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ORIGINAL PAPER

Eliminating expression of erucic acid-encoding loci allows the identification of "hidden" QTL contributing to oil quality fractions and oil content in *Brassica juncea* (Indian mustard)

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Abstract Oil content and oil quality fractions (viz., oleic, linoleic and linolenic acid) are strongly influenced by the erucic acid pathway in oilseed Brassicas. Low levels of erucic acid in seed oil increases oleic acid content to nutritionally desirable levels, but also increases the linoleic and linolenic acid fractions and reduces oil content in Indian mustard (*Brassica juncea)*. Analysis of phenotypic variability for oil quality fractions among a high-erucic Indian variety (Varuna), a low-erucic east-European variety (Heera) and a zero-erucic Indian variety (ZE-Varuna) developed by backcross breeding in this study indicated that lower levels of linoleic and linolenic acid in Varuna are due to substrate limitation caused by an active erucic acid pathway and not due to weaker alleles or enzyme limitation. To identify compensatory loci that could be used to increase oil content and maintain desirable levels of oil quality fractions under zero-erucic conditions, we performed Quantitative Trait Loci (QTL) mapping for the above traits on two independent F1 doubled haploid (F1DH) mapping populations developed from a cross between Varuna and Heera. One of the populations comprised plants segregating for erucic acid content (SE) and was used earlier for construction of a linkage map and QTL mapping of several yield-influencing traits in *B. juncea*. The second population consisted of zero-erucic acid individuals (ZE) for which, an Amplified Fragment Length Polymorphism (AFLP)-based framework linkage map was constructed in the present study. By QTL mapping for oil quality fractions and oil content in the ZE population, we detected novel loci contributing to the above traits. These loci did not co-localize with mapped locations of the *fatty acid desaturase 2* (*FAD2), fatty acid desaturase 3* (*FAD3)* or *fatty acid elongase* (*FAE)* genes unlike those of the SE population wherein major QTL were found to coincide with mapped locations of the *FAE* genes. Some of the new loci identified in the ZE population could be detected as 'weak' contributors (with LOD < 2.5) in the SE population in which their contribution to the traits was "masked" due to pleiotropic effects of erucic acid genes. The novel loci identified in this study could now be used to improve oil quality parameters and oil content in *B. juncea* under zero-erucic conditions.

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Introduction

Brassica species, by virtue of their close relationship with the model plant, *Arabidopsis thaliana* and their economic potential as major oilseed and vegetable crops are a popular

resource for analyzing the genetics of complex traits in crop plants. Oil content and oil quality are two such quantitative traits that are major components of crop improvement programs in oilseed Brassicas. Oil quality of *Brassica juncea* (a major oilseed crop of the Indian subcontinent) and related Brassica species, viz., *B. napus* (extensively grown in Canada and Europe) and *B. rapa*, is determined by relative proportions of the constituent fatty acids, viz., erucic (C22:1), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acid. Many studies have shown significant correlations between erucic acid levels, oil content and other oil quality fractions of *B. napus* (Chen and Beversdorf 1990; Ecke et al. 1995), *B. rapa* (Tanhuanpaa et al. 1996, 1998) and *B. juncea* seeds (Cheung et al. 1998; Lionneton et al. 2002; Sharma et al. 2002; Ramchiary et al. 2007). A schematic representation of the biosynthetic inter-relationships of various oil quality fractions is given in Fig. 1. While reduced erucic acid levels in Brassica seeds lead to a nutritionally desirable increase in oleic acid (C18:1) content (Boulter 1983; Chang and Huang 1998), it also increases the linoleic (C18:2) and linolenic (C18:3) acid fractions. Increased oleic acid content in oil enhances its suitability as a cooking medium (Barker et al. 2007) but high levels of unsaturated fatty acids (viz., linolenic acid) increase susceptibility of the oil to oxidation, thereby reducing its shelf life (Browse et al. 1998; Lauridsen et al. 1999). Significantly, we have observed that under zero-erucic conditions, the seed oil content is reduced by as much as 3% in *B. juncea* (unpublished data). The economic ramifications of this reduction in oil content are enormous given the fact that *B. juncea* is cultivated over 6 million hectares of land in India.

Candidate genes associated with synthesis of the above oil quality fractions and oil content have been identified earlier using induced mutants (in *Arabidopsis*) and contrasting variability in germplasm of oilseed Brassicas wherever available (Horiguchi et al. 2001; Tanhuanpaa and Schulman 2002; Delourme et al. 2006; Li et al. 2006).

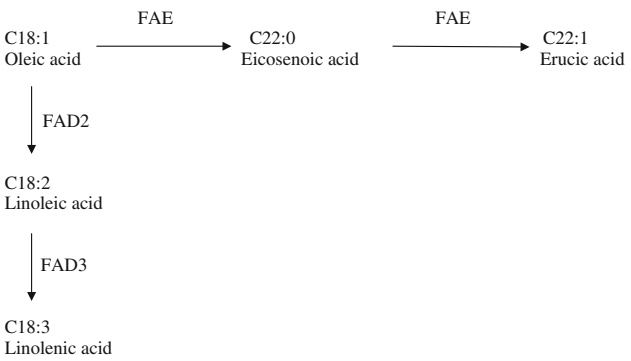

Fig. 1 Overview of the oil quality components of the fatty acid biosynthetic pathway (cytosolic) and associated candidate genes. FAE, fatty acid elongase; FAD2, fatty acid desaturase 2; FAD3, fatty acid desaturase 3

Identification of additional genes or loci that contribute to subtle, continuous variability for the above traits in the crop under zero-erucic conditions would be most useful in designing novel strategies to maintain nutritionally desirable levels of oleic, linoleic and linolenic acid fractions and also increase oil content. We therefore analyzed, in the present study, segregation of oil quality fractions and oil content using two sets of F1 doubled haploid (DH) mapping populations—one segregating for erucic acid content and the other consisting of only zero-erucic lines. These populations were developed from the F1DH progeny of a cross between a high-erucic Indian line, Varuna, and a low-erucic, east-European type line, Heera (Pradhan et al. 2003). By QTL mapping for oil quality parameters and oil content under zero-erucic conditions, we were able to identify new loci for the above traits none of which co-localized with mapped locations of candidate genes viz., *FAD2*, *FAD3* or *FAE*. These loci, which remained "hidden" in the population segregating for erucic acid due to strong pleiotropic effects of the erucic acid genes, could now be successfully integrated in crop improvement programs to increase oil content and manipulate the oil composition of *B. juncea*.

Materials and methods

Plant material

A population of 1,200 DH lines was generated from the F1 progeny of a cross between the high-erucic Indian variety, Varuna and the canola-quality, east-European line, Heera following the protocol for haploid generation described earlier for *B. juncea* (Mukhopadhyay et al. 2007). Two independent sets of mapping populations were resourced from the above DH lines. One set (Population SE), *S*egregating for *E*rucic acid content, consisted of 123 individuals used previously for the development of a detailed linkage map of *B. juncea* and analysis of yield-influencing loci (Pradhan et al. 2003; Ramchiary et al. 2007; Panjabi et al. 2008). Another set (Population ZE), comprising 110 *Z*ero *E*rucic-acid plants, was identified from the original population of 1,200 DH plants and used in the present study. A Varuna line with zero-erucic (ZE) acid phenotype (henceforth referred to as ZE-Varuna) was developed by backcrossing an F1DH zero erucic plant with the Varuna parent for five generations (BC5) followed by self-pollination for four generations (F4).

Measurement of oil quality fractions and oil content

Analysis of harvested seeds obtained from the multi-site field experiments conducted by Ramchiary et al. (2007)

using the 123 SE lines indicated high heritability for oil quality fractions and high to medium heritability for oil content over different environments (data not shown). Hence, the F1DH plants were grown under field conditions in Delhi during the winter season (October–March). Seeds were harvested and trait measurements were done over two growing seasons to improve the sensitivity and accuracy of analysis. For analysis of fatty acid composition, observations were taken from three replicates. Each replicate comprised 200 mg of selfed seeds pooled from three plants of each genotype/line. Measurement of erucic, oleic, linoleic and linolenic acid fractions was done by preparation of fatty acid methyl esters (FAMES) following Thies (1971). Samples were analyzed using gas chromatography on a Perkin Elmer Autosystem XLGC. Oil content was determined using a Foss Near Infra-red Reflectance Spectroscopy (NIRS) systems analyzer following Mika et al. (2003).

Construction of a linkage map for the ZE population

DNA was isolated from well-expanded leaves of zero-erucic lines following the protocol described earlier (Rogers and Bendich 1994). An AFLP-based linkage map of the zero-erucic lines was prepared using 408 polymorphic bands generated using 20 primer pairs (5 *Pst*I/*Taq*I, 6 *Eco*RI/*Taq*I and 9 *Eco*RI/*Mse*I) identified earlier for whole genome selection (WGS) in *B. juncea* (Pradhan et al. 2003). Two microsatellite markers and ten gene-specific, PCR-based Intron Polymorphism (IP) markers derived from *A. thaliana* and *Brassica* sequences (Panjabi et al. 2008) were added to this linkage map to reduce gaps. Mapping was done using the program JoinMap version 2.0 (Stam 1993; Stam and Van Ooijen 1996) following the mapping criteria described by Pradhan et al. (2003).

Amplification, cloning and mapping of *FAD2* and *FAD3* genes of *B. juncea*

Primer sequences used for amplification of the *B. rapa*-specific *FAD2* genes (*FAD2-1*) from *B. juncea* are: FAD2RAPA1 5′-CCGCTCGAGAGAACCAGAGAGATT CATT ACCA-3′ and FAD2RAPA2 5′-CGCCATGGTTC CACATAGATACACTCCTCC-3′. Primers used for amplification of the *B. nigra*-specific *FAD2* genes (*FAD2-2*) from *B. juncea* are as follows: FAD2NIG1 5′-GCGGGATCCCA CTGCGTTTGCTGGCTT-3′ and FAD2NIG2 5′-GCGAAG CTTCAGAGGACGAGGATAAGCCATTA-3′. PCR primers for amplification of the *FAD3* gene were designed from conserved regions of the gene sequences available for *A. thaliana* and various *Brassica* species and are as follows: FAD3FP 5′-ATGGTTGTTGCTATGGACCAGCG-3′; FAD3RP 5′-TTAATTGATTTTAGATTTGTCAGAAGC ATA-3′. The amplified fragments from the *B. juncea* cultivars, Varuna and Heera, were gel purified using the MinElute Gel Extraction kit (Qiagen) and cloned into the *Srf*I site of pCR-Script Amp SK(+) (Stratagene) or in the Zero Blunt TOPO PCR Cloning kit (Invitrogen life technologies) according to manufacturers' instructions. All DNA manipulations were performed using standard protocols. Cloned fragments were sequenced with the universal forward and reverse primers and gene-specific internal sequencing primers using the ABI PRISM 310 Genetic Analyzer (Applied Biosystems). Genomic sequences flanking the *FAD3* genes were isolated using the Universal Genome Walker kit (Clontech) following manufacturer's instructions. Allele-specific PCRs based on sequence polymorphisms and/or length polymorphism between the amplified fragments of the *FAD2* and *FAD3* genes from the two cultivars were used to map these genes on the linkage maps generated earlier for the SE population (Pradhan et al. 2003; Panjabi et al. 2008) using the JoinMap version 2.0 program (Stam 1993; Stam and Van Ooijen 1996).

Trait value calculations and QTL mapping

The traits evaluated by QTL mapping included *A*bsolute levels of *O*leic acid (A-O), *A*bsolute levels of *l*ino*l*eic acid (A-LL) and *A*bsolute levels of *l*ino*len*ic acid (A-LLN) (expressed as percent of total fatty acid in seed), Oleic Desaturation Ratio (ODR), *c*onversion rate for the formation of *l*ino*len*ic acid (C-LLN) and *oil* content in zero erucic lines (OIL). ODR and conversion rates for linolenic acid were calculated using the formula

$$\text{ODR or Conversion rate} = \text{Product}/\text{Substrate}.$$

Since the presence of erucic acid in the fatty acid biosynthetic pathway would influence the availability of oleic acid (substrate for desaturation reactions leading to the formation of linoleic and linolenic acid; Fig. 1), the erucic (C22:1) and eicosenoic (C20:1) acid fractions of seed oil were also considered for calculating conversion rates. Thus, ODR for linoleic acid formation was given by (C18:2 + C18:3)/(C18:1 + C18:2 + C18:3 + C20:1 + C22:1) and conversion rate for linolenic acid formation was calculated as C18:3/(C18:1 + C18:2 + C18:3 + C20:1 + C22:1). QTL mapping of the above traits on the SE and ZE populations was performed by composite interval mapping (CIM; Zeng 1993, 1994) using the software package WinQTL Cartographer version 2.5 (Wang et al. 2001–2005) as described earlier (Ramchiary et al. 2007). Genome wide threshold values (=0.05) estimated from 1,000 permutations of trait data across all genetic intervals were used for declaring the presence of a QTL (Churchill and Doerge 1994; Doerge and Churchill 1996).

Results

Oil quality components in parental lines (Varuna and Heera), Zero-erucic Varuna, SE and ZE populations: phenotypic variability and metabolic interactions

The mean values of oil quality components in the *B. juncea* parental lines, Varuna and Heera and the ZE-Varuna line are summarized in Fig. 2. The two parents have comparable levels of the primary substrate (represented by the total of erucic, oleic, linoleic and linolenic acid fractions; Fig. 2a) but differ significantly in the oleic and linoleic acid fractions in addition to the contrasting phenotype for erucic acid. No significant differences were observed between ZE-Varuna and Heera for oleic acid content, primary levels of linoleic acid (i.e., sum of the linoleic and linolenic fractions) and ODR values (Fig. 2). These observations indicate that lower levels of linoleic acid in Varuna were due to substrate limitation caused by the functional erucic acid pathway and not due to weaker alleles or enzyme limitation for oleic acid desaturation.

Linolenic acid levels in Varuna were only a little higher than that of Heera in spite of a significant difference in the amount of the precursor, linoleic acid (Fig. 2a). The ZE-Varuna plants had higher levels of linolenic acid and lower linoleic acid content as compared to Heera (Fig. 2a). ZE-Varuna also has higher levels of linoleic and linolenic acid in comparison to Varuna implying that substrate limitation for *FAD3*-mediated desaturation reduced leads to reduced linolenic acid content in Varuna. Conversion rates of Varuna, Heera and ZE-Varuna for linolenic acid formation indicated that Varuna had stronger alleles for linoleic acid desaturation than Heera (Fig. 2b). Increased accumulation of linolenic acid in the ZE-Varuna plants was, therefore, due to the incorporation of favorable alleles from Heera (lack of the erucic acid pathway) as well as Varuna (for desaturation of linoleic acid).

The distribution of oleic, linoleic and linolenic acid fractions among the 123 and 110 individuals of the SE and ZE populations, respectively are summarized in Fig. 3. Data on absolute values of various fatty acid fractions in seed is given in Supplementary Tables 1 and 2 for the ZE and SE populations, respectively. Data on broad sense heritability of the traits and correlations between traits for both the populations is given in Supplementary Tables 3 and 4, respectively. All the three fractions are quantitative traits based on the distribution pattern of their phenotypes in both the populations. Oleic acid levels in the SE population followed a binomial distribution pattern indicating that it is mainly influenced by two loci. There is a conspicuous shift in the phenotypic range towards the higher parent (Heera) value for oleic and linoleic acid fractions in individuals of the ZE population implying that linoleic acid formation is strongly influenced by substrate limitation in the SE population. In contrast, the range of linolenic acid levels is comparable in the SE and ZE population plants. The zero erucic individuals of the SE population were randomly distributed among all categories of linolenic acid fractions indicating that linolenic acid formation is not influenced by substrate limitation in the SE population (Supplementary Table 2).

Development of a linkage map for zero erucic F1DH lines of *B. juncea*

A framework linkage map was constructed with 408 AFLP, 2 microsatellites and 10 IP markers using 110 zero-erucic individuals comprising the ZE population (Supplementary Figure 1). The ten A genome linkage groups (LGs) of *B. juncea* have been designated as A1–A10 and the eight LGs representing the B genome have been designated as B1–B8 following internationally accepted guidelines for

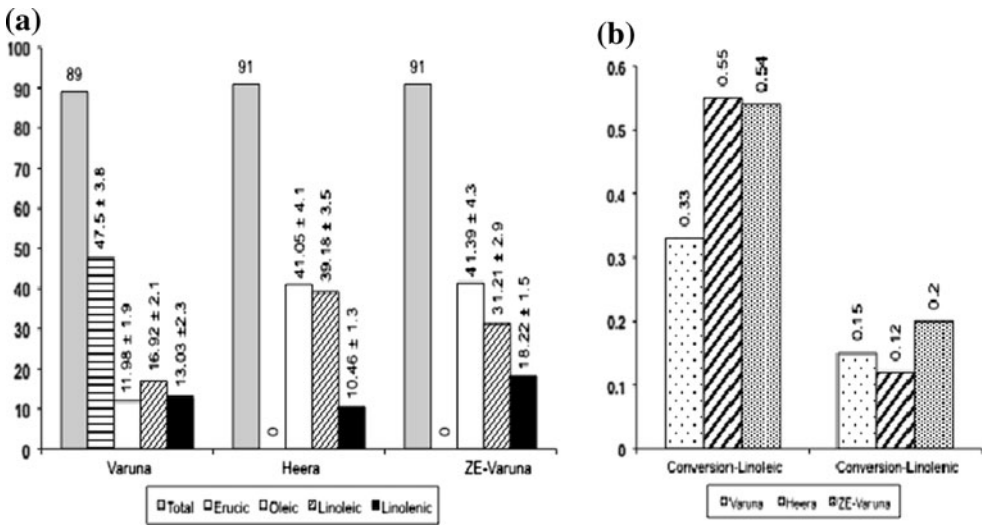

Fig. 2 Distribution of the phenotypic variability for oil quality components (oleic acid, linoleic acid and linolenic acid) in the parental lines, Varuna and Heera and the ZE-Varuna line vis-à-vis (**a**) absolute levels (values represent percent of the fatty acid fraction in seed oil along with their standard deviation) and (**b**) conversion rates

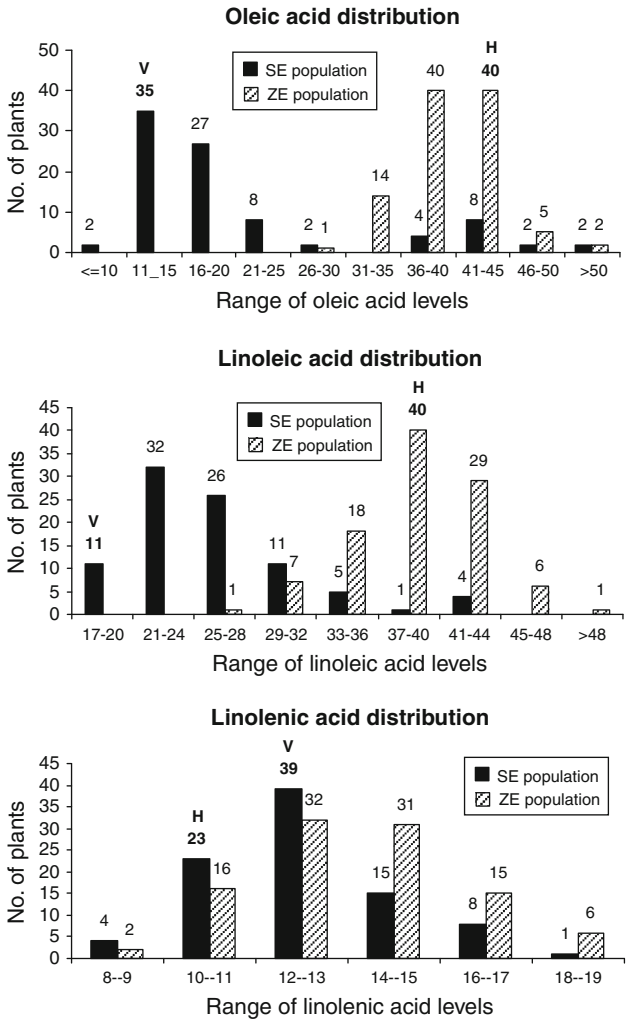

Fig. 3 Distribution of phenotypic variability for absolute levels of oleic, linoleic and linolenic acid fractions (represented as percent of fatty acid fraction in seed oil) in the SE and ZE populations. Parent values (*V* Varuna, *H* Heera) are indicated for each component

nomenclature of the Brassica genome linkage groups (http://www.brassica.info/resource/maps/lg-assignments.php). The total map length was 1,614 cM with an average marker interval of 3.84 cM. Since this population comprised only those individuals that had a zero-erucic phenotype, two linkage groups (B3 and A8) containing the *FAE* genes governing erucic acid content (Gupta et al. 2004) were split due to lack of polymorphic markers in the corresponding regions. Linkage mapping at a LOD threshold of 5.0, therefore, resulted in 20 linkage groups instead of 18, which is the haploid chromosome number of *B. juncea*. The split fragments of B3 and A8 comprised only three markers each and have not been represented as independent linkage groups in the linkage map. The placement of markers in all the linkage groups was mostly identical to that reported earlier for the SE population (Pradhan et al. 2003).

Isolation and mapping *FAD2* and *FAD3* genes of *B. juncea*

The *FAD2* and *FAD3* genes are associated with desaturation reactions leading to the formation of linoleic acid and linolenic acid, respectively (Fig. 1). To analyze their potential role in influencing variability in oil quality components of *B. juncea*, we attempted to isolate and map these genes. The full-length *FAD2* genes of *B. juncea* were PCR amplified from Varuna and Heera using the *B. rapa* (*FAD2-1*) and *B. nigra* (*FAD2-2*) gene-specific primers. Each primer combination amplified a single fragment in the *B. juncea* cultivars. Nucleotide sequences of various *FAD2* genes from *B. juncea* cultivars are available under accession numbers HM147242, HM147243, HM138372 and HM138373 in the Genbank database. Allele-specific primers were designed for each paralog based on identified SNPs and used to genotype the mapping population. One of the genes (*FAD2-1*) mapped on linkage group A5 (data not shown) while the other (*FAD2-2*) was localized on B1 (Fig. 4).

PCR amplification of the *FAD3* gene generated multiple fragments in Varuna and Heera. One of these fragments (*FAD3-R1*) showed length polymorphism and mapped on A5 linkage group (Supplementary Figure 1). Sequencing of other PCR products identified at least two more paralogs of the *FAD3* gene either of which, however, did not show any polymorphism between Varuna and Heera in their entire gene sequence. Genome walking in the 5′ and 3′ regions was performed for these genes and SNPs were identified in one of the fragments (*FAD3-N1*). Allele-specific primers were designed for this gene and the gene was mapped on linkage group B1 (Fig. 4). The other homologues of the *FAD3* gene could not be mapped due to lack of any polymorphism up to 3 kb of the 5′ and 3′ flanking genomic sequences obtained by genome walking (data not shown). Nucleotide sequences for *FAD3* genes isolated from *B. juncea* cultivars are available in GenBank database under accession numbers HM138370 and HM138371.

QTL mapping for oil quality fractions in the ZE and SE populations

QTL analysis of the five oil quality component traits, A-LLN, A-LL, A-O, ODR and C-LLN, for the ZE and SE populations was performed at a LOD threshold of 2.5. A total of 15 QTL for the ZE population and 17 QTL for the SE population were detected for the above traits (Tables 1, 2; Fig. 4). These QTL were distributed over six linkage groups (A3, A7, A9, A10, B7, B8) in the ZE population while those for the SE population were spread over eight linkage groups (A1, A2, A7, A8, A9, B1, B3, B6). In both the SE and ZE populations, all the QTL detected using

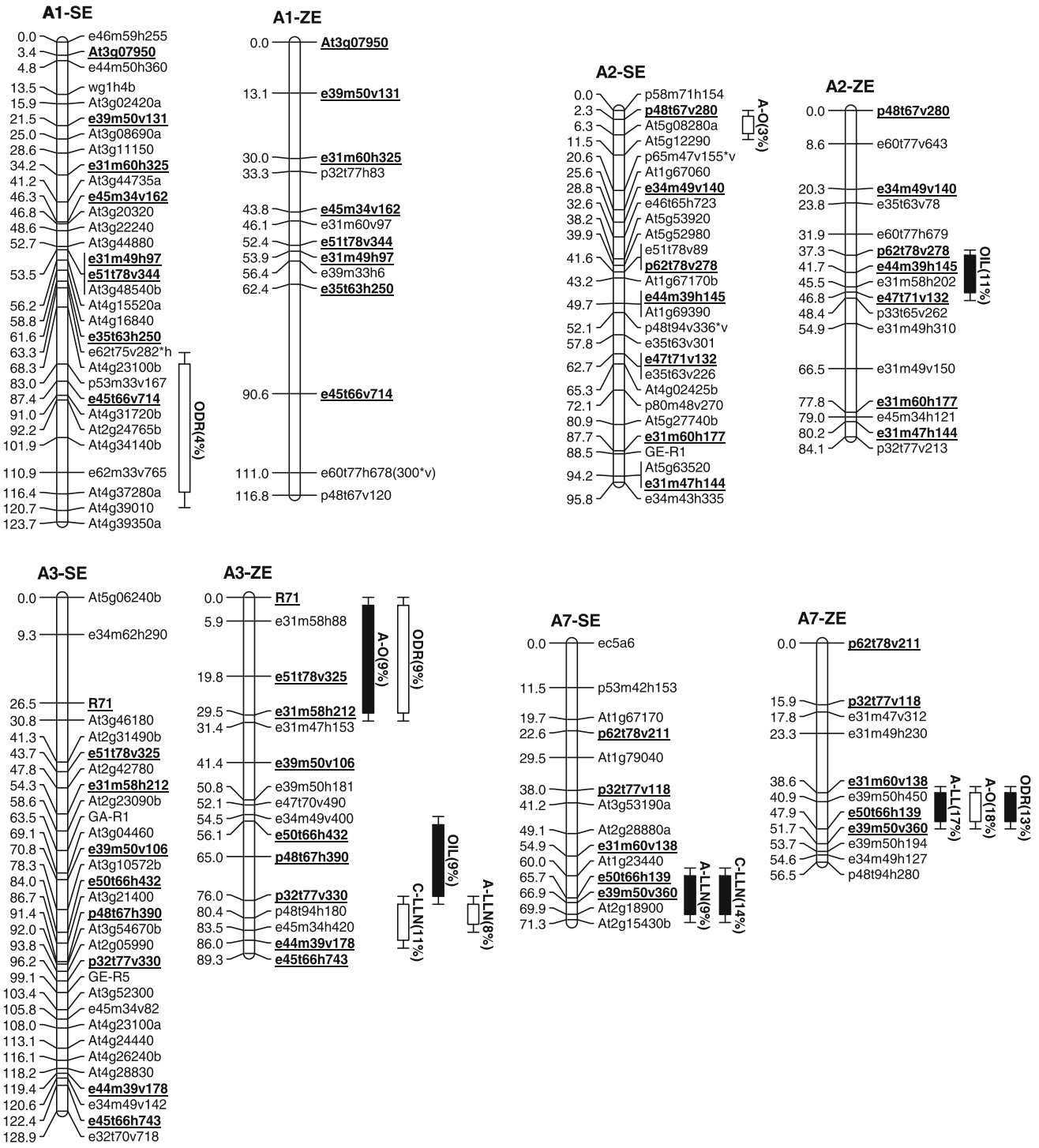

Fig. 4 Distribution of QTL for various oil quality components in the SE and ZE populations and for oil content in the ZE population. Polymorphic AFLP, microsatellite and intron polymorphism (IP) markers that are common between the two populations are in *bold* and *underlined*. Linkage groups are designated as A1–A10 and B1–B8 following international guidelines for the nomenclature of linkage groups derived from the *B. rapa* (**A**) and *B. nigra* (**B**) genomes. Linkage groups A8 and B3 in the ZE population are partial representations. Number in parenthesis in the QTL name refers to the R^2 value. Genetic interval (QTL region) shown in the figure is the confidence interval. Trait enhancing alleles are represented by empty (for Varuna) or solid (for Heera) QTL bars. *A-LLN* absolute linolenic, *A-LL* absolute linoleic, *A-O* absolute oleic, *ODR* oleic desaturation ratio, *C-LLN* conversion-linolenic, *OIL* oil content (only for ZE population)

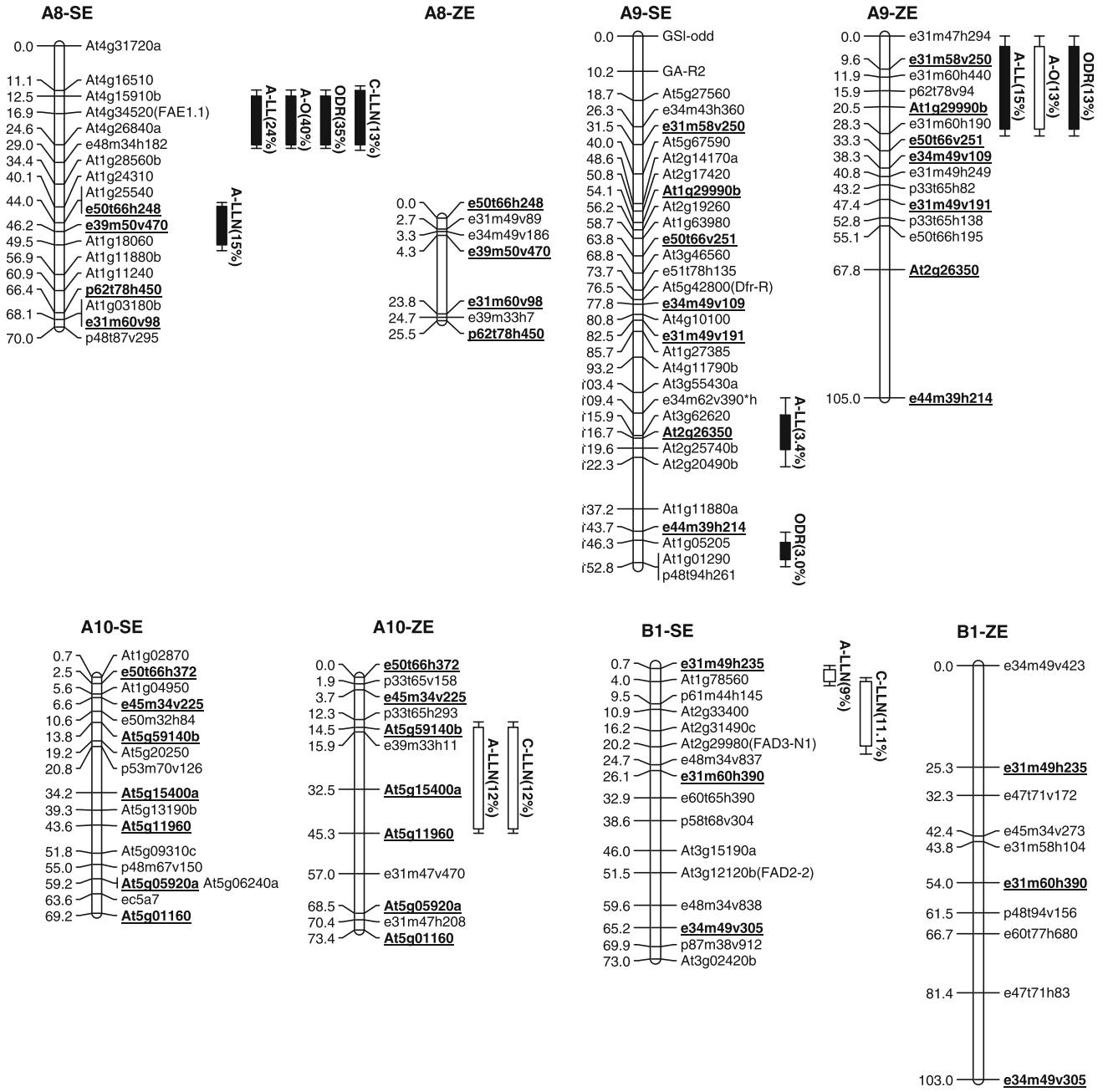

Fig. 4 continued

absolute levels were also detected using conversion ratios (Table 1). On using conversion ratios, one additional locus was detected for the C-LLN and ODR phenotypes in the SE population and one new locus was detected in the C-LLN trait of the ZE population.

None of the QTL detected for oil quality fractions and oil content were common between the two populations (Table 1; Fig. 4). In the absence of erucic acid variation, new loci were detected for oleic, linoleic and linolenic acid content. Interestingly, 5 of the 15 new oil quality QTL detected in the ZE population were also found as weak contributors (with LOD value < 2.5) in the SE population indicating that strong effects of the erucic acid pathway decreased the power of detection of these loci to trait variability (Supplementary Figure 2). These loci could only be detected under zero erucic conditions in the ZE population. Four of these QTL on linkage groups A10, B7 and B8 were associated with linolenic acid content while one on linkage group A7 was for ODR.

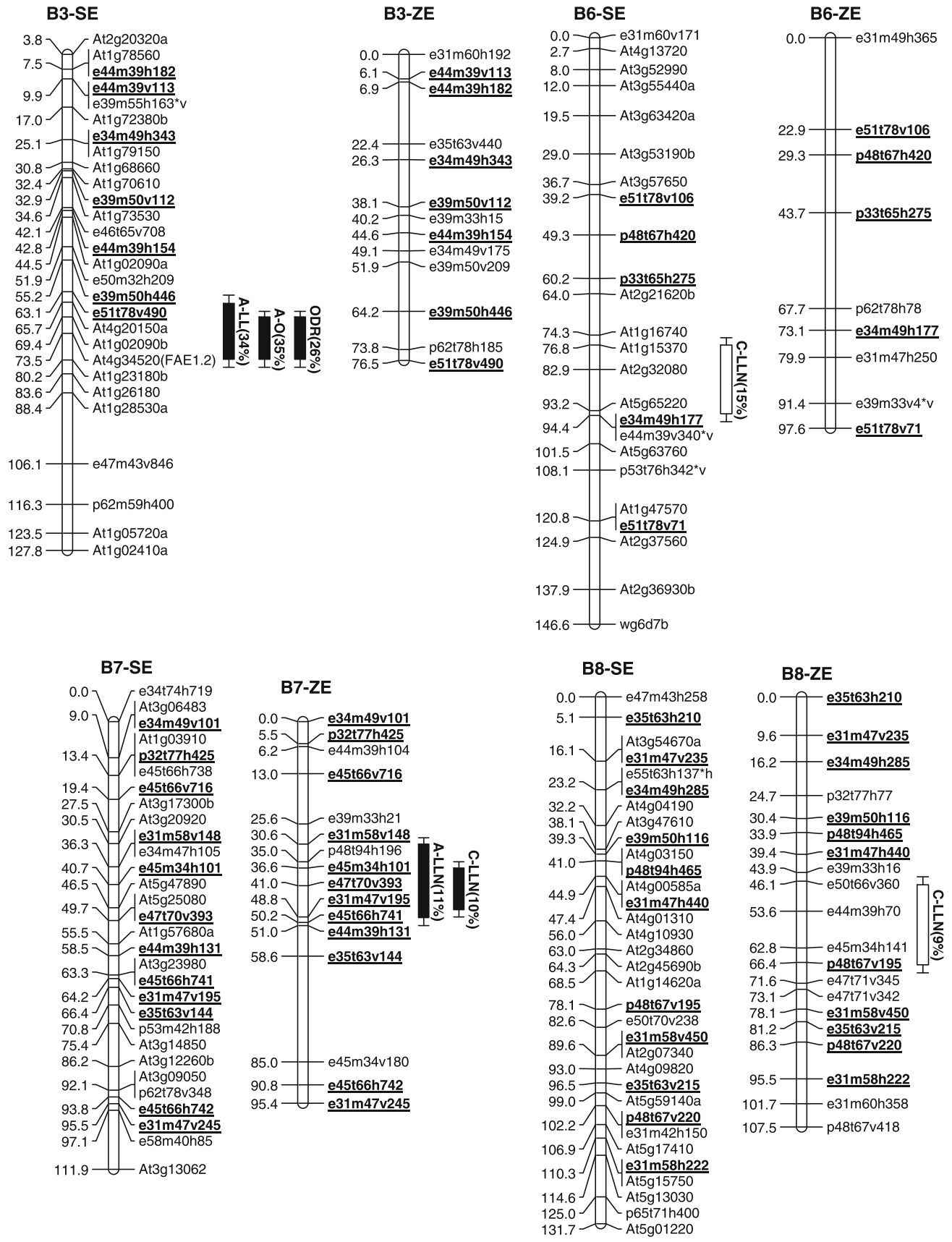

Fig. 4 continued

Table 1 QTL analysis of oil quality fractions and oil content: genome-based distribution of QTL and source of trait enhancing alleles (in parenthesis; *V* Varuna, *H* Heera) in the SE and ZE populations

	Position of QTL (cM) in ZE population							Position of QTL (cM) in SE population						
	A-LLN	A-LL	A-O	ODR	C-LLN	OIL	Total	A-LLN	A-LL	A-O	ODR	C-LLN	OIL[b]	Total
A1	–	–	–	–	–	–	0	–	–	–	83.0–116.4 (V)	–	–	1
A2	–	–	–	–	–	37.3–48.4 (H)	1	–	–	0.0–6.3 (V)	–	–	–	1
A3	76.0–83.5 (V)	–	0.0–31.4 (H)	0.0–31.4 (V)	76.0–87.4 (V)	56.1–76.0 (H)	5	–	–	–	–	–	–	0
A7	–	38.6–47.9 (H)	38.6–47.9 (V)	38.6–47.9 (H)	–	–	3	60.0–70.0 (H)	–	–	–	60.0–69.9 (H)	–	2
A8	–	–	–	–	–	–	0	40.1–49.5 (H)	12.5–24.6 (H)[a]	12.5–24.6 (H)[a]	12.5–24.6 (H)[a]	11.1–24.6 (H)[a]	10.0–34.2 (V)[a,b]	6
A9	–	0.0–28.3 (H)	0.0–28.3 (V)	0.0–28.3 (H)	–	–	3	–	109.4–122.3 (H)	–	146.3–152.8 (H)	–	–	2
A10	14.5–45.3 (V)	–	–	–	14.5–45.3 (V)	–	2	–	–	–	–	–	–	0
B1	–	–	–	–	–	–	0	0.0–4.0 (V)	–	–	–	4.0–20.2 (V)	–	2
B3	–	–	–	–	–	–	0	–	65.7–80.2 (H)[a]	69.4–80.2 (H)[a]	69.4–80.2 (H)[a]	–	63.3–81.3 (V)[a,b]	4
B6	–	–	–	–	–	–	0	–	–	–	–	76.8–94.4 (V)	–	1
B7	30.6–51.0 (H)	–	–	–	36.6–48.8 (H)	–	2	–	–	–	–	–	–	0
B8	–	–	–	–	46.1–68.7 (V)	–	1	–	–	–	–	–	–	0
Total	3	2	3	3	4	2	**17**	3	3	3	4	4	2	**19**

[a] Corresponds to mapped locations of the *FAE* genes

[b] Derived from Ramchiary et al. (2007)

Table 2 QTL analysis of oil quality components in the SE and ZE populations and oil content in ZE population. Genetic interval (QTL region) shown in the table is the confidence interval

Trait	QTL	LG	LOD	Additive effect	R^2	Flanking markers	
						Left	Right
ZE population							
Linolenic—Absolute	A-LLN	A3	2.68	0.65	7.49	e45m34h420	p32t77v330
		B7	3.82	−0.77	10.94	e31m58v148	e44m39h131
		A10	4.11	0.81	11.76	5g 59140b	5g 11960
Linoleic—Absolute	A-LL	A9	4.92	−1.6	14.5	e31m47h294	e31m60h190
		A7	5.63	−1.65	16.91	e31m60v138	e50t66h139
Oleic—Absolute	A-O	A9	5.08	1.61	13.29	e31m47h294	e31m60h190
		A3	3.13	−1.28	8.82	e31m47h153	R71
		A7	5.90	1.78	17.62	e31m60v138	e50t66h139
ODR	ODR	A9	4.65	−0.02	12.46	e31m47h294	e31m60h190
		A3	3.18	0.01	8.8	e31m47h153	R71
		A7	4.85	−0.02	13.08	e31m60v138	e50t66h139
Linolenic—conversion	C-LLN	B8	3.22	0.01	9.03	e50t66v360	p62t78h284
		A3	3.75	0.01	10.69	e34m49v142	p32t77v330
		B7	3.46	−0.01	9.66	e45m34h101	e31m47v195
		A10	4.32	0.01	12.26	5g 59140b	5g 11960
Oil	OIL	A3	2.58	−1.16	8.45	p32t77v330	e50t66h432
		A2	3.40	−1.16	11.31	p62t78v278	e51t78v378
SE population							
Linolenic—Absolute	A-LLN	A7	3.11	−0.63	8.89	1g 23440	2g 18900
		B1	3.09	0.61	8.63	e62m33h107	1g 78560b
		A8	4.99	−0.8	14.64	1g 24310	1g 18060
Linoleic—Absolute	A-LL	A9	2.57	−1.02	3.38	e34m62v390h	2g 20490b
		B3	17.21	−3.25	33.9	4g 20150a	1g 23180b
		A8	13.38	−2.7	23.6	4g 15910b	4g 26840a
Oleic—Absolute	A-O	B3	21.18	−6.82	34.89	1g 02090b	1g 23180b
		A2	2.58	1.91	2.75	p58m71h154	5g 08280a
		A8	22.86	−7.22	39.48	4g 15910b	4g 26840a
ODR	ODR	A9	2.66	−0.01	2.72	1g 05205	1g 01290
		B3	17.34	−0.03	25.96	1g 02090b	1g 23180b
		A1	2.67	0.01	3.77	p53m33v167	4g 37280a
		A8	21.08	−0.04	35.26	4g 15910b	4g 26840a
Linolenic—conversion	C-LLN	B6	5.24	0.01	14.79	1g 15370	e44m39v340v
		A7	5.56	−0.01	14.37	1g 23440	2g 18900
		B1	4.51	0.01	11.08	1g 78560b	2g 29980
		A8	5.16	−0.01	12.89	4g 16510	4g 26840a

A-LLN absolute-linolenic, *A-LL* absolute-linoleic, *A-O* absolute-oleic, *ODR* oleic desaturation ratio, *C-LLN* conversion rate-linolenic, *OIL* oil content

Clustering of QTL for different traits was more prominently seen in the ZE population than the SE population. In the ZE population, QTL for A-O/A-LL/ODR and A-LLN/C-LLN phenotypes were found to co-localize in most of the linkage groups indicating that final amounts of the oleic, linoleic and linolenic acid fractions were governed only by the desaturation potential of enzymes and not by any other interacting loci (Tables 1, 2; Fig. 4). The negative correlation between the A-O phenotype with ODR and A-LL phenotypes was reflected in the source of the trait enhancing alleles for the above traits on A3, A7 and A9 linkage groups of the ZE population (Table 1; Fig. 4). Mapping of the oleic and linoleic acid content QTL to the same genetic interval with opposite allelic effects indicates that the same functional genes are responsible for variation in both oleic and linoleic acid levels. Significantly, no

overlapping QTL were detected in the ZE population for the A-LL trait with the A-LLN or C-LLN phenotypes implying that phenotypic variation of linolenic acid is not dependent on the amount of available precursor (linoleic acid). None of the oil quality QTL of the ZE population co-localized with mapped locations of the *FAD2* and *FAD3* genes.

In the SE population, co-localization of major QTL was observed on B3 and A8 for the A-O, A-LL and ODR phenotypes, which collectively accounted for 75, 58 and 61% of the phenotypic variance (R^2), respectively, for the above traits (Fig. 4; Tables 1, 2). One C-LLN QTL ($R^2 = 13\%$) on A8 was also found to be overlapping with the A-LL trait in the SE population. All these regions coincided with mapped locations of the *FAE* genes (Gupta et al. 2004) which catalyze elongation of oleic acid (C18:1) to erucic acid (C22:1) (Fig. 1). In addition, prominent overlaps were seen for the C-LLN ($R^2 = 14\%$) and A-LLN ($R^2 = 9\%$) QTL on linkage group A7 (Fig. 4). The C-LLN QTL on linkage group B1 ($R^2 = 11\%$) was located in proximity to one of the *B. juncea FAD3* genes (*FAD3-N1*) mapped in this study.

Twelve of 15 QTL (80%) of the ZE population and 11 of 17 QTL (65%) of the SE population were located on *B. rapa*-derived chromosomes (Table 1). Analysis of the relative contributions of the trait enhancing alleles from the two parents (Varuna and Heera) to oil quality also revealed differences between the two populations. Of the 15 QTL identified in the ZE population, 8 trait enhancing alleles (53%) were contributed by Varuna and 7 (47%) were derived from Heera (Table 1). In contrast, the corresponding figures for the 17 QTL of the SE population were 5 (29%) and 12 (71%) for Varuna and Heera, respectively (Table 1). Low levels of linoleic and linolenic acid and high oleic acid content in a zero erucic background are more desirable for superior oil quality. Therefore, desirable alleles for oil quality traits would be those that *reduce* the linoleic and linolenic acid fractions (and not the trait enhancing alleles discussed above) and trait-enhancing alleles that increase oleic acid content.

Oil content variability in parental lines and ZE-Varuna and QTL mapping in the ZE population

Seeds of the parental lines, Varuna (high-erucic) and Heera (zero-erucic), are characterized by oil content of 43 and 40%, respectively. In contrast, oil content in the ZE-Varuna line is reduced to around 36%. Oil content in the SE population ranged from 38 to 50% (Ramchiary et al. 2007) while in the ZE population, it ranged from 25 to 42%. In earlier studies on the SE population (Ramchiary et al. 2007), two major QTL for oil content that collectively account for ~25% of the trait variation, were found to overlap with mapped locations of the *FAE* genes on linkage groups A8 and B3. However, at both these loci, trait-enhancing alleles for oil content were derived from the high-erucic parent, Varuna. In contrast, QTL mapping for oil content in the ZE population detected two novel loci on linkage groups A2 and A3, which did not co-localize with the *FAE* genes (Tables 1, 2; Fig. 4). Both these loci are derived from the A genome and collectively explain ~19% phenotypic variance for the trait. At both these loci, trait-enhancing alleles for oil content are derived from the low-erucic parent, Heera (Table 1).

Discussion

Most of the major QTL detected for oleic, linoleic and linolenic acid fractions in the SE population co-localized with mapped locations of the *FAE* genes substantiating pleiotropic effects of the erucic acid pathway which leads to substrate limitation for the FAD2 and FAD3-mediated desaturation reactions in *B. juncea*. Similar results were reported earlier in *B. rapa* (Tanhuanpaa et al. 1996, 1998) and *B. napus* (Chen and Beversdorf 1990). Our analysis of oil quality fractions in a zero-erucic acid population led to the identification of new loci, none of which co-localized with mapped locations of the *FAE, FAD2* or *FAD3* genes. Many of these QTL remained "hidden" or undetected in the SE population due to strong effects of the *FAE* genes. Their contribution to trait variability is detected only under zero-erucic conditions (Supplementary Figure 2). Most of the desirable alleles for oil quality traits are derived from the A genome. These alleles could now be used to improve the oil composition of *B. juncea* under zero-erucic conditions. Requirement of the essential fatty acids, viz., linoleic and linolenic acid among the vegetarian Indian population is largely met through consumption of vegetable oils. Mutants, unless regulatory in nature, would more likely lead to a complete elimination of these fatty acids from seed oil. Therefore, it is essential to maintain these fatty acids at a level of 10–12%, which can potentially be achieved by using existing natural adaptive variability and loci identified in the present study. Introgression of Varuna alleles from overlapping QTL identified on linkage groups A7 and A9 for oleic and linoleic acid content would increase oleic acid and reduce linoleic acid under zero-erucic conditions (Tables 1, 2; Fig. 4). Reduction in linolenic acid content under zero-erucic conditions can be achieved by introgressing Heera alleles from QTL identified on A3, A10 and B3 linkage groups and the Varuna allele from B7 (Tables 1, 2; Fig. 4). However, it is also important to note that context dependency or the genetic background of the loci might strongly influence their contribution to the trait(s). Therefore, it would be difficult

to give a quantitative prediction of the levels of fatty acids that can be obtained in seed oil by using these loci.

Earlier studies from our laboratory and other groups had demonstrated tight linkage of the erucic acid (*FAE*) loci with oil content of *Brassica* seeds (*see* "Introduction"). Reduced erucic acid levels lead to a concomitant reduction in oil content in all the Brassica species analyzed till date (Ramchiary et al. 2007 and references therein). Increased oil content found in high-erucic *Brassica* species has been attributed to an increase in molecular mass of oil due to elongation of oleic acid (an 18-carbon molecule) to erucic acid (a 22-carbon molecule) (Ecke et al. 1995; Burns et al. 2003). Although reduction in erucic acid content is nutritionally desirable, the economic ramifications of the concurrent reduction in oil content are significant. Identification of new loci for oil content would resolve the problem of linkage drag identified in *B. juncea* due to clustering of antagonistic QTL for low erucic acid and high oil content (Ramchiary et al. 2007). QTL mapping of oil content in the ZE population identified two novel compensatory loci for this trait on linkage groups A2 and A3. Oil content QTL on the A3 linkage group were observed in different mapping populations of *B. napus* in earlier studies (Zhao et al. 2006 and references therein). However, due to lack of common markers, we could not establish their overlap (if any) with the *B. juncea* QTL identified in the present study. Both the trait-enhancing alleles for oil content in the ZE population are derived from the zero-erucic parent, Heera. Our analysis of oil content in the parental lines (Varuna and Heera) and in the zero-erucic Varuna line (ZE-Varuna) also confirms the above observations. Oil content of the ZE-Varuna line is reduced to ~36% in contrast to that of the zero-erucic parent, Heera (~40%) and the high-erucic parent, Varuna (~43%) implying that under zero-erucic conditions, Heera has stronger alleles for oil content. These loci could now be mobilized for increasing oil content of mustard as they could compensate for reduction in oil yield under zero-erucic conditions, which is an important consideration for developing canola-quality lines and hybrids in mustard-growing areas of India.

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