Evaluation of quality traits and their genetic variation in global collections of *Brassica napus* L

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Abstract

Evaluating quality traits is important to the selection of elite lines in *Brassica napus* L. In this study, the quality traits of 488 global collections of B. napus L were evaluated for two consecutive years under central Chinese growing conditions, and a series of phenotypic data was obtained. The measured total glucosinolate content (GLC) and erucic acid content (ERU) values for 95.5% of the accessions were consistent with the original values, and large variations in quality traits were found among these accessions, thus enabling selection for these characters. In general, Chinese accessions tended to have a higher oil content (OC) than foreign accessions, while compared with winter and spring accessions, semi-winter accessions tended to have the highest OC. The mean GLC and ERU of Chinese rapeseed accessions showed gradual downward trends over time, and the genotypic variation in ERU accounted for 98.44% of the total variation, which was the highest among all 10 of the quality traits. Additionally, the heritability for ERU was largest among all 10 of the quality traits. Significant correlations were observed between different traits; OC had significantly (P < 0.01) negative correlation coefficients with oleic acid content, whereas OC had significantly (P < 0.01) positive correlation coefficients with ERU. Principal component analysis revealed that there was no clear boundary among materials of different geographic origins and different ecotypes according to the first two principal coordinates, respectively. This information about variations in quality traits revealed in this study could identify parents for improved rapeseed breeding.

Keywords: Brassica napus L, genetic diversity, genetic variation, plant breeding, quality trait

Introduction

Oilseed rape (OSR) (*Brassica napus* L.) is one of the major global oil crops. Its oil is used as green fuel, for human consumption, to feed animals, and in the chemical and pharmaceutical industry (Friedt and Snowdon, 2009).

Current elite 'canola' breeding materials are believed to be derived from a limited number of crosses between the original genotypes with these quality traits and concurrent breeding lines (Stefansson *et al.*, 1961; Krzymanski, 1974; Becker *et al.*, 1999; Ecke *et al.*, 2010), which has ultimately led to a low level of diversity in *B. napus* quality traits. Because crop improvement programmes depend on the existence of sufficient genetic variability, heritability, correlation and genetic gain during selection (Khan *et al.*, 2006; Aytaç and Kınacı, 2009), it is important to better understand the variation in the seed quality traits of *B. napus*.

Korber *et al.* (2012) studied seedling development in a *B. napus* diversity set and its relationship to agronomic performance using 518 inbreds and observed differences in phenotypic diversity among the eight examined germ-plasm types. Chen *et al.* (2014) evaluated yield and

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agronomic traits and their genetic variation using 488 collections of *B. napus* and found differences in the phenotypic diversity of both among different ecotype accessions and among materials of different geographic origins, however, the phenotypic diversity of quality traits remains unclear.

The main objective of *B. napus* breeding is to increase the seed oil content (OC) and improve the fatty acid profile to meet food and industrial demands (Ahmad et al., 2015), and correlation studies can inform the design of a breeding programme aimed at the simultaneous improvement of multiple traits (Kumar, 2013). In addition to the effects of genetics, the environment is another vital factor that influences B. napus quality traits (Gunasekera et al., 2006; Zhang et al., 2013). To better understand and effectively utilize B. napus germplasm in China, it is necessary to evaluate global collections of the species for quality traits and to study their genetic variation under local environmental conditions. The objectives of the present study were (i) to assess the phenotypic and genotypic variation in 10 quality traits in 488 global collections of *B. napus*; (ii) to compare the variation in quality traits among different crop groups, i.e. winter, semi-winter and spring rapeseed; (iii) to assess changes in the quality traits of Chinese breeding lines and cultivars over time; (iv) to analyse the genotypic variability by environment interactions; (v) to detect and analyse correlations among the investigated traits and (vi) to group the material using principal component analysis (PCA). Our findings could provide useful information for selecting parental lines for OSR breeding programmes, and the data obtained in this study could be used in our subsequent studies of associations among the molecular and phenotypic traits of B. napus.

Materials and methods

Plant materials and field trials

A total of 488 global collections of *B. napus* conserved at the Oil Crops Middle-term Genebank of China (OMGC) were used in this study. The global collections exhibited abundant genetic diversity as they originated from 23 countries on four continents, including 245 accessions from China, 26 accessions from Japan, 23 accessions from the Americas, 166 accessions from Europe, 20 accessions from Australia, and eight accessions from other regions (online Supplementary Table S1).

All field trials were managed following standard agricultural practices. The experimental location, experimental design, land preparation, sowing, fertilizing, plant spacing, and harvesting programme were the same as in Chen *et al.* (2014).

Measurement of 10 quality traits and data collection

A total of 10 quantitative seed characters (OC, protein content (PC), total glucosinolate content (GLC), palmitic acid content (PAL), stearic acid content (STE), oleic acid content (OLE), linoleic acid content (LNL), linolenic acid content (LIN), eicosenoic acid content (EIC), and erucic acid content (ERU)) were used to assess the quality of 488 *B. napus* collections, and their abbreviations are presented in Table 1.

After ripening and drying, the harvested materials were threshed, and approximately 3 g of threshed seeds were used to determine seed OC, PC, total GLC and fatty acid composition according to the conventional method of analysis detailed in the WinISI III operations manual using a Foss NIRS (near-infrared spectrometer) 5000 (Foss NIR Systems, Inc., http://www.foss-nirsystems.com). Calibration and validation procedures were performed using ISI software, version 1a.1 (Infra Soft International), as described by Wiedemann *et al.* (1998).

Statistical analysis

Analysis of variance was performed for individual years, and error variances were tested for homogeneity (Gai, 2000). A combined analysis of variance of the 2-year trial data was performed for the quantitative characters, and the phenotypic character data were analysed by determining the mean, minimum (min), maximum (max), standard deviation (S.D.) and frequency distribution in the SPSS 16.0 computer package (SPSS, 2007). The least significant difference (LSD) at the 5 and 1% levels was used to compare differences between means (Gai, 2000), and the multi-trait means of different group accessions were compared using one-way ANOVAs (analysis of variances) at a 0.05 and 0.01 level of probability followed by Duncan's test and a Student's t-test in SPSS 16.0 (Gai, 2000; SPSS, 2007). The relationships among traits were determined by a Pearson's correlation analysis using the accession means (Fisher, 1924).

Calculation of the best linear unbiased prediction (BLUP) value and heritability for each quality trait

As the 10 quality traits of the 488 accessions were investigated over 2 years with three replications, an R script (www.eXtension.org/pages/61006) based on a linear model described by Merk *et al.* (2012) was used to obtain the BLUP of each trait for each line. The variance components of genotype (var_{genotype}), the genotype and year interaction (var_{genotype/year}), the replication and year PAL

STE

OLE

INL

LIN

EIC

ERU

 $P = 0.000^{**}$

 $P = 0.000^{**}$

2012						
Trait	Mean ± S.D. 2011	Range 2011	Mean ± S.D. 2012	Range 2012	Correlation coefficient (r) 2011/2012	<i>P</i> -value ^a 2011/2012
OC	40.989 ± 1.7413	35.21-46.85	40.964 ± 1.8309	36.38-48.57	0.741**	<i>P</i> = 0.668 ns
PC	23.132 ± 1.2105	19.99–26.87	23.028 ± 1.1557	19.55-27.60	0.533**	$P = 0.044^*$
GLC	67.057 ± 17.5984	18.90-96.09	70.115 ± 18.7120	19.86–105.83	0.972**	$P = 0.000^{**}$

 3.691 ± 0.7009

 2.464 ± 0.5478

 33.340 ± 16.0971

 16.202 ± 3.3143

 7.855 ± 2.3376

 10.133 ± 3.6854

26.314 ± 18.5181

 Table 1.
 Means, S.D., ranges and correlation coefficient of 10 quality traits of 488 B. napus accessions evaluated in 2011 and 2012

Note: oil content (%; OC), protein content (%; PC), total glucosinolate content (µmol/g; GLC), palmitic acid content (%; PAL), stearic acid content (%; STE), oleic acid content (%; OLE), linoleic acid content (%; LNL), linolenic acid content (%; LIN), eicosenoic acid content (%; EIC), and erucic acid content (%; ERU). Abbreviations are the same in tables 2–4. ns, not significant.

^a*P*-value comparison of paired-sample means.

 3.760 ± 0.7006

 1.647 ± 0.5150

 34.788 ± 16.1602

 15.255 ± 3.3933

 7.965 ± 2.4241

 9.358 ± 3.3800

 27.227 ± 19.0538

*, **significant at P < 0.05 and 0.01 probability levels, respectively.

2.30-5.93

0.11-4.39

10.57-68.46

9.36-23.71

0.52-15.90

0.80-22.91

0.50-54.09

interaction (var_{replication/year}) and the residual error (var_{residual}) of the 10 quality traits were obtained by running the R script. The heritability of each quality trait was calculated using the formula:

$$\begin{aligned} Heritability &= \mathrm{var}_{\mathrm{genotype}} / (\mathrm{var}_{\mathrm{genotype}} + \mathrm{var}_{\mathrm{genotype}/\mathrm{year}} / 2 \\ &+ \mathrm{var}_{\mathrm{residual}} / 4) \end{aligned}$$

РСА

Following the method of Chen *et al.* (2014), the numerical data of all 10 quality traits were converted to an ordinal scale with values from 1 to 10, i.e. from the first class $[Xi < (X - 2\sigma)]$ to the 10th grade $[Xi > (X + 2\sigma)]$, with increments of 0.5 σ between each level; *X* represents the mean value of all samples, and σ is the S.D. (Pecetti *et al.*, 1992). Accessions were classified by PCA (Sneath and Sokal, 1973) on a matrix of the rank frequencies of all 10 quality traits. PCA was performed with the computer program NTSYS-pc (Rohlf, 1998).

Results

Evaluation of 10 quality traits

A series of phenotypic quality trait data (488 accessions \times 10 traits \times 3 replications \times 2 years = 29,280 data points) were obtained, and we found a wide range of variation in the 10 quality traits among the evaluated accessions (Fig. 1

(a)–(j)). For example, OC varied from 36.75 to 47.55%; GLC varied from 20.20 to 100.96μ mol/g of seeds; OLE varied from 9.80 to 67.37%; ERU varied from 0.74 to 54.37%.

0.961**

0.707**

0.982**

0.960**

0.976**

0.935**

0.992**

2.25 - 5.78

0.72-4.43

9.03-66.28

10.42-23.99

0.50-16.73

1.96-24.86

0.71-54.64

According to the double-low rapeseed standard, low (0) ERU is <1% and low (0) GLC is <30 µmol/g of seeds. The 2-year means (data not shown) observed for erucic acid and total GLC of the 488 accessions were compared with the original germplasm values (online Supplementary Table S1), which were defined as those determined for the first germplasm collected in the OMGC. The results showed that, of 488 accessions, the observed mean ERUs were not consistent with the original values (the original low erucic acid value and the measured high erucic acid value) in 18 lines (3.7%); the observed mean and original GLC values (the original low GLC value and the measured high GLC value) did not match in 14 lines (2.9%); and the observed means of the above two traits were not consistent with the original values in 10 lines (2.0%). In other words, the observed means of the above two traits were consistent with the original values in 466 lines (95.5%) in 488 accessions. All inconsistent germplasm information came from abroad and was mostly foreign early germplasm.

The overall means of the major traits across genotypes for each of the 2 years are shown in Table 1, and there were highly significant (P < 0.01) correlations between the 2 years in all 10 traits. The trait with the largest correlation coefficient between the 2 years was ERU, whereas the trait with smallest value was PC. A comparison of the paired-sample means between the 2 years showed that, except for OC and PC, all of the traits showed highly



Fig. 1. Frequency distribution of 488 *B. napus* L accessions by (a) OC, (b) PC, (c) total GLC, (d) PAL, (e) STE, (f) OLE, (g) LNL, (h) LIN, (i) EIC, and (j) ERU. Values are the means of the evaluations in 2011 and 2012.

significant (P < 0.01) differences between the 2 years. For example, the mean GLC in 2012 was significantly higher than that in 2011, while the mean OLE in 2011 was significantly higher than in 2012.

In this study, all accessions were assigned three different germplasm types, namely winter OSR (166), spring OSR

(122), and semi-winter OSR (210). The means and LSD comparisons of the 10 quality traits of accessions from different ecotypes across the 2 years are presented in online Supplementary Table S2. Through careful analysis, we found that the mean OC of the semi-winter OSR accessions was significantly higher (P < 0.01) than that of the winter or

spring OSR; the mean GLC of the winter OSR was significantly higher (P < 0.01) than that of the semi-winter or spring OSR; and the mean ERU of the spring OSR was significantly lower (P < 0.01) than that of the winter or semi-winter OSR. Additionally, our results demonstrated that the mean OLE of the spring OSR was significantly higher (P < 0.01) than that of the winter or semi-winter OSR.

Means and LSD comparisons of the quality traits of accessions from different geographic origins across the 2 years are presented in online Supplementary Table S3. Due to the dominance of Chinese and European materials, the differences in the accessions of these two geographic origins were compared, and the results showed that the mean OC of the Chinese accessions was significantly higher (P<0.01) than that of the European accessions while the mean PC and GLC values of the Chinese accessions were significantly lower (P<0.01) than those of the European accessions. Additionally, in terms of fatty acid composition, our results demonstrated that, except for the mean LNL, there were no significant differences (P>0.05) in the mean PAL, STE, OLE, LIN, EIC and ERU values between the Chinese and European accessions.

The means and LSD comparisons of the quality traits of Chinese rapeseed accessions from different breeding periods across the 2 years are presented in Table 2. Over time, the mean OC of the accessions first increased and then decreased before increasing again; relative to 2001-2010, the mean was only significantly (P < 0.01) lower from 1950 to 1970. For PC and GLC, the accession means first decreased and then increased slightly before decreasing again. From 1950 to 1970, the mean was significantly (P < 0.01) higher than that of other periods, while from 2001 to 2010, it was significantly (P < 0.01) lower than in other periods.

In terms of fatty acid composition, the mean PAL, STE, OLE and LNL values showed a gradual upward trend over time. From 2001 to 2010, the mean was significantly (P < 0.01) higher than that of other periods, while from 1950 to 1970 and from 1971 to 1980, the mean was significantly (P < 0.01) lower than that of other periods. There was no significant (P > 0.05) change in the mean LIN in different breeding periods. For EIC and ERU, the mean accession values showed a gradual downward trend with time. From 2001 to 2010, the mean was significantly (P < 0.01) lower than that of other periods to 1970 and 1971 to 1980, the mean was significantly (P < 0.01) lower than that of other periods, while from 1950 to 1970 and 1971 to 1980, the mean was significantly (P < 0.01) higher.

Mining germplasm resources with excellent quality traits

In evaluating the consistency of the data between the 2 years, analysis of the means of 488 *B. napus* accessions in 2011 and 2012 showed that three accessions (6024-1,

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Breeding time	Ζ	(%)	Trait/mean ±	S.D.								
			OC	PC	GLC	PAL	STE	OLE	LNL	LIN	EIC	ERU
2001–2010	36	14.7	41.86 ± 2.51 a A	22.46 ± 1.18 c B	28.21 ± 8.67 c C	4.39± 0.29 a A	2.58± 0.24 a A	60.47 ± 6.73 a A	18.78 ± 1.33 a A	7.58± 1.57 a A	3.68± 1.65 c D	2.53 ± 6.89 d D
1991–2000	65	26.5	41.27± 1.41 ab AB	22.89± 0.95 b B	67.67 ± 14.68 b B	3.83 ± 0.74 b B	2.06 ± 0.51 b B	35.29 ± 15.83 b B	15.92 ± 3.44 b B	7.99 ± 2.83 a A	9.87 ± 3.67 b C	25.04 ± 18.96 c C
1981–1990	47	19.2	41.61 ± 1.43 a AB	22.71 ± 0.97 bc B	67.19 ± 15.77 b B	3.64 ± 0.66 bc BC	2.06 ± 0.49 b B	33.50± 16.28 b BC	15.20± 3.19 bc BC	7.46± 2.20 a A	9.89± 3.10 b BC	28.24 ± 19.15 c BC
1971–1980	99	26.9	41.39 ± 1.85 ab AB	22.90± 0.94 b B	71.66 ± 9.38 ab AB	3.47± 0.54 cd C	1.80 ± 0.40 c C	27.08 ± 13.54 c CD	14.26 ± 2.73 c CD	7.64 ± 2.08 a A	11.19± 2.75 a AB	34.56 ± 15.57 b AE
1950–1970	31	12.7	40.69 ± 1.23 b B	23.72 ± 1.16 a A	76.49 ± 8.45 a A	3.23 ± 0.17 d D	1.57 ± 0.23 d C	20.88 ± 5.93 d D	13.05 ± 1.21 d D	7.91 ± 1.28 a A	12.02 ± 1.99 a A	41.34 ± 4.70 a A
Different lower	case le	stters inc	licate a signific	ant difference	s at $\alpha = 0.05$, ar	nd different up	percase lette	rs indicate a sig	gnificant differe	ence at $\alpha = 0$.	.01.	

Table 2. Means, S.D. and LSD comparison of 10 quality traits of Chinese accessions with different breeding times across 2 years

Source of variation	df	OC	PC	GLC	PAL	STE	OLE	LNL	LIN	EIC	ERU
Among blocks	2	0.46 ns	1.49	0.03 ns	0.73**	2.99*	0.31**	0.51**	0.19**	0.11*	0.06*
Environment (year)	1	0.00 ns	0.12**	0.68**	0.23**	28.24**	0.20**	1.87**	0.05**	1.11**	0.06**
Genotype	487	66.52**	48.79**	94.91**	91.75**	40.70**	96.48**	91.81**	95.81**	89.23**	98.44**
Env × Geno (year × Geno)	487	9.92**	14.9**	1.42**	1.84**	7.03**	0.89**	1.91**	1.22**	3.16**	0.43**
Error	1950	23.09	34.70	2.95	5.45	21.04	2.12	3.91	2.73	6.39	1.02

Table 3. Proportion of sum of squares to total sum of squares for 10 quality traits of 488 B. napus accessions across 2 years

ns, not significant.

*, **significant at P < 0.05 and 0.01 probability levels, respectively.

87-9 and G142) had OC values higher than 46.40%; five accessions (No 23 Rape, 363, Target, Legend and Huyou 18) had PC values higher than 25.20%; 34 accessions (including two additional, unlisted materials) had GLC values higher than 30 µmol/g; two accessions (Yixuan 159 and H62) had PAL values lower than 2.50%; two accessions (Chuanyou 96-98 and Chengkoushengli) had STE values lower than 1.00%; four accessions (Zheyou 17, Zhongshuang 11, Huyou 18 and Hongyou 3) had OLE values higher than 63.00%; one accession (Cresor) had LNL values higher than 23.70%; two accessions (Samurai and 93355) had LIN values higher than 15.00% and EIC values greater than 20.00%; 42 accessions (not listed) had ERU values lower than 1.00%; two accessions (Yixuan 159 and H62) had ERU values higher than 50.00%; and 28 accessions (not listed) not only had ERU values lower than 1.00% but GLC values lower than 30 µmol/g. These 28 accessions were double-low rapeseed germplasm.

Genotypic variability

To determine whether and how the environment influences these traits, we converted the proportional sums of squares to the total sum of squares for different sources of variation and tested the level of significance. Our results showed that the differences between the 2 years (environments) were significant (P<0.01) for PC, GLC, PAL, STE, OLE, LNL, LIN, EIC and ERU (Table 3) but not significant (P>0.05) for OC. Differences among *B. napus* accessions were also significant (P<0.01) for all characters under investigation, and genotype×year (environment) interactions were significant (P<0.01) for all quality traits.

Genotype clearly contributed a large portion of the variation in all characters (40.70–98.44%); genotypic variations in OC and GLC accounted for 65.22 and 94.91% of the total variations, respectively. The value of the genotypic variation in ERU was the largest of all 10 phenotypic traits,

while year contributed the largest portion of the variation in STE (28.24%).

The heritability of 10 quality traits

By running the R script described above using 29,280 data points, the variance components and heritability of the 10 quality traits were determined and are shown in online Supplementary Table S4. The heritability of the PC trait was lowest; those of the STE and OC traits were smaller; and the heritability values of the other seven quality traits (GLC, PAL, OLE, LNL, LIN, EIC and ERU) were larger than 0.950.

Relationships among 10 quality characters

The complete correlation coefficients among the 10 quality traits under investigation are presented in Table 4. The objective was to explore which traits are well associated and meaningful for breeding. The OC correlation coefficients were significantly (P < 0.01) negative against PC, GLC, PAL, OLE, LNL and LIN, whereas they were significantly (P < 0.01) positive against EIC and ERU. Of the above correlation coefficients, the absolute value of the correlation coefficient between OC and PC was the largest (-0.589^{**}) . GLC was positively and significantly correlated with ERU ($r = 0.627^{**}$), and PAL had a significantly positive correlation with LNL ($r=0.967^{**}$). There were negative and significant correlations between PAL and ERU (r= -0.952^{**}), and the correlation coefficients between OLE and LNL ($r=0.861^{**}$) were positive and significant. There were negative and significant correlations between OLE and ERU ($r = -0.955^{**}$) as well as between LNL and ERU $(r = -0.946^{**}).$

РСА

Associations among 488 *B. napus* accessions were obtained from a PCA with the 10 quality traits and the different

	OC	PC	GLC	PAL	STE	OLE	LNL	LIN	EIC	ERU
OC										
PC	-0.589**									
GLC	-0.194**	0.334**								
PAL	-0.174**	-0.080	-0.536**							
STE	-0.171**	-0.100*	-0.640**	0.861**						
OLE	-0.137**	-0.143**	-0.709**	0.846**	0.852**					
LNL	-0.249**	0.032	-0.506^{**}	0.967**	0.863**	0.861**				
LIN	-0.160**	0.003	-0.094*	0.619**	0.417**	0.331**	0.561**			
EIC	0.181**	0.034	0.636**	-0.439**	-0.609**	-0.804^{**}	-0.525^{**}	0.093*		
ERU	0.159**	0.116*	0.627**	-0.952**	-0.880**	-0.955**	-0.946**	-0.560**	0.617**	

Table 4. Correlation coefficients among 10 quality characters of 488 B. napus accessions evaluated in 2011 and 2012

*, **Significant at the P < 0.05 and 0.01 probability levels, respectively.

ecotypes as the first two principal coordinates (first principal component (PC1), and second principal component (PC2)) (online Supplementary Fig. S1). The first two PCA components provided a reasonable summary of the data and explained 42.5% of the total variation. The PC1 was the most important and explained 26.9% of the total variance, and 15.6% of the variation among accessions was attributed to the PC2. Winter OSR accessions were distributed in lower part of the plot; spring OSR accessions were mainly distributed in the middle part of the plot; and semi-winter OSR accessions were mainly distributed in the winter, spring and semi-winter OSR accessions were distributed in the middle of the plot, so there was no clear boundary among the three ecotypes.

Associations among the 488 *B. napus* accessions were obtained from a PCA with the 10 quality traits and the different geographic origins as the first two principal coordinates (PC1 and PC2) (online Supplementary Fig. S2). European and Chinese accessions accounted for 84.2% of the total number of accessions. European accessions were distributed in the lower part of the plot, whereas Chinese accessions were distributed in the upper part of the plot, and the two distributions partially intersected. The accessions from Japan, America, Australia and other regions, which accounted for 15.8% of the total, were distributed in both broken parts of the plot. In other words, there was no clear boundary among the materials of different geographic origins.

Discussion

Improving the quality and increasing the OC are significantly important in OSR breeding and production, but the phenotypic variation in quality traits is continuous and influenced by different alleles. In this study, we found a wide range of variation in 10 quality traits among the evaluated accessions, and these results, especially the identification of excellent germplasm, will provide important support for the improvement of *B. napus* quality.

Out of 10 quality traits, three (OC, PC and EIC) showed unimodal normal distributions while the other seven traits (GLC, PAL, STE, OLE, LNL, LIN and ERU) showed bimodal normal distributions. The bimodal normal distributions of seven quality traits may be due to artificial selection; the bimodal distribution of ERU could have resulted from artificial selection in two directions (low erucic acid and edible oil quality breeding or high erucic acid oil industry breeding).

Of 488 accessions, the observed means for the two traits (ERU and GLC) of 466 lines (95.5%) were consistent with the original values, indicating the reliability of the study results. In an additional 22 lines (4.5%), the observed means of the two traits (ERU and GLC) were inconsistent with the original values. Errors in the original germplasm data was the first reason for this mismatch, while the second was the failure to strictly isolate those germplasms during the regeneration process, which led to a change in the above two traits.

All 10 quality traits were highly significantly (P < 0.01) correlated between the 2 years, demonstrating the replicability of our experimental results. Environment (year) contributed the largest portion of the variation to STE (28.24%) out of the 10 phenotypic traits (Table 3). Online Supplementary Table S4 shows that the heritability of the STE trait was smallest of the seven fatty acid components, which indicated that this character (STE) was most influenced by the environment (year). Using 282 double haploid lines from Sollux and Gaoyou, Zhao *et al.* (2008) found high heritabilities for OC and all fatty acids ($b^2 = 0.82-0.94$), except for STE ($b^2 = 0.38$), which is consistent with our results.

Although the effect of the total variation in the environment on OC can be ignored (0.00% ns), the interaction between genotype and environment was larger (9.92%**) as was the contribution of the error to the total variation (23.09%) (Table 3). Online Supplementary Table S4 shows that the heritability of the OC trait was 0.815. Han (1990) and Zhao *et al.* (2008) determined the heritability of this trait (OC) in *B. napus* to be 0.820 and 0.900, respectively, and our result is consistent with Han (1990) and approximates that of Zhao *et al.* (2008).

In this study, the average OC of the semi-winter OSR accessions was significantly higher (P < 0.01) than that of the winter and spring types (online Supplementary Table S2). The high OC of the semi-winter types was closely related to the ecological adaptability of the varieties. This experiment was carried out in the semi-winter ecological zone, and semi-winter OSR accessions are adapted to the temperate climate in China, where they are preferred over the winter and spring types due to their considerably higher potential OC (Kimber and McGregor, 1995; Friedt and Snowdon, 2009). Similarly, the mean OC of the Chinese accessions was higher than that of other accession sources, which may also be attributed to ecological adaptability.

Our results showed that the average GLC and ERU of the Chinese accessions declined continuously from 1950–1970 to 2001–2010 and especially decreased significantly (P < 0.01) from 1991–2000 to 2001–2010, which was closely related to the history of Chinese double-low rapeseed breeding. It has been suggested that in this period, Chinese breeders began to focus on improving the quality of *B. napus*, and this goal was indeed achieved during this period. As for OC, the mean OC of the Chinese accessions did not change significantly (P > 0.05) from 1950–1970 to 2001–2010 (Table 2), suggesting that it is difficult to improve this trait (OC) but also that there is great room for improvement.

The results of previous studies of the relationship between OC and OLE were inconsistent. Using high erucic acid B. napus, Nath et al. (2009) found that OC was significantly negatively correlated with oleic acid and positively correlated with ERU; the significant positive correlation between OC and ERU were also confirmed by Klassen (1976). While Mollers and Schierholt (2002), Zhao et al. (2008), and Dimov and Mollers (2010) observed a positive correlation between OC and OLE using low erucic acid B. napus, a Pearson's correlation analysis of quality traits showed that OC had a significantly (P < 0.01) negative correlation with OLE and a positive correlation with ERU (Table 4). These results were in accordance with those of some previous reports (Klassen, 1976; Nath et al., 2009; Abideen et al., 2013) and inconsistent with those of others (Mollers and Schierholt, 2002; Zhao et al., 2008; Dimov and Mollers, 2010). This may have been because of the B. napus accessions selected for our study. Mollers and Schierholt (2002), Zhao et al. (2008) and Dimov and Mollers (2010) used zero erucic acid material, while Nath et al. (2009) and Abideen et al. (2013) used high erucic acid material. In this study, most of the materials were high erucic acid accessions.

Previous researchers have consistently found a significantly negative correlation between OLE and ERU (Khan *et al.*, 2008; Islam *et al.*, 2009; Tonguc and Erbas, 2012), and a Pearson's correlation analysis of quality traits in this study showed significant negative correlations between OLE and ERU ($r = -0.955^{**}$). The results were consistent with previously reported results (Khan *et al.*, 2008; Islam *et al.*, 2009; Tonguc and Erbas, 2012).

Previous conclusions about the relationship between oleic acid and LNL are also inconsistent. Using high erucic acid B. napus, Liu (1981) and Zhou and Liu (1987) confirmed a significant positive correlation between OLE and LNL, while Mollers and Schierholt (2002), Zhao et al. (2008) and Dimov and Mollers (2010) found a negative correlation between OLE and LNL using low erucic acid B. napus. In this study, Pearson's correlation analysis of quality traits also found positive and significant correlation coefficients between OLE and LNL $(r=0.861^{**})$ (Table 4), which is in accordance with some previously reported results (Liu, 1981; Zhou and Liu, 1987) and inconsistent with others (Mollers and Schierholt, 2002; Zhao et al., 2008; Dimov and Mollers, 2010). This result may have been due to most of the materials being high erucic acid accessions, which were also used by Liu (1981) and Zhou and Liu (1987).

Table 4 also shows that GLC was significantly positively correlated with ERU ($r = 0.627^{**}$), but these results were inconsistent with the independent inheritance of the two traits (GLC and ERU) (Zhou and Liu, 1989). This may be attributable to artificial selection for orientation in OSR breeding.

In this study, PCA showed that there was no obvious distinction among accessions from different ecotypes and geographic origins (Figures S1 and S2). Chen *et al.* (2014) reported that three major groups (winter OSR accessions, spring OSR accessions and semi-winter OSR accessions) could be distinguished when projecting the accessions onto the first two PCAs, but the result was inconsistent with Chen *et al.* (2014). This may be related to the amount of information about the 10 quality traits. Although there are apparently 10 quality traits (OC and PC were classified as the first kind, GLC as the second, and the composition of the seven fatty acids as the third).

Conclusions

Near-infrared evaluations of *B. napus* accessions showed a wide range of variation in quality traits. In general, Chinese accessions tended to have a higher OC than foreign accessions, and among winter and spring accessions, semiwinter accessions tended to have the highest OC. Over time, the mean total GLC and ERU of Chinese rapeseed accessions showed a gradual downward trend. Genotypic variation in ERU accounted for 98.44% of the total variation, which was the highest among all 10 of the quality traits, and heritability for ERU was also the largest among all 10 of the quality traits. Environment (year) contributed the largest portion of the variation in STE (28.24%) among all 10 of the quality traits. OC significantly (P < 0.01) negatively correlated with OLE, whereas it was significantly (P < 0.01)positively correlated with ERU. PCA revealed no clear boundary among the study materials along the first two principal coordinates of different geographic origins and different ecotypes. High variations in quality traits were found among these accessions, indicating that it is possible to select for these characters. Moreover, the phenotypic quality trait data obtained in this study could be used in our subsequent genome-wide association studies in B. napus.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S1479262117000089.

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References

- Abideen SN, Nadeem F and Abideen SA (2013) Genetic variability and correlation studies in *Brassica napus* L. genotypes. *International Journal of Innovation and Applied Studies* 2: 574–581.
- Ahmad S, Sadaqat HA, Tahir MHN and Awan FS (2015) An insight in the genetic control and interrelationship of some quality traits in *Brassica napus. Genetics and Molecular Research* 14: 17941–17950.
- Aytaç Z and Kınacı G (2009) Genetic variability and association studies of some quantitative characters in winter rapeseed (*Brassica napus* L.). African Journal of Biotechnology 8: 3547–3554.
- Becker HC, Löptien H and Röbbelen G (1999) Breeding: an overview. In: Gómez-Campo C (ed.) *Biology of Brassica Coenospecies*. Amsterdam: Elsevier, pp. 413–460.
- Chen BY, Xu K, Li J, Li F, Qiao JW, Li H, Gao GZ, Yan GX and Wu XM (2014) Evaluation of yield and agronomic traits and their genetic variation in 488 global collections of *Brassica napus* L. *Genetic Resources and Crop Evolution* 61: 979–999.
- Dimov Z and Mollers C (2010) Genetic variation for saturated fatty acid content in a collection of European winter oilseed rape material (*Brassica napus*). *Plant Breeding* 129: 82–86.
- Ecke W, Clemens R, Honsdorf N and Becker HC (2010) Extent and structure of linkage disequilibrium in canola quality winter

rapeseed (*Brassica napus* L.). *Theoretical and Applied Genetics* 120: 921–931.

- Fisher RA (1924) The distribution of the partial correlation coefficient. *Metron* 3: 329–332.
- Friedt W and Snowdon RJ (2009) Oilseed rape. In: Vollmann J and Rajcan I (eds) *Handbook of Plant Breeding. Oil Crops*, vol. 4. New York: Springer, pp. 91–126.
- Gai JY (2000) *Experimentation Method*. Beijing, China: China Agriculture Press.
- Gunasekera CP, Martin LD, Siddique KHM and Walton GH (2006) Genotype by environment interactions of Indian mustard (*Brassica juncea* L.) and canola (*B. napus L.*) in Mediterranean-type environments. I. Crop growth and seed yield. *European Journal of Agronomy* 25: 1–12.
- Han JX (1990) Genetic study on oil content in *Brassica napus* L. Oil Crops China 12: 1–6 (in Chinese with English abstract).
- Islam MS, Rahman SL and Alam MS (2009) Correlation and path coefficient analysis in fat and fatty acids of rapeseed and mustard. *Bangladesh Journal of Agricultural Research* 34: 247–253.
- Khan FA, Ali S, Shakeel A, Saeed A and Abbas G (2006) Genetic variability and genetic advance analysis for some morphological traits in (*Brassica napus* L.). *Journal of Agriculture Research* 44: 83–87.
- Khan S, Farhatullah, Khalil IH, Munir I, Khan MY and Ali N (2008) Genetic variability for morphological traits in F_{3:4} Brassica populations. Sarhad Journal of Agriculture 24: 217–222.
- Kimber DS and McGregor DI (1995) The species and their origin, cultivation and world production. In: Kimber D and McGregor DI (eds) *Brassica Oilseeds: Production and Utilization*. Wallingford: CABI Publishing, pp. 1–9.
- Klassen AJ (1976) Relationship between quality and quantity of oil in *Brassica* species. *Plant Science* 56: 427–428.
- Korber N, Wittkop B, Bus A, Friedt W, Snowdon RJ and Stich B (2012) Seedling development in a Brassica napus diversity set and its relationship to agronomic performance. *Theoretical and Applied Genetics* 125: 1275–1287.
- Krzymanski J (1974) Rapeseed breeding for better oil and meal quality in Poland. In: Proceedings of the 4th International Rapeseed Conference, Giessen, Germany, pp. 49–55.
- Kumar S (2013) Genetic analysis of oil content and quality parameters in Indian mustard (*Brassica juncea* (L.) Czern and Coss). *Scholarly Journal of Agricultural Science* 3: 299–304.
- Liu XY (1981) Fatty acid content of Chinese rapeseed germplasm resource. *Chinese Journal of Oil Crop Science* 3: 38–41.
- Merk HL, Yarnes SC, Van Deynze A, Tong N, Menda N, Mueller LA, Mutschler MA, Loewe SA, Myers JR and Francis DM (2012) Trait diversity and potential for selection indices based on variation among regionally adapted processing tomato germplasm. *Journal of the American Society for Horticultural Science* 137: 427–437.
- Mollers C and Schierholt A (2002) Genetic variation of palmitate and oil content in a winter oilseed rape doubled haploid population segregating for oleate content. *Crop Science* 42: 379–384.
- Nath UK, Wilmer JA, Wallington EJ, Becker HC and Möllers C (2009) Increasing erucic acid content through combination of endogenous low polyunsaturated fatty acids alleles with *Ld-LPAAT+Bn-fae1* transgenes in rapeseed (*Brassica napus* L.). *Theoretical and Applied Genetics* 118: 765–773.

Rohlf FJ (1998) *NTSYS-PC Numerical Taxonomy and Multivariate Analysis System*. New York: Exeter Software.

Pecetti L, Annicchiarico P and Damania AB (1992) Biodiversity in a germplasm collection of durum wheat. *Euphytica* 60: 229–238.

- Sneath PHA and Sokal RR (1973) *Numerical Taxonomy: The Principles and Practices of Numerical Classification.* San Francisco: W. F. Freeman and Co., p. 573.
- SPSS Inc. (2007) SPSS Base 16.0 User's Guide. Chicago: SPSS Inc.
- Stefansson RR, Hougen FW and Downey RK (1961) Note on the isolation of rape plants with oil seed from erucic acid. *Canadian Journal of Plant Science* 41: 218–219.
- Tonguc M and Erbas S (2012) Evaluation of fatty acid compositions and some seed characters of common wild plant species of Turkey. *Turkish Journal of Agriculture and Forestry* 36: 673–679.
- Wiedemann SCC, Hansen WG, Snieder M and Wortel VAL (1998) NIR calibration in practice. *Analusis* 26: 38–42. doi: 10.1051/ analusis:199826040038.
- Zhang HP, Berger JD and Milroy P (2013) Genotype × environment interaction studies highlight the role of phenology in specific adaptation of canola (*Brassica napus*) to contrasting Mediterranean climates. *Field Crops Research* 144: 77–88.
- Zhao JY, Dimov Z, Becker HC, Ecke W and Möllers C (2008) Mapping QTL controlling fatty acid composition in a doubled haploid rapeseed population segregating for oil content. *Molecular Breeding* 21: 115–125.
- Zhou YM and Liu HL (1987) Studies on the heritance of major fatty acid composition in the oil of rapeseed (*Brassica napus* L.). *Acta Agronomic Sinica* 13: 1–10.
- Zhou YM and Liu HL (1989) Relationships between major quality traits in *Brassica napus* L. *Journal of Huazhong Agricultural* University 8: 97–101.