JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Correlations between Tocopherol and Fatty Acid Components in Germplasm Collections of *Brassica* Oilseeds

Yuanlong Li,[†] Nazim Hussain,[†] Lingmin Zhang,[†] Xiaoyang Chen,[§] Essa Ali,[†] and Lixi Jiang*[,†]

[†]Key Laboratory of Crop Germplasm Resources of Zhejiang Province, College of Agriculture and Biotechnology of Zhejiang University, 866 Yu-Hang-Tang Road, Hangzhou 310058, People's Republic of China

[§]Institute of Crop Science, Jinhua Academy of Agricultural Sciences, 138 Shuanglong Nan Street, Jinhua 321017, People's Republic of China

Supporting Information

ABSTRACT: To date, little is known about the correlations among the tocopherol (T) and fatty acid (FA) components in rapeseed oils. In the current study, a germplasm collection of landraces from the species *Brassica juncea*, *Brassica rapa*, and *Brassica napus* and a collection of low erucic acid (EA) breeding lines from *B. napus* were analyzed for FA and T contents. In the groups comprising landraces, the most notable correlation was the significantly positive one between α-T and the sum of C18:1 and C18:2, whereas neither positive correlations were found between α-T and C18:3 nor were positive correlations observed between α-T and very long chain FAs (VLCFA). Hardly any association between γ-T and FA components was observed, indicating the possible function of α-T beyond its antioxidant property. The complexity of correlation between T and FA components in *Brassica* oils may arise from the role of α-T in the FA metabolism of endoplasmic reticulum (ER).

KEYWORDS: *tocopherols, fatty acids, correlation, Brassica oilseeds*

INTRODUCTION

Brassica is a genus of plants in the family of Brassicaceae including important sources of edible oils or biofuels such as *Brassica napus* (AACC, 2*n* = 38), *Brassica rapa* (AA, 2*n* = 20), and *Brassica juncea* (AABB, 2*n* = 36). *Brassica* oilseeds (*B. napus*), along with soybean (*Glycine max* L.), oil palm (*Elaeis guineensis jacg*), and cottonseed (*Gossypium hirsutum* L.), are the most important sources of plant oils worldwide (million tons).[1] The nutritional characteristics of *Brassica* oils are mainly determined by the proportion of their main constituent fatty acids (FAs), such as erucic acid (EA) (C22:1), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3).

The oils derived from *Brassica* oilseeds of traditional varieties (non-canola type) differ significantly from most other vegetable oils by the high content of very long chain fatty acids (VLCFA, C > 20), such as C22:1 and C20:1 (eicosenoic acid), which together account for 45−60% of the total FAs.[2,3] Among the unsaturated FAs, C22:1 is nutritionally undesirable and associated with health problems,[4,5] whereas C18:3 is easily oxidized, giving rise to off-flavor and rancid odor, causing a shortened shelf life.[6,7] In contrast, C18:1 and C18:2 are the most desirable FA components in edible seed oils due to their health and cooking benefits. The high C18:1 oil can be heated to a higher temperature without smoking, making it more suitable as a cooking oil compared with polyunsaturated fatty acids (PUFA).[8] PUFA, such as C18:2, are essential components in animal feed, but derived from plants only.[9] Neither animals nor humans have the necessary desaturases to convert C18:1 into C18:2 and C18:3.[10]

In addition to desirable FAs (high C18:1 and C18:2), vitamin E (tocochromanols) content is another important quality index to be considered in *Brassica* oilseeds. Vitamin E is a powerful fat-soluble antioxidant, indispensable for the protection of PUFA against oxidative deterioration in both plants and animals.[11] It has beneficial effects in the prevention of cardiovascular diseases.[12] In animals, vitamin E deficiency has severe consequences, including neurological dysfunction and muscular dystrophy.[12,13] Because vitamin E is synthesized only by plants, it is a very important dietary nutrient for all mammals.[14]

The term "tocochromanol" refers to two kinds of compounds: tocopherols (Ts) and tocotrienols. Both compounds exhibit vitamin E activity. However, tocotrienols tend to be less common in seed oils than Ts. Ts are synthesized in plastids and exist in four forms, α-, β-, γ-, and δ-T, differing from each other in both molecular structure and biological effectiveness.[15,16] In general, α-T is the predominant form of T in photosynthetic tissues, whereas, β-, γ-, and δ-T tend to predominant in seeds of most plants.[17] Among the four isoforms, α-T has the highest vitamin E efficacy (100%), whereas γ-T has 8−19% relative activity of vitamin E.[18] Together, they play an important role in protection of sensitive FAs against oxidation.[14,19]

Increasing the C18:1 and C18:2 FAs as well as total T and α-/γ-tocopherol (α-/γ-T) ratio is an important goal for breeding programs of *Brassica* oilseeds. However, there is a paucity of studies on the correlations among the T and FA components in *Brassica* oilseeds. In the current study, a comprehensive analysis on the correlation of these seed qualitative components was conducted on the basis of a large collection of landraces without intensive directional selection in

Received: October 6, 2012
Revised: December 8, 2012
Accepted: December 9, 2012
Published: December 9, 2012

Table 1. Description of the Plant Materials Used for the Analysis[a]

name	des	name	des	name	des	name	des	name	des	name	des
Bj		Rat	P/L	Dia	P/L	Sha	P/L	Cht2	P/L	WA	P/L
Att1	P/L	RYK1	P/L	FJ	P/L	She	P/L	Dar1	P/L	751	C/L
Bha1	P/L	RYK2	P/L	Gan	P/L	Ska	P/L	Dar2	P/L	AH	C/L
Bhk	P/L	Sar	P/L	Gha	P/L	SQ	P/L	HA3	P/L	GY	C/L
Bun1	P/L	SN	P/L	Gil	P/L	Sul	P/L	Har4	P/L	HB	C/L
Cha1	P/L	Sun1	P/L	Guj	P/L	Sun2	P/L	Isl5	P/L	HLJ	C/L
Chh	P/L	Swt1	P/L	HA1	P/L	Swa1	P/L	KA	P/L	HN	C/L
CM	P/L	Veh	P/L	HA2	P/L	Swa2	P/L	Kha3	P/L	IO31	C/L
DIK	P/L	JCY	C/L	Han2	P/L	Swa3	P/L	Koh2	P/L	IO75	C/L
Fai	P/L	JSD	C/L	Han3	P/L	Tar	P/L	Lak2	P/L	IO78	C/L
Ghi	P/L	KMG	C/L	Har2	P/L	BF9	C/L	Lak3	P/L	IO79	C/L
Han1	P/L	KYC	C/L	Har3	P/L	BZH	C/L	Mar	P/L	IO84	C/L
Har1	P/L	LYK	C/L	Has	P/L	DSJ	C/L	Mii	P/L	LJX	C/L
HS1	P/L	PGQ	C/L	Haz	P/L	GDH	C/L	Nar	P/L	R-line	C/L
Hun1	P/L	XCL	C/L	HS2	P/L	HSD	C/L	Now1	P/L	XY	C/L
Isl1	P/L	***Br***		Hun2	P/L	LSY	C/L	Now2	P/L	Arw	N/B
Isl2	P/L	Ast	P/L	Hun3	P/L	Mic	C/L	Now3	P/L	Wes	N/B
Isl3	P/L	Att2	P/L	Jas	P/L	RAT	C/L	Oka1	P/L	FY2	C/B
Isl4	P/L	Bak	P/L	Jug	P/L	SRB	C/L	Oka2	P/L	HYQ	C/B
Jhe	P/L	Bal	P/L	Kar	P/L	TPY	C/L	Pes	P/L	IO135	C/B
Kha1	P/L	Ban1	P/L	Kha2	P/L	XCY	C/L	Raw1	P/L	IO2	C/B
Khr	P/L	Bat1	P/L	KK	P/L	YYJ	C/L	Raw2	P/L	IOC	C/B
Khu	P/L	Bha2	P/L	Lak1	P/L	***Bn***		Ris	P/L	MJ	C/B
Koh1	P/L	BSA	P/L	Mag	P/L	AK	P/L	Swa4	P/L	ZD619	C/B
Man	P/L	Bun2	P/L	Mas	P/L	Ban2	P/L	Swa5	P/L	ZS6	C/B
Mat	P/L	Bun3	P/L	Mim	P/L	Ban3	P/L	Swt2	P/L	ZS72	C/B
MC	P/L	Cha2	P/L	Nag	P/L	Bat2	P/L	Tal	P/L	ZS758	C/B
Pas1	P/L	Chl	P/L	Nig1	P/L	Bat3	P/L	Tan	P/L	ZS9	C/B
PB	P/L	Chs	P/L	Nig2	P/L	Cha3	P/L	Tax	P/L	Fal	D/B
Raj	P/L	Chu	P/L	Pas2	P/L	Cht1	P/L	TN	P/L	Ard	F/B

[a]Abbreviations: *Bj*, *Brassica juncea* L.; *Br*, *Brassica rapa* L.; *Bn*, *Brassica napus* L.; des, description; N, Canada; C, China; D, Germany; F, France; P, Pakistan; L, landrace; B, breeding line.

the three *Brassica* oil crop species as well as a collection of breeding lines of low EA content of *B. napus*.

MATERIALS AND METHODS

Plant Materials. A total of 177 genotypes were collected mainly from the Gene Bank of Chinese Brassica Oilseeds (Institute of Oil Crops, Chinese Academy of Agricultural Sciences) and the Department of Plant Breeding and Genetics, Khyber Pakhtunkhwa Agricultural University, Pakistan, representing a great genetic diversity from different geographical locations. To construct a collection of germplasm, in which the number of genotypes distributed in catalogues of different levels of a qualitative trait accords with the Normal Distribution Pattern, we assigned the 177 genotypes into the first collection of landraces and the second collection of breeding lines (low EA type). The first collection consists of 162 landraces without intensive directional selection belonging to three *Brassica* species, namely *B. juncea* (group 1, G1), *B. rapa* (group 2, G2), and *B. napus* (group 3, G3), whereas the second collection is composed of 15 low EA breeding lines/varieties belonging to the species *B. napus* (group 4, G4). The names and descriptions of species, origins, and types of plant materials are listed in Table 1.

Field Experiment. The plant materials were grown at the Experimental Farm of Zhejiang University, Hangzhou, China, in the year 2010/2011. All of the plant materials were planted in randomized complete block design with three replications. All of the agronomic practices followed the recommendations of local agricultural practice in the region of the Lower Reach of the Yangtze Valley. The flowers were selfed. Only the seeds from the selfed flowers on the uppermost part of a main raceme or of the uppermost side branch were harvested and analyzed for seed qualitative traits.

Assessment of Fatty Acids. FAs were analyzed referring to the method of Katavic et al.[20] and Zou et al.[21] with modifications. Approximately 200 mg of seeds was ground and 50 mg of the meal was taken into a 12 mL screw-top glass tube with 2 mL of extracting solution (chloroform/isopropanol, 2:1, v/v) and kept for 2 h (vortex vigorously for 30 s per 30 min) in the dark at room temperature and then centrifuged at 2500 rpm for 5 min. The supernatant of 500 μL was transferred into a new tube with 2 mL of 1% MeOH/H_2SO_4 (v/v), capped tightly, and kept in a water bath (80 °C) for 1 h. After the tubes had cooled to room temperature, 2 mL of 0.9% NaCl was added. Extraction of FA methyl esters was done three times by adding 1 mL of hexane each time, followed by vortexing and centrifugation (2500 rpm for 2 min). The supernatant per three extractions, containing the FA methyl esters, was transferred into a new tube. One milliliter of the supernatant was transferred into the GC vials. Two microliters of the sample was autoinjected into the gas chromatograph (GC, 6890N, Agilent, USA) system, equipped with a fused silica capillary column Rtx-Wax (30 m × 0.25 mm × 0.50 μm, Restek, USA) and a FID detector, with an initial column temperature of 160 °C, held for 1 min, and then increased to 240 °C at the rate of 4 °C/min, and then held for 16 min to the end. For the identification and quantification of the main FA components in rapeseed, a total of 11 standards of corresponding FA methyl esters (AccuStandard and NU-CHEK, USA) were used. The content of each component was expressed as grams per kilogram seed, and the total fatty acid (total FA) content was calculated as the sum of these 11 components, namely, C14:0 (myristic acid), C16:0 (palmitic acid), C18:0 (stearic acid), C18:1, C18:2, C18:3, C20:0 (arachidic acid), C20:1, C22:0 (docosanoic acid), C22:1, and C24:0 (tetracosanoic acid), of which the last five components belong to the VLCFA group. The FA compositions

dx.doi.org/10.1021/jf3042837 | J. Agric. Food Chem. 2013, 61, 34−40

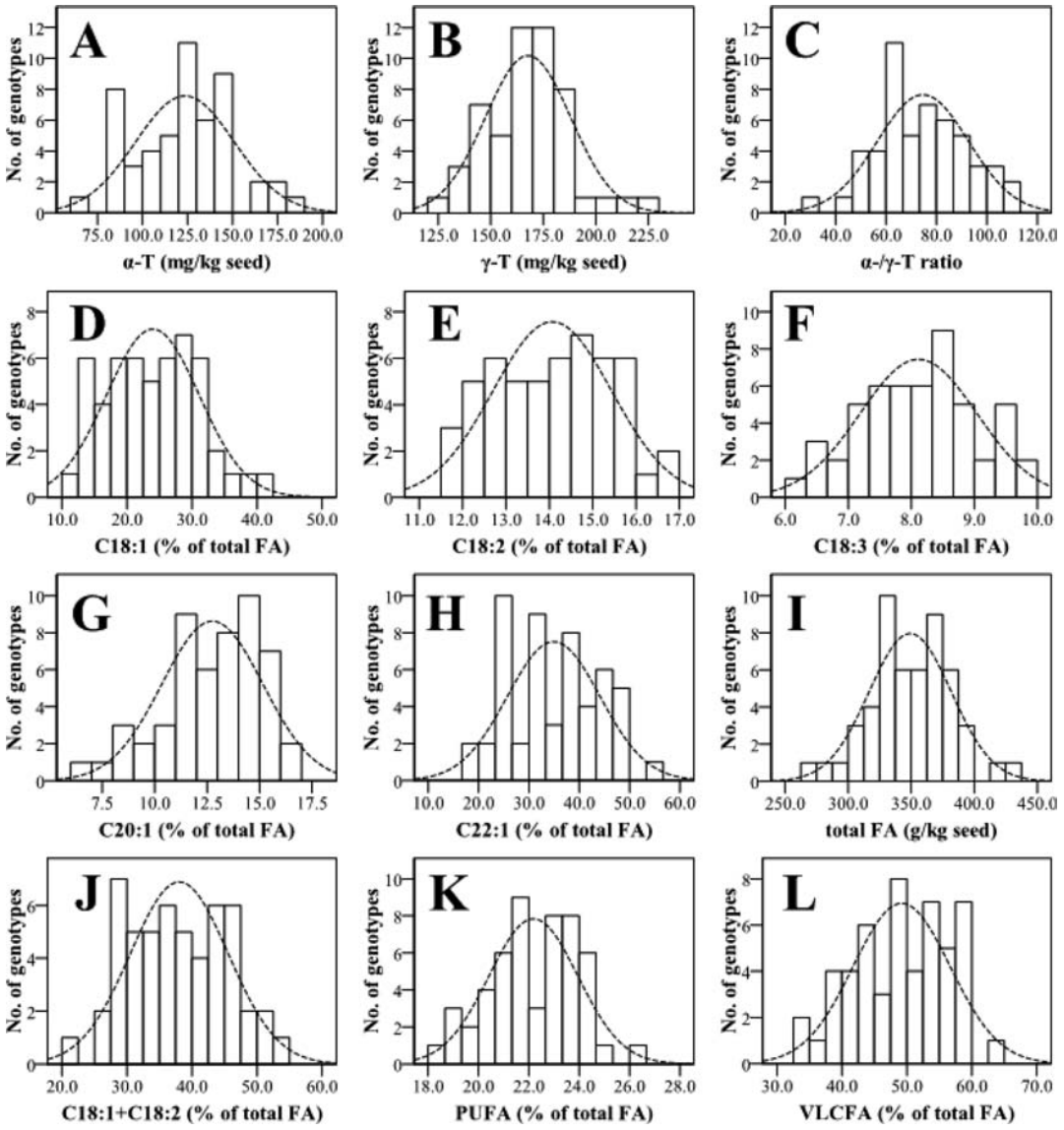

Figure 1. Distribution of (A) α- and (B) γ-T content (mg kg^{-1} seed), (C) α-/γ-T ratio, and FA composition (% of total FA): (D) C18:1, (E) C18:2, (F) C18:3, (G) C20:1, (H) C22:1, (J) C18:1 + C18:2, (K) PUFA, (L) VLCFA, and (I) total FA (g kg^{-1} seed) of 52 genotypes (*Brassica napus* L.) in G3 of the first collection.

(percent) refer to the percentage ratio of each component to the total FA.

Analysis of Tocopherols. Analyses of Ts (α-T, β-T, γ-T, and δ-T) were performed according to the methods of KamalEldin et al.[22] and Egesel et al.[23] with some modifications. In brief, approximately 75 mg of seeds was weighed using an electronic balance (Sartorious BS-124S) and transferred into a 2 mL skirted screw-cap microtube (Sangon Biotech, China) with 1.5 mL of *n*-hexane. Seeds were ground by bead mill homogenizer (Bead Ruptor-24, Omni, USA) at 8 m/s for 30 s. For the extraction of Ts, samples were stored in the dark for 15 h at room temperature and then centrifuged at 5000 *g* for 7 min. An aliquot of 1 mL of the clear supernatant was filtered through a disposable filter holder (0.45 μm, PTFE membrane filter) and stored in 1 mL HPLC vials. For T quantification, a normal-phase high-performance liquid chromatograph (HPLC, 600, Waters, USA), equipped with a Zorbax Rx-SIL column (4.6 mm × 250 mm × 5 μm, Aglient, USA) and a fluorescence detector (λex = 295 nm, λem = 330 nm), was used. Twenty microliters of the sample was autoinjected into the system. The mobile phase was hexane/*tert*-butyl methyl ether (94:6, v/v) at a flow rate of 1 mL/min. Identification and quantification of all the T isomers were done using calibration curves of standards from Supelco (USA). The contents of T isomers were expressed in milligrams per kilogram seed, and the total T was calculated as the sum of the four isomers.

Statistical Analysis. Data were classified with Win-Excel. The test of distribution normality and the Pearson's correlation coefficients among the T and FA components were calculated from the means over three biological replications by using the SPSS 17.0 statistical package (SPSS Inc., Chicago, IL, USA).

RESULTS

Description of Seed Qualitative Traits. We analyzed the seed qualitative traits, namely, T content (mg kg^{-1} seed), total T (mg kg^{-1} seed), FA composition (% of total FA), and total FA content (g kg^{-1} seed) in the germplasm collections of the *Brassica* oil crops. The distribution number for the selected qualitative traits, namely, α- and γ-T contents (mg kg^{-1} seed), α-/γ-T ratio, and FA compositions (% of total FA), namely C18:1, C18:2, C18:3, C20:1, C22:1, C18:1+C18:2, PUFA, VLCFA, and total FA (g kg^{-1} seed), are shown in Figure 1 (G3) and Supplemental Figures S1−S3 of the Supporting Information (G1, G2, and G4). Briefly, except for C20:1 in G3

dx.doi.org/10.1021/jf3042837 | J. Agric. Food Chem. 2013, 61, 34−40

Table 2. Means and Coefficients of Variances (CVs) of the T and FA Components in the First Germplasm Collection of Landraces (G1 of *B. juncea*, G2 of *B. rapa*, and G3 of *B. napus*) and the Second Germplasm Collection of Low EA Breeding Lines from *B. napus* (G4)[a]

	Ts (mg kg^{-1} seed)				FAs (% of total FA)							FAs (g kg^{-1} seed)
group	α-T	γ-T	δ-T	total T	C16:0	C18:0	C18:1	C18:2	C18:3	C20:1	C22:1	total FA
First Collection (162 Landraces of G1−G3)												
mean	97.86	175.01	3.05	276.82	2.73	1.21	18.94	14.80	8.46	10.09	41.61	347.53
CV	28.09	13.95	53.37	12.35	18.52	15.53	30.56	14.99	14.85	25.29	17.71	12.20
G1 (44 Landraces of *B. juncea*)												
mean	76.13	179.77	3.89	260.44	2.60	1.10	14.29	17.84	9.58	7.69	44.15	308.49
CV	26.13	15.02	35.23	14.06	6.58	9.54	10.32	7.49	15.81	10.79	5.06	8.85
G2 (66 Landraces of *B. rapa*)												
mean	92.13	177.59	3.73	274.17	2.31	1.24	18.08	13.34	7.99	9.60	45.21	372.25
CV	13.35	13.90	36.69	9.31	7.58	14.06	14.24	6.43	9.29	12.40	7.85	10.37
G3 (52 Landraces of *B. napus*)												
mean	123.52	167.71	1.47	294.04	3.35	1.26	23.96	14.07	8.11	12.75	34.90	349.19
CV	21.97	12.03	53.60	11.70	9.61	17.46	29.54	9.64	11.34	18.68	26.10	9.24
G4 (15 Breeding Lines of *B. napus*)												
mean	129.63	175.66	2.75	308.75	4.44	2.01	60.27	20.91	8.43	1.66	1.15	350.82
CV	35.30	16.16	54.17	18.42	17.50	21.72	4.96	12.32	14.13	60.68	107.35	21.88

[a]Abbreviations: CV, coefficient of variance (%).

(Figure 1), all of the other qualitative traits selected agreed with the Normal Distribution Pattern in the first collection. On the other hand, except for qualitative traits such as α-T, C20:1, C22:1 and VLCFA, the rest of the qualitative traits in the second collection (G4) distributed normally (Supporting Information, Supplemental Figure S4).

The parameters that reflect the mean and variations of the seed qualitative traits in the first (G1, G2 and G3) and second (G4) collections are summarized in Table 2. In the first collection, the total FA in 1 kg of seeds varies from 246.25 to 473.73 g, with an average of about 347.53 g. The coefficient of variance (CV) for total FA is about 12.20%. On the other hand, the total T in 1 kg of seeds varies between 150.61 and 361.02 mg, with an average of about 276.82 mg. The CV of total T is similar to that of the total FA. As a whole collection, the seed qualitative traits, such as δ-T, C18:1, α-T, and C20:1, had much greater CV values than other traits. Among the three groups (G1, G2, and G3), the means of α-T, C18:1, and C20:1 in G3 were 62.25, 67.67, and 65.80% higher than in G1 and 34.07, 32.52, and 32.81% higher compared with G2, respectively. The means of δ-T and C22:1 in G1 and G2 were similar, more than 153.74 and 26.50% higher than that in G3, respectively. Moreover, the means of PUFA (C18:2 and C18:3) in G2 and G3 were almost similar but much smaller than that of G1, whereas the means of γ-T were nearly the same among all three groups (Table 2). In the second collection (G4), those parameters relating to tocopherols were not much different from those in the first collection (G1, G2, and G3). However, due to the directional selection for low EA, the contents of other FA components, in particular C18:1 and C18:2, increased substantially (Table 2).

Correlations between T and FA Components. The result of correlation analysis of these four groups is presented in Table 3. In the first collection (G1, G2, and G3), α-T was correlated significantly positive with α-/γ-T ratio, C18:2, and C18:1+C18:2 in all three groups, whereas the correlations of α-T with some FA components were significantly positive for two of these three groups, such as with C18:1 (r = 0.309* in G1, r = 0.425** in G3, but r = NS in G2), with C20:1 (r = NS in G1, r = 0.379** in G2, and r = 0.299* in G3), and with PUFA (r = NS in G1, r = 0.273* in G2, and r = 0.360** in G3). On the other hand, either negative or no association of α-T was found with γ-T, C18:3, C22:1, and VLCFA. No correlations between α-T and total FA were found in any of the three groups. Moreover, γ-T showed no association with the majority of the seed qualitative traits. The only significant associations of γ-T (either positive or negative) with the T and FA components included total FA (r = 0.254* in G2) and C18:3 (r = −0.324* in G1). In contrast to α-T, the α-/γ-T ratio also associated with total FA in G2 (r = −0.313*); on the other hand, it showed no correlations with C18:2 and C18:3 in G1, with C20:1 and PUFA in G2, and with C20:1 in G3. Besides the correlations between Ts and FAs, associations of FA components with each other were also found, either positive or negative. Highlighting the significance of associations, it can be inferred from the results that the correlations among these components followed the same trend within all three groups (G1, G2, and G3). For example, C18:1 showed positive correlation with C20:1 and negative correlation with C18:3, C22:1, and VLCFA. C18:2 associated negatively with C22:1 and VLCFA (data not shown).

The correlations of T and FA components in G4 were analyzed and are shown in Table 3. In this group, γ-T is correlated significantly positive with total FA (r = 0.600*), and the α-/γ-T ratio was positively associated with C18:2 (r = 0.590*) and PUFA (r = 0.593*). Within FA components, only a small number of associations were found, that is, the significantly positive correlations between PUFA and C18:2 and C18:3 and the significantly negative correlations between C18:1 and C18:2 and PUFA (data not shown).

DISCUSSION

Brassica oilseeds are among the successful examples in which the seed quality has been significantly improved. Previous efforts were given to optimize the composition of the oil FA and lower glucosinolates in seed cake. More recently, oilseed breeders became interested in the increase of seed total T and the elevation of the proportion of α-T.[23−25] There has been a paucity of studies on the correlations of contents among the T and FA components, which should be drawn on the basis of

dx.doi.org/10.1021/jf3042837 | J. Agric. Food Chem. 2013, 61, 34−40

Table 3. Correlations among T Content (Milligrams per Kilogram Seed), α-/γ-T Ratio, FA Composition (Percent of Total FA), and Total FA Content (Grams per Kilogram Seed) in the Germplasm Collections of *Brassica* Oil Crops (G1, G2, G3, and G4)[a]

Pearson's correlation	γ-T	α-/γ-T	C18:1	C18:2	C18:3	C20:1	C22:1	total FA	C18:1+C18:2	PUFA	VLCFA
G1 (44 Landraces of *B. juncea*)											
α-T	0.181 NS	0.877**	0.309*	0.301*	−0.426**	−0.147 NS	−0.024 NS	−0.094 NS	0.371*	−0.224 NS	−0.099 NS
γ-T		−0.292 NS	0.029 NS	0.148 NS	−0.324*	−0.125 NS	0.102 NS	0.052 NS	0.104 NS	−0.271 NS	0.137 NS
α-/γ-T			0.313*	0.214 NS	−0.282 NS	−0.090 NS	−0.070 NS	−0.143 NS	0.324*	−0.130 NS	−0.156 NS
G2 (66 Landraces of *B. rapa*)											
α-T	−0.243*	0.774**	0.163 NS	0.435**	−0.056 NS	0.379**	−0.391**	−0.207 NS	0.291*	0.273*	−0.340**
γ-T		−0.783**	−0.103 NS	−0.070 NS	0.156 NS	0.024 NS	0.056 NS	0.254*	−0.120 NS	0.046 NS	0.076 NS
α-/γ-T			0.160 NS	0.316**	−0.174 NS	0.204 NS	−0.265*	−0.313*	0.251*	0.116 NS	−0.244*
G3 (52 Landraces of *B. napus*)											
α-T	0.023 NS	0.867**	0.425**	0.511**	−0.070 NS	0.299*	−0.495**	0.108 NS	0.496**	0.360**	−0.523**
γ-T		−0.456**	−0.058 NS	−0.077 NS	0.184 NS	0.063 NS	0.032 NS	0.147 NS	−0.069 NS	0.037 NS	0.054 NS
α-/γ-T			0.423**	0.521**	−0.157 NS	0.255 NS	−0.479**	0.033 NS	0.496**	0.322*	−0.513**
G4 (15 Breeding Lines of *B. napus*)											
α-T	0.066 NS	0.884**	−0.278 NS	0.412 NS	0.194 NS	−0.423 NS	−0.165 NS	0.145 NS	0.145 NS	0.419 NS	−0.304 NS
γ-T		−0.381 NS	0.275 NS	−0.372 NS	−0.239 NS	0.321 NS	0.334 NS	0.600*	−0.086 NS	−0.403 NS	0.302 NS
α-/γ-T			−0.429 NS	0.590*	0.258 NS	−0.509 NS	−0.275 NS	−0.132 NS	0.151 NS	0.593*	−0.384 NS

[a]Significance: NS, nonsignificant ($P > 0.05$); *, correlation is significant at $P \leq 0.05$; **, correlation is significant at $P \leq 0.01$.

large germplasm collections. Dolde et al.[26] analyzed the correlations between FA and T components in canola, soybean, and sunflowers. Their conclusion that total T and degree of unsaturation in canola seeds were not correlated was based on only nine canola varieties with genetically altered FA composition. We analyzed a collection of 162 landraces from *B. napus*, *B. rapa*, and *B. juncea* and a collection of 15 low EA breeding lines of *B. napus* for the contents of T and FA components in seeds. In any of the first three *Brassica* groups (G1, G2, and G3) comprising landraces without directional selections, the most notable correlation was the significantly positive one between α-T and C18:1+C18:2, whereas hardly any association between γ-T, the dominant form of vitamin E in seeds, and a FA variety was found. In the second collection (G4) consisting of only low EA breeding lines, a significantly positive correlation between γ-T and total FA was noted, whereas the α-/γ-T ratio showed no negative associations with any of the other seed qualitative traits (Table 3). The results suggest that there is no contradiction in breeding goals to simultaneously integrate desired qualitative traits such as high α-T and γ-T, and high C18:1 and C18:2 into the same elite commercial variety.

In plants, de novo synthesis of FAs occurs in plastids,[27,28] and their subsequent modifications proceed in plastids through the "prokaryotic pathway" or in the endoplasmic reticulum (ER) via the "eukaryotic pathway".[29−32] The desaturation of C18 compounds is conducted by both eukaryotic and prokaryotic pathways, using the ER-localized FA desaturases (FAD2 and FAD3) and the plastid-localized FA desaturases (FAD6, FAD7, and FAD8), respectively,[29] whereas the formation of VLCFA is catalyzed by the fatty acid elongase (FAE) complex, a membrane-bound multienzyme complex exclusively localized in the ER.[32] Like FAs, Ts are also synthesized in plastid (mostly chloroplast) and can be transported along with FAs between the plastid and ER through two-way channels.[33−36]

To date, the relationship between these two compounds in plants remains unclear. Previously, the associations between Ts and FAs in oils were mostly attributed to the T function as antioxidant in stabilizing unsaturated FAs.[11,37] If this is the only truth, then it would be rather difficult to explain our results that in the collection of landraces (G1, G2, and G3) the α-T correlated significantly positive (or no significant correlations) with C18:1, C18:2, and the sum of C18:1 and C18:2 and significantly negative (or no significant correlations) with C18:3 and VLCFA, but, contrastingly, the γ-T, the most predominant T form in *Brassica* oils,[23] had nonsignificant correlations with either C18:1, C18:2, the sum of C18:1 and C18:2, or VLCFA. We also verified the conclusions made in other studies that there is neither relationship between γ-T and C18:1 nor between γ-T and C18:2.[38] It is likely that α-T, which coexists with FAs, affects the ER FA metabolism by inhibiting FAD3 and FAE, but it may not affect the FA metabolism in plastids (Figure 2). Recent advances in plant molecular biology revealed the involvement of Ts in regulating the change from C18:2 to C18:3 in the ER in response to low-temperature stress in *Arabidopsis*.[39] The interaction between Ts and the nonplastid FA metabolism was further reported by subsequent studies.[40]

With these findings in mind, we are of the opinion that the function of Ts in regulating FA formation may be beyond the role as antioxidants. The complexity of correlation between T and FA components in *Brassica* oils may arise from the interaction between α-T and the ER FA metabolism. The

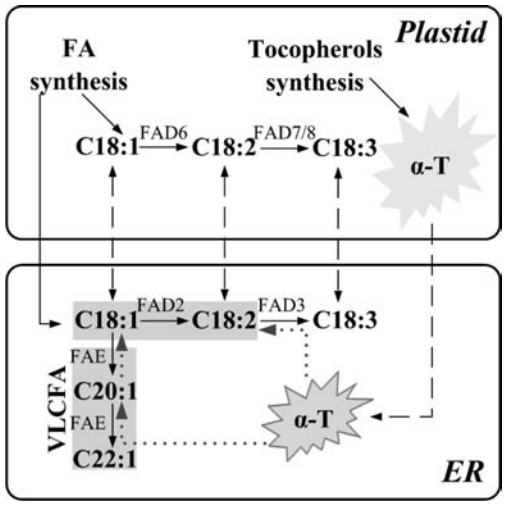

Figure 2. Location of C18:1, C18:2, C18:3, and VLCFA formation and probable relationship between α-T and FA biosynthesis in ER.

mechanisms that underlie the statistical correlations are interesting and suggest cues for deep investigations.

■ ASSOCIATED CONTENT

Supporting Information

Distribution number for the selected qualitative traits, namely, α- and γ-T contents (mg kg^{-1} seed), α-/γ-T ratio, and FA compositions (% of total FA): C18:1, C18:2, C18:3, C20:1, C22:1, C18:1+C18:2, PUFA, VLCFA, and total FA (g kg^{-1} seed). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +86-0571-8898 2905. Fax: +86-0571-8898 2130. E-mail: jianglx@zju.edu.cn.

Funding

The work of our laboratory was sponsored by the Natural Science Foundation of China (Grants 30971700 and 31171463) and Zhejiang Province (Grant Z3100130) and the Fundamental Research Funds for the Central Universities (2012FZA6011 and 2012XZZX012).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Prof. Xiaoming Wu of Institute of Oil Crop in Wuhan for providing us a part of the rapeseed germplasm and Xiaodan Wu, Jinhui Li, and Mei Li, technicians at the Institute of Agrobiology and Environmental Sciences, for their assistance in operating equipment.

■ REFERENCES

(1) http://faostat.fao.org.

(2) Jonsson, R. Erucic acid heredity in rapeseed (*Brassica napus* L. and *Brasscia campestris* L.). *Hereditas* **1977**, *86*, 159−170.

(3) Nath, U. K.; Wilmer, J. A.; Wallington, E. J.; Becker, H. C.; Mollers, C. Increasing erucic acid content through combination of endogenous low polyunsaturated fatty acids alleles with *Ld-LPAAT* plus *Bn-fae1* transgenes in rapeseed (*Brassica napus* L.). *Theor. Appl. Genet.* **2009**, *118*, 765−773.

(4) Beare, J. L.; Craig, B. M.; Youngs, C. G.; Campbell, J. A. Effects of saturated fat in rats fed rapeseed oil. *Can. J. Biochem. Physiol.* **1963**, *41*, 605−612.

(5) Laryea, M. D.; Jiang, Y. F.; Xu, G. L.; Lombeck, I. Fatty acid composition of blood lipids in chinese children consuming high erucic acid rapeseed oil. *Ann. Nutr. Metab.* **1992**, *36*, 273−278.

(6) Roebbelen, G.; Nitsch, A. Genetical and physiological investigations on mutants for polyenoic fatty acids in rapeseed, *Brassica napus* L. I. Selection and description of new mutants. *Z. Pflanzenzuecht.* **1975**, *75*, 93−105.

(7) Prevot, A.; Perrin, G.; Laclaverie, G.; Auge, Ph.; Coustille, J. L. A new variety of low-linolenic rapeseed oil; characteristics and room-odor tests. *J. Am. Oil Chem. Soc.* **1990**, *67*, 161−164.

(8) Warner, K.; Mounts, T. L. Frying stability of soybean and canola oils with modified fatty acid compositions. *J. Am. Oil Chem. Soc.* **1993**, *70*, 983−988.

(9) Burr, G.; Burr, M. M.; Miller, E. S. On the fatty acids essential in nutrition. III. *J. Biol. Chem.* **1932**, *97*, 1−9.

(10) Wallis, J. G.; Watts, J. L.; Browse, J. Polyunsaturated fatty acid synthesis: what will they think of next? *Trends Biochem. Sci.* **2002**, *27*, 467−473.

(11) Goffman, F. D.; Bohme, T. Relationship between fatty acid profile and vitamin E content in maize hybrids (*Zea mays* L.). *J. Agric. Food Chem.* **2001**, *49*, 4990−4994.

(12) Bramley, P. M.; Elmadfa, I.; Kafatos, A.; Kelly, F. J.; Manios, Y.; Roxborough, H. E.; Schuch, W.; Sheehy, P. J. A.; Wagner, K. H. Vitamin E. *J. Sci. Food Agric.* **2000**, *80*, 913−938.

(13) Schneider, C. Chemistry and biology of vitamin E. *Mol. Nutr. Food Res.* **2005**, *49*, 7−30.

(14) KamalEldin, A.; Appelqvist, L. A. The chemistry and antioxidant properties of tocopherols and tocotrienols. *Lipids* **1996**, *31*, 671−701.

(15) Schultz, G. Biosynthesis of α-tocopherol in chloroplasts of higher plants. *Fett Wiss. Technol.−Fat Sci. Technol.* **1990**, *92*, 86−91.

(16) Marwede, V.; Gul, M. K.; Becker, H. C.; Ecke, W. Mapping of QTL controlling tocopherol content in winter oilseed rape. *Plant Breed.* **2005**, *124*, 20−26.

(17) DellaPenna, D. A decade of progress in understanding vitamin E synthesis in plants. *J. Plant Physiol.* **2005**, *162*, 729−737.

(18) DellaPenna, D.; Last, R. L. Progress in the dissection and manipulation of plant vitamin E biosynthesis. *Physiol. Plant.* **2006**, *126*, 356−368.

(19) Pongracz, G.; Weiser, H.; Matzinger, D. Tocopherols − antioxidants in nature. *Fett Wiss. Technol.−Fat Sci. Technol.* **1995**, *97*, 90−104.

(20) Katavic, V.; Reed, D. W.; Taylor, D. C.; Giblin, E. M.; Barton, D. L.; Zou, J. T.; Mackenzie, S. L.; Covello, P. S.; Kunst, L. Alteration of seed fatty acid composition by an ethyl methanesulfonate-induced mutation in *Arabidopsis thaliana* affecting diacylglycerol. *Plant Physiol.* **1995**, *108*, 399−409.

(21) Zou, J. T.; Katavic, V.; Giblin, E. M.; Barton, D. L.; MacKenzie, S. L.; Keller, W. A.; Hu, X.; Taylor, D. C. Modification of seed oil content and acyl composition in the Brassicaceae by expression of a yeast sn-2 acyltransferase gene. *Plant Cell* **1997**, *9*, 909−923.

(22) KamalEldin, A.; Gorgen, S.; Pettersson, J.; Lampi, A. M. Normal-phase high-performance liquid chromatography of tocopherols and tocotrienols − comparison of different chromatographic columns. *J. Chromatogr., A* **2000**, *881*, 217−227.

(23) Egesel, C. O.; Gul, M. K.; Kahriman, F.; Ozer, I.; Turk, F. The effect of nitrogen fertilization on tocopherols in rapeseed genotypes. *Eur. Food Res. Technol.* **2008**, *227*, 871−880.

(24) Goffman, F. D.; Becker, H. C. Genetic variation of tocopherol content in a germplasm collection of *Brassica napus* L. *Euphytica* **2002**, *125*, 189−196.

(25) Marwede, V.; Schierholt, A.; Mollers, C.; Becker, H. C. Genotype X environment interactions and heritability of tocopherol contents in canola. *Crop Sci.* **2004**, *44*, 728−731.

(26) Dolde, D.; Vlahakis, C.; Hazebroek, J. Tocopherols in breeding lines and effects of planting location, fatty acid composition, and

temperature during development. *J. Am. Oil Chem. Soc.* **1999**, *76*, 349−355.
(27) Harwood, J. L. Fatty-acid metabolism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1988**, *39*, 101−138.
(28) Rawsthorne, S. Carbon flux and fatty acid synthesis in plants. *Prog. Lipid Res.* **2002**, *41*, 182−196.
(29) Harwood, J. L. Recent advances in the biosynthesis of plant fatty acids. *Biochim. Biophys. Acta, Lipids Lipid Metab.* **1996**, *1301*, 7−56.
(30) Ohlrogge, J. B.; Jaworski, J. G. Regulation of fatty acid synthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1997**, *48*, 109−136.
(31) Baud, S.; Lepiniec, L. Regulation of de novo fatty acid synthesis in maturing oilseeds of *Arabidopsis*. *Plant Physiol. Biochem.* **2009**, *47*, 448−455.
(32) Baud, S.; Lepiniec, L. Physiological and developmental regulation of seed oil production. *Prog. Lipid Res.* **2010**, *49*, 235−249.
(33) Soll, J.; Kemmerling, M.; Schultz, G. Optical manipulation reveals strong attracting forces at membrane contact sites between endoplasmic reticulum and chloroplasts. *Arch. Biochem. Biophys.* **1980**, *204*, 544−550.
(34) Soll, J.; Schultz, G.; Joyard, J.; Douce, R.; Block, M. A. Localization and synthesis of prenylquinones in isolated outer and inner envelope membranes from spinach-chloroplasts. *Arch. Biochem. Biophys.* **1985**, *238*, 290−299.
(35) DellaPenna, D.; Pogson, B. J. Vitamin synthesis in plants: tocopherols and carotenoids. *Annu. Rev. Plant Biol.* **2006**, *57*, 711−738.
(36) Andersson, M. X.; Goksor, M.; Sandelius, A. S. Optical manipulation reveals strong attracting forces at membrane contact sites between endoplasmic reticulum and chloroplasts. *J. Biol. Chem.* **2007**, *282*, 1170−1174.
(37) KamalEldin, A.; Andersson, R. A multivariate study of the correlation between tocopherol content and fatty acid composition in vegetable oils. *J. Am. Oil Chem. Soc.* **1997**, *74*, 375−380.
(38) Richards, A.; Wijesundera, C.; Salisbury, P. Genotype and growing environment effects on the tocopherols and fatty acids of *Brassica napus* and *B. juncea*. *J. Am. Oil Chem. Soc.* **2008**, *85*, 159−168.
(39) Maeda, H.; Sage, T. L.; Isaac, G.; Welti, R.; DellaPenna, D. Tocopherols modulate extraplastidic polyunsaturated fatty acid metabolism in *Arabidopsis* at low temperature. *Plant Cell* **2008**, *20*, 452−470.
(40) Song, W.; Maeda, H.; DellaPenna, D. Mutations of the ER to plastid lipid transporters *TGD1*, *2*, *3* and *4* and the ER oleate desaturase *FAD2* suppress the low temperature-induced phenotype of *Arabidopsis* tocopherol-deficient mutant *vte2*. *Plant J.* **2010**, *62*, 1004−1018.