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QTL mapping of yield-associated traits in *Brassica juncea*: meta-analysis and epistatic interactions using two different crosses between east European and Indian gene pool lines

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Abstract Genetic analysis of 12 yield-associated traits was undertaken by dissection of quantitative trait loci (QTL) through meta-analysis and epistatic interaction studies in *Brassica juncea*. A consensus (integrated) map in *B. juncea* was constructed using two maps. These were VH map, developed earlier in the laboratory by using a DH population from the cross between Varuna and Heera (Pradhan et al. in Theor Appl Genet 106:607–614, 2003; Ramchiary et al. in Theor Appl Genet. 115:807–817, 2007; Panjabi et al. in BMC Genomics 9:113, 2008), and the TD map, developed in the present study using a DH population of 100 lines from the cross between TM-4 and Donskaja-IV. The TD map was constructed with 911 markers consisting of 585 AFLP, 8 SSR and 318 IP markers covering a total genome length of 1,629.9 cM. The consensus map constructed by using the common markers between the two maps contained a total of 2,662 markers and covered a total genome length of 1,927.1 cM. Firstly, QTL analysis of 12 yield-associated traits was undertaken for the TD population based on three-environment phenotypic data. Secondly, the three-environment phenotypic data for the same 12 quantitative traits generated by Ramchiary et al. (2007) were re-analyzed for the QTL detection in the VH map. Comparative analysis identified both common and population-specific QTL. The study revealed the presence of QTL clusters on LG A7, A8 and A10 in both TD and VH maps. Meta-analyses resolved 187 QTL distributed over nine linkage groups of TD and VH maps into 20 meta-QTL. Maximum resolution was recorded for the LG A10 wherein all the 54 QTL were mapped to a single meta-QTL within a confidence interval of 3.0 cM. Digenic epistatic interactions of QTL in both TD and VH maps revealed substantial additive × additive interactions showing a higher frequency of Type 1 and Type 2 interactions than Type 3 interactions. Some of the loci interacted with more than one locus indicating the presence of higher order epistatic interactions. These findings provided some detailed insight into the genetic architecture of the yield-associated traits in *B. juncea*.

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Introduction

Brassica juncea (mustard) is a major oilseed crop of south Asia. It is a natural allopolyploid (AABB) between two diploid species, namely *B. rapa* (AA) and *B. nigra* (BB). There are two gene pools in *B. juncea*, the east European and the Indian gene pool (Pradhan et al. 1993; Srivastava et al. 2001) and hybrids between the lines belonging to the two gene pools are heterotic for yield (Pradhan et al. 1993;

Jain et al. 1994; Sodhi et al. 2006). Several desirable traits, particularly high oil content, yellow seed coat color, 'Canola' quality along with many other yield-enhancing traits such as high silique density and seed number are present in east European lines, and traits such as higher seed size and longer silique are present in Indian germplasm. However, previous attempts to bring together the desirable traits from both the gene pools through pedigree breeding have been largely unsuccessful as the east European lines are ill-adapted to the agronomic conditions prevailing in mustard growing regions of south Asia. Hence, development of more productive lines and hybrids will, therefore, require precise transfers of gene(s)/QTL through marker-assisted selection (MAS) from one gene pool to the other without sacrificing the loci involved in heterosis.

Successful application of QTL analysis in MAS and QTL cloning, however, depends on the reliability and accuracy of QTL analysis. Since the chromosomal QTL intervals are quite often large and may include many loci, the introgression of favorable QTL through MAS can create the problem of linkage drag. Other factors that make QTL-based trait introgression unpredictable is the presence of genotype–environment (G × E) and epistatic interactions. Thus, confining QTL to narrow genetic intervals along with a more or less complete picture of the genetic control of a trait is the most critical requirement for the success of MAS and QTL cloning. Reliability in QTL work can be achieved through the use of multiple populations tested under diverse agronomic conditions. Only major consensus QTL with consistency across populations and environments could be put into broad use in marker-assisted breeding.

Detailed QTL analysis of yield-associated traits in *B. juncea* was earlier carried out in a doubled haploid (DH) population of a cross between an Indian gene pool line Varuna and an east European line Heera (Ramchiary et al. 2007). The analysis showed that the contribution of the A genome toward agronomically important traits in *B. juncea* was more than that of the B genome and many QTL were found to be clustered in a few linkage groups (LGs). We report in this study the construction of a high density consensus (integrated) map in *B. juncea* using two different maps. One was the Varuna × Heera map (hereafter referred to as the VH map) (Pradhan et al. 2003; Ramchiary et al. 2007; Panjabi et al. 2008). The second map developed in the present study used a DH population derived from F₁ of cross between an Indian gene pool line TM-4 and an east European line Donskaja-IV (hereafter referred to as the TD map). The TD map was subjected to multi-environment QTL analysis for the same 12 yield-associated traits earlier analyzed using the VH map (Ramchiary et al. 2007). Comparative QTL analysis between the two QTL maps identified many

consistent QTL regions and also population-specific QTL regions in *B. juncea*. Using the integrated map, consensus QTL regions with refined confidence intervals on different LGs were identified by meta-analysis. Genetic control of the different yield-associated traits was further investigated for the existence of epistatic interactions.

Materials and methods

Plant material, field experiments and trait measurements

The TD mapping population used in the present study consisted of 100 DH lines developed by microspore culture (Mukhopadhyay et al. 2007) from the F₁ of cross between TM-4 (an Indian cultivar) and Donskaja-IV (an east European cultivar).

The TD population and parents were grown in three locations in India, namely Delhi in the year 2006–2007 (normal winter growing season, short day condition), Bharatpur, Rajasthan, in the year 2007–2008 (normal winter growing season and short days with temperatures being higher than in Delhi during seed maturity) and Leh, Jammu and Kashmir, in the year 2008 (summer season, long day condition). The lines were planted in a randomized complete block design with three replications. Each treatment was planted in three rows with a row to row distance of 40 cm and plant to plant distance of 10 cm. Five competitive plants from the middle row were used for phenotyping.

The 12 yield-associated traits evaluated were plant height (*Plht*), primary branches (*Pbr*), secondary branches (*Sbr*), main shoot length (*Msl*), siliques on the main shoot (*Sqms*), siliques on a plant (*Sqp*), silique length (*Sql*), seeds in a silique (*Ssq*), thousand seed weight (*Tsw*), silique density (*Sqdy*), days to flowering (*Df*) and seed oil content (*Oil*). Traits were measured following Ramchiary et al. (2007).

Construction of TD linkage map and development of a consensus map

Leaf samples were harvested from the field-grown plants of TD population and total genomic DNAs were isolated by the CTAB method following Rogers and Bendich (1994). A total of 911 markers consisting of 585 AFLP, 318 IP (intron polymorphism) and 8 SSR markers were used to construct the TD map using the program JoinMap version 4.0 (Van Ooijen 2006) with the parameters followed by Pradhan et al. (2003). Enzyme combinations EcoR1/Mse1, EcoR1/Taq1 and Pst1/Taq1 were used for AFLP generation

following Pradhan et al. (2003). IP and SSR markers were tested and used following Panjabi et al. (2008) and Ramchiary et al. (2007), respectively. Markers were distributed over 18 LGs covering a total length of 1,629.9 cM with an average interval size of 3.7 cM. LGs were identified as A1–A10 and B1–B8 following Panjabi et al. (2008) (Supplementary Table S1 and Supplementary Fig. S1). Linkage groups were established with a minimum LOD threshold of 5.0 and the recombination fractions were translated into map distances with the Kosambi function (Kosambi 1944). The LGs were drawn with MapChart 2.2 (Voorrips 2002).

Development of a consensus map in *B. juncea* was carried out by merging TD map developed in the present study with the VH map developed earlier (Ramchiary et al. 2007; Panjabi et al. 2008). Initially, a consolidated VH map was constructed by merging the marker genotyping data of the maps constructed by Ramchiary et al. (2007) and Panjabi et al. (2008). This consolidated VH map consisted of 2,169 markers: 1,290 AFLPs, 70 RFLPs, 708 IP, 69 SSRs, 28 gene markers and 4 SCAR/CAPS markers. The total coverage of the map was 1,902.9 cM (Supplementary Table S2 and Supplementary Fig. S1). The consolidated VH and the TD maps developed in this study were further used for developing a consensus map in *B. juncea*.

The consensus map was constructed by combining the linkage information of the different markers in the TD and VH maps using the *combine groups for map integration* function of JoinMap 4.0 following the regression mapping algorithm. A total of 418 markers (278 AFLPs, 132 IP and 8 SSRs) were identified as common markers between the two maps. In both the maps, no significant change in the ordering of markers was observed. Heterogeneity of recombination frequencies was non-significant ($P > 0.01$) for the majority (90.7 %) of the common marker pairs. The resulting consensus map contained a total of 2,662 markers, covering a total length of 1,927.1 cM (Supplementary Table S3 and Supplementary Fig. S1). The consensus map will be referred to as 'INT' map.

Statistical analyses and QTL mapping

Trait means, ANOVAs, correlations and heritabilities for all the traits evaluated in both TD and VH populations were determined using the SPAR 2.0 software (2005). A framework map with an average marker spacing of one marker approximately every 5 cM was used for scanning the genome for detection of QTL. QTL analyses were performed in both TD and VH populations. Additive QTL were detected with QTL Cartographer 2.5 by composite interval mapping (Wang et al. 2005). Genome was scanned for QTL at a walk speed of 2 cM. Statistical significance of QTL for each trait was assessed by permuting each data set

1,000 times, with a significance level of 0.05 (Churchill and Doerge 1994; Doerge and Churchill 1996). For each trait, a minimum LOD value of 2.5 was used for the identification of putative QTL and multiple QTL peaks detected within 10 cM of each other were regarded as a single QTL. QTL nomenclature was as proposed by Ramchiary et al. (2007).

Meta-analysis was performed on the QTL clusters of each linkage group using Biomercator 2.1 (Arcade et al. 2004). Genetic maps and the data files describing QTL (trait names, LOD scores and R^2) of both VH and TD populations were loaded into Biomercator 2.1 for meta-analysis. The Akaike information criterion (AIC) was used to select the QTL model and that with lowest AIC value was considered a significant model indicating the number of meta-QTL.

QTL Network 2.1 (Yang et al. 2007) was used for detecting epistatic interactions in both TD and VH populations. The analyses were based on a mixed linear model approach with 1 cM walk speed and a testing window of 10 cM. In addition, 2D genome scans were also performed for detecting epistatic effects among loci with or without individual additive main effects. Threshold for the presence of QTL was generated by performing 1,000 permutations.

Results

Phenotypic variation in parents and mapping populations

TM-4 and Donskaja-IV are highly contrasting for most of the yield-associated traits as revealed by the mean values of the 12 traits evaluated in the three environments (Table 1). It was observed that the expression pattern of many of the component traits was different between the short day winter conditions at Delhi and Bharatpur and the long day high-altitude condition at Leh. A comparison of phenotypic variation pattern between the parents in the TD mapping population (TM-4 and Donskaja-IV) (present study) and VH mapping population (Varuna and Heera) (Ramchiary et al. (2007) for the 12 quantitative traits in two distinct environments (Delhi and Leh) revealed that trait expression pattern by Indian gene pool lines Varuna and TM-4 was similar and distinct from that of the east European lines, Heera and Donskaja-IV, in both the environments (data not shown).

In the TD mapping population, all the 12 traits showed continuous variation in all the three environments (Supplementary Fig. S2). Transgressive segregation in both the directions was recorded for most of the traits indicating the presence of favorable alleles in both the parents (Table 1;

Table 1 Descriptive statistics of the parents, range observed for phenotypic variations in the TD mapping population for 12 yield-associated traits in three environments, Delhi (DEL), Bharatpur (BHP) and Leh (LEH) and their broad-sense heritability estimates

Trait	Mean trait values of TM-4 (±SD)			Mean trait values of Donskaja-IV (±SD)			Range of phenotypic values of mapping population			Heritability (%)		
	DEL	BHP	LEH	DEL	BHP	LEH	DEL	BHP	LEH	DEL	BHP	LEH
Plant height (Plht)	129.3 ± 16.0	162.6 ± 11.0	63.4 ± 5.4	255.0 ± 20.6	204.7 ± 9.7	127.1 ± 10.9	111.1–261.3	129.0–253.2	66.2–137.6	94.0	90.0	60.0
Primary branches (Pbr)	3.5 ± 1.1	4.5 ± 1.3	3.7 ± 0.6	12.9 ± 2.3	10.3 ± 1.2	4.0 ± 1.1	3.3–13.9	3.8–10.7	2.4–5.5	88.0	85.0	22.0
Secondary branches (Sbr)	11.1 ± 2.4	9.2 ± 3.3	7.8 ± 2.0	24.8 ± 10.4	11.3 ± 1.2	8.9 ± 4.2	9.5–40.0	5.4–21.5	2.3–9.0	65.0	46.0	18.0
Main shoot length (Msl)	67.7 ± 9.3	76.3 ± 10.9	35.5 ± 4.8	25.9 ± 7.5	19.7 ± 1.7	73.2 ± 8.0	21.1–85.7	12.0–84.5	34.1–87.3	85.0	88.0	66.0
Siliques per main shoot (Sqms)	45.5 ± 5.0	42.5 ± 5.5	19.6 ± 4.8	30.8 ± 8.9	24.3 ± 4.9	55.4 ± 6.3	23.3–74.9	17.6–71.6	20.6–63.6	67.0	66.0	59.0
Siliques per plant (Sqp)	348.8 ± 41.8	363.7 ± 110.2	114.5 ± 40.9	744.9 ± 256.3	520.3 ± 82.5	356.0 ± 167.1	361.8–1424.9	171.1–676.9	78.9–315.0	44.0	39.0	26.0
Silique length (Sql)	6.3 ± 0.2	4.7 ± 0.3	5.1 ± 0.4	4.2 ± 0.4	4.3 ± 0.2	4.5 ± 0.4	3.6–7.5	2.0–5.4	3.9–6.8	95.0	95.0	72.0
Seeds per silique (Ssq)	18.7 ± 1.2	16.3 ± 2.0	16.1 ± 3.4	6.6 ± 2.0	11.3 ± 0.5	17.6 ± 1.8	8.4–24.3	5.0–22.3	12.6–20.5	89.0	86.0	29.0
Thousand seed weight (Tsw)	2.7 ± 0.3	2.0 ± 0.6	3.1 ± 0.3	2.3 ± 0.3	2.2 ± 0.1	3.0 ± 0.2	1.3–3.2	0.7–2.5	2.0–4.1	86.0	79.0	84.0
Silique density (Sqdy)	0.7 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	1.2 ± 0.3	1.2 ± 0.3	0.8 ± 0.1	0.5–1.8	0.4–2.9	0.4–0.7	48.0	92.0	46.0
Days to flower (Df)	49.0 ± 2.1	52.3 ± 0.9	–	98.7 ± 5.0	114.3 ± 4.9	–	34.3–118.3	49.3–94.5	–[a]	97.0	97.0	–
Oil (Oil)	41.2 ± 1.2	40.2 ± 3.8	40.6 ± 2.0	46.7 ± 0.7	–	45.4 ± 0.8	32.8–45.2	26.5–42.1	39.8–52.9	74.0	74.0	83.0

[a] Denotes no data available

Supplementary Fig. S2). Moderate to high broad-sense heritability was observed for most of the traits in all the three environments (Table 1). The highest heritability estimate of 94 % was recorded for plant height in the Delhi environment. Both positive and negative correlations among the traits were observed in all the three environments (Supplementary Table S4). There was significant negative correlation among the traits such as siliques on a plant (*Sqp*), seeds in a silique (*Ssq*) and thousand seed weight (*Tsw*) and also between silique density (*Sqdy*) and seed in a silique (*Ssq*) and thousand seed weight (*Tsw*). These traits are considered to be directly associated with yield and hence these negative correlations are suggestive of competition among the sinks for the assimilates.

QTL analysis

TD population

QTL analysis by Windows QTL Cartographer 2.5 detected 84 significant QTL in the three environments (Delhi, Bharatpur and Leh). These QTL were distributed over 17 of the 18 LGs, LG A1 being the only exception (Supplementary Table S5 and Supplementary Fig. S3). A total of 66 (78.6 %) QTL were detected as single-environment QTL, 15 (17.9 %) in two environments and 3 (3.6 %) in all the three environments. Donskaja-IV contributed trait-enhancing alleles for a total of 44 QTL and TM-4 for 40 QTL. Of the 84 significant QTL detected, 52 were identified as major QTL accounting for more than 10 % of the total phenotypic variation explained (R^2 value) in at least one of the three environments.

VH population

For an unbiased comparison of the QTL map of TD population with the QTL map of VH population, the trait values of the 12 yield-associated traits of VH population were retrieved from the published work of Ramchiary et al. (2007) and were re-analyzed using the consolidated VH map developed in the present study. The mapping criteria followed was the same as that for the QTL analysis of the TD population.

As compared to 65 QTL detected by Ramchiary et al. (2007), the re-analysis using consolidated VH map identified 94 QTL over three environments (Delhi, Gwalior and Leh) (Supplementary Table S6 and Supplementary Fig. S3). However, there was an overall agreement on the detection of major QTL between the previous analyses of Ramchiary et al. (2007) and the analyses carried out on the consolidated VH map in this study. The increase in the

number of QTL was primarily due to an increase in the minor QTL as the LOD threshold value was reduced to 2.5 in the present analysis. Of the 94 QTL identified, 75 (79.8 %) were detected as one-environment QTL, 12 (12.8 %) in the two environments and 7 (7.4 %) as three-environment. Of the 94 significant QTL detected, 37 were identified as major QTL explaining more than 10 % of the total phenotypic variance.

Comparison of two QTL maps

Comparison of QTL data of both the maps (TD and VH maps) revealed that the A genome in general contributed more QTL than the B genome (Table 2). Of the 84 QTL detected in the TD map, 48 were detected from the A genome and the remaining 36 from the B genome. Similarly, in the VH QTL map, 54 were detected in the A genome LGs and 40 in the B genome LGs. High frequency of single-environment QTL in both the populations suggested that more than 75 % of the QTL express in response to specific environment where the populations are grown.

Chromosomal distribution of yield-associated QTL revealed that the contribution of LG A10 was most significant in both the maps. The LG A10 harbors 14 major QTL for 12 quantitative traits in the TD map and 12 major QTL for 11 quantitative traits in the VH map. High QTL contribution was also observed for LGs A9 and B7 in both the maps. Three LGs, namely A1, A4 and B5, were found to be devoid of any major yield contributing QTL. These were followed by LGs A6, B1 and B2, which had one to two major QTL. While no QTL could be detected in the LG A5 in the VH map, we could detect five QTL in the same LG in the TD map. LG A7 contained six major QTL in the VH map, while only one major QTL was detected on the LG in the TD map. Significant difference in the number of QTL detected was also observed for LG B3, 12 in the VH map and only 2 in the TD map.

There was clustering of QTL in many LGs in both TD and VH maps. Most prominent clustering signifying multifunctional QTL region was observed in the LG A10 in both the maps (Supplementary Fig. S3). This multifunctional QTL region in the LG A10 contains at least one major QTL for all the 12 yield-associated traits that were studied. Similar multifunctional QTL regions were detected in LG B7 in the TD map and LG A7 in the VH map. In all these linkage groups, where there was clustering of QTL, there was association of trait-enhancing allele of one trait with trait-depressing alleles of the other traits, both being contributed by the same parent.

Table 2 Comparative chromosomal distribution of QTL and their effects in TD and VH maps based on 12 yield-associated traits

TD Map — Number of QTL

Linkage group	Plht	Pbr	Sbr	Msl	Sqms	Sqp	Sql	Ssq	Tsw	Sqdy	Df	Oil	Total
A1	-	-	-	-	-	-	-	-	-	-	-	-	0
A2	-	1_D[a]	1_D	-	-	-	-	-	-	-	-	-	2
A3	-	-	-	-	-	-	$2_{T,D}$	-	1_T	1_T[a]	-	1_D[a]	5
A4	-	-	-	-	-	-	-	-	-	-	1_T	-	1
A5	1_D[a]	-	-	-	-	-	1_D	1_D[a]	-	-	1_D	1_T	5
A6	-	-	-	-	-	-	-	-	1_D	-	-	-	1
A7	1_T	1_T	1_D	-	-	-	1_D	1_D	1_T	-	-	-	6
A8	2_T	-	-	1_T[a]	1_T[a]	1_T[a]	-	-	1_T	-	-	1_T[a]	7
A9	-	1_D[a]	$2_{T,D}$[b]	-	-	1_D[a]	-	1_T[a]	1_T[a]	1_D[a]	-	-	7
A10	1_D	1_D[a]	1_D	$2_{T,D}$[a]	1_D[a]	1_D[a]	2_T	1_T[a]	1_T[a]	1_D	1_D[a]	1_T[a]	14
Total (A)	5	3	6	3	2	3	6	4	6	3	3	4	48
B1	-	-	-	-	1_T[a]	-	-	-	-	-	-	-	1
B2	1_D[a]	-	-	-	1_T[a]	-	-	-	-	-	-	-	2
B3	-	-	-	-	-	-	-	-	-	-	-	2_D[b]	2
B4	-	1_D	1_D	-	1_T[a]	1_D[a]	-	1_T	1_D	-	-	1_D[a]	7
B5	-	-	-	-	-	1_T	-	-	-	-	-	1_T	2
B6	1_T	-	-	-	-	-	-	-	-	1_D	1_T[a]	-	3
B7	2_D[b]	1_D[a]	-	$2_{T,D}$[b]	1_T	-	1_T[a]	1_T[a]	$2_{T,D}$[b]	1_D	1_D[a]	1_T[a]	14
B8	-	-	1_T[a]	-	1_T	1_T	1_T[a]	-	1_D	-	-	-	5
Total (B)	4	2	2	2	6	3	2	2	4	2	2	5	36
Total (A+B)	9	5	8	5	8	6	8	6	10	5	5	9	84

VH Map — Number of QTL

Linkage group	Plht	Pbr	Sbr	Msl	Sqms	Sqp	Sql	Ssq	Tsw	Sqdy	Df	Oil	Total
A1	-	-	-	-	-	-	-	1_H	1_V	-	-	-	2
A2	-	-	-	1_V	1_V	-	-	-	-	-	-	-	2
A3	-	-	1_H	1_V	-	1_H	-	-	2_V	-	-	-	5
A4	-	-	-	1_H	-	-	-	-	1_V	-	1_H	-	3
A5	-	-	-	-	-	-	-	-	-	-	-	-	0
A6	1_V[a]	-	1_V	-	-	-	1_V[a]	1_V	-	-	1_H	-	5
A7	1_V	1_V[a]	1_H	-	-	-	1_V	1_H	1_V[a]	1_V[a]	1_V[a]	1_H[a]	9
A8	-	-	-	-	-	$2_{V,H}$	1_V	-	2_V	-	1_V	2_V[b]	7
A9	-	1_H[a]	1_V[a]	-	-	-	2_V[b]	1_V[a]	-	1_H[a]	1_H[a]	-	7
A10	1_V[a]	-	$2_{V,H}$[b]	-	$3_{V,H}$[c]	2_V[b]	1_V	-	1_V[a]	1_H[a]	1_H	1_V	14
Total (A)	3	2	6	3	4	5	6	4	8	4	5	4	54
B1	-	-	-	-	-	-	1_V	-	2_V	-	-	-	3
B2	1_H	-	-	-	-	-	-	-	-	-	-	-	1
B3	-	-	-	-	$3_{V,H}$[d]	1_H[a]	2_V[b]	1_H	2_V[b]	3_H[b]	-	-	12
B4	-	-	1_H	-	1_H[a]	2_H[b]	1_V	-	-	-	-	-	5
B5	-	-	-	1_H	-	-	-	-	-	-	1_H	-	2
B6	1_H	1_H	-	-	-	-	-	-	-	-	-	-	2
B7	-	1_H	-	1_H	-	1_H[a]	1_V	1_H[a]	1_V	-	1_H[a]	2_V[b]	9
B8	2_H[b]	-	-	-	1_H	1_H[a]	1_V	-	-	-	1_H	1_H	6
Total (B)	4	2	1	2	4	4	6	2	5	3	3	4	40
Total (A+B)	7	4	7	5	8	9	12	6	13	7	8	8	94

T, D, V and H: QTL with trait-enhancing allele from TM-4, Donskaja-IV, Varuna and Heera, respectively

Plht plant height, Pbr primary branches, Sbr secondary branches, Msl main shoot length, Sqms siliques per main shoot, Sqp siliques per plant, Sql silique length, Ssq seeds per silique, Tsw thousand seed weight, Sqdy silique density, Df days to flower, Oil seed oil content

[a] Detected as major QTL

[b] One of the two QTL is detected as major QTL

[c] Two of the three QTL are detected as major QTL

[d] One of the three QTL are detected as major QTL

Meta-analysis of QTL

Meta-analysis of QTL was performed for the LGs A3, A7, A8, A9, A10, B3, B4, B7 and B8. Considering the QTL detected in each environment as an independent QTL, a total of 187 QTL ranging from 11 in the LG A3 to 54 in the LG A10 were subjected to meta-analysis by projecting the QTL map of the TD population onto that of the VH population. At the best fit AIC value, all the 187 QTL distributed over nine LGs were integrated into 20 meta-QTL (Table 3). Maximum resolution was observed in the LG A10 wherein all the 54 QTL were integrated into one meta-QTL. The confidence interval of this meta-QTL was resolved to 3.0 cM showing a six times reduction in the confidence interval from the largest interval of 17.9 cM. This meta-QTL is flanked by the markers At5g16330 (29.0 cM) and At5g14660 (35.2 cM) (Supplementary Fig. S1 and Fig. S4).

Detection of epistatic QTL

Digenic epistatic interactions of QTL for the 12 yield-associated traits was carried out for the TD and the VH populations using QTL Network 2.1 (Supplementary Tables S7 and S8). Following Li et al. (2001), digenic epistatic interactions were classified into three categories: (1) interaction between two QTL with significant additive effects (Type 1), (2) interaction between one locus with significant additive effect and a locus without significant additive effect (Type 2) and (3) interaction between two loci without significant additive effects (Type 3).

TD population

A total of eight pairs of epistatic QTL involving 14 loci were detected in six of the 12 traits analyzed (Supplementary Table S7). Of the 14 loci involved in epistatic interactions, 9 were observed to be QTL with significant additive effects exhibiting four pairs of Type 1 and two pairs of Type 2 interactions (Table 4). Silique density (*Sqdy*) exhibited three pairs of epistatic interactions, while other five traits, namely plant height (*Plht*), main shoot length (*Msl*), silique length (*Sql*), seeds in a silique (*Ssq*) and days to flower (*Df*) exhibited one pair of interaction each. The QTL in the confidence interval of 44.4–50.1 cM in the linkage group B7 was found to be involved in two digenic interactions for silique density. Phenotypic variance explained by epistasis in TD population was low and ranged from 0.7 % for seeds in a silique (*Ssq*) to 3.3 % for silique density (*Sqdy*).

VH population

All the quantitative traits except the number of secondary branches and number of siliques on a plant showed digenic epistatic interactions in the VH population. A total of 60 loci were found to be involved in 32 pairs of digenic interactions (Supplementary Table S8) in 10 of the 12 quantitative traits analyzed. Of the 60 loci involved in digenic interactions, 26 loci were QTL with significant additive effects wherein 13 pairs of interacting loci showed Type 1 and four pairs showed Type 2 interaction (Table 4). It was interesting to observe that all the four pairs of epistatic interaction for thousand seed weight were of Type 1 (Fig. 1), followed by seed oil content in which three of the four digenic interactions were of Type 1. Epistatic interaction analysis also showed involvement of a locus in more than one digenic interaction. This type of interaction was observed for the trait of primary branches (*Pbr*) (the locus in the genetic interval of 6.8–10.9 cM in the LG A7), thousand seed weight (*Tsw*) (the locus in the genetic interval of 21.5–33.8 cM in the LG A3 and the locus in the genetic interval of 16.3–24.7 cM in the LG A4) and seed oil content (*Oil*) (the locus in the genetic interval of 25.7–32.5 cM in the LG A10). Phenotypic variance explained by epistasis for various yield-associated traits ranged from 2.3 % for seeds in a silique to 13.6 % for silique length.

Discussion

The two DH mapping populations (TD and VH) used in this study have been derived from two different bi-parental crosses wherein one parent is from the Indian gene pool and the other from east European gene pool of *B. juncea*. The VH population is derived from a cross between two extremely diverse parents Varuna and Heera, while the TD population is derived from a cross between two moderately diverse parents TM-4 and Donskaja-IV (Srivastava et al. 2001). The most striking difference between the two east European parents used in the study is that Heera originated through interspecific crosses, whereas Donskaja-IV is a natural east European type line of *B. juncea* (Srivastava et al. 2001). Hence, a detailed genetic dissection by QTL analysis using these two mapping populations was expected to uncover a whole gamut of genetic diversity and allow identification of consensus as well as population-specific QTL that might exist in the two gene pools.

In the earlier QTL analysis of the VH population, Ramchiary et al. (2007) had shown that the A genome made more significant contribution to productivity in *B. juncea*. QTL dissection of the TD population for the yield-influencing traits also revealed a similar trend of high contribution from the A genome compared to that by the B genome. We also observed high frequency of single-environment QTL in both the populations. One of the reasons for this observation could be the differential expression of

Table 3 Meta-QTL for yield-influencing traits identified in *B. juncea*

LG	Number of input QTL[a]	Consensus QTL model (k)	AIC[b] value	Position (cM) of M-QTL(s)	CI of M-QTL (cM) From	CI of M-QTL (cM) To	CI of M-QTL (cM) Length	CI of smallest QTL	CI of largest QTL	Mean CI of input QTL	Coefficient of reduction in CI from mean of input QTL to M-QTL	Coefficient of reduction in CI from CI of largest QTL to M-QTL	Coefficient of reduction in CI from CI of smallest QTL to M-QTL
A3	11	2	101.8	45.1	34.9	55.3	20.4	6.9	13.7	9.3	0.5	0.7	0.3
				111.1	91.1	131.1	40.0	3.8	10.2	6.5	0.2	0.3	0.1
A7	20	2	172.4	8.3	4.3	12.2	7.9	1.1	15.0	6.6	0.8	1.9	0.1
				51.8	39.7	63.9	24.2	5.0	12.1	8.4	0.3	0.5	0.2
A8	18	2	166.9	24.3	18.9	29.6	10.7	4.4	11.1	7.4	0.7	1.0	0.4
				61.5	52.1	70.8	18.7	5.5	17.2	10.2	0.5	0.9	0.3
A9	17	2	168.2	66.9	60.7	73.1	12.4	1.3	10.8	6.1	0.5	0.9	0.1
				117.7	103.5	131.7	28.2	5.2	12.1	8.7	0.3	0.4	0.2
A10	54	1	389.7	32.2	30.7	33.7	3.0	0.9	17.9	7.3	2.4	6.0	0.3
B3	16	3	158.4	27.3	15.4	39.2	23.8	3.0	15.1	8.7	0.4	0.6	0.1
				81.4	74.5	88.3	13.8	4.3	13.6	8.2	0.6	1.0	0.3
				111.4	101.8	121.0	19.2	7.4	13.4	9.7	0.5	0.7	0.4
B4	12	2	110.3	57.1	47.6	66.6	19.0	7.1	24.4	14.9	0.8	1.3	0.4
				116.0	103.3	128.8	25.5	6.2	10.3	8.0	0.3	0.4	0.2
B7	27	3	283.5	20.9	13.1	28.8	15.7	3.2	20.0	9.1	0.6	1.3	0.2
				71.9	61.7	82.2	20.5	4.4	9.9	6.5	0.3	0.5	0.2
				128.3	120.2	136.3	16.1	2.7	20.0	9.5	0.6	1.2	0.2
B8	12	3	118.5	29.7	15.5	42.1	26.6	7.2	–[c]	–	–	–	–
				77.3	67.1	87.4	20.3	3.9	19.4	11.0	0.5	1.0	0.2
				121.8	109.5	134.0	24.5	5.0	16.9	10.6	0.4	0.7	0.2

CI confidence interval (calculated as 1-LOD drops on the left and right side of the peak of the QTL)

[a] QTL detected in each environment were considered as independent

[b] AIC value: Akaike information criterion

[c] Only one QTL in this meta-QTL

Table 4 Number and type of epistatic interactions identified in the TD and VH mapping populations

Trait	Mapping population	Number of loci[a]	Type of epistasis[b]			Total number of interactions	Range of the AA effects[e]		$V_p(e)$[f]
			I	II	III		Minimum	Maximum	
Plht	TD	2	0	1	0	1	–[c]	–	0.0105
	VH	4	0	1	1	2	−5.2481	−3.8904	0.0252
Pbr	TD	–[d]	–	–	–	–	–	–	–
	VH	3	2	0	0	2	−0.5402	0.1839	0.0911
Msl	TD	2	1	0	0	1	–	–	0.0146
	VH	4	0	0	2	2	1.5350	2.4953	0.0643
Sqms	TD	–	–	–	–	–	–	–	–
	VH	10	1	1	3	5	−1.8794	1.7725	0.1124
Sql	TD	2	0	1	0	1	–	–	0.0176
	VH	10	0	1	4	5	−0.1902	0.1754	0.1364
Ssq	TD	2	0	0	1	1	–	–	0.0070
	VH	4	1	0	1	2	−0.2757	0.3598	0.0227
Tsw	TD	–	–	–	–	–	–	–	–
	VH	6	4	0	0	4	0.0717	0.1431	0.1018
Sqdy	TD	5	2	0	1	3	0.0287	0.0437	0.0326
	VH	6	1	1	1	3	−0.0361	0.0238	0.0804
Df	TD	2	1	0	0	1	–	–	0.0097
	VH	6	1	0	2	3	−1.4477	−0.9725	0.0277
Oil	TD	–	–	–	–	–	–	–	–
	VH	7	3	0	1	4	−0.3600	0.4600	0.0321

Plht plant height, *Pbr* primary branches, *Sbr* secondary branches, *Msl* main shoot length, *Sqms* siliques per main shoot, *Sqp* siliques per plant, *Sql* silique length, *Ssq* seeds per silique, *Tsw* thousand seed weight, *Sqdy* silique density, *Df* days to flower, *Oil* oil

[a] Number of loci involved in digenic epistatic interactions

[b] Epistatic interaction between (I) two loci with significant main effects, (II) a locus with significant main effect and a locus without significant main effect, and (III) two loci without significant main effects

[c,d] No range could be specified and/no epistatic interactions were identified

[e] Positive values of AA indicate that trait value is increased by parental association of the alleles for the two interacting loci and vice versa

[f] $V_p(e)$: percentage of phenotypic variance explained by epistasis

genes in the environments differing in photoperiod (short day conditions in Delhi, Gwalior and Bharatpur and long day conditions in the high altitude Leh), as the east European parents (Donskaja-IV and Heera) used in these two bi-parental populations are photoperiod sensitive. Comparative QTL analysis in the present study revealed the presence of QTL clusters on A7, A8 and A10 in both VH and TD maps and linkage group B7 in TD map, suggesting tight linkage or pleiotropy. QTL hotspots have previously been described by Quijada et al. (2006), Udall et al. (2006), Radoev et al. (2008), Basunanda et al. (2010) and Shi et al. (2009) in *B. napus*. To distinguish between linkage and pleiotropy is difficult and calls for an increase in the number of segregating individuals and/or a greater marker saturation in the region of interest (Tuberosa et al. 2002). The observation of population-specific QTL, particularly in the linkage groups A5 and B3, revealed the existence of new genes in different bi-parental populations. Besides, some new yield-influencing QTL have been located

independently in the east European line Donskaja-IV by comparative QTL analysis. A major QTL for the number of secondary branches and another one for the number of siliques on a plant were located in the LG A2. Two major QTL have been located for seed oil content in Donskaja-IV, one each in the LG A3 and LG B3. These loci could possibly be transferred to Indian germplasm without negative effects for increasing oil content.

The present study confirms that in terms of yield QTL, the most important linkage group is A10 with major QTL for all the 12 yield-influencing traits in a multi-functional QTL region in both the maps. The two maps also showed similarity for the source of trait-enhancing alleles for this linkage group. For example, Indian parents, TM-4 and Varuna, contributed trait-enhancing alleles in the QTL for silique length, number of seeds in a silique, thousand seed weight and seed oil content, while Donskaja-IV and Heera contributed trait-enhancing alleles in the QTL for plant height, number of primary branches, number of siliques on
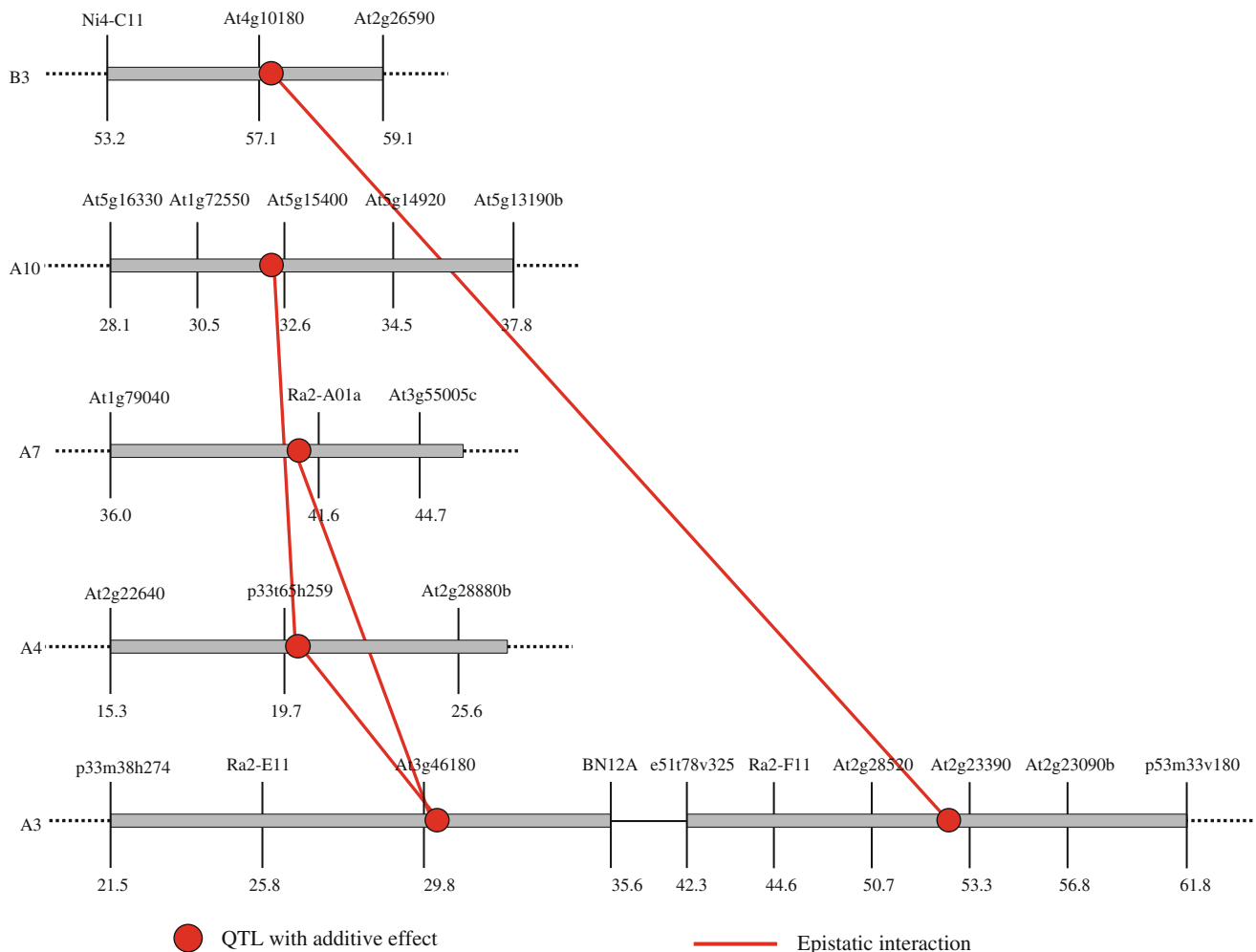
Fig. 1 Network of epistatic QTL for thousand seed weight (*Tsw*) on linkage groups A3, A4, A7, A10 and B3 in VH population detected by QTL Network 2.1. All the QTL were detected as significant additive QTL either by Windows QTL Cartographer 2.5 or QTL Network 2.1 or both

a plant, silique density and days to flower. These observations confirm that yield improvement in Indian gene pool has been achieved primarily through selection for longer siliques, larger seeds and higher oil content.

Meta-QTL analysis of linkage group A10 for the QTL hotspot region containing 54 QTL from VH and TD maps identified one consensus meta-QTL. This meta-QTL mapped to an interval of 3.0 cM showing six-fold reduction in the confidence interval than their original QTL maps. The smaller confidence interval makes it possible to focus research efforts in this narrow multi-functional QTL region to identify causal polymorphisms using higher resolution mapping strategies that permit easy identification of candidate genes. The concept of meta-analysis has been applied in many crop plants such as rice, cotton, soybean, wheat, maize and cocoa for several traits (Swamy et al. 2011) including those in *Brassica napus* (Shi et al. 2009; Hanocq et al. 2007; Lü et al. 2008; Chardon et al. 2004; Rong et al. 2007). It provides a new method for comparing

QTL from different maps and revealing the consensus QTL through the use of a consensus map. The meta-QTL with the smallest confidence interval (CI) and having a consistent and large effect could, therefore, be useful in MAS and cloning of QTL.

A search for candidate genes through in silico analysis of the meta-QTL region (At5g14660–At5g16330) on LG A10 of *B. juncea* yielded a total of 101 genes in *B. rapa* (R-block) with orthologs in *Arabidopsis thaliana*. Of these 101 genes annotated on the Brassica database, 12 genes were observed to be related to yield, agronomic and other morphological traits. These genes affect the number of branches (At5g15100), flowering time (At5g15160, At5g15230, At5g15840, At5g15850, At5g16260 and At5g16320), plant height (At5g15230), seed weight and seed yield (At5g 15230) (Shi et al. 2009; Ding et al. 2012). Some of the genes have been shown to be involved in photomorphogenesis and regulation of timing of transition from vegetative to reproductive phase (At5g15160), flower and ovule

development (At5g15800), establishment of the whorled pattern of floral organs (At5g15710) and seed oil synthesis (At5g15530, At5g16230 and At5g16240).

The study on epistatic interaction has demonstrated substantial additive × additive epistatic effects indicating that epistasis plays an important role in trait variation. The TD population identified 8 digenic interactions as against 32 digenic interactions identified in the VH population. In general, a higher frequency of digenic interactions of Type 1 and Type 2 (23 interactions) were detected than Type 3 interactions (17 interactions) in the present study (Table 4). Our results are in agreement with the work of Zhao et al. (2005) on *B. napus* which showed higher frequency of Type 1 and Type 2 interactions for the oil content and in variance to the results of Radoev et al. (2008) which detected higher frequency of Type 3 digenic interaction for yield-associated traits in a DH population in *B. napus*. Loci without significant additive effects involved in Type 2 and Type 3 interactions have also been labeled as 'modifiers' and are thought to act as genetic buffers providing functional redundancy for species to survive perturbations (Yang et al. 2007; Greenspan 2001; Radoev et al. 2008; Yu et al. 1997; Luo et al. 2001; Li et al. 2008; Melchinger et al. 2007) or are genes having too small additive effects to be detected by currently available algorithms to be declared as significant QTL (Zhao et al. 2005).

Some of the loci involved in epistatic interactions (one locus for silique density in the TD population and four loci for number of primary branches, thousand seed weight and seed oil content in the VH population) interacted with more than one locus (Supplementary Tables S7 and S8). These multi-locus interactions have been proposed to constitute higher order epistatic interactions (Zhao et al. 2005). Hence, the epistatic interactions observed in the present study could actually be an underestimation.

It was also observed that all the trait-enhancing alleles of additive QTL for the thousand seed weight were contributed by the Indian parent in the VH population (Supplementary Table S6). It was also observed that six out of the seven main effect QTL (Supplementary Table S8) were involved in epistatic interactions, and the QTL in the linkage group A3 in the genetic interval 21.5–35.8 cM and that in the linkage group A4 in the genetic interval 16.3–24.7 cM were involved in epistatic interactions with more than one locus. Hence, for the maximum gain particularly for the seed size, all the main effect QTL involved in epistatic interactions should be introgressed simultaneously from the Indian genotypes to the east European genotypes.

Our results reveal the complex pattern of QTL and their interaction effects underlying the yield-influencing traits in *B. juncea*. Moreover, by analyzing the marker and QTL data from two different mapping populations, we have been able to identify common QTL and QTL which are unique to genetic backgrounds. The integrated map with high density of markers and utilizing the advantages of different marker systems is anticipated to serve as a reference map for breeding yield traits in *B. juncea*.

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