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ORIGINAL PAPER

Identification of quantitative trait loci (QTL) for oil and protein contents and their relationships with other seed quality traits in *Brassica juncea*

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Abstract A detailed RFLP-genomic map was used to study the genetics of oil, seed and meal protein and sum of oil and seed/meal protein contents in a recombinant doubled-haploid population developed by crossing black- and yellow-seeded *Brassica juncea* lines. Two yellow seed color genes (SC-B4, SC-A6) and one QTL for erucic acid content (E_{1b}) showed pleiotropic effect for oil, protein and sum of oil and seed/meal protein contents. Six (O-A1, O-A6, O-A9, O-B3, O-B4, O-B5) and five (SP-A1, SP-A9, SP-B4, SP-B6, SP-C) QTLs were significant for oil and seed protein contents, respectively. Tight linkage of three of these QTLs (SP-A1, SP-A9, SP-B4, O-A1, O-A9, O-B4), with opposite effects, poses challenge to the plant breeders for simultaneous improvement of negatively correlated (r = –0.7**) oil and seed protein contents. However, one QTL for oil content (O-B3) and two for seed protein content (SP-B6, SP-C) were found to be unlinked, which offer the possibility for simultaneous improvement of these two traits. QTLs significant for meal protein (MP-A1, MP-A6, MP-A9, MP-B5, MP-B6) were significant at least for oil, seed protein or sum of oil and seed/meal protein contents (T-A6, T-A7, T-B4, T-B5). Sum of oil and seed protein contents and sum of oil and meal protein contents had a perfect correlation, as well as same epistatic interactions and QTLs with similar additive effect. This indicates that protein in seed or meal has practically the same meaning for breeding purposes. Epistatic interactions were significant for the quality traits, and their linkage reflected association among the traits.

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Introduction

The oilseed crops *Brassica napus*, *Brassica rapa*, and *Brassica juncea* are one of the most important sources of vegetable oil in the world. In North America, canola quality (low erucic acid, low glucosinolates) *B. rapa* and *B. napus* are the main *Brassica* oilseed crops. However, *B. juncea*, which is widely grown in Indian Subcontinent, has high potential of growing in Canada due to its many good characteristics over *B. rapa* and *B. napus*, e.g., heat resistance, drought tolerance, higher yield potential, blackleg resistance, shattering resistance, early maturity, etc. (Downey 1990; Woods et al. 1991). This species has recently been converted into canola type and is becoming increasingly popular

in North America and Australia (Burton et al. 2003; Rakow 2003).

The major components of *Brassica* seed are water, protein, oil, and residue composed mainly of carbohydrates, fiber, ash, minerals, etc. After extraction of oil from *Brassica* seed, the left over meal/seed cake contain >40% protein with an excellent amino acid profile (Newkirk et al. 1997; Krzymanski 1998). High seed oil and meal protein are the most important objectives in *Brassica* oilseed breeding programs, including *B. juncea* breeding. It is well known that seed oil and seed protein contents are negatively correlated in the *Brassicas* (Grami and Stefansson 1977b; Zhao 2002). However, the relationships among oil, seed/meal protein, and residual contents, especially the effect of changes in the content of one component on the content of other components, are very important for better understanding of genetics of oil and seed/meal protein contents (Si et al. 2003).

Oil and protein contents are controlled by a large number of genes with mainly additive and epistatic gene action (Grami and Stefansson 1977a; Zhao 2002). The contents are influenced by environment (Si et al. 2003), seed color (Rahman et al. 2001), and erucic acid content (Cheung and Landry 1998). Therefore, identification of quantitative trait loci (QTLs) for oil, seed/meal protein, and sum of oil and seed/meal protein contents would expand our knowledge of the genetic control of these traits. The identified QTLs might also uncover their relationships with other seed quality traits, e.g., seed color, erucic acid, glucosinolates, etc. The molecular markers tightly linked to the QTLs could be used to identify the desired plants for increased oil and protein contents.

Using a recombinant doubled-haploid (DH) population segregating for different plant morphological and seed traits, a comprehensive RFLP-based genomic map of *B. juncea* has been constructed (Mahmood et al. 2003a) and QTLs for seed color (Mahmood et al. 2005a), erucic acid content (Mahmood et al. 2003a), aliphatic glucosinolates (Mahmood et al. 2003b), and yield components (Mahmood et al. 2005b) have been identified. The same segregating DH population was employed to identify QTLs associated with oil, seed/meal protein, and sum of oil and seed/meal protein contents. Further, correlations between different seed quality traits, viz. oil, seed/meal protein, sum of oil and seed/meal protein contents, erucic acid content, aliphatic glucosinolates, and seed color, were studied in this DH population to elucidate the linkage relationships of the correlated traits with their identified QTLs.

Materials and methods

Plant material

A black/brown-seeded, non-canola quality (high erucic acid, high glucosinolates), low in oil *B. juncea* cv. RLM-514 (designated as BSP) of Indian origin was reciprocally crossed with a yellow-seeded, canola quality (low erucic acid, low glucosinolates), high in oil *B. juncea* breeding line (designated as YSP), and a segregating population of 112 DH lines was produced (Thiagarajah and Stringam 1993; Mahmood et al. 2003a).

Experimentation and data collection

In 1999, a trial was conducted at the Edmonton Research Station, Alberta, Canada, using three replications. However, data were taken from only two of these replications. Each plot consisted of four rows, 6 m long and 0.3 m apart. In 2000, three trials, each with two replications, were conducted in Alberta (the Edmonton Research Station, Ellerslie and Kelsey). Each plot consisted of three rows, 4 m long and 0.3 m apart. The design of the experiment was randomized complete block, the details of which have been described earlier (Mahmood et al. 2003a, 2005b). Self-pollinated seeds of the DH and parental lines from green house were seeded at all locations. All plots, except at the Edmonton Research Station in 1999, were manually harvested. Seed sample of each plot was used for measurement of seed oil and protein contents at 5% moisture using NIR (Daun et al. 1994). Meal protein and residual contents were calculated as:

$$\text{Meal Protein} = \text{Seed protein} \times (100 - \text{Oil})^{-1} \times 100$$

$$\text{Residue} = 100 - (\text{Oil} + \text{Seed Protein} + 5).$$

The coefficient (r_{ij}) of correlation between the quality traits was calculated as

$$r_{ij} = \sigma_{ij}/\sigma_i\sigma_j$$

where σ_{ij} is the covariance of the traits i and j, respectively, and σ_i and σ_j are standard deviations for the traits i and j, respectively. Computations were done using SAS/STAT 6.0 (SAS Inc. 1989).

Quantitative trait loci analysis

A detailed RFLP linkage map has been constructed using these DH lines (Mahmood et al. 2003a). The genomic map consisted of 300 linked (organized into 18

linkage groups and seven unlinked segments, covering a total map distance of 1,564 cM) and 16 unlinked loci at a LOD value of 3 (Mahmood et al. 2003a). Least significant means across individual and mean environments were used for QTL analysis. MapQTL (Version 3.0) (Van Ooijen and Maliepaard 1996) was employed for QTL analysis using the MQM approach (Jansen and Stam 1994). A LOD value of 2.4 was chosen as the threshold to declare a putative QTL. The details have been described earlier (Mahmood et al. 2003a, b).

Epistasis analysis

A two-way ANOVA was used to determine the digenic espistasis affecting a quantitative trait in an unreplicated population (Li et al. 1997) as described by Mahmood et al. (2005a). Multiple regression model (Li et al. 1997) was used to eliminate false positive interactions due to background genetic effects of segregating QTLs. The details have been described earlier (Li et al. 1997; Mahmood et al. 2005a).

Estimation of number of genes controlling a quantitative trait

The number of genes (k) controlling the seed quality traits was calculated by following the method described by Snap et al. (1984):

$$k = \{(\text{Range}/2)\}^2/\text{DH variance}.$$

Results

Trait statistics

The yellow- and black/brown-seeded parents differed significantly for seed oil, seed protein, sum of oil and meal protein contents, but not for meal protein content (Table 1). Analysis of variance suggested the presence of significant variation for these traits in the recombinant DH population. Furthermore, transgressive segregation was observed in the population for these traits (data not shown).

Table 1 Oil and protein contents of the black- and yellow-seeded parents of *Brassica juncea* in the mean environment

	OIL	SP	MP	MSUM	SSUM	RESI
YSP	43.02	26.61	46.69	89.72	69.63	25.37
BSP	40.61	27.70	46.63	87.23	68.30	26.70
Difference	2.41**	–1.09*	0.06^{NS}	2.49**	1.33**	–1.33**

All units are in %. *YSP* yellow-seeded parent, *BSP* black-seeded parent, *SP* seed protein content, *MP* meal protein content, *MSUM* sum of oil and meal protein contents, *SSUM* sum of oil and seed protein contents, *RESI* residual contents, *NS* non-significant

*, **Significant at a probability level of 0.05 and 0.01, respectively

Initially, we performed QTL and epistasis analysis on seed oil, seed protein, meal protein, sum of oil and seed protein, sum of oil and meal protein, and residual contents. However, during the course of this study, we found that the latter three traits, viz. sum of oil and seed protein, sum of oil and meal protein, and residual contents, had perfect correlation with each other, as well as all these traits were governed by the same QTLs and epistatic interactions. Therefore, results of only sum of oil and meal protein contents would be presented and discussed further unless it is important to state the other two traits.

Correlation between seed quality traits

Total aliphatic and 3-butenyl glucosinolates had significant positive association with oil, sum of oil and meal protein contents (Table 2). Erucic acid content was positively and significantly correlated with oil, meal protein, sum of oil and meal protein contents (Table 2). The correlation of yellow seed color with oil, meal protein, and sum of oil and meal protein contents was significant and positive, but it was significant and negative with seed protein content (Table 2). Oil content showed significant negative correlation with seed and meal protein contents but significant positive correlation with sum of oil and meal protein contents. Meal protein content was positively and significantly associated with seed protein and sum of oil and meal protein contents (Table 2).

Quantitative trait loci analysis

Six QTLs were identified for oil content (O-A1, O-A6, O-A9, O-B3, O-B4, and O-B5), four for sum of oil and meal protein contents (T-A6, T-A7, T-B4, and T-B5), and five for each of seed (SP-A1, SP-A9, SP-B4, SP-B6, and SP-C) and meal protein contents (MP-A1, MP-A6, MP-A9, MP-B5, and MP-B6) (Table 3). Approximately, half of those QTLs originated from the A genome and half from the B genome. Most of the QTLs identified for the quality parameters were consistent across the environments (Table 3). The distribution of the QTLs on the *B. juncea* genome is shown in Fig. 1. Of the six and five QTLs significant for oil and seed protein contents, respectively (Table 3), three common/tightly linked QTLs (O-A1, O-A9, O-B4 and

Table 2 Coefficient of correlation between different seed quality traits in *Brassica juncea*

	BE	TA	EA	COL[a]	OIL	MP	SP	MSUM
PE	–0.75**	0.40**	0.07	–0.02	0.02	0.07	0.05	0.07
BE		0.34**	0.18**	–0.01	0.17**	0.03	–0.08*	0.16**
TA			0.33**	–0.03	0.26**	0.15**	–0.05	0.31**
EA				0.1*	0.3**	0.28**	0.02	0.44**
COL[a]					0.45**	0.1**	–0.20**	0.45**
OIL						–0.13**	–0.69**	0.70**
MP							0.81**	0.62**
SP								0.04

PE 2-propenyl glucosinolate, *BE* 3-butenyl glucosinoalte, *TA* total aliphatic glucosinolates, *EA* erucic acid content, *COL* seed color, *MP* meal protein content, *SP* seed protein content, *MSUM* sum of oil and meal protein contents. *, ** Significant at a probability level of 0.05 and 0.01, respectively, at a degree of freedom (*df*) of 886. However, where seed color was involved, degree of freedom was 664, as seed color was assessed over three environments (Mahmood et al. 2005a)

[a] The sign of the coefficient correlation was reversed keeping in view the assessment of seed color (by index of light reflectance) using a HunterLab Mininscan Spectrometer (Mahmood et al. 2005a)

Table 3 Quantitative trait loci for quality traits of *Brassica juncea* in the mean environment

Trait[a]	QTL[b]	Flanking loci	Dis (cM)[d]	S Int (cM)[e]	LOD[f]	ADD (%)[g]	σ_P^2 [j]	Totσ_P^2 [k]	Env[l]
OIL	O-A1	wg1g5-ec2d11a	0	15	4.71	0.84[h]	9.7		4
	O-A6	ec3c8dNM-ec3f4eNM	0	45	4.25	0.90	9		4
	O-A9	tg1g9cNM-ec3d3	5	26	4.57	–0.90[i]	10.2		4
	O-B3	wg2a11a-wg6f10bNP	10	23	3.26	–0.83	8.1		4
	O-B4	wg7b6cNM-G9F8T7	5	45	7.93	1.29	17.6		4
	O-B5	wg3c5aNM-ec2e5c	0	12	5.08	–0.90	10.7	65.3	4
SP	SP-A1	wg6c6b-wg1g5	10	28	7.75	–0.94	20.6		4
	SP-A9	wg9d5b-tg1g9cNM	0	64	7.28	0.78	15.2		4
	SP-B4	wg7b6cNM-G9F8T7	0	47	8.54	–0.82	17.0		4
	SP-B6	wg7f10aNM-wg2d9b	5	16	4.76	0.60	8.9		3
	SP-C[c]	tg6c5-wg7f8a	10	8	2.79	–0.51	6.9	68.6	2
MP	MP-A1	wg6c6b-wg1g5	10	8	4.01	–0.96	12.4		4
	MP-A6	ec3c8dNM-ec3f4eNM	0	25	3.24	0.76	8.1		4
	MP-A9	wg9d5b-tg1g9cNM	0	40	3.98	0.82	10		4
	MP-B5	ec2e5c-wg8b1b	5	13	3.64	–0.8	9.5		4
	MP-B6	wg7f10aNM-wg2d9b	5	9	3.04	0.73	7.7	47.7	3
MSUM	T-A6	ec3c8dNM-ec3f4eNM	0	30	5.74	1.44	14.5		4
	T-A7	wg5d9d-ec4f10aNP	0	16	3.69	–1.083	9.1		4
	T-B4	177N18T7b-wg7e6bNP	5	8	2.42	0.94	6.1		2
	T-B5	wg3c5aNM-ec2e5c	5	28	6.21	–1.53	18.1	47.8	4

[a] *MP* meal protein content, *SP* seed protein content, *MSUM* sum of oil and meal protein contents

[b] Name of a QTL based on the name of a trait followed by the linkage group number (Mahmood et al. 2003a)

[c] Unlinked segment in the genome of *Brassica juncea* (Mahmood et al. 2003a)

[d] *Dis* distance of the QTL from the first flanking marker

[e] *S Int* support intervals (peak of a QTL before fixing it) for QTL were determined at a LOD value of 2.4

[f] *LOD* LOD value associated with detected QTL

[g] *ADD* additive effect (%) associated with detected QTLs

[h] Positive additive effect showed that YSP alleles increased trait expression in comparison to BSP alleles at associated QTLs

[i] Negative additive effect showed that BSP alleles increased trait expression in comparison to YSP alleles at associated QTLs

[j] σ_P^2 % of total phenotypic variance explained by a QTL

[k] Totσ_P^2 % of total phenotypic variance explained by all QTLs

[l] *ENV* number of environment(s) in which the QTL was significant

SP-A1, SP-A9, SP-B4) affected both traits, however, with opposite additive effects (Table 3, Fig. 1). Similarly, three common/tightly linked QTLs (O-A6, O-B4, O-B5 and T-A6, T-B4, and T-B5) affected both oil and sum of oil and meal protein contents, and the linked/common QTLs had similar additive effects (Table 3,

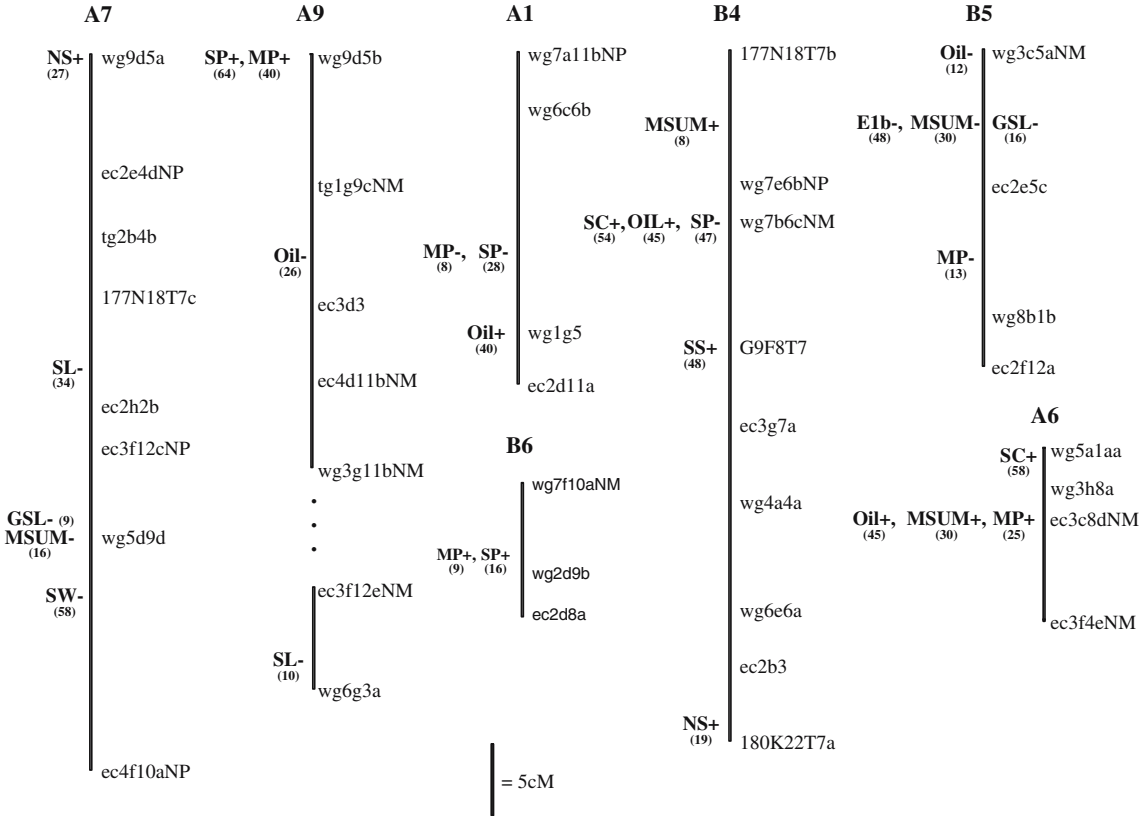

Fig. 1 Distribution of QTLs for seed quality and quantitative traits in *Brassica juncea*. *A* and *B* refer to the A and B genomes of *Brassica juncea*, and numeric number refers to the linkage group in the A or B genome (Mahmood et al. 2003a). ... shows the missing part of the linkage group A9 between its two segments. Abbreviations along the linkage groups show the positions of QTLs for different traits. Support interval at a LOD value of 2.4 for each QTL is given in brackets. *Negative* and *positive* signs show negative and positive (i.e. *opposite*) additive effects associated with the QTLs. *NS* number of siliques per main raceme, *SL* siliqua length, *SW* 1,000-seed weight, *SS* number of seeds per siliqua (Mahmood et. 2005b), E_{1b} = erucic acid (Mahmood et al. 2003a), *SC* seed color (Mahmood et al. 2005a), *GSL* total aliphatic glucosinolates (Mahmood et al. 2003b), *MSUM* sum of oil and meal protein contents, *MP* meal protein content, *SP* seed protein content

Fig. 1). Of the five QTLs for meal protein content, three (MP-A1, MP-A9, and MP-B6) were tightly linked with the QTLs for seed protein content (SP-A1, SP-A9, and SP-B6), and the linked/common QTLs had similar additive effects (Table 3, Fig. 1). Four of the QTLs for meal protein content (MP-A1, MP-A6, MP-A9, and MP-B5) had tight linkage with the QTLs for oil content (O-A1, O-A6, O-A9, and O-B5) (Fig. 1); however, two of them (MP-A6, MP-B5) had additive effects similar to those of the QTLs for oil content (O-A6, O-B5) (Table 3, Fig. 1), and two of them (MP-A1, MP-A9) had opposite additive effects compared to the additive effects of the QTLs for oil content (O-A1, O-A9) (Table 3, Fig. 1). Two QTLs for meal protein content (MP-A6, MP-B5) significantly affected sum of oil and meal protein contents (T-A6, T-B5), and the linked/common QTLs had similar additive effects (Table 3, Fig. 1).

Epistasis analysis

Significant epstatic interactions were found for all the quality traits (Table 4). In general, higher number of epistatic interactions was observed between A × B genome loci than A × A genome loci, and least number of interactions for B × B genome loci (Table 4). Compared to the effects of the QTLs (Table 3), the epistatic interactions were less consistent across the environments (Table 4). Three, six, eleven, and eight significant epistatic interactions were identified for oil, meal protein, seed protein, and sum of oil and meal protein contents, respectively (Table 4). The distribution of linked/common epistasis for the quality traits is shown in Fig. 2. One (A2 × A5) of the three epistatic interactions observed for oil (Table 4) was linked to one of the epistatic interactions for seed protein (Fig. 2), with the genotypic class 1Y/2Y having the

Table 4 Epistatic interactions for quality traits in *Brassica juncea* in the mean environment

Trait[a]	Loc1[b]	Loc2[b]	R^{2e}	ENV[f]	Digenic genotypes[g]			
					1B/2B (%)	1Y/2B (%)	1B/2Y (%)	1Y/2Y (%)
Oil	Wg1g6a (A2)[c]	ec4h9a (A5)	10.96	2	44.75[h] ab	43.30 bc	43.03 c	45.24 a
	Wg8g3 (A9)	tg2b4b (A7)	8.01	2	42.95 b	45.69 a	44.24 ab	43.77 b
	Wg4d7a (B4)	wg4c4b (A7)	9.03	2	44.33 b	47.89 a	43.67 b	43.43 b
SP	Wg6b4aNP (A2)	ec2b3a (A5)	15.00	3	22.74 bc	24.24 a	23.84 ab	22.33 c
	ec2e4cNM (A5)	ec3c8e (A3)	11.44	2	24.21 a	22.72 b	22.63 b	24.01 a
	Wg6f12aNP (A5)	tg1g9a (A8)	9.82	3	23.75 a	22.66 b	22.60 b	24.07 a
	ec2e4cNM (A5)	ec4d11a (B7)	10.34	2	24.38 a	22.56 b	23.78 ab	23.99 a
	Wg4c4b (A7)	wg6e1dNM (B6)	13.69	3	23.71 b	23.62 b	25.15 a	22.00 c
	ec5g4a (A9)	ec4h9b (A8)	12.70	3	24.12 a	23.34 a	22.11 b	24.05 a
	Wg7a11aNP (B2)	ec2h2d (D)[d]	9.78	2	24.19 a	22.99 ab	22.31 b	23.82 a
	ec2f1d (B3)	wg6c6b (A1)	7.25	2	23.81 a	24.04 a	22.31 b	24.71 a
	ec2b3b (B4)	wg8a11b (A1)	9.51	3	21.86 b	24.11 a	24.01 a	23.63 a
	Wg4d7a (B4)	wg1g10 (A3)	7.52	2	21.67 b	23.40 a	24.23 a	23.68 a
	ec3f4f (B4)	ec3g7c (A10)	7.76	2	22.13 b	23.51 a	24.42 a	23.41 a
MP	wg7f5a (A2)	wg6c3dNP (A5)	11.79	2	40.76 b	43.53 a	41.79 b	40.94 b
	ec2d9b (A2)	ec2h2a (B3)	9.79	3	40.66 b	42.96 a	42.21 ab	40.93 b
	ec3f4a (A5)	wg2g11a (A8)	8.18	3	42.52 a	40.90 b	41.19 ab	42.72 a
	wg4c4b (A7)	wg6e1dNM (B6)	8.04	3	42.35 b	42.10 b	43.95 a	40.53 c
	ec3g7a (B4)	wg6h1 (A9)	9.65	4	41.46 b	41.62 b	44.13 a	40.86 b
	wg6b4c (B4)	ec3g7c (A10)	9.36	3	14.17 b	42.60 ab	43.48 a	41.52 b
MSUM	ec2d9b (A2)	ec2h2a (B3)	11.37	2	83.43 b	87.56 a	87.23 a	85.93 a
	wg6f12aNP (A5)	ec2c12cNM (A9)	10.26	2	83.90 b	87.50 a	87.37 a	86.11 a
	ec3c8dNM (A6)	wg4d7b (A4)	7.02	2	88.57 a	85.39 b	84.00 b	84.99 b
	163I15T7a (A6)	wg7d9 (A6)	9.04	4	87.88 a	87.50 a	83.38 b	85.00 b
	wg7e6bNP (B4)	wg3h8a (A6)	11.44	3	89.91 a	83.42 c	86.21 b	85.09 bc
	wg4d7a (B4)	wg4c4b (A7)	9.06	4	85.67 b	89.32 a	86.54 b	85.21 b
	ec3g7a (B4)	wg7h2b (A9)	7.70	4	86.36 ab	87.99 a	87.50 a	84.80 b
	wg4d7a (B4)	ec3g12c(B4)	8.63	0	87.67 a	83.85 b	84.49 b	86.30 ab

[a] *MP* meal protein content, *SP* seed protein content, *MSUM* sum of oil and meal protein contents

[b] *Loc1* and *Loc2* Loci 1 and 2, respectively, involved in epistatic interaction

[c] Linkage group on which the interacting locus is present

[d] Unlinked segment in the genome of *Brassica juncea* (Mahmood et al. 2003a)

[e] R^2 variation explained by the epistatic interaction

[f] *ENV* environment(s) in which the epistatic interaction was significant

[g] *Y and B* homozygous genotypes of YSP (yellow-seeded parent) and BSP (black/brown-seeded parent) alleles, respectively, at the interacting loci, e.g. Y1/Y2 represents homozygous genotypes of YSP at interacting loci 1 and 2, respectively

[h] Means (%) associated with different digenic genotypes in digenic interactions. For each interaction, digenic means followed by same letter are statistically non-significant

highest mean for oil content and lowest mean for seed protein content (Table 4). Oil and sum of oil and meal protein contents had a common/tightly linked epistasis (B4 × A7) (Fig. 2) with genotypic class 1Y/2B having the highest means for oil and sum of oil and meal protein contents (Table 4). Meal protein also showed linked epistasis for oil (A2 × A5), seed protein (A2 × A5, A5 × A8, A7 × B6, and B4 × A10), and sum of oil and meal protein contents (A2 × B3, B4 × A9) (Fig. 2), and means of meal protein and other traits associated with different genotypes for these epistasis were in agreement with the type of association (i.e. negative or positive) of meal protein with oil, seed protein, and sum of oil and meal protein contents (Tables 2, 4).

Seed color and quality traits

Contents of oil and protein in the brown- and yellow-seeded DH lines are presented in Table 5. Yellow seeds had significantly higher oil content but lower seed protein content than brown/black seeds. No significant difference was found for meal protein content between these two seed color types (Table 5). However, sum of oil and meal protein contents were significantly higher in yellow seeds than in black/brown seeds (Table 5).

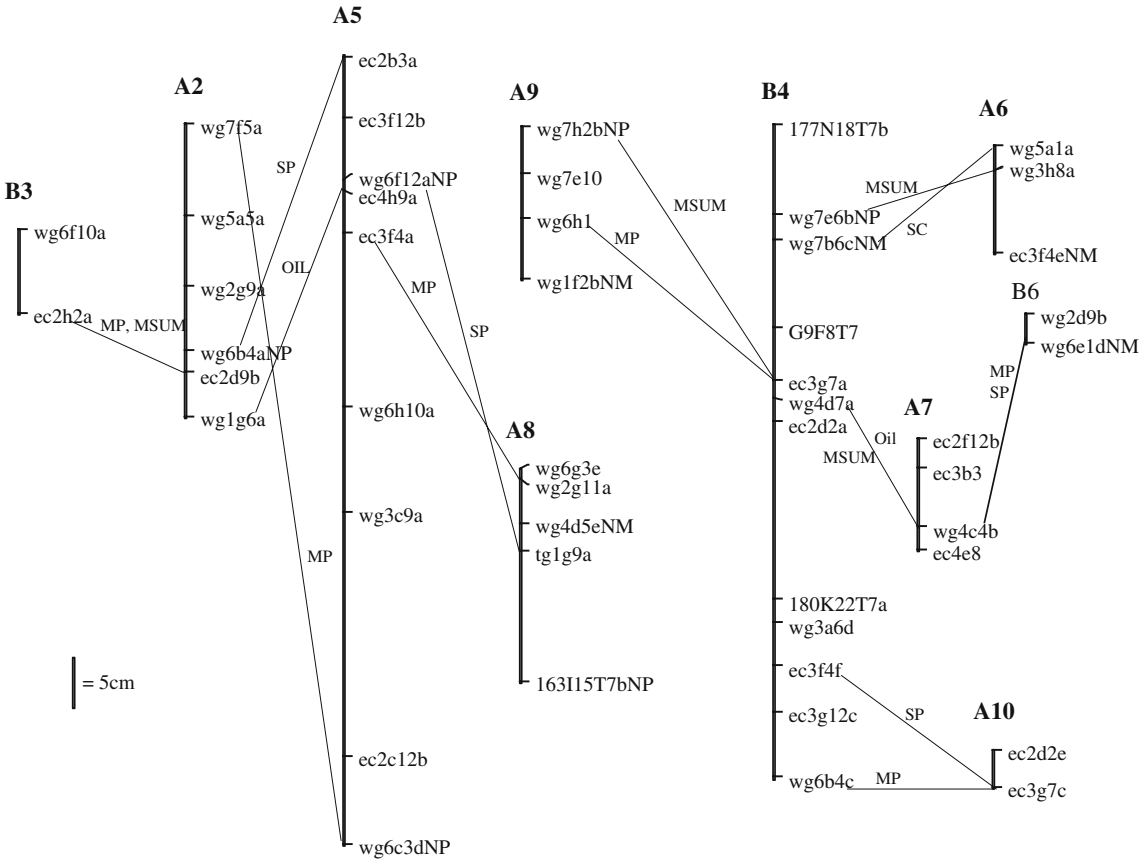

Fig. 2 Distribution of linked/common epistasis for seed quality traits in *Brassica juncea*. *A* and *B* refer to the A and B genomes of *Brassica juncea*, and numeric number refers to the linkage group in the A or B genome (Mahmood et al. 2003a). Lines connected the loci involved in epistasis. Abbreviations on the lines show the traits involved in epistasis. *SC* seed color (Mahmood et al. 2005a). *MSUM* sum of oil and meal protein contents, *MP* meal protein content, *SP* seed protein content

Table 5 Oil and protein contents of black/brown- and yellow-seeded DH lines of *Brassica juncea* in the mean environment

		OIL	SP	MP	MSUM	SSUM	RESI
Yellow	Mean ± S.E.	47.6 ± 0.53	22.3 ± 0.42	42.5 ± 0.53	90.1 ± 0.58	69.9 ± 0.32	25.1 ± 0.32
	Range	44.2–52.2	19.1–25.3	37.9–47.7	83.5–94.6	66.2–72.3	22.7–28.8
Black	Mean ± S.E.	43.4 ± 0.24	23.7 ± 0.20	41.8 ± 0.27	85.3 ± 0.34	67.1 ± 0.20	27.9 ± ‘0.20
	Range	35.6–48.1	20.0–29.1	35.9–47.6	73.1–91.2	59.8–70.4	27.7–33.8
Difference		+4.2**	–1.4*	$+0.7^{NS}$	+4.8**	+2.8**	–2.8**

All units are in %. *SP* seed protein content, *MP* meal protein content, *MSUM* sum of oil and meal protein contents, *SSUM* sum of oil and seed protein contents, *RESI* residual contents

*, ** Significant at a probability level of 0.05 and 0.01, respectively

Number of genes and quantitative trait loci for quality traits

The number of genes estimated for the quality traits confirmed their polygenic nature (Table 6). Highest number of genes was estimated for oil, sum of oil and seed protein, and sum of oil and meal protein contents (Table 6). However, the number of genes estimated by traditional quantitative genetic analysis did not match with the number of the QTLs identified by molecular analysis for any of the traits. Most striking difference was found for sum of oil and seed protein and sum of oil and meal protein contents (Table 6).

Discussion

This study presents a comprehensive report on the relationships of oil, seed, and meal protein, sum of oil and meal protein contents, as affected by erucic acid

Table 6 Number of genes estimated following traditional quantitative genetic analysis and QTLs significant (in parenthesis) at a LOD value of 2.4 for different seed quality traits in *Brassica juncea* in different environments

Trait[a]	Environments[b]			
	ERS99	ERS00	EL00	KE00
Oil	12 (6)	10 (6)	10 (6)	14 (6)
SP	9 (5)	9 (5)	7 (4)	7 (3)
MP	8 (5)	5 (5)	8 (5)	8 (4)
SSUM	13 (4)	9 (3)	11 (4)	13 (3)
MSUM	13 (4)	7 (3)	10 (4)	13 (3)

[a] *SP* seed protein content, *MP* meal protein content, *SSUM* sum of oil and seed protein contents, *MSUM* sum of oil and meal protein contents

[b] *ERS99* Edmonton Research Station 1999, *ERS00* Edmonton Research Station 2000, *EL00* Ellersile 2000, *KE00* Kelsey 2000

content, seed color, aliphatic glucosinolates and yield components, and the distribution of their common/linked QTLs and epistatic interactions on the A- and B-genomes of *B. juncea*. The tight linkage of the seed color QTLs (SC-A6 and SC-B4) (Mahmood et al. 2005a) with the QTLs for oil, seed and meal protein, and sum of oil and meal protein contents (Table 3, Fig. 1), as well as the tight linkage of epistasis for seed color (Mahmood et al. 2005a), with the epistasis for sum of oil and meal protein contents (A6 × B4) (Table 4, Fig. 2) strengthens earlier suggestions of pleiotropic effect of yellow seed color genes on oil and protein contents in the *Brassica* seeds (Jönsson and Bengtsson 1970). Erucic acid content was positively and significantly correlated with oil content (Table 2), and one of the two QTLs (E_{1b}) affecting erucic acid content (Mahmood et al. 2003a) was found tightly linked with one of the QTLs for oil content (Fig. 1). Similar results were also reported by Cheung and Landry (1998) in *B. juncea*. However, in case of *B. napus*, Ecke et al. (1995) reported that two QTLs for erucic acid content were linked with two of the QTLs for oil content. This suggests that in *B. juncea*, either only one of the two QTLs for erucic acid content was linked with one of the QTLs for oil content or regions surrounding one of the QTLs for erucic acid content were monomorphic for oil QTL in the present and previous studies (Cheung and Landry 1998).

Total aliphatic and 3-butenyl glucosinolates had positive correlation with oil and sum of oil and meal protein contents (Table 2). However, none of the QTLs for 3-butenyl glucosinolates (Mahmood et al. 2003b) were found to have any linkage with the QTLs for other seed quality traits. Therefore, the observed correlations appeared to be due to sampling error in the population used in the present study. In contrast, one of the QTLs for total aliphatic glucosinolates (GSL-B5) (Mahmood et al. 2003b) was tightly linked with the QTLs for oil and sum of oil and meal protein contents, while another QTL GSL-A7 had close linkage with the QTL for sum of oil and meal protein contents (Table 3, Fig. 1). However, none of these QTLs had been recommended for marker-assisted selection for lowering aliphatic glucosinolates in *B. juncea* (Mahmood et al. 2003b).

It is evident from the QTL analysis that some of the QTLs were affecting both seed oil and protein contents but in an opposite manner (Table 3). The strong negative association between seed oil and protein content (Table 2), their linked/common QTLs and epistasis with opposite effects (Tables 3, 4; Figs. 1, 2) found in the present and previous studies (Zhao 2002) poses potential challenges to plant breeders for simultaneous improvement of both oil and protein contents in the *Brassicas* (Grami and Stefansson 1977b; Zhao 2002). However, independent segregation of one QTL for oil content (O-B3) and two QTLs for protein content (SP-B6, SP-C) (Table 3) provides opportunity for simultaneous improvement of these two traits in *B. juncea*.

Meal protein is affected by seed protein, oil, and sum of oil and meal protein contents (i.e. meal protein = seed protein × $(100\text{-oil})^{-1}$ × 100). This is reflected in the present study by linkage of the QTLs (where all of the QTLs significant meal protein were also significant at least for oil, seed protein, or sum of oil and meal protein contents) (Table 3, Fig. 1) and epistasis for meal protein with other traits (Table 4, Fig. 2). The positive association (Table 2) of meal protein content with seed protein and sum of oil and meal protein contents was justified by similar additive effects of their linked QTLs (Tables 3, 4; Figs. 1, 2). The weak association between meal protein and oil contents was probably due to the fact that half of their linked QTLs had similar additive effects and half of them had opposite additive effects (Table 2, Fig. 1). The strong association between meal and seed protein contents is further strengthened by their four linked epistatic interactions with similar effects for both meal and seed protein contents (Table 4, Fig. 2), while weakly associated oil and meal protein contents had only one linked epistatic interaction with opposite effects for oil and meal protein contents (Table 4, Fig. 2).

Significant positive correlation between oil and sum of oil and meal protein contents (Table 2) was supported by their linked/common epistasis and QTLs with similar additive effects (Tables 3, 4; Fig. 1, 2).

Sum of oil and seed protein and sum of oil and meal protein contents had perfect positive correlation (data

not shown), same epistatic interactions (data not shown), and same QTLs with similar additive effects (data not shown). This suggests that estimating protein content in seed or meal will have the same meaning for simultaneous improvement of oil and protein contents in the *Brassica* seeds. However, meal protein is important in elaborating the relations among oil, residual, seed, and meal protein contents, especially when efforts are focused on improving oil with improved meal protein content. This can be explained by using a hypothetical model of *Brassica* seed with 40% oil, 25% seed protein, 41.67% meal protein, 30% residue at a moisture content of 5%. In this case, when oil content changed from 40 to 50%, it was found that (a) meal protein content remained the same if an increase in one unit of oil came at the expense of ~0.415 units of seed protein and ~0.585 units of residue, (b) meal protein content would decrease if an increase in one unit of oil came at the expense of more than 0.415 units of seed protein and less than 0.585 units of residue, (c) meal protein content would increase if an increase in one unit of oil came at the expense of less than 0.415 units of seed protein and more than 0.585 units of residue.

In the present study, improving black seed to yellow resulted in an increase of oil content by 4.2 units, and this increase in oil was at the expense of 1.4 units of seed protein and 2.8 units of residue (i.e. per unit increase in oil caused a reduction in seed protein and residual contents by 0.33 and 0.67 units, respectively) (Table 5). Using the aforementioned calculations, where hypothetical data were used, it was found that a unit increase in oil would increase meal protein content by 0.16 units, and thus, a sum improvement of meal protein content by 0.67 units in yellow seed was expected. The net improvement of meal protein content was 0.7 units in the present study (Table 5). This suggests that increasing oil in the *Brassica* seed does not necessarily decrease the meal protein provided that such an increase is compensated substantially (at least >59%) by residual contents.

It is generally believed that conversion of black seed to yellow results in lowering fiber (residual) contents (Rahman et al. 2001). Such decrease in fiber (residual) contents should increase both oil and seed protein contents, as all these seed components compete for the same source of carbon in the metabolic process. Our studies in *B. juncea* showed that converting black seed color to yellow had decreased not only residual contents but also seed protein contents (Table 5). Woods (1980) also reported 0.4% lower seed protein but 2.8% higher oil in yellow seeds than in black seeds in a population of *B. juncea* segregating for seed color. The present and previous studies show the complexity of oil, protein and fiber (residue) synthesis and potential challenges to plant breeders to improve oil content without compromising meal protein content.

Breeding for sum of oil and protein contents is recommended for simultaneous improvement of both oil and protein contents (Grami and Stefansson 1977b). In the present study, QTLs for sum of oil and meal protein contents (Table 3) are also recommended for marker-assisted selection. T-B4 and T-A6 were tightly linked with the QTLs for seed color (SB-B4, SC-A6) (Mahmood et al. 2005a) and T-B5 with the QTL for erucic acid (E_{1b}) (Mahmood et al. 2003a) (Fig. 1); these QTLs will be automatically selected while developing yellow-seeded, canola quality *B. juncea*. It is interesting to note that QTL/s for seed color and erucic acid had positive and negative pleiotropic effects, respectively, on sum of oil and meal protein contents (Table 3, Fig. 1) (Mahmood et al. 2003a, 2005a). Therefore, while reducing erucic acid content in *B. juncea*, sum of oil and meal protein contents would be compromised. However, improving seed color from black to yellow would result in increasing sum of oil and meal protein contents. T-A7 is the only QTL for sum of oil and meal protein contents that can be suggested for marker-assisted selection. The QTL T-A7 was consistently significant in all environments for oil, seed and meal protein contents (with similar additive effect), however, at a LOD value less than two (data not shown). The QTL T-A7 was linked with the QTL SW-A7 for 1,000-seed weight (Mahmood et al. 2005b) and both the QTLs had similar (negative) additive effect (Fig. 1). This strengthens the previous findings of positive impact of heavier seed on sum of oil and meal protein contents (Dawn and DeClerq 1988). The QTLs like SP-B6 & SP-C for seed protein content and O-B3 for oil content (Table 3) are also recommended for MAS because of their independent segregation (Fig. 1). The epistasis such as B4 × A7 for oil and sum of oil and meal protein contents, B4 × A9 and A2 × B3 for meal protein, and sum of oil and meal protein contents (Table 4) could potentially be considered for marker-assisted selection.

Butruille et al. (1999) reported that the number of QTLs detected for days to flowering, plant height, 1,000-seed weight and seed yield in *B. napus* were in the range of number of effective factors calculated by using biometrical methods. However, no such pattern was found in the present study (Table 6). Such relationships are ambiguous. In QTL analysis, change in critical LOD value can change the number of QTLs significant for a parameter. Furthermore, in practice, it is difficult to meet all the underlying assumptions for

calculating the number of genes for a trait, i.e., equal effect of individual genes, absence of epistasis, opposite extreme of segregating populations containing all increasing and decreasing alleles in a small sample, etc.

Significant genotypes × environment interactions were found for the quality traits (data not shown), however, without any significant change in the ranking of the DH lines in different environments. The correlations between the rankings of the DH lines in any two environments were positive and significant, with co-efficient of correlation 0.69** to 0.96**. This suggests that once the quality traits are fixed, these can be maintained in different environments.

References

Burton W, Salisbury P, Potts D (2003) The potential of canola quality *Brassica juncea* as an oilseed crop for Australia. In: Proceeding of the 11th international rapeseed congress, vol 1. Copenhagen, pp 5–7

Butruille DV, Guries RP, Osborn TC (1999) Linkage analysis of molecular markers and quantitative trait loci in populations of inbred backcross lines of *Brassica napus* L. Genetics 153:949–964

Cheung WY, Landry BS (1998) Molecular mapping of seed quality traits in *Brassica juncea* L. Czern. and Coss. Acta Hortic 459:139–147

Daun JK, Clear KM, Williams P (1994) Canadian grain commission, Grain Research Lab, Winnipeg, Manitoba, R3C 3G8. JAOCS 71:10

Dawn JK, DeClercq DR (1988) Quality of yellow and dark seeds in *Brassica compestris* canola varieties of Candle and Tobin. JACCS 65:122–126

Downey RK (1990) *Brassica* oilseed breeding-acheivements and opportunities. Plant Breed Absct 60:1165–1170

Ecke W, Uzunova M, Weißleder K (1995) Mapping the genome of rapeseed (*Brassica napus* L.). II. Localization of genes controlling erucic acid synthesis and seed oil content. Theor Appl Genet 91:972–977

Grami B, Stefansson BR (1977a) Gene action for protein and oil content in summer rape. Can J Plant Sci 57:625–631

Grami B, Stefansson BR (1977b) Genetics of protein and oil content in summer rape: Heritability, numbers of effective factors, and correlations. Can J Plant Sci 57:937–943

Jansen RC, Stam P (1994) High resolution of quantitative traits into multiple trait via interval mapping. Genetics 136:1447–1455

Jönsson R, Bengtsson R (1970) Yellow-seeded rape and turnip rape. I. Influence of breeding for yellow seeds upon yield and quality parameters. Sver Utsadesforen Tidskr 80:149–155

Krzymanski J (1998) Agronomy of oilseed *Brassicas*. Acta Hortic 459:55–60

Li Z, Pinson SRM, Park WD, Paterson AH, Stansel JW (1997) Epistasis for three grain yield components in rice (*Oryza sativa* L.). Genetics 145:453–465

Mahmood T, Ekuere U, Yeh F, Good AG, Stringam GR (2003a) RFLP linkage analysis and mapping genes controlling the fatty acid profile of *Brassica juncea* using reciprocal DH populations. Theor Appl Genet 107:283–290

Mahmood T, Ekuere U, Yeh F, Good AG, Stringam GR (2003b) Molecular mapping of seed aliphatic glucosinolates in *Brassica juncea*. Genome 46:753–760

Mahmood T, Rahman MH, Stringam GR, Raney JP, Good AG (2005a) Molecular markers for seed color in *Brassica juncea*. Genome 48:755–760

Mahmood T, Rahman MH, Stringam GR, Yeh F, Good AG (2005b) Molecular markers for yield components in *Brassica juncea*—do these assist in breeding for high seed yield? Euphytica 144:157–167

Newkirk RW, Classen HL, Tyler RT (1997) Nutritional evaluation of low glucosinolate mustard meals (*Brassica juncea*) in broiler diets. Poult Sci 76:1272–1277

Rahman MH, Joersbo M, Poulson MH (2001) Development of yellow-seeded *Brassica napus* of double low quality. Plant Breed 120:473–478

Rakow G (2003) The potential of *Brassica juncea*, *B. carinata* and *Sinapis alba* as new oilseed crops in Canada. In: Proceeding of the 11th international rapeseed congress, vol 1, Copenhagen, p1

SAS Institute Inc. (1989) SAS/STAT. Cary, NC, USA

Si P, Mailer JM, Galwey N, Turner DW (2003) Influence of genotype and environment on oil and protein concentrations of Canola (*Brassica napus* L.) grown across southern Australia. Aust J Agric Res 54:397–407

Snap JW, Wright AJ, Simpson E (1984) Methods for estimating genes number for quantitative character using doubled haploid lines. Theor Appl Genet 67:143–149

Thiagarajah MR, Stringam GR (1993) A comparison of genetic segregation in traditional and microspore-derived populations of *Brassica juncea* L. Czern and Coss. Plant Breed 111:330–334

Van Ooijen JW, Maliepaard C (1996) MapQTL™ Version 3.0: software for the calculation of QTL position on genetic maps. CRO-DLO, Wageningen

Woods DL, Capcara JJ, Downey RK (1991) The potential of mustard (*Brassica juncea*) as an edible crop on the Canadian prairies. Can J Plant Sci 71:195–198

Woods DL (1990) The association of yellow seed coat with other characters in mustard *Brassica juncea*. Crucifer News L 5:23–24

Zhao J (2002) QTLs for oil content and their relationships to other agronomic traits in an European x Chinese oilseed rape population. PhD thesis, faculty of agricultural sciences, George-August Uni Göttingen, Germany