Theor Appl Genet (2003) 107:283–290
DOI 10.1007/s00122-003-1244-x

T. Mahmood · U. Ekuere · F. Yeh · A. G. Good · G. R. Stringam

RFLP linkage analysis and mapping genes controlling the fatty acid profile of *Brassica juncea* using reciprocal DH populations

Received: 8 July 2002 / Accepted: 5 November 2002 / Published online: 28 March 2003

Abstract An RFLP linkage map, comprising 300 linked and 16 unlinked loci, was constructed using reciprocal DH populations of *Brassica juncea*. The linked loci were organized into 18 linkage groups and seven unlinked segments, covering a total map distance of 1,564 cM. The A and B genomes were identified. The χ^2 test showed that 96.1% of the common intervals in the two populations differed non-significantly for recombination fractions, thus strongly suggesting the absence of sex-based differences for recombination fractions in *B. juncea*. Two QTLs, E_{1a} and E_{1b}, significantly affected erucic acid content, and individually explained 53.7% and 32.1%, respectively, and collectively 85.8% of the phenotypic variation in the population. The QTLs E_{1a} and E_{1b} showed epistasis, and the full model including epistasis explained nearly all of the phenotypic variation in the population. The QTLs E_{1a} and E_{1b} were also associated with contents of oleic, linoleic and linolenic acids. Three additional QTLs (LN_2, LN_3 and LN_4) significantly influenced linolenic acid content. The QTL LN_2 accounted for 35.4% of the phenotypic variation in the population. Epistatic interactions were observed between the QTLs E1a and LN_2. The stability of the detected QTLs across years and locations, and breeding strategies for improving the fatty acid profile of *B. juncea*, are discussed.

Keywords *Brassica juncea* · RFLP · Recombination fraction · QTL · Marker-assisted selection

Communicated by J. Dvorak

T. Mahmood
Lembke Research Ltd, P.O Box 2499, Morden, MB, R6M 1C2

U. Ekuere · A. G. Good
Department of Biological Sciences, University of Alberta, Edmonton, Alberta, T6G 2E1

F. Yeh
Department of Renewable Resources, University of Alberta, Edmonton, Alberta, T6G 2H1

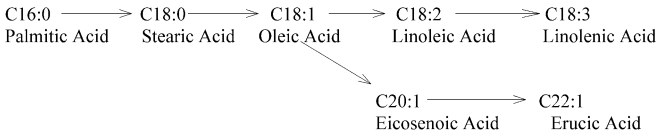
G. R. Stringam (✉)
Department of AFNS, University of Alberta, Edmonton, Alberta, T6G 2P5
e-mail: gary.stringam@ualberta.ca
Tel.: +780-492-3869
Fax: +780-492-4265

Introduction

In North America, *Brassica rapa* and *Brassica napus* are the two major species currently grown as canola (erucic acid <%2, and glucosinolate <30 micromoles/g of oil-free seed). However, because of its superiority to existing canola species for specific agronomic traits (i.e. high yield potential, drought tolerance and superior blackleg resistance), condiment *Brassica juncea* is of interest to plant breeders and has been converted into a canola type (Love et al. 1990; Woods et al. 1991). Conventional canola oil has 55–65% oleic acid, 14–18% linoleic acid and 8–12% linolenic acid. *B. juncea* oil has 50–60% erucic acid, 10–15% oleic acid, 10–15% linoleic acid and 14–16% linolenic acid. High levels of erucic acid are associated with health problems (Beare et al. 1963). Oleic and linoleic acids are considered to be neutral fatty acids, and one of the major objectives in many *Brassica* breeding programs is to maximize their content. Linolenic acid is undesirable because its three double-bond structure pre-disposes it to oxidation, resulting in off-flavors and reduced shelf life (Galliard 1980). To convert non-canola type *B. juncea* into a canola type, the entire fatty acid profile requires alteration, as was the case with *B. napus* (Woods et al. 1991).

Marker-assisted selection (MAS) is an important tool for plant breeders to increase the efficiency of a breeding program, especially for traits controlled by many genes. Thus, MAS would particularly be valuable in improving the fatty acid profile and agronomic traits of *B. juncea*. QTLs associated with erucic acid content have been identified in *B. napus* (Jourdren et al. 1996; Thormann et al. 1996) and *B. juncea* (Cheung et al. 1998), and the gene (FAE1, fatty acid elongase) has been cloned in *B. napus*

(Roscoe et al. 1996). A major QTL significantly affecting linolenic acid content in *B. napus* (Thormann et al. 1996) has also been cloned in *Arabidopsis* (FAD3, omega-3 desaturase) (Arondel et al. 1992). In *B. napus*, genes controlling erucic acid content also control the inheritance of eicosenoic acid content acting dominantly (Kondra and Stefansson 1965). A simplified biosynthetic pathway for major fatty acids (modified from Downey and Rakow 1987) is shown below:



In designing crossing schemes to introgress specific traits, the recombination frequencies observed in a particular cross can be affected by factors such as the distance of loci from centromeres, intra- or inter-specific crosses (Paterson et al. 1990), genetics (Baker et al. 1976), environment (Elliot 1955) and sex-dependence (Lagercrantz and Lydiate 1995). In the presence of sex-dependent differences, the incorrect choice of the direction of parents in a cross can seriously affect MAS (Young and Tanksley 1989). Although most plants are hermaphroditic, distinguishable differences in recombination frequencies in male and female meioses are present in crops such as maize (*Zea mays*) (Robertson 1984), wheat (*Triticum aestivum*) (Wang et al. 1995) and *Brassica oleracea* (Kearsey et al. 1996). Earlier investigations for such differences have been limited to specific segments of chromosomes, because of the availability of only morphological markers and difficult chiasma formation studies (Robertson 1984). With available molecular markers, these problems have been resolved, and whole genomes can be studied for such differences (Wang et al. 1995; Kearsey et al. 1996; Kelly et al. 1997).

The primary objective of the present study was to develop a detailed RFLP genetic map of *B. juncea*, to find QTLs associated with the fatty acids in *B. juncea*, and analyze sex-dependent differences in recombination frequencies. These QTLs can then be utilized for marker-assisted selection to improve the fatty acid profile of *B. juncea*.

Materials and methods

Mapping population

The mapping population originated from a cross and its reciprocal between two *B. juncea* lines, a non-canola highly inbred Indian cultivar RLM-514 (designated as the high-erucic acid, high-glucosinolate parent, HEP) and a canola quality inbred line (low-erucic acid, low-glucosinolate parent, LEP). Sixty one doubled-haploid (DH) lines were produced from a single F_1 plant (original cross) (S population), and 51 from seven F_1 plants (reciprocal cross) (R population) (Thiagaragah and Stringam 1993). The R and S populations together were designated as the C population.

Phenotypic data

In 1999, a field trial was conducted at the Edmonton Research Station, Alberta, Canada, using three replications. Each plot consisted of four rows, 6-m long and 0.3-m row-spacing. In 2000, trials were conducted at three sites, the Edmonton Research Station, Ellerslie and Kelsey, all in Alberta. In 2000, two replications were seeded, and the plot size was reduced to three rows, 4-m long and 0.3-m row-spacing due to shortage of self-pollinated seed. A randomized complete block design was used at each site. Taking into consideration the large size of the experiment, each replication was randomly divided into four sets. Two parental lines and 112 DH lines were randomly nested into four sets. Each set contained RLM-514 (HEP) as a check. Thus, one set contained 30 entries and three sets 29 entries each. Each set contained the same DH lines in each replication but in a different arrangement due to randomization. The data were analyzed according to the model:

$$Y_{ijkm} = \mu + R_i + S_j + RS_{ij} + L(S)_{k(j)} + \in_{ijkm},$$

where R = replications, i = 1 to 3, S = sets, j = 1 to 4, L = lines, and k = 1 to 30.

Self-pollinated seeds from parental and DH lines were analyzed for their fatty acid profile by using the ISO 5508 (1990) method.

Construction of linkage map

Self-pollinated seed of the DH and parental lines were grown in the greenhouse for DNA extraction. DNA extraction, restriction enzyme digestion, gel electrophoresis and alkaline transfer were carried out as described by Sharpe et al. (1995). RFLP clones (names starting with ec, wg, tg) were provided by T.C. Osborn, University of Wisconsin, USA. RFLP probe d3t7 was developed by A.G. Good. The remainder of the RFLP probes were ESTs described by Sillito et al. (2000). Of 229 probes screened for polymorphism using DNA digested with five restriction enzymes (*Eco*RI, *Bam*H, *Hin*dIII, *Xba*I and *Eco*RV), 132 were used to map RFLPs in the two populations on the basis of easily scorable bands (data not shown). Linkage analysis was carried out using Mapmaker version 3.0 (Lander et al. 1987). Initially, an LOD score of 5 and a distance of 10 cM were used to form the initial linkage groups. "Order", "Sequence" and "Try" commands were used to construct marker positions of individual groups. In order to bridge the large gaps between markers, LOD score and distance were reduced to 3 and 40 cM, respectively. Double cross-overs, especially in short intervals, were checked for scoring errors. The Kosambi mapping function (Kosambi 1944) was used to convert recombination frequencies into map distances.

QTL analysis

MapQTL (version 3.0) (Van Ooijen and Maliepaard 1996) was employed for QTL analysis using the MQM approach (Jansen and Stam 1994). This approach has two steps. The first step involves finding putative QTLs using interval mapping. A LOD value of 2.4 was chosen as the threshold to declare the presence of a putative QTL. In the second step, markers close to QTLs were selected as co-factors, thus leading towards a multiple-QTL model.

Results

Genetic map

Three hundred and sixteen loci were scored from the 132 informative probes. However, some of the loci were not used in the two populations due to a larger proportion of data missing from one population. Overall, 276 loci could be mapped in the R population and 307 in the S population,

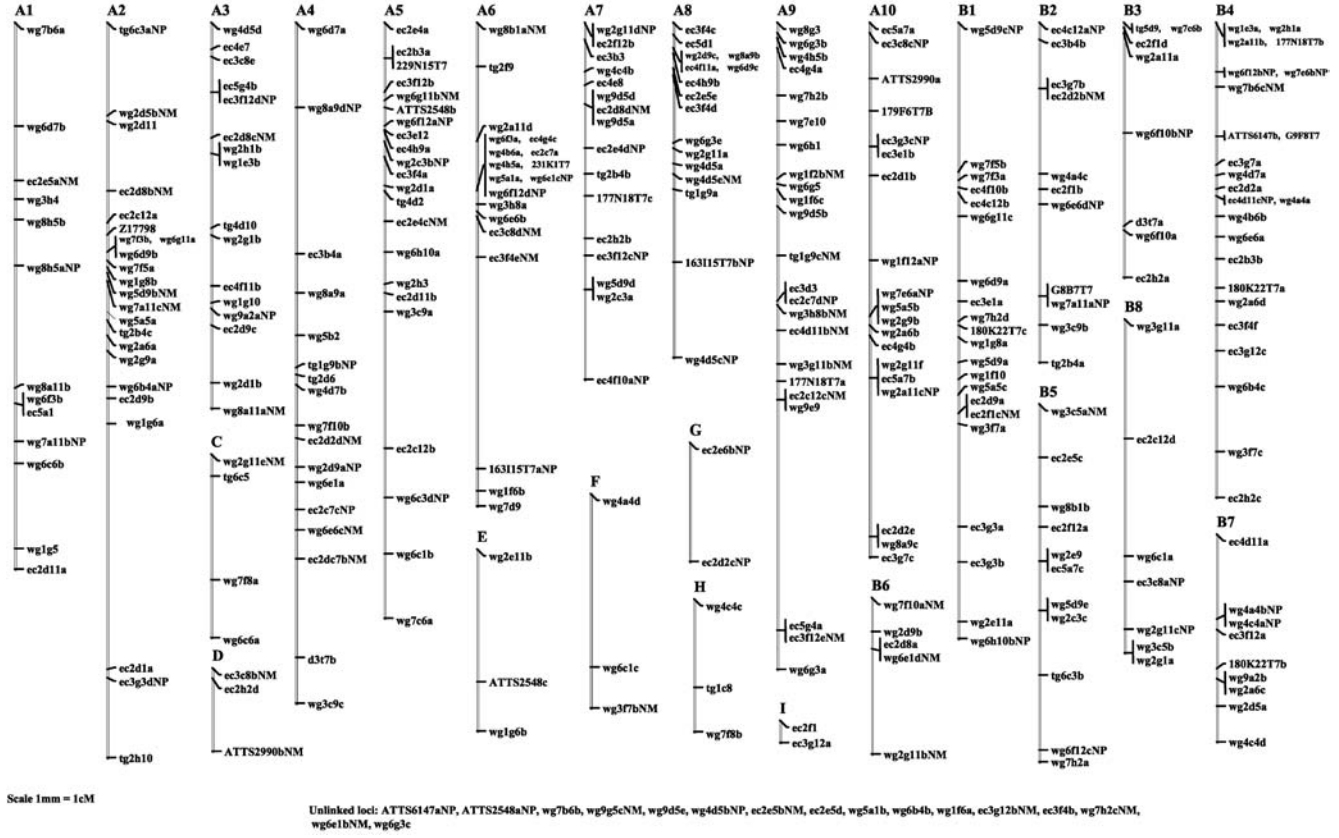


Fig. 1 Genomic linkage map of *B. juncea*. *A1–A10* = A genome corresponding to *N1–N10* of *B. napus* (Butruille et al. 1999), *B1–B8* = B genome, C-I = unlinked segments

and 267 loci were common in the two populations. The maps derived from the two populations were homogeneous (discussed below); hence, the two populations were combined to obtain a single map (Fig. 1). Of 316 loci mapped in the C population, 280 were assembled into 18 linkage groups (A1–A10, B1–B8), 20 into seven small segments (C-I) and 16 remained unlinked (Fig. 1). The total map distance was 1,564 cM, with an average distance of 5.2 cM per locus. The majority of RFLP loci were characterized by two alleles; however, 85 of 316 loci were scored as null for HEP or LEP due to missing bands. The symbols NP (null for HEP) or NM (null for LEP) were assigned to these loci (Fig. 1). On average, 2.4 loci were scored per probe. Of 132 probes used, 35 gave one polymorphic locus and 97 were duplicated. Of the 97 probes, ten depicted intra-chromosomal duplication and 87 were involved in inter-chromosomal duplication.

Reciprocal recombination differences

The difference in the frequency of meiotic recombination in F_1s from the original cross and its reciprocal was investigated in three different ways: (1) on an individual interval basis, (2) on a whole linkage group basis, and (3) on a whole genome basis.

There were 233 intervals flanked by loci common in the S and R populations. The χ^2 test of heterogeneity (α = 5%) was applied to determine the homogeneity of the two maps (Table 1). Ten DH lines were randomly removed from the S population to adjust its size to that of the R population. All χ^2 values were non-significant for whole-genome and individual intervals except nine intervals (3.9%). All linkage groups and unlinked segments, except unlinked segment E, exhibited non-significant χ^2 values (Table 1). This strongly suggested that the two maps derived from the S and R populations were essentially the same, and could be integrated into a single map.

Identification of the A and B genomes

B. juncea, an amphidiploid (AABB), contains the genomes of two diploid ancestors, *Brassica rapa* (AA) and *Brassica nigra* (BB) (U 1935). The A genome (N1-N10) of *B. napus* (Butruille et al. 1999) was used to identify the A genome (A1–A10) of *B. juncea* (Fig. 2a). The B genome was named as B1–B8, and its homoeologous relationships are shown in Fig. 2a. B3 has only one locus common with A5. Gene order was conserved in most cases; however, rearrangements were also observed. Frequent rearrangements could be seen in intra-genomic comparisons (Fig. 2b). No

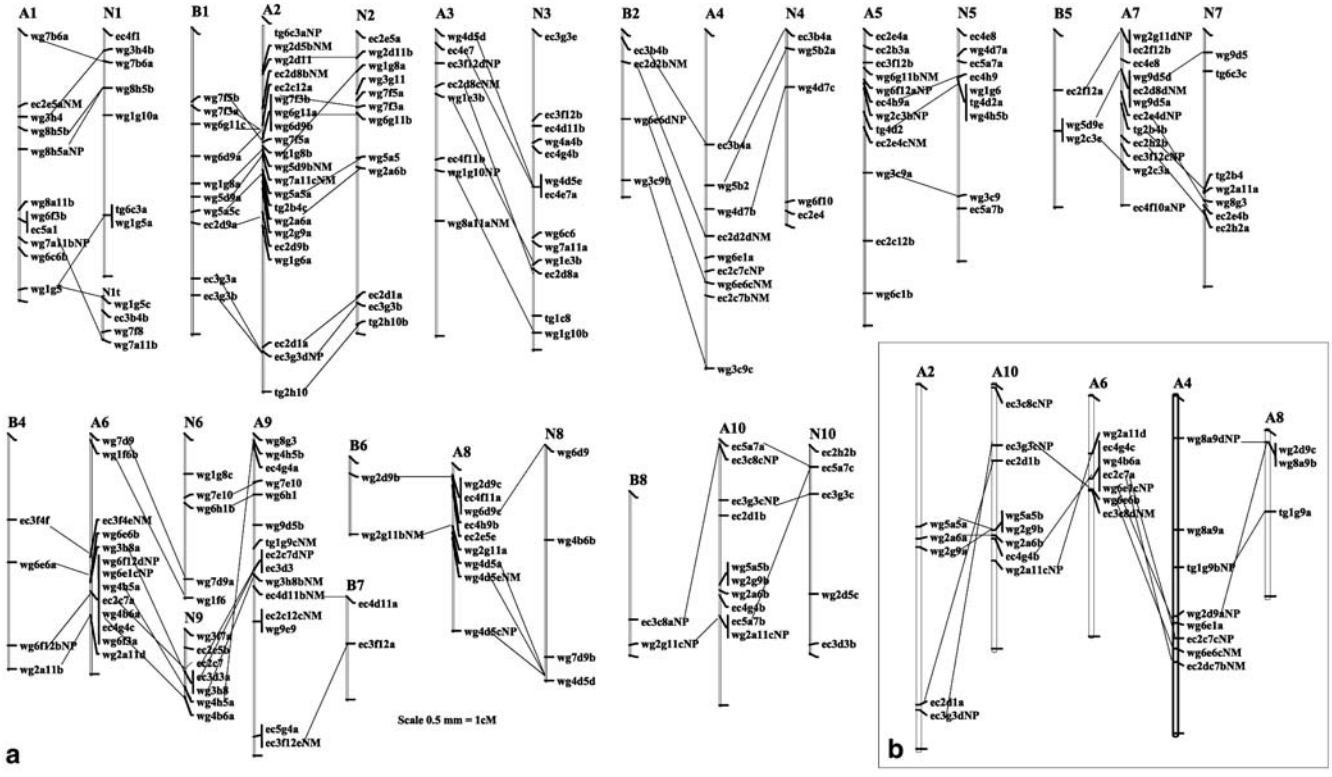


Fig. 2 Identification of A genome, inter- (**a**) and intra-genomic relationships (**b**) in *B. juncea*. *N1–N10* = A genome of *B. napus* (Butruille et al. 1999), *A1–A10* = B genome of *B. juncea* (corresponding to *N1–N10*), *B1–B8* = B genome of *B. juncea*

Table 1 Chi-square test for heterogeneity of two maps developed from populations derived from a cross and its reciprocal in *B. juncea*

LK[a]	(χ^2) Dev.[b]	(χ^2) Het.[c]	*df*[d]	Intervals significantly differing for recombination fraction in two populations
A2	1.09	24.8	19	wg2g9a-wg6b4aNP, wg1g6a-ec2d1a
A4	2.86	22.54	12	wg6d7a-wg8a9dNP, wg5b2-tg1g9bNP
A10	3.06	10.58	11	179F6T7B-ec3g3cNP
B2	0.69	11.08	7	wg6e6dNP-G8B7T7
B4	0.05	21.09	22	ec3f4f-ec3g12c
B5	0.00	14.41	9	wg3c5aNM-ec2e5c
!E	0.47	4.18	1	ATTS2548c-wg1g6b
Overall	0.30	209.86	232	

[a] LK = linkage group (χ^2)
[b] Dev = chi-square value for deviation from the 1:1 ratio
[c] (χ^2) Het = chi-square value for heterogeneity
[d] *df* = degrees of freedom
! = unlinked segment

generalization regarding the distances between the conserved loci could be made in the comparisons.

Inheritance of, and QTLs associated with, erucic, oleic linoleic and linolenic acids

The HEP, LEP and F_1 had erucic acid contents of 50.8%, 0.6% and 34.5% respectively. The 112 DH lines were distributed into three classes: low (0–5%), medium (25–40%) and high (40–55%) with 22, 55 and 35 lines respectively, and followed a 1:2:1 ratio (χ^2 = 3.05, p = 0.25–0.10). For oleic acid, the low (7–11%), medium (11–25%) and high (25–48%) classes followed a 28:63:21 (i.e. 1:2:1) ratio (χ^2 = 2.63, p = 0.25–0.5). For linoleic acid, a 1:2:1 (22:58:32) ratio (χ^2 = 1.93. p = 0.25–0.5) was observed for low (<18%), medium (18–22%) and high (>22%) classes. This suggested that contents of erucic, oleic and linoleic acids were determined by two genes acting in an additive manner. The DH population followed a near-normal distribution for linolenic acid, and exhibited transgressive segregation. This indicated that linolenic acid was inherited as a quantitative trait (data not shown).

Table 2 Genetics of QTLs for fatty acids in *B. juncea* in the mean environment

Acid	QTL	LK	Dis. (cM)	Flanking loci	LOD	σ_P^{2a}	Tot. σ_P^{2b}	Add.	Hom.	Epistasis	
										($E_{1a} \times E_{1b}$)	($E_{1a} \times LN_2$)
Erucic	E_{1a}	A8	0	ec4h9b-ec2e5e	38.26	53.7	85.8	•–12.15	BN15, BN7	!0.98	–
	E_{1b}	B5	5	wg3c5aNM-ec2e5c	27.16	32.1		–9.25			
Oleic	E_{1a}	A8	0	ec4h9b-ec2e5e	22.17	42.9	70.9	♦8.04		0.97	–
	E_{1b}	B5	0	wg3c5aNM-ec2e5c	16.65	28.0		6.32			
Linoleic	E_{1a}	A8	0	ec4h9b-ec2e5e	18.60	36.4	68.3	3.25		0.85	–
	E_{1b}	B5	5	wg3c5aNM-ec2e5c	16.02	31.9		2.98			
Linolenic	E_{1a}	A8	0	ec4h9b-ec2e5e	8.60	15.1	76.4	1.11		0.38	
	E_{1b}	B5	5	wg3c5aNM-ec2e5c	7.43	14.2		1.05			
	LN_2	A4	5	wg4d7b-wg7f10b	14.02	35.4		–1.70	BN14		0.60
	LN_3	A7	0	tg2b4c-wg2a6a	4.48	7.5		0.76			
	LN_4	A5	0	tg4d2-ec2e4cNM	2.52	4.2		–0.55			

[a] σ_P^2 = % of total phenotypic variance explained by individual loci
[b] Tot. σ_P^2 = % of total phenotypic variance explained by all QTLs
LOD = Lod value associated with detected QTL; Add. = additive effect associated with detected QTL; Dis. = distance of the QTL from the first flanking marker; Hom. = homology to the other known loci, BN15, BN7, BN14 = linkage groups in the genetic map of *B. napus* (Thormann et al. 1996). •negative additive effect showed that contributing alleles orignated from HEP. ! variation (R^2, using two-way ANOVA) explained by epistasis. ♦positive additive effect showed that LEP alleles contributed to parameters at associated QTLs

Table 3 Summary inheritance of eicosenoic acid content in *B. napus*

Back cross	Genotypes	Eicosenoic acid (%)		Observed	Expected	χ^2 value	*p*
		Mean	Range				
(Low × F_1) Liho-Z × (Liho-Z × Nug-E)	$e_1e_1e_2e_2$	0.5	0–2	13	1	1.85	0.10–0.25
	{$E_1e_1e_2e_2$ $e_1e_1E_2e_2$ $E_1e_1E_2e_2$}	11.6	7–15	59	3		
(High × F_1) Nug-E × (Liho-Z × Nug-E)	$E_1e_1E_2e_2$ $E_1E_1E_2e_2$ $E_1e_1E_2E_2$ $E_1E_1E_2E_2$	15.3	11–22	79			

E, e = dominant and recessive alleles respectively controlling eicosenoic acid content, χ^2 value = chi-square value, *p* = probability, Nug-E, Liho-Z, Liho-Z x Nug-E = parents and F_1 used in the study. Nug-E ($E_1E_1E_2E_2$), Liho-Z ($e_1e_1e_2e_2$), and F_1 ($E_1e_1E_2e_2$) (i.e. Liho-Z × Nug-E) had erucic acid contents of 36.1%, 0% and 22.4% respectively, and eicosenoic acid contents of 13.3%, 1.8% and 15.4%, respectively (after Kondra and Steffansson 1965)

Two QTLs (E_{1a} and E_{1b}) significantly affected erucic, oleic and linoleic acids (Table 2), and together explained 85.8%, 70.9% and 68.3% of the phenotypic variation for the fatty acids, respectively, based on the the mean value over all environments. The QTLs showed strong epistasis, and this model explained approximately 98%, 97% and 85% of the variation for erucic, oleic and linoleic acids, respectively (Table 2).

The RFLP loci ec4h9b and wg3c5aNM were tightly linked to E_{1a} and E_{1b} respectively. Locus E_{1a} (ec4h9b) showed a highly significant distortion ($\chi^2 = 8.06$, $p < 0.005$) towards the HEP in the S population but followed a 1:1 ratio ($\chi^2 = 0.75$, $p = 0.25$–0.50) in the R population. Locus E_{1b} (wg3c5aNM) followed a 1:1 ratio in both the S and R populations ($\chi^2 = 0.33$, $p = 0.5$–0.75 and $\chi^2 = 0.017$, $p = 0.99$–1.00, respectively). Erucic acid content segregation followed a qualitative inheritance model. The genotypic classes were designated as follows: for the low class $e_{1a}e_{1a}e_{1b}e_{1b}$ (alleles from the LEP), for the high class $E_{1a}E_{1a}E_{1b}E_{1b}$ (alleles from the HEP) and for the intermediate class either $E_{1a}E_{1a}e_{1b}e_{1b}$ or $e_{1a}e_{1a}E_{1b}E_{1b}$. The loci E_{1a} (ec4h9b) and E_{1b} (wg3c5aNM) differed significantly for their contribution to erucic acid content (data not shown).

Five QTLs (E_{1a}, E_{1b}, LN_2, LN_3 and LN_4) significantly affected linolenic acid (Table 2). The proportion of the total phenotypic variation explained individually by these QTLs varied from 4.2 to 35.4%, and collectively 76.4% based on the mean value over all environments. Epistasis existed between E_{1a} and E_{1b}, and between E_{1a} and LN_2. The epistatic models explained more variation than that explained by a multilocus model (Table 2).

It is noteworthy that the QTLs (E_{1a}, E_{1b}) associated with oleic, linoleic and linolenic acids were the same as those associated with erucic acid. However, for oleic, linoleic and linolenic acids, the QTLs acted in an opposite manner, i.e. E_{1a} and E_{1b} decreased, while e_{1a} and e_{1b} increased the contents of oleic, linoleic and linolenic acids (Table 2).

Eicosenoic acid

In the biosynthetic pathway of fatty acids, oleic acid serves as the precursor and eicosenoic acid as the

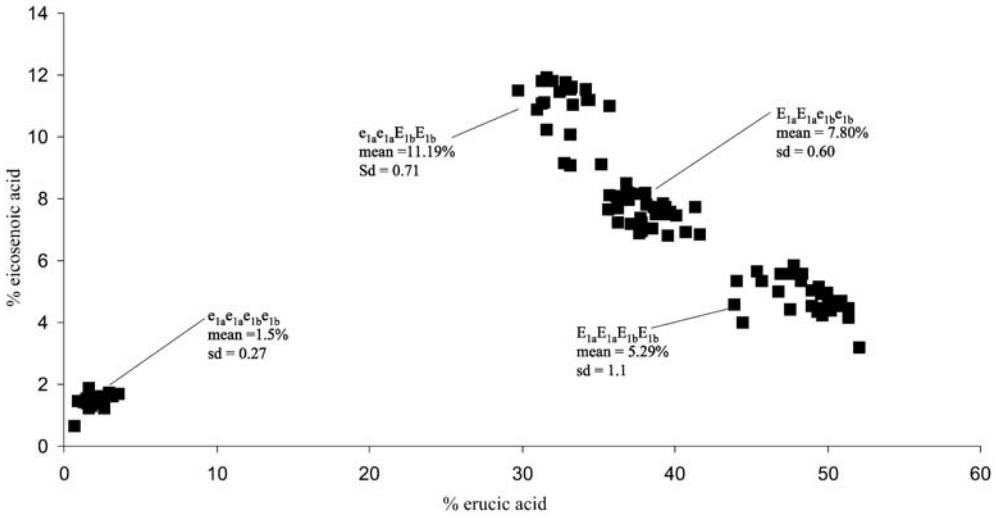


Fig. 3 Relationship between erucic and eicosenoic acid at varying erucic acid contents in a *B. juncea* DH populations segregating for erucic acid. $E_{1a}E_{1a}E_{1b}E_{1b}$, $e_{1a}e_{1a}E_{1b}E_{1b}$ and $E_{1a}E_{1a}e_{1b}e_{1b}$, and $e_{1a}e_{1a}e_{1b}e_{1b}$ show the eicosenoic acid content associated with high, medium and low classes of erucic acid respectively

intermediate product for erucic acid. Kondra and Stefansson (1965) reported that eicosenoic acid content was controlled by the same genes as for erucic acid, however, the genes showed complete dominance for eicosenoic acid content (Table 3).

The distribution of eicosenoic acid at different erucic acid contents in the present study indicated that the eicosenoic acid values formed clusters/groups, rather than following a continuous distribution pattern (Fig. 3). These four groups corresponded to four genotypes, $E_{1a}E_{1a}E_{1b}E_{1b}$ (high erucic acid), $e_{1a}e_{1a}e_{1b}e_{1b}$ (low erucic acid) (parental group) $E_{1a}E_{1a}e_{1b}e_{1b}$ and $e_{1a}e_{1a}E_{1b}E_{1b}$ (intermediate groups). These groups had a very small range and standard deviation, and differed significantly from each other (Fig. 3). Levels of erucic and eicosenoic acids had both a positive and significant correlation ($r = 0.31$**). Omitting the group associated with genotype $e_{1a}e_{1a}e_{1b}e_{1b}$, eicosenoic acid was negatively and significantly correlated with erucic acid ($r = -0.81$).

Discussion

The present study suggests that each allele controlling erucic acid content has more or less an equal potential of initiating the first step of chain elongation from 18:1 to 20:1. The difference in potential of producing erucic acid content is exhibited in the second elongation step, i.e. from 20:1 to 22:1. Thus, alleles with high potential of producing erucic acid convert most of the eicosenoic acid to erucic acid, and vice versa. The absence of effective alleles prevents the elongation step from 18:1 to 20:1. This is why zero erucic-acid cultivars have only traces of eicosenoic acid. The present study explains why the group with genotype $e_{1a}e_{1a}E_{1b}E_{1b}$ had a higher eicosenoic acid content than that of the group associated with genotype $E_{1a}E_{1a}e_{1b}e_{1b}$ because E_{1b} contributed less, as compared to E_{1a}, towards total erucic-acid content. The present report also suggests why low erucic-acid parents in our study and in Kondra and Stefansson's (1965) study had similar amounts of erucic acid, and why high erucic-acid parents in both studies had a different eicosenoic-acid content (i.e. because of different alleles of the erucic-acid gene with a different potential for producing erucic acid in the parents). In the present study, it was possible to clearly differentiate medium (i.e. low medium and high medium) and high classes both for erucic and eicosenoic acids using marker genotypes at the E_1 loci. In the earlier study (Kondra and Stefansson 1965), medium and high classes for eicosenoic acid were mixed, and taken as a single class (Table 3).

Our study appears to be the first to evaluate sex-based differences of recombination fractions in *B. juncea*. By using a χ^2 test ($\alpha = 5\%$) 3.9% (9/233) intervals were found to be significantly different for recombination fractions in the R and S populations (Table 1). Therefore, our results strongly suggest the absence of sex-based differences of recombination in *B. juncea*. Our results are further supported by the findings of Kelly et al. (1997) in *B. napus*. However, contrasting results have been published for *B. nigra* (Lagercrantz and Lydiate 1995) and *B. oleracea* (Kearsey et al. 1996). Indistinguishable and distinguishable patterns of recombination in different *Brassicas* might be species-specific (polyploidy level, phylogenetic origin) (Lagercrantz and Lydiate 1995).

The findings of the present study have important implications in genetic analysis and breeding strategies. Since recombination in the present study was independent of the direction of the cross, either parent could be used as male or female in a breeding program. Moreover, integrated maps of *B. juncea* could be developed from different crosses without consideration of male or female meioses. Map-based cloning requires that loci be mapped very finely and precisely in a particular interval. Our results suggest that the direction of the cross would not, therefore, affect the fine-mapping for map-based cloning in *B. juncea*.

Cheung et al. (1997) reported complex rearrangements in the *B. juncea* genome, suggesting that the *B. juncea* genome has undergone numerous rearrangements after polyploid formation. Axelsson et al. (2000) found complete colinearity of the genetic map of re-synthesized *B. juncea*, not only when compared to that of naturally occurring *B. jucea* but also to those of its diploid progenitor species, *B. rapa* and *B. nigra*. They concluded that the genomes of *B. juncea* and its diploid progenitor have remained essentially unchanged since polyploid speciation. Colinearity, with few rearrangements, was observed when the *B. juncea* genetic map was compared to that of *B. napus* (Butruille et al. 1999), and the A genome to the B genome in the present study (Fig. 2a). Complex re-arrangements could be noticed in the intra-genomic comparison for the A genome (A10, A2, A6, A4, A8) (Fig. 2b). This strengthens the earlier suggestion (Axelsson et al. 2000) that the complex rearrangements observed by Cheung et al. (1997) might have resulted from the inability to distinguish between inter- and intra-genomic comparisons. Lack of complete colinearity in the present study might be attributed to different plant materials, probes and experimental conditions in our studies. The re-synthesized amphidiploid *B. juncea* that was used in the Axelsson study (Axelsson et al. 2000) had not been propagated through self-pollinated seeds. Rearrangements, in the form of loss or gain of DNA segments, have been observed in a re-synthesized amphidiploid *B. juncea* following a few generations of self-pollination (Song et al. 1995).

For fatty acid analysis, our results confirmed the previous findings of a 2-gene model for the inheritance of erucic acid content with additive gene action in *B. juncea* (Kirk and Hurlstone 1983) and in *B. napus* (Chen and Beversdorf 1990). In a *B. napus* DH population segregating for erucic acid, Chen and Beversdorf (1990) found that the two genes for erucic acid controlled the accumulation of oleic acid. This is in agreement with the results in the present study. There are no published reports in *B. juncea* of the inheritance of linoleic acid content in populations segregating for erucic acid. We propose that the same two genes controlling erucic acid content also affect the quantity of linoleic acid. This is supported by our observation that the same two QTLs were associated with the levels of these two acids. However, as far as inheritance of the contents of erucic, linoleic and oleic acids is concerned, the genes acted in an opposite manner. This is confirmed by a significant negative correlation (r = 0.90–0.96) of erucic acid with oleic and linoleic acids, and a significant positive correlation between oleic and linoleic acids (r = 0.86). In the biosynthesis of these acids, oleic acid serves as the precursor for erucic and linoleic acids. When the erucic acid pathway is active, most of the oleic acid is converted into erucic acid and a small percentage desaturated to linoleic acid, thus resulting in a strong negative correlation of erucic acid with oleic and linoleic acids. Linolenic acid segregated as a quantitative trait. Five QTLs for linolenic acid were found, two of which were the same as for erucic acid. It appears that there are at least three additional genes controlling linolenic acid content in *B. juncea*. Similar findings have been reported in *B. napus* (Chen and Beversdorf 1990).

In the present study, no maternal effects were observed for any fatty acid in *B. juncea* (data not shown). Maternal effects have been reported in *B. napus* for oleic, linoleic and linolenic acids (Thomas and Kondra 1973; Diepenbrock and Wilson 1987; Pleines and Friedt 1989). This suggests that the maternal effects in the *Brassicas* may be species specific.

Epistatic relationships between the genes controlling linoleic acid content have been reported in *B. napus* (Rajcan et al. 1999); and, in the present study, we have shown that epistatic relationships exist between genes controlling additional fatty acids in *B. juncea*.

This study reports an unequal contribution of genes controlling erucic acid content in *B. juncea*. However, both an equal and unequal contribution of genes controlling erucic acid have been reported for *B. napus* (Krzymanski and Downey 1969; Jourdren et al. 1996). Krzymanski and Downey (1969) identified five alleles in *B. napus*, namely e, E^a, E^b, E^c and E^d, acting in an additive manner and contributing erucic acid contents of <1, 10, 15, 30 and 3.5%, respectively.

G × E interactions were observed for the fatty acids, however, the interactions had little impact on the ranking of DH lines in different environments, as the correlations among the ranking of the lines in any two environments for any fatty acid were always positive and significant ($r > 0.85$). Of the QTLs mapped for linolenic acid, LN4 could not be identified in the Ellerslie 2000 trial and the Kelsey 2000 trial. Also, the QTL could not be detected in a fixed interval on linkage group A5. The QTL LN3 could be detected in all environments, but its position on linkage group A2 changed in different environments. Effects of three QTLs (E_{1a}, E_{1b} and LN_2) were highly consistent across different environments, and could be very effectively deployed for MAS for the fatty acids in *B. juncea*.

Acknowledgements We express thanks to Dr. Reinhold Mayerhofer, for his valuable help in data analysis and interpretation, and Dr. T. C. Osborn, for providing RFLP probes. Financial support from the Canadian Wheat Board, Alberta Canola Producers Commission, and Dow Agro Sciences is gratefully acknowledged.

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