
New South Wales				
Moree	336 (161)^{c}	18 (14)^{def}	358 (97)^{h}	712 (415)^{j}
Tamworth	203 (72)^{b}	12 (15)^{de}	339 (197)^{h}	554 (243)^{ij}
Wagga Wagga	196 (60)^{ab}	13 (8)^{def}	311 (127)^{h}	520 (151)^{ij}
Western Australia				
Katanning	186 (61)^{ab}	31 (15)^{fg}	364 (183)^{h}	581 (217)^{ij}
Esperance	146 (48)^{ab}	17 (15)^{def}	272 (169)^{h}	435 (282)^{i}
Wongan Hills	207 (58)^{b}	11 (16)^{d}	399 (54)^{h}	617 (317)^{ij}
Mingenew	191 (43)^{ab}	27 (23)^{defg}	308 (93)^{h}	526 (221)^{ij}
Merredin 9154)^{ij}	193 (48)^{ab}	21 (15)^{defg}	306 (94)^{h}	521
South Australia				
Straun	146 (72)^{ab}	37 (22)^{g}	434 (269)^{h}	617 (343)^{ij}
Bordertown	157 (56)^{ab}	31 (17)^{fg}	329 (95)^{h}	517 (92)^{ij}
Turretfield	108 (29)^{a}	30 (2)^{efg}	250 (10)^{h}	388 (42)^{i}
LSD	93	18	190	272
p	0.001	0.001	0.160	0.016
B. juncea				
Moree	254 (130)^{k}	13 (9)^{m}	372 (150)^{n}	639 (178)^{o}
Merredin	152 (71)^{l}	16 (7)^{m}	347 (121)^{n}	515 (167)^{o}
LSD	95	11	86	178
p	0.004	0.613	0.495	0.109

Averages values based on 90 samples ($n = 90$) of *B. napus* representing 17 genotypes, and 22 samples ($n = 22$) of *B. juncea* representing 11 genotypes, grown in different locations in 2002, are shown. The standard deviation is shown in parenthesis. Values in columns, for the same species, followed by different letters are significantly different ($p < 0.05$)

contained lower levels of 18:1 (average of 48%) and higher levels of 18:2 and 18:3 (average values of 29 and 13%, respectively). Eucic acid (22:1) was absent or occurred only as a minor component (<1%) with the exception of one sample of *B. napus* (TQ 008) which contained 5.3% of 22:1 and 7.5% of 20:1 (data not shown). For *B. napus*, the concentration of 18:1 negatively correlated with those of 18:2 ($R^2 = 0.59$) and 18:3 ($R^2 = 0.69$). For *B. juncea*, a negative correlation existed between 18:1 and 18:2 ($R^2 = 0.72$).

Genotype effects on the FA composition of canola oil have been reported [15]. The *B. napus* genotypes examined in the present study belonged to one of the following types: Clearfield, triazine-tolerant, or conventional. There were no significant genotype effects on the FA composition of the oils from the Clearfield or triazine tolerant genotypes, with the exception of Hyden (18.3%) and Surpass 501TT (21.9%), where the content of 18:2 was significantly different (LSD = 3.55) (Table 6). Much greater variation was observed among the genotypes belonging to the conventional trait, particularly for 18:3, which ranged from 7.9 to 12.3%. In contrast, the FA composition of *B. juncea* was not significantly affected by genotype, with the exception of relatively minor differences in the concentration of 18:0 (range of 1.8–2.6%).

Table 7 shows the effects of environment on the FA composition of *B. napus* and *B. juncea* oils. The environment significantly ($p < 0.05$) influenced the FA composition of the *B. napus* oils with the exception of 16:0 (range of 4.4–4.9%). The greatest variation was observed between the sites Esperance and Merredin, both of which were within the state of Western Australia. The seeds grown in Esperance contained the highest concentration of 18:3 (14%) and the lowest concentration of 18:1 (56.6%), whereas those grown in Merredin contained the lowest concentration of 18:3 (8.5%) and the highest concentration of 18:1 (62.2%). The concentration of 18:2 was also appreciably higher in the Esperance samples than the Merredin samples. Pritchard et al. [19] found that the concentration of 18:3 in Australian-grown canola increased with lower maximum spring temperature and higher rainfall. Esperance was cooler and experienced higher rainfall during the growing season than Merredin did, and the observed differences in FA between the two locations could have resulted from a combination of these two factors. It has been reported that lower environmental temperatures tend to increase polyunsaturated fatty acid production in rapeseed embryos [20]. Previous studies have also shown that lower environmental temperatures stimulate enzymic desaturation of 18:1–18:2 in low-linolenic flax [21] and sunflower [22]. However, the subsequent desaturation step from 18:2 to 18:3 in flaxseed was unaffected by temperature [21]. There was no significant difference in the 18:3 levels in the *B. juncea* genotypes grown in the locations of Moree and Merredin. However, the Moree samples had significantly ($p < 0.05$) higher levels of 18:2 (33.6%) and lower levels of 18:1 (41.9%) compared to 30.6 and 46.7%, respectively, in the Merredin samples.

Positional Distribution of Fatty Acids

For the *B. napus* genotypes, a linear relationship existed between the concentration (weight %) of the unsaturated

Table 5 Fatty acid composition (weight %) of *B. napus* and *B. juncea* seed oils

	FA Composition (weight %)											
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	24:0
B. napus	0.2	4.4	0.3	2.3	61.4	19.1	9.6	0.7	1.4	0.2	0.2	0.1
Range	0.1–1.3	4.2–5.4	0.2–0.4	1.2–2.5	47.3–65.9	16.8–25.6	6.8–19.8	0.1–0.8	0.4–8.8	0–0.3	0–6.4	0–0.9
%CV	77.6	5.7	12.6	19.4	6.4	6.4	12.8	14.6	116.9	31.5	354.4	123.3
B. juncea	0.2	3.8	0.3	2.5	48	28.8	13.1	0.7	1.6	0.2	0.4	0.4
Range	0.1–1.1	3.6–5.3	0.2–0.7	1.3–2.7	35.3–50.7	26.6–37.9	10.9–19.1	0.2–0.7	0.4–4.7	0.1–0.4	0–0.7	0–5.8
%CV	78	14.5	38.6	12.9	8.7	8.7	13.7	15.5	62.0	25.5	141.7	238.8

Values shown are averages for 90 *B. napus* samples ($n = 90$) representing 17 genotypes, and 22 *B. juncea* samples ($n = 22$) representing 11 genotypes, grown in different locations in 2002

Table 6 The effect of genotype on selected fatty acids of *B. napus* and *B. juncea* seed oils

	FA composition (weight %)				
	16:0	18:0	18:1	18:2	18:3
B. napus Clearfield					
45C74	4.9 (0.2)^{a}	2.0 (0.1)^{bc}	60.6 (0.7)^{ef}	19.8 (0.4)^{hijkl}	10.6 (0.8)^{mno}
45C75	4.7 (0.1)^{a}	2.1 (0.1)^{bc}	60.7 (0.9)^{ef}	19.2 (0.5)^{hijk}	11.1 (0.4)^{no}
ATR Grace	4.5 (0.2)^{a}	1.9 (0.2)^{bc}	60.4 (2.0)^{ef}	19.4 (0.8)^{hijk}	11.5 (1.1)^{no}
Surpass 603 CL	4.6 (0.3)^{a}	2.0 (0.3)^{bc}	61.9 (1.1)^{efg}	20.4 (0.6)^{ijkl}	8.6 (1.1)^{mn}
B. napus triazine tolerant					
Beacon	4.6 (0.3)^{a}	1.3 (0.9)^{b}	60.5 (0.7)^{ef}	19.7 (0.8)^{hijk}	11.6 (0.6)^{no}
BLN2673TT	4.9 (0.2)^{a}	1.9 (0.1)^{bc}	61.1 (1.6)^{efg}	19.5 (0.8)^{hijk}	10.6 (1.0)^{mno}
BLN2676TT	4.9 (0.2)^{a}	1.8 (0.2)^{bc}	60.9 (1.6)^{efg}	19.0 (1.0)^{hijk}	11.5 (1.1)^{no}
Hyden	4.6 (0.2)^{a}	2.1 (0.1)^{bc}	62.1 (1.7)^{fg}	18.3 (0.9)^{hi}	10.8 (1.1)^{mno}
Surpass 501 TT	4.8 (0.3)^{a}	1.8 (0.2)^{bc}	60.6 (0.9)^{ef}	21.9 (1.0)^{l}	9.2 (0.9)^{mno}
T11 Pinnacle	4.6 (0.1)^{a}	2.0 (0.2)^{bc}	60.3 (0.5)^{ef}	20.7 (1.1)^{jkl}	11.4 (1.2)^{no}
TQ 008	5.2 (0.1)^{a}	1.8 (0.1)^{bc}	48.2 (0.5)^{d}	19.0 (1.0)^{hijk}	11.1 (0.8)^{no}
B. napus conventional					
Ag Castle	4.6 (0.1)^{a}	2.0 (0.1)^{bc}	62.2 (0.9)^{fg}	19.7 (0.6)^{hijk}	9.6 (0.8)^{mno}
Ag Outback	4.6 (0.2)^{a}	2.0 (0.1)^{bc}	65.0 (0.5)^{g}	17.9 (1.3)^{h}	7.9 (0.5)^{m}
Lantern	4.4 (0.1)^{a}	2.2 (0.5)^{c}	60.8 (4.9)^{ef}	19.3 (2.4)^{hijk}	11.5 (3.0)^{no}
Mystic	4.8 (0.5)^{a}	2.2 (0.3)^{c}	59.7 (3.1)^{ef}	21.2 (1.4)^{kl}	8.7 (2.0)^{mn}
Rainbow	4.5 (0.2)^{a}	2.0 (0.5)^{bc}	57.8 (4.8)^{e}	20.9 (2.1)^{jkl}	12.3 (3.5)^{o}
RQ011	4.5 (0.2)^{a}	2.2 (0.1)^{c}	60.9 (0.8)^{efg}	20.4 (0.6)^{ijkl}	10.1 (0.6)^{mno}
LSD	0.8	0.8	4.1	2.1	3.1
p	0.020	0.023	0.045	0.001	0.001
B. juncea					
JN004	4.2 (0.9)^{pq}	2.3 (0.1)^{stu}	44.2 (3.1)^{wxy}	33.4 (3.0)^{ab}	12.6 (0.6)^{cde}
JN028	4.8 (0.1)^{q}	2.2 (0.2)st	40.8 (5.7)^{w}	32.2 (0.7)^{zab}	12.9 (1.2)^{cde}
JO006	4.4 (0.7)^{pq}	2.5 (0.1)^{uv}	45.8 (2.7)^{wxy}	31.0 (3.6)^{zab}	13.4 (1.4)^{cde}
JO009	4.6 (0.9)^{q}	2.1 (0.2)^{s}	42.3 (4.3)^{wx}	32.9 (3.2)^{ab}	15.0 (0.1)^{de}
JP009	4.6 (0.7)^{q}	2.2 (0.1)st	42.8 (1.9)^{wxy}	33.4 (2.0)^{ab}	13.9 (1.4)^{cde}
JP056	4.6 (0.8)^{q}	2.6 (0.2)^{v}	42.1 (9.6)^{wx}	33.9 (5.7)^{ab}	13.3 (2.5)^{cde}
JP059	4.7 (0.9)^{q}	2.5 (0.2)^{uv}	46.5 (4.7)^{xy}	31.1 (3.2)^{zab}	12.3 (0.1)^{cd}
JP063	4.7 (0.9)^{q}	2.4 (0.1)^{tuv}	48.0 (0.3)^{y}	28.8 (3.0)^{z}	12.9 (0.3)^{cde}
JQ008	3.7 (0.1)^{p}	2.3 (0.1)^{stu}	48.0 (0.3)^{y}	30.5 (0.3)^{za}	12.7 (0.1)^{cde}
JQ009	4.3 (1.0)^{pq}	2.3 (0.1)^{stu}	46.2 (4.3)^{xy}	32.4 (3.6)^{zab}	11.4 (0.7)^{c}
JQ010	4.3 (1.1)^{pq}	1.8 (0.8)^{r}	40.8 (3.3)^{w}	34.8 (0.8)^{b}	15.1 (5.7)^{e}
LSD	0.7	0.2	5.2	3.9	2.7
p	0.506	0.011	0.162	0.322	0.273

Minor component FA are not shown. Values shown are averages based on 90 samples ($n = 90$) of *B. napus* representing 17 genotypes, and 22 samples ($n = 22$) of *B. juncea* representing 11 genotypes, grown in different locations in 2002. The standard deviation is shown in parenthesis. Values in columns, for the same species, followed by different letters are significantly different ($p < 0.05$)

Table 7 The effect of genotype on selected fatty acids of *B. napus* and *B. juncea* seed oils

	FA Composition (weight %)				
	16:0	18:0	18:1	18:2	18:3
B. napus					
New South Wales					
Moree	4.9 (0.2)^{a}	2.3 (0.3)^{c}	61.3 (3.9)^{de}	19.7 (2.6)^{fg}	8.9 (1.3)^{ij}
Tamworth	4.7 (0.2)^{a}	2.0 (0.2)^{bc}	61.8 (2.1)^{e}	19.8 (1.5)^{fg}	9.4 (1.6)^{ijk}
Wagga Wagga	4.4 (0.2)^{a}	2.0 (0.2)^{bc}	60.4 (3.4)^{de}	19.9 (0.6)^{fg}	11.3 (2.3)^{ijkl}
Western Australia					
Katanning	4.7 (0.3)^{a}	2.0 (0.2)^{bc}	61.5 (1.0)^{de}	19.6 (3.4)^{fg}	10.1 (0.9)^{ijk}
Esperance	4.5 (0.1)^{a}	1.8 (0.8)^{bc}	56.6 (8.0)^{d}	21.8 (0.8)^{h}	14.0 (5.1)^{l}
Wongan Hills	4.5 (0.3)^{a}	2.0 (0.2)^{bc}	60.4 (0.6)^{de}	19.7 (1.0)^{fg}	10.9 (0.8)^{ijk}
Mingenew	4.8 (0.2)^{a}	1.4 (0.7)^{b}	59.2 (0.8)^{de}	20.8 (1.0)^{gh}	11.5 (1.0)^{jkl}
Merredin	4.4 (0.1)^{a}	2.4 (0.1)^{c}	62.2 (2.3)^{e}	19.9 (1.0)^{fg}	8.5 (1.4)^{i}
South Australia					
Straun	4.7 (0.3)^{a}	1.8 (0.2)^{bc}	61.3 (1.3)^{de}	19.3 (1.2)^{f}	10.8 (1.2)^{ijk}
Bordertown	4.8 (0.2)^{a}	1.9 (0.6)^{bc}	60.4 (0.7)^{de}	20.2 (1.2)^{fg}	11.1 (1.0)^{ijkl}
Turretfield	4.5 (0.1)^{a}	1.7 (0.6)^{bc}	58.8 (1.8)^{de}	20.7 (0.8)^{gh}	11.9 (0.4)^{kl}
LSD	1.1	0.7	4.9	1.3	2.9
p	0.782	0.005	0.005	0.007	0.001
B. juncea					
Moree	4.9 (0.4)^{m}	2.3 (0.2)^{o}	41.9 (3.5)^{p}	33.8 (2.1)^{r}	12.7 (1.3)^{t}
Merredin	4.0 (0.4)^{n}	2.3 (0.4)^{o}	46.7 (3.3)^{q}	30.6 (2.2)^{s}	13.8 (2.0)^{t}
LSD	0.3	0.1	2.1	1.7	1.2
p	0.001	0.821	0.001	0.001	0.372

Values shown are averages based on 90 samples ($n = 90$) of *B. napus* representing 17 genotypes, and 22 samples ($n = 22$) of *B. juncea* representing 11 genotypes, grown in different locations in 2002. Minor component acids are not shown. The standard deviation is shown in parenthesis. Values in columns, for the same species, followed by different letters are significantly different ($p < 0.05$)

FA 18:1 ($R^2 = 0.85$), 18:2 ($R^2 = 0.69$), and 18:3 ($R^2 = 0.79$) in the *sn*-2 position and the total triacylglycerol (Fig. 2). The relationship was also valid for the *B. juncea* genotypes where the R^2 values for 18:1, 18:2, and 18:3 were 0.7, 0.79, and 0.93, respectively (Fig. 3). This showed that as the total concentration in oil of a given unsaturated FA increased, its concentration at the *sn*-2 position also increased. The same relationship has been shown to exist for other oilseeds including soybean [23], maize [24], and *Brassica* species [25]. The few samples that deviated from this linear relationship had FA compositions markedly different from each other in respect of one or more unsaturated FA. They included *B. napus* genotypes Lantern, Rainbow, Surpass501TT, and the *B. juncea* genotype JP056.

FA are incorporated to the *sn*-2 position by the reaction of acetyl-CoAs of the substrate fatty acids with lysosphosphatidic acid. This reaction is catalyzed by the enzyme lysosphosphatidic acid acyltransferase (LPAAT). The reason for unsaturated FA to preferentially esterify at the *sn*-2

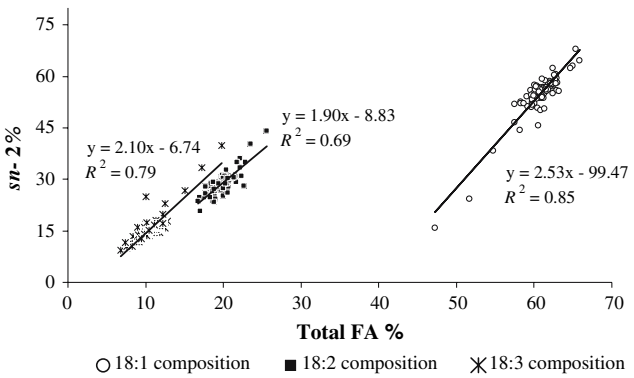

Fig. 2 Relationship between the FA concentration (weight %) in the total oil and triacylglycerol *sn*-2 position for *B. napus*

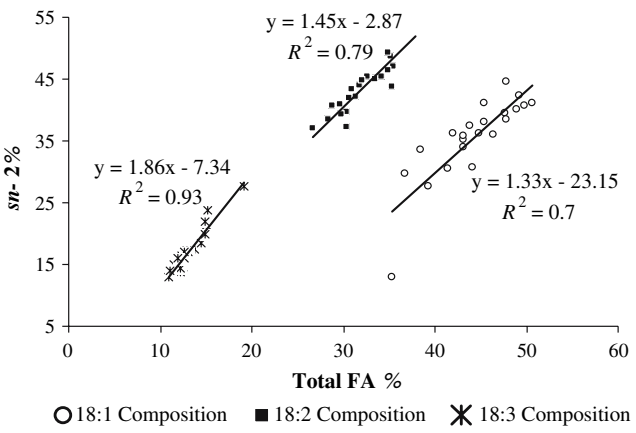

Fig. 3 Relationship between the FA concentration (weight %) in the total oil and triacylglycerol *sn*-2 position for *B. juncea*

Table 8 Correlations between selected fatty acid and tocopherol concentrations

	Correlation Coefficient (R^2)		
	α-Tocopherol	γ-Tocopherol	Total
B. napus			
18:1	0.06	0.06	0.06
18:2	(0.03)	0.03	0.18
18:3	(0.16)	(0.27)*	(0.26)*
B. juncea			
18:1	0.27	0.02	(0.11)
18:2	0.16	0.01	0.09
18:3	(0.55)*	(0.43)*	(0.45)*

Values shown are correlation coefficients from linear extrapolation based on 90 samples ($n = 90$) of *B. napus* representing 17 genotypes, and 22 samples ($n = 22$) of *B. juncea* representing 11 genotypes, grown in different locations in 2002. Negative correlations are shown in parenthesis

* $p < 0.05$

position may be attributed to greater selectivity of LPAAT towards unsaturated FA. Indeed, it has been shown that, for a wide variety of species including rapeseed, LPAAT exhibits substrate selectivity for unsaturated FA over saturated FA [26].

It is possible that the positional distribution of unsaturated FA within the triacylglycerol molecule influences their susceptibility to oxidative deterioration. We have observed that canola oils with more or less identical FA composition exhibited significantly different oxidative stability when used for deep-frying (unpublished data). This could possibly have been due to different positional distribution of the polyunsaturated FA components of the oil. However, on the basis of the above-mentioned results, as the FA composition of the canola oils tested were the same, the positional distribution of the FA would also have been the same. This effectively excluded positional distribution as the reason for the observed differences in the oxidative stability of the canola oils tested. This result implies that any observed differences in the functional properties between canola oils with the same or closely related FA composition cannot be attributed to the positional distribution of their constituent FA.

Correlations Between FA Composition and Tocopherol Levels

The concentration of 18:3 in the *B. napus* genotypes was negatively correlated ($p < 0.05$) with the concentration of γ-tocopherol as well as total tocopherol (Table 8). This negative correlation was also observed for α-tocopherol. Linear regression of the 18:3 concentration against the total tocopherol concentration for each environment also suggested an inverse relationship between the two variables. Significant correlations were not observed between the remaining unsaturated fatty acids (18:1 and 18:2) and the tocopherols.

Few studies have paid attention to possible relationships between tocopherol content and FA composition of vegetable oils. Dolde et al. [6] investigated commercial and experimental breeding lines of canola, sunflower and soybean oil, observed a highly positive correlation between 18:3 and total and individual (δ- and γ-) tocopherol concentrations in both conventional and genetically modified soybean oils, but not in canola oil. They speculated that the relationship they observed was a consequence of the very narrow gene pool of the soybean phenotype used in their study. A similar relationship was found by Almonor et al. [8] in a study also conducted on a limited number of soybean genotypes. Furthermore, the relationship was based on the percentage composition of tocopherols rather than absolute amounts. McCord et al. [10] who included a wider range of soybean samples in their study found that although reduced 18:3 lines had lower total tocopherol content than normal lines, there was significant variation in the tocopherol content among lines. In addition, some reduced 18:3 lines had tocopherol contents similar to those of normal 18:3 lines. They suggested that it should be possible to develop low 18:3 soybean cultivars that have acceptable tocopherol content.

The apparent absence of a positive correlation between 18:3 and tocopherol in vegetable oils is somewhat surprising because tocopherols are effective antioxidants in oil, and it may be expected that the plant reacts to increased polyunsaturation by producing more tocopherols to protect the oil from oxidation. The absence of a direct relationship supports the proposition put forward by Dolde et al. [6] that tocopherol concentration and FA composition are not causally related but influenced similarly by independent external variables such as temperature or soil type. This has implications for current efforts being made to develop designer vegetable oils with enhanced levels of highly unsaturated FA such DHA. The apparent inability of the plant to respond by producing higher levels of tocopherols may make such oils highly susceptible to oxidative deterioration. This problem may be overcame by selecting genotypes with inherently higher tocopherol content for development of DHA-enriched vegetable oils.

Acknowledgments We thank Dr. Rod Mailer (Department of Primary Industries, NSW) and Wayne Burton (Department of Primary Industries, VIC) for seed samples and Dr. Graham Hepworth (University of Melbourne) for statistical analyses. This project was funded by Food Science Australia.

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