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ORIGINAL PAPER

Mapping of yield influencing QTL in *Brassica juncea*: implications for breeding of a major oilseed crop of dryland areas

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Abstract Quantitative trait loci (QTL) analysis of yield influencing traits was carried out in *Brassica juncea* (AABB) using a doubled haploid (DH) mapping population of 123 lines derived from a cross between Varuna (a line representing the Indian gene pool) and Heera (representing the east European gene pool) to identify potentially useful alleles from both the parents. The existing AFLP based map of *B. juncea* was further saturated with RFLP and SSR markers which led to the identification of the linkage groups belonging to the A (*B. rapa*) and B (*B. nigra*) genome components of *B. juncea*. For QTL dissection, the DH lines were evaluated at three different environments and phenotyped for 12 quantitative traits. A total of 65 QTL spread over 13 linkage groups (LG) were identified from the three environments. QTL analysis showed that the A genome has contributed more than the B genome to productivity (68% of the total QTL detected) suggesting a more prominent role of the A genome towards domestication of this crop. The east European line, Heera, carried favorable alleles for 42% of the detected QTL and the remaining 58% were in the Indian gene pool line, Varuna. We observed clustering of major QTL in a few linkage groups, particularly in J7 and J10 of the A genome, with QTL of different traits having agronomically antagonistic allelic effects co-mapping to the same genetic interval. QTL analysis also identified some well-separated QTL which could be readily transferred between the two pools. Based on the QTL analysis, we propose that improvement in yield could be achieved more readily by heterosis breeding rather than by pure line breeding.

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Introduction

Crops that have been subjected to detailed genetic analysis and intensive breeding (e.g. tomato and corn) as well as those which are vital for global food security such as rice and wheat have been targeted for extensive molecular mapping and QTL analyses in recent years (Asins 2002). However, crops restricted to certain areas of the tropics and subtropics have, by and large, remained out of the purview of involved genetic mapping work. *Brassica juncea*, the Indian oilseed mustard, is one such crop. The crop is grown in around six million hectares in the north-western region of India during the winter growing season either under completely rainfed or limited protective irrigation conditions. The average yields of the best check varieties in India are around 2.2 tonnes per hectare. Any improvement in yield of *B. juncea* would augment the

income of farmers inhabiting dry land areas. Yield increase might also make this crop a viable alternative in the irrigated areas of north-western India where the continuous wheat-rice cycles are steadily depleting ground water resources.

B. juncea (AABB) is an allopolyploid species containing the genomes of two diploid species namely, *B. rapa* (AA) and *B. nigra* (BB). We have shown earlier that there are two distinct and genetically diverse gene pools in *B. juncea*, the east European and the Indian gene pools (Srivastava et al. 2001). In our extensive initial field experiments (unpublished), we observed that the two gene pools possess many contrasting agronomic traits and that the *B. juncea* lines belonging to the Indian gene pool show more *B. rapa* (AA)-like characterstics whereas those belonging to the east European gene pool show closeness to *B. nigra* (BB) for many agronomic and yield influencing traits (Table 1). The east European lines, generally tall and late in maturity, are ill-adapted to winter growing conditions of the Indian subcontinent and therefore, are poor yielders. However, these lines possess many desirable yield enhancing component traits. Extensive attempts by our group to combine the positive agronomic traits from both gene pools through pedigree breeding did not lead to any superior selection that surpassed the yield of the Indian gene pool parent. In comparison, we found that hybrids between the lines belonging to the two gene pools were heterotic for both vegetative growth and yield (Pradhan et al. 1993). Subsequently, we developed two hybrids namely DMH-1 using a CMS system (Sodhi et al. 2006) and DMH-11 using molecular methods (Jagannath et al. 2001, 2002) for pollination control.

QTL analysis of major component traits pertaining to yield could be helpful in understanding the reason behind the failure to obtain useful recombinants through pedigree breeding. This kind of analysis could help locate the favorable alleles and determine their organization and, consequently, help breeders in devising strategies for transferring these loci from one gene pool to the other. In this study, we report a detailed QTL analysis of a cross between an Indian line, Varuna and an east European line, Heera for 12 important agronomic traits related to yield and domestication of this crop. For this purpose, the linkage map of *B. juncea* described earlier (Pradhan et al. 2003) was further saturated by addition of SSR and RFLP markers. The SSR and RFLP markers helped us to distinguish the linkage groups belonging to the A and B genomes of *B. juncea*. Our analysis shows that many of the major QTL are clustered on a few linkage groups and that there is an antagonistic association of allelic effects among QTL of different traits co-mapping to the same genetic interval. Based on these findings, we discuss whether pedigree breeding or heterosis breeding would be the method of choice for further yield enhancement in mustard.

Table 1 Agronomic trait values of Indian and East European *B. juncea* germplasm in comparison with two diploid progenitor parents *B. rapa* and *B. nigra*

Agronomic traits[a]	Indian *B. juncea*[b]	East European *B. juncea*[c]	*B. rapa*[d]	*B. nigra*[e]
Plant height (*Plht*)	177.1 ± 7.9	267.5 ± 53.3	151.5 ± 19.4	225.9 ± 15.6
Number of primary branches (*Pbr*)	5.6 ± 0.4	10.4 ± 3.3	7.5 ± 1.7	12.4 ± 2.1
Number of secondary branches (*Sbr*)	12.7 ± 3.1	22.2 ± 3.8	7.9 ± 3.5	20.6 ± 2.0
Main shoot length (*Msl*)	79.6 ± 6.4	38.1 ± 21.5	63.9 ± 9.4	36.6 ± 2.4
Siliqua per main shoot (*Sqms*)	50.4 ± 5.2	37.1 ± 9.6	58.1 ± 11.9	39.0 ± 2.4
Siliqua density (*Sqdy*)	0.63 ± 0.06	1.13 ± 0.31	0.92 ± 0.18	1.06 ± 0.07
Siliqua per plant (*Sqp*)	462.5 ± 96.1	753.0 ± 116.6	355.1 ± 106.2	748.5 ± 60.4
Siliqua length (*Sql*)	6.2 ± 0.8	4.5 ± 0.4	5.1 ± 0.7	2.2 ± 0.2
Seeds per siliqua (*Ssq*)	13.5 ± 0.8	13.9 ± 2.7	20.1 ± 4.8	7.2 ± 0.9
Test weight (*Tsw*)	5.2 ± 1.0	2.3 ± 0.4	2.5 ± 0.3	1.3 ± 0.2
Days to flower (*Df*)	52.3 ± 1.4	101.5 ± 25.2	65.7 ± 11.5	89.8 ± 8. 7
Days to mature (*DM*)	149.0 ± 5.2	169.9 ± 5.8	150.0 ± 7.9	163.8 ± 11.8

[a] Agronomic trait values were recorded from a field experiment conducted at Delhi during 2004–05. Nine lines and/or varieties of the above mentioned four types of Brassicas were grown in a replicated trial with three replications. Five competitive plants from each line /replication were used for recording the quantitative data

[b] Indian *B. juncea* lines were Varuna, Pusa Bold, RH-30, Rajat, Kranti, RN-247, Pusa Jai Kissan, PBY-1 and Proagro

[c] East European (Exotic) *B. juncea* lines were Cutlass, Zem-84-500, BJ-ATC-94394, Skorospieka-II, Heera, Donskaja-IV, Kranodonskaja, Vinimiik-II and Niesopajcosji

[d] *B. rapa* lines were BSH-1, Pusa Kalyani, Ecko, DYS-1, Tora Type (BS), Autralian Campestris, Torch, R500 and Agena

[e] *B. nigra* lines were IC257, CV-BRAI-044-81, ATC-93845, ATC-93961, ATC-93960, ATC-93967, ATC-9384155, CV-VINCE and ATC-90745

Materials and methods

Plant materials

A population of 123 doubled haploid (DH) lines was developed from the F1 of a cross between an Indian variety, Varuna, and the canola quality mustard line, Heera. This population was used earlier for the development of a *B. juncea* map (Pradhan et al. 2003). Phenotypic data on different quantitative traits were obtained by growing the parents and the mapping population in three different environments in India namely: Delhi (normal winter growing season, short day condition); Gwalior, Madhya Pradesh (normal winter growing season, short day condition but temperatures higher than Delhi during seed maturity); Leh, Jammu and Kashmir (summer season, long day condition). The lines were planted in a randomized complete block design with three replications. Each line was planted in three rows with a row length of 3 m per replication. Phenotyping was done from 15 competitive plants (five from each replication) and the mean of these 15 observations was used as the trait value.

Trait measurement

The twelve traits evaluated included plant height (*Plht*), days to flowering (*Df*), primary branches (*Pbr*), secondary branches (*Sbr*), main shoot length (*Msl*), siliqua on the main shoot (*Sqms*), siliqua on a plant (*Sqp*), siliqua density (*Sqdy*), siliqua length (*Sql*), seeds in a siliqua (*Ssq*), test weight (*Tsw*) and seed oil content (*oil*). Plant height was measured from the ground to the tip of the main shoot. Days to flowering was recorded when about 50% of the plants in a plot had at least one flower open. Primary and secondary branches were the number of branches arising out of the main shoot and primary branches, respectively. Main shoot length was measured from the base of the last primary branch to the tip of the main shoot. Siliqua on the main shoot and siliqua on a plant were the number of well-filled, normal siliqua in the main shoot and the whole plant, respectively. Siliqua density was calculated as the number of siliqua per unit length of the main shoot. Siliqua length was measured from base to the tip of the siliqua. Seeds in a siliqua were counted as the number of well-developed seeds in a siliqua. Test weight was measured as 1,000-seed weight. Seed oil content was estimated using near infrared spectroscope (NIRS) following Mika et al. (2003).

Linkage map construction

DNA was isolated from well-expanded leaves of field grown plants following Rogers and Bendich (1994). RFLP clones, coded as "pW" (earlier referred to as "wg" and "tg") and "pX" (earlier referred to as "ec") (Parkin et al. 1995; Sharpe et al. 1995) were provided by T. C. Osborn, University of Wisconsin. Detection of polymorphism, Southern hybridization and genotyping were done following Pradhan et al. (2003). For mapping SSR markers, primers for 335 microsatellites—108 from *B. rapa*, 98 from *B. nigra* and 129 from *B. napus* (available in the Brassica DB database: www.brassica.info/)—were custom synthesized (Microsynth). DNA amplification and genotyping of the mapping population was carried out following Padmaja et al. (2005). New markers were added to the existing map using the program JoinMap version 2.0 (Stam 1993; Stam and Van Ooijen 1996).

QTL mapping

Trait means, ANOVAs, correlations and heritabilities were determined using the SPAR 1 (1991) software. A framework map with an average marker density of approximately 10 cM was used for QTL dissection of quantitative traits. All the traits were analyzed via composite interval mapping (CIM; Zeng 1993, 1994) using the software package WinQTL Cartographer version 2.5 (Wang et al. 2001–2005). Tests for the presence of QTL were performed at 2 cM intervals using a 10 cM window and five background cofactors, which were selected via forward regression analysis (Model 6). For declaring the presence of a QTL, genome wide threshold values ($\alpha = 0.05$) were estimated from 1,000 permutations of trait data across all genetic intervals (Churchill and Doerge 1994; Doerge and Churchill 1996).

Results

Identification of the linkage group belonging to the A and B genomes

Further saturation of the existing *B. juncea* map (Pradhan et al. 2003) was carried out by the addition of more AFLP, RFLP, SSR and gene markers. Screening 90 RFLP clones detected polymorphism between the two parents for 36 of the tested clones. These 36 clones detected 39 RFLP loci which were mapped to the *B. juncea* genome. Of the 335 SSRs of *B. rapa*, *B. nigra* and *B. napus* origin that were tested, 222 SSRs showed amplification products from *B. juncea*. However, polymorphism between Varuna and Heera was detected only in 66 SSRs (19 from *B. rapa*, 34 from *B. nigra* and 13 from *B. napus*). These 66 SSRs generated 69 loci, which mapped to all the 18 LGs of the existing *B. juncea* map (Table 2). The high-density *B. juncea* map currently available in our laboratory has 1,448 markers comprising 1,297 AFLP, 72 RFLP, 69 SSR and ten gene markers covering a total length of 1,840.1 cM (Table 2). It is available in Fig. S1 of electronic supplementary material

Table 2 Characteristics of the *B. juncea* map constructed with 1,448 markers

New nomenclature of LGs	Existing nomenclature of LGs[a]	Total number of markers	AFLP	RFLP	SSR	Gene markers	Length (cM)	Genome assigned	Corresponding A genome LGs[b] of *B. napus*
J1	LG4	75	63	8	4	–	128.9	A	N1
J2	LG10	94	83	9	2	–	97.4	A	N2
J3	LG6	123	103	9	11	–	117.5	A	N3
J4	LG16	60	53	5	2	–	55.0	A	N4
J5	LG13	84	74	6	3	1	100.6	A	N5
J6	LG8	41	36	4	1	–	91.9	A	N6
J7	LG14	65	56	5	3	1	68.8	A	N7
J8	LG17	74	66	4	2	2	73.8	A	N8
J9	LG1	114	106	3	3	2	129.7	A	N9
J10	LG18	42	36	3	3	–	72.0	A	N10
J11	LG15	31	27	0	2	2	66.4	B	–
J12	LG12	92	88	1	2	1	149.4	B	–
J13	LG3	83	71	5	6	1	123.6	B	–
J14	LG11	65	59	3	3	–	77.7	B	–
J15	LG5	153	145	2	6	–	125.4	B	–
J16	LG7	36	32	2	2	–	129.3	B	–
J17	LG9	104	99	0	5	–	102.1	B	–
J18	LG2	112	100	3	9	–	130.6	B	–
Total		1,448	1,297	72	69	10	1,840.1		

[a] Pradhan et al. (2003)

[b] Butruille et al. (1996), Udall et al. (2006), Quijada et al. (2006)

(ESM). The majority of the *B. rapa* and *B. napus* specific SSR markers mapped exclusively to LG4, LG6, LG10, LG13, LG14 and LG17 while the majority of the SSR markers of *B. nigra* origin mapped exclusively to LG2, LG3, LG5, LG9, LG11 and LG15 of *B. juncea* map (Pradhan et al. 2003). LG16 and LG18 contained SSR markers of both *B. napus* and *B. nigra* origin, LG7 and LG12 contained SSR markers of both *B. rapa* and *B. nigra* origin and LG1 contained SSR markers originating from all three species. Comparison of common RFLP and SSR markers between our map of *B. juncea* and published maps of *B. napus* (Butruille et al. 1999; Piquemal et al. 2005; Quijada et al. 2006; Udall et al. 2006) and *B. rapa* (Teutonico and Osborn 1994) revealed that LG1, LG4, LG6, LG8, LG10, LG13, LG14, LG16, LG17 and LG18 of our map corresponded to N9, N1, N3, N6, N2, N5, N7, N4, N8 and N10, respectively of the *B. napus* map. These were designated as the A genome LGs (Table 2 and Fig. S2 of ESM). The remaining eight LGs (LG2, LG3, LG5, LG7, LG9, LG11, LG12 and LG15) of our map were identified as the B genome LGs (Table 2). Following the internationally standard nomenclature of linkage groups belonging to U's triangle, the A genome LGs of *B. juncea* were designated as J1–J10 corresponding to N1–N10, respectively of the *B. napus* map. The remaining eight LGs of the B genome were designated as J11–J18 as shown in Table 2.

Quantitative trait variation in parents and mapping population

The parents used for developing the recombinant DH population were diverse with respect to the majority of the quantitative traits studied. Varuna showed larger seed size, greater siliqua and main shoot length, flowered earlier and had shorter height. In comparison, Heera had more siliqua on a single plant as well as the main shoot, more seeds in a siliqua, more primary and secondary branches, was longer in maturity duration and had greater plant height (Table 3). Transgressive segregation was recorded in the DH mapping population for all the quantitative traits. Distribution analysis of all the 12 quantitative traits from the three environments (Delhi, Leh and Gwalior) showed a nearly normal distribution (data not shown). Broad sense heritability was observed to be high for plant height, siliqua length, seeds in a siliqua, test weight and days to flower in all the environments. The rest of the traits showed moderate to low broad sense heritability (Table 3).

The correlation between traits was estimated by regressing phenotypic values of one trait on those of another trait. There were differences in the trend (both degree and direction) among the three environments; plant height, in general, showed positive correlations with primary branches, siliqua on the main shoot, siliqua on a single plant, siliqua

Table 3 Mean phenotypic trait values of the parents and the observed range and broad sense heritability in the DH mapping population from three environments (Delhi, Gwalior and Leh)

Traits	Mean trait value of parent Varuna			Mean trait value of parent Heera			Range in the mapping population			Broad sense heritability		
	Delhi	Gwalior	Leh	Delhi	Gwalior	Leh	Delhi	Gwalior	Leh	Delhi	Gwalior	Leh
Plht	168	178	112	215	218	139	133–273	143–272	79–171	90.0	83.7	67.0
Pbr	6	5	4	7	6	4	5–17	4–10	3–6	89.6	46.4	40.6
Sbr	12	11	7	12	3	8	6–13	2–19	3–13	65.4	44.2	38.4
Msl	81.3	90.8	73.5	69.6	79.9	73.1	22.9–91.1	40.3–90.3	46.2–94.0	89.2	70.3	59.9
Sqms	56	50	38	61	67	56	27–84	37–70	27–56	71.7	54.3	58.0
Sqp	390	267	150	653	256	325	293–1132	161–507	88–434	44.5	24.9	37.2
Sql	7.2	8.4	8.5	4.5	4.6	5.1	3.8–7.1	4.1–7.9	4.9–8.9	86.3	89.6	80.5
Ssq	12	14	16	16	15	19	10–21	10–12	13–23	69.2	75.1	57.5
Tsw	6.3	5.3	4.5	2.3	2.3	2.5	1.9–6.4	2.2–5.6	1.9–4.6	94.0	92.7	75.2
Sqdy	0.69	0.54	0.45	0.74	0.84	0.76	0.39–1.73	0.53–1.07	0.42–0.82	81.6	53.2	76.4
Df	54	58	52	65	66	62	55–117	44–97	47–57	93.5	96.1	65.2
Oil	43.9	44.0	39.9	39.6	40	37.5	38.2–50.3	37.9–50.6	35.6–45.3	75.3	67.8	68.7

Plht plant height, *Pbr* primary branches, *Sbr* secondary branches, *Msl* main shoot length, *Sqms* siliqua on the main shoot, *Sqp* siliqua on a single plant, *Sql* siliqua length, *Ssq* seeds in a siliqua, *Tsw* thousand seed weight, *Sqdy* siliqua density, *Df* days to flowering, *Oil* seed oil content

density and days to flower. Among the yield components, primary and secondary branches showed positive correlations with siliqua on a single plant and siliqua density, whereas the main shoot length showed positive correlations with siliqua on the main shoot, siliqua on a single plant and siliqua length, but negative correlation with siliqua density. On the other hand, siliqua on a single plant showed positive correlation with siliqua density. Siliqua length showed positive correlation with test weight but was found to be negatively correlated with siliqua on the main shoot, siliqua on a single plant and siliqua density (Table S1 of ESM).

Mapping and identification of trait improving QTL alleles

QTL analysis of the 12 quantitative traits was carried out using a LOD threshold of 2.8. The confidence interval for QTL detected taking 1 LOD support limit for 12 quantitative traits ranged from 4.2 to 28 cM. A total of 65 significant QTL spread over 13 linkage groups (LG) were detected from all the three environments (Fig 1 and Table S2 of ESM). Of the 65 QTL, nine were detected in all the three environments (*Plht1*, *Plht6*, *Sql7*, *Tsw8*, *Sqdy4*, *Df2*, *Df3*, *Oil2* and *Oil7*), 15 in two environments (*Plht4*, *Plht5*, *Sqp3*, *Sql3*, *Sql6*, *Ssq1*, *Ssq2*, *Tsw1*, *Tsw4, Tsw5*, *Sqdy3*, *Sqdy5*, *Df1*, *Df4* and *Oil1*) and the remaining 41 were detected in only one environment. Nine QTL, the highest number, were detected for siliqua length and these QTL (*Sql1–9*) were distributed over eight LGs, followed by eight QTL for test weight (*Tsw1–8*) distributed over six LGs. All the QTL of four quantitative traits namely, primary (*Pbr1–4*) and secondary branches (*Sbr1–4*), main shoot length (*Msl1–4*) and siliqua on the main shoot (*Sqms1–4*) were detected in single environments (Fig. 1 and Table S2).

Of the 65 QTL identified, 34 (53%) had trait enhancing alleles from the Indian parent Varuna and 31 (47%) had trait enhancing alleles from the east European parent Heera (Table 4). However, considering shorter height and early flowering as agronomically desirable traits, a total of 38 and 27 favorable alleles were identified from Varuna and Heera, respectively. Among the yield contributing traits, the nine QTL (*Sql1–9*) detected for siliqua length and the eight QTL (*Tsw1–8*) for test weight had all the trait enhancing alleles from Varuna while the six QTL (*Sqp1–6*) for siliqua on a single plant had all the trait enhancing alleles from Heera. The remaining yield contributing traits had favorable alleles from both the parents, Varuna having more favorable alleles for main shoot length (*Msl1*, *Msl2* and *Msl4*), plant height (*Plht3*, *Plht4*, *Plht5* and *Plht6*) and days to flower (*Df1*, *Df3* and *Df4*) and Heera having more favourable alleles for secondary branches (*Sbr1*, *Sbr2* and *Sbr4*), seeds in a siliqua (*Ssq1*, *Ssq3* and *Ssq4*) and siliqua density (*Sqdy1*, *Sqdy4*, *Sqdy4* and *Sqdy5*) (Fig. 1 and Table S2 of ESM).

Genomic distribution of QTL for the 12 quantitative traits indicated that the A genome contributed 66% (43) while the B genome contributed the remaining 34% (22) of QTL (Table 4). It was also observed that the Indian parent Varuna contained the majority of the trait enhancing QTL alleles (25 out of 43 QTL, 58%) from the A genome while the east European parent Heera contained the majority of the trait enhancing QTL alleles (13 out of 22 QTL, 59%) from the B genome (Table 4).

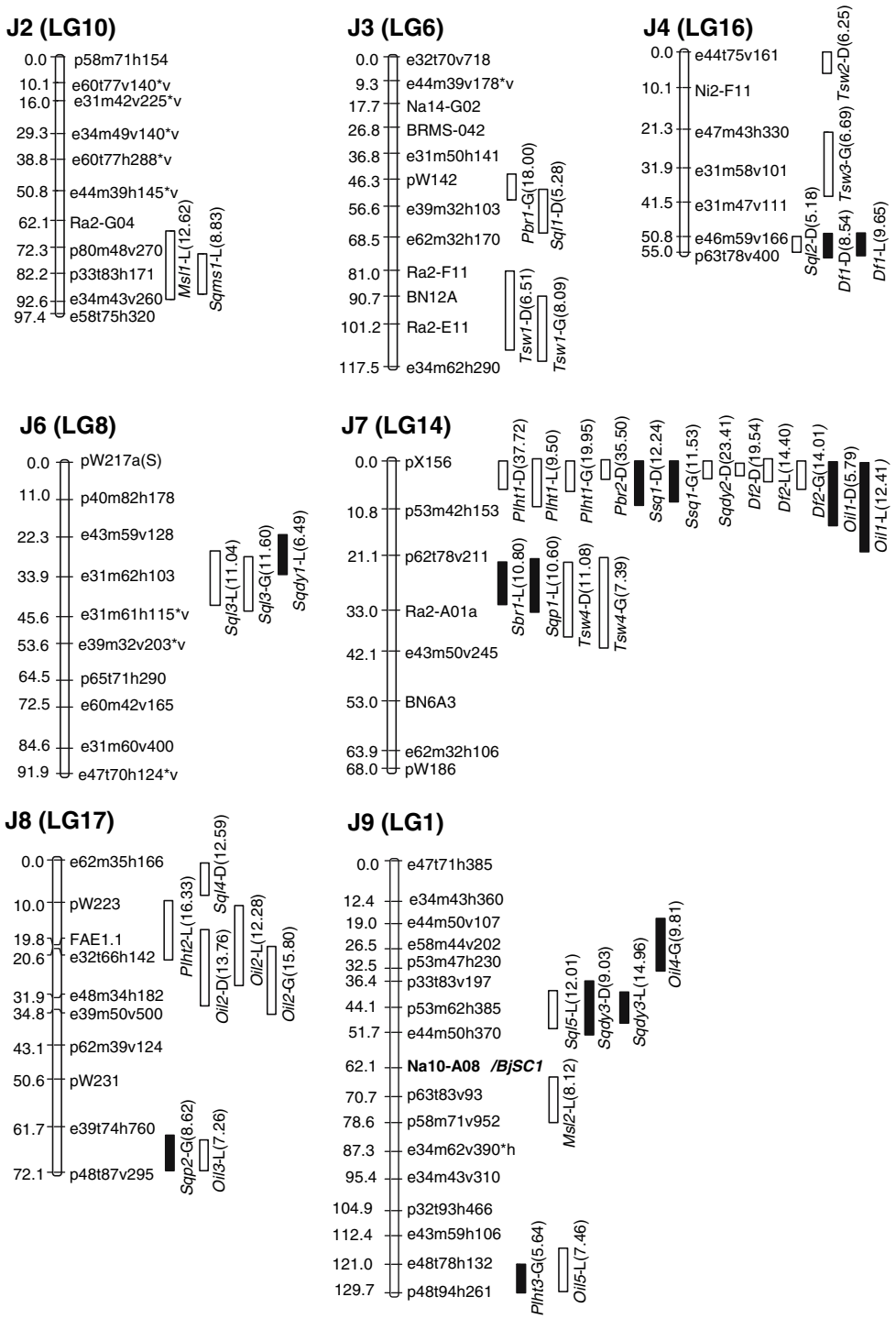
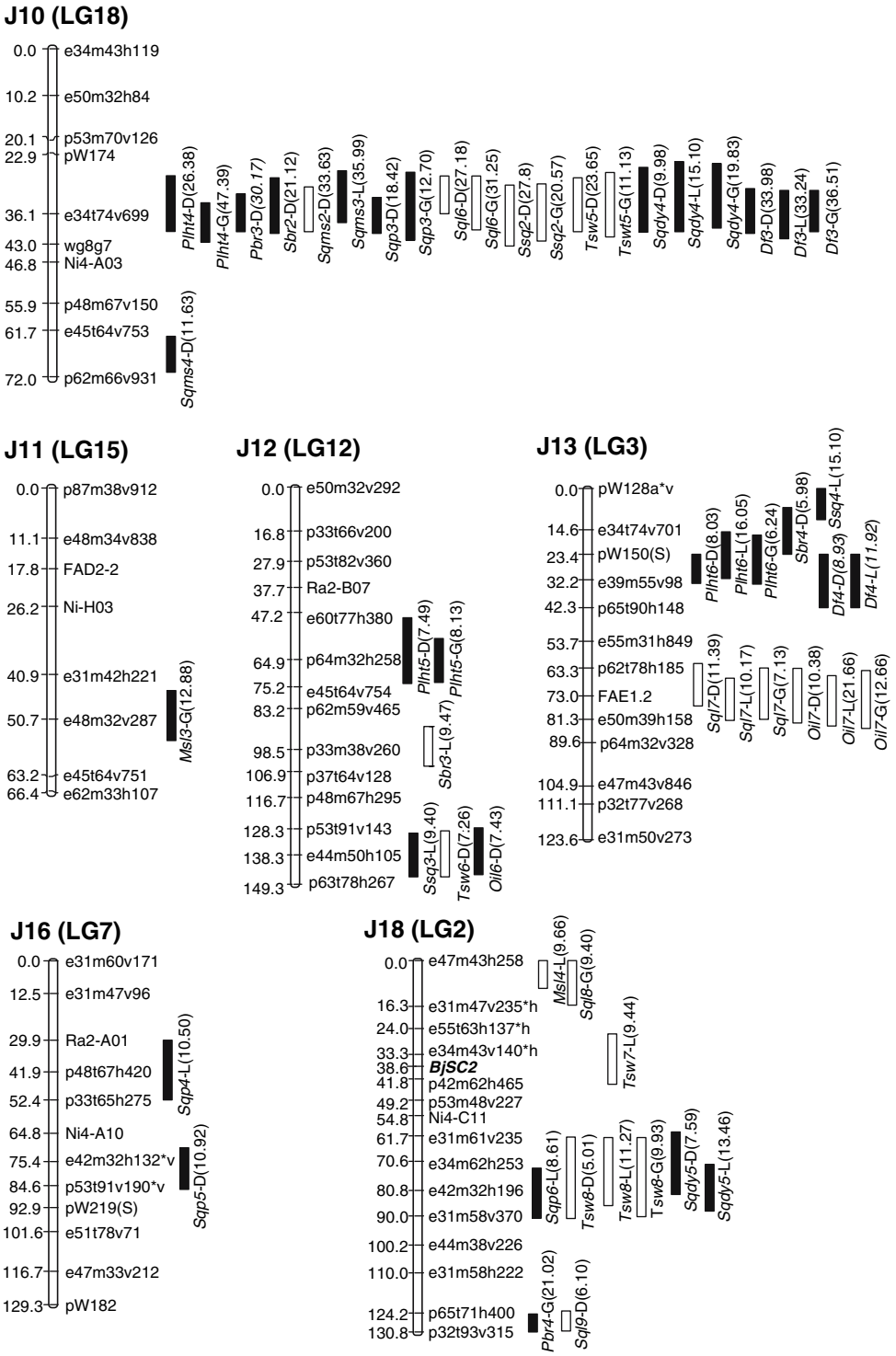


Fig. 1 The framework map showing quantitative trait loci (QTL) for yield components and other agronomic traits detected in the F1DH mapping population. The traits shown in the maps are: plant height (*Plht*), days to flowering (*Df*), primary branches (*Pbr*), secondary branches (*Sbr*), main shoot length (*Msl*), siliqua on the main shoot (*Sqms*), siliqua on a plant (*Sqp*), siliqua density (*Sqdy*), siliqua length (*Sql*), seeds in a siliqua (*Ssq*), test weight (*Tsw*) and seed oil content (*oil*). The linkage groups are designated as J1–J18 with the corresponding previous nomenclature followed by Pradhan et al. (2003) in the parentheses. QTL were designated using the trait name initials (see above) followed by the number identifying the QTL number for the trait. The letter following the hyphen indicates the environment (*D* Delhi, *L* Leh and *G* Gwalior) where the QTL was detected. The figure in the parenthesis is the R^2 value of the QTL. The allele that increased the trait value is indicated by empty (Varuna) and solid (Heera) QTL bars. *BjSC1* in J9 and *BjSC2* in J18 are two mapped loci for seed coat colour and FAE1.1 in J8 and FAE1.2 in J13 are two loci for erucic acid trait

Organization of QTL in the genome

Of the 13 LGs containing the 65 QTL, a clustering of QTL was observed in all the LGs except in J11 and J16 (Fig 1). The most pronounced QTL clustering was observed in J7 (9 QTL) and J10 (12 QTL). In comparison, the QTL mapped in J3, J4, J8 and J9 belonging to the A genome and J12, J13 and J18 belonging to the B genome were more dispersed. The most significant clustering was observed in J10 where 11 out of 12 QTL were found to cluster within one interval (22–43 cM) (Fig 1). QTL detected in this interval were the major QTL for plant height (*Plht4*), primary branches (*Pbr3*), secondary branches (*Sbr2*), siliqua on the main shoot (*Sqms2* and *Sqms3*), siliqua length (*Sql6*), seeds in a siliqua (*Ssq2*), test weight (*Tsw5*) and days to flower (*Df3*), explaining more than 20% of the phenotypic variance for each QTL. Similarly in J7, six QTL detected for plant height (*Plht1*), primary branches (*Pbr2*), seeds in a siliqua

Fig. 1 continued



(*Ssq1*), siliqua density (*Sqdy2*), days to flower (*Df2*) and oil content (*Oil1*) were found to cluster within one interval (0–21 cM). These two narrow clusters of J7 (0–21 cM) and J10 (22–43 cM) detected sister QTL for plant height (*Plht1* in J7 and *Plht4* in J10), primary branches (*Pbr2* in J7 and *Pbr3* in J10), seeds in a siliqua (*Ssq1* in J7 and *Ssq2* in J10), siliqua density (*Sqdy2* in J7 and *Sqdy4* in J10) and days to flower (*Df2* in J7 and *Df3* in J10) showing the opposite allelic effects. For example, the Varuna allele of *Plht1* in J7 increased the plant height while the Varuna allele of *Plht4* in J10 decreased the plant height.

In all the LGs where clustering of QTL was observed, there was association of trait enhancing alleles of one trait with trait depressing alleles of other traits both being contributed by the same parent. This antagonistic association was most prominent in J7, J9 and J10 of the A genome and J18 of

Table 4 Genomic distribution of 65 QTL identified for 12 quantitative traits in three environments and their distribution between the two parents for trait enhancing allele

Sl No	Trait	A genome			B genome			Total QTL
		Number of QTL	Number of trait enhancing alleles contributed by each parent		Number of QTL	Number of trait enhancing alleles contributed by each parent		
			Varuna	Heera		Varuna	Heera	
1	Plant height (*Plht*))	4	2	2	2	0	2	6
2	Primary branches (*Pbr*)	3	2	1	1	0	1	4
3	Secondary branches (*Sbr*)	2	0	2	2	1	1	4
4	Main shoot length (*Msl*)	2	2	0	2	1	1	4
5	Siliqua on the main shoot (*Sqms*)	4	2	2	0	0	0	4
6	Siliqua on a plant (*Sqp*)	3	0	3	3	0	3	6
7	Siliqua length (*Sql*)	6	6	0	3	3	0	9
8	Seeds in a siliqua (*Ssq*)	2	1	1	2	0	2	4
9	Test weight (*Tsw*)	5	5	0	3	3	0	8
10	Siliqua density (*Sqdy*)	4	1	3	1	0	1	5
11	Days to flower(*Df*)	3	1	2	1	0	1	4
12	Seed oil Content (*Oil*)	5	3	2	2	1	1	7
	Total	43	25	18	22	9	13	65

the B genome (Fig. 1). In J8 of the A genome and J13 of the B genome, the FAE 1 genes responsible for the erucic acid trait variation (Gupta et al. 2004), the low erucic acid alleles from Heera, were found to be associated with unfavorable alleles for yield components such as siliqua length (*Sql7* in J13), oil content (*Oil2* in J8 and *Oil7* in J13) and plant height (*Plht2* in J8). A similar unfavorable association was also recorded in J9 and J18 between yellow seed coat color alleles from Heera (Padmaja et al. 2005) and some of the yield component QTL (*Msl2* and *Sql5* in J9 and *Tsw7* in J18) (Fig. 1). We also observed some yield related QTL containing favorable alleles from Varuna and Heera located independently without any unfavorable association. For example, the QTL influencing main shoot length (*Msl1*), siliqua on the main shoot *(Sqms1)* in J2, primary branches (*Pbr1*), siliqua length (*Sql1*) and test weight (*Tsw1*) in J3 and test weight (*Tsw2* and *Tsw3*) in J4 (Fig. 1) were found to be independent with favorable alleles from Varuna while the QTL influencing main shoot length (*Msl3*) in J11 and siliqua on a single plant (*Sqp4* and *Sqp5*) in J16 (Fig. 1) were observed to be independently mapped with favorable alleles from Heera.

Discussion

B. rapa (AA) as compared to *B. nigra* (BB) has made more significant contribution to the domestication of *B. juncea*

The present QTL analysis showed that the A genome has contributed 66% of the total QTL identified for the twelve agronomically important traits in *B. juncea*. The contribution of the A genome is therefore more extensive to the domestication of *B. juncea* as a crop. The identification of more favorable alleles from the Indian gene pool parent Varuna (38 out of 65) as compared to the east European pool represented by Heera also suggests that more stringent selection for superior alleles has been applied in the domestication of Indian *B. juncea* types than the east European types. It was also observed that the Indian types contained more trait enhancing alleles from the A genome while the east European types contained more trait enhancing alleles from the B genome. It is proposed that the Indian and east European gene pools are the result of independent processes of domestication. The divergence observed in the two pools (Pradhan et al. 1993; Srivastava et al. 2001; Table 1) could be due to both the founder effect and divergent selection. The RFLP data and phylogenetic evidence provided by Song et al. (1988) also supports the view that *B. juncea* has polyphyletic origin.

QTL analysis in *B. juncea* reveals clustering of major loci in a few linkage groups with mutually antagonistic effects

Based on our previous knowledge, any breeding effort in *B. juncea* has to take two major factors into consideration: one that the two gene pools are highly divergent and contain contrasting agronomic traits, the other that variability within each gene pool for major component traits is rather limited (Table 1). A third important factor revealed in this study—clustering of major QTL in a few linkage groups

(J7, J9, J10 and J18; Fig. 1)—will have to be contended with in future breeding efforts. This finding suggests that domestication of *B. juncea* might have occurred quite rapidly through the fixation of only a few genomic regions.

The most intriguing situation exists in linkage groups J10 and J7 belonging to the A genome. Out of the 12 QTL detected on J10, 11 mapped to a narrow interval of 22–46 cM. Most of the QTL in this LG are major loci explaining high phenotypic variance and the effects of these QTL also influence the pattern of correlation (positive or negative) of these traits. J10 is homologous to N10 of *B. napus* in which clustering of QTL related to yield and other agronomic traits have been reported recently by Quijada et al. (2006). We also observed opposite allelic effects of sister QTL between J7 and J10, which mapped in a clustered manner. A detailed dissection of these two genomic regions through fine mapping and comparative genomics might lead to isolation and characterization of key genes controlling agronomically important traits in *Brassica*.

In addition to clustering of the QTL, we also observed very strong antagonistic association of QTL of trait enhancing allele of one trait with the trait depressing allele of another trait contributed by the same parent. This situation is particularly obvious on J7, J9 and J10 belonging to the A genome and J18 belonging to the B genome. Looking at the distribution of major QTL and the antagonistic nature of the clustered QTL, it can be visualized that obtaining desired recombinants with favorable alleles of both the parents through conventional pedigree methods would be difficult. This could explain the failure of our earlier attempts to combine useful traits of east European and Indian types through pedigree breeding.

Clustering of QTL and antagonistic association of QTL alleles has been reported earlier in many plant species e.g. in rice (Xiao et al. 1998), corn (Ragot et al. 1995) and tomato (Tanksley et al. 1996). This could be either due to tight linkage or pleiotropy. In cases where deleterious association is due to tight linkage, marker assisted disruption through fine mapping of the target regions could be followed for the elimination of linkage drag.

Transfer of quality traits from east European gene pool line Heera to Indian gene pool varieties

The east European gene pool line Heera is the major donor source for canola quality traits and yellow seed coat color trait for the Indian gene pool lines. QTL analysis presented in this study reveals strong negative association between low erucic acid alleles in Heera with high QTL allele for oil content in J8 belonging to the A genome and with high QTL alleles of oil content and siliqua length in J13 belonging to the B genome. A similar problem of linkage drag could be observed around the two loci identified earlier for the seed coat color in J9 (*BjSc1* in Fig. 1) and J18 (*BjSc2* in Fig. 1) (Padmaja et al. 2005).

It is clear that it would be difficult to develop a productive low erucic acid Indian cultivar even through conventional backcross breeding using the east European line Heera as a donor. The negative association of low erucic acid QTL alleles with high oil content QTL alleles has been reported earlier in *B. juncea* (Cheung et al. 1997) and *B. napus* (Ecke et al. 1995; Qiu et al. 2006). In case the QTL of these two traits are tightly linked, a marker assisted disruption approach or a transgenic approach for decreasing erucic acid content would be more appropriate strategies for developing lines that are low in erucic acid but have high oil content. It has also been suggested that the erucic acid content of the seed oil is itself a major determinant of seed oil content in *B. napus* (Ecke et al. 1995; Burns et al. 2003). In this pleiotropic situation, it would be prudent to look for low erucic acid individuals among the segregants containing compensatory genes for high oil content.

Various studies on QTL mapping reported earlier had shown linkage related problems for breeding of quality traits in *Brassica* species. Recently, Quijada et al. (2006) showed negative association of low glucosinolate QTL with one of the seed yield QTL in linkage group N2 of *B. napus*. N2 of *B. napus* is homologous to J2 of the A genome which has been shown in this study to contain many favorable yield related QTL i.e. main shoot length and siliqua in the main shoot from a high glucosinolate parent Varuna. The information available from this QTL mapping study will be of great help in the elimination of linkage drag while transferring the low glucosinolate trait from Heera to the lines of the Indian gene pool.

Breeding opportunities in *B. juncea* for yield enhancement

In comparison to pedigree breeding, heterosis breeding using east European × Indian type crosses has been more successful (Pradhan et al. 1993). *B. juncea* hybrids DMH-1 and DMH-11 developed in our laboratory have shown yield heterosis in the range of 19–40% over the best Indian and national check varieties (Sodhi et al. 2006). However, some of the yield component traits, particularly seed size and siliqua length, are poor in the hybrid due to low trait values of these characters in the east European parent used as a combiner in these hybrids. From the present QTL study, it seems possible to improve the east European combiners through transfer of some of the favorable QTL from Varuna, which are lying independently or without antagonistic effects on some of the linkage groups by using marker-assisted backcross breeding. These include QTL for main shoot length (*Msl1*) and siliqua on the main shoot (*Sqms1*) on J2, test weight (*Tsw1*), primary branches (*Pbr1*) and siliqua length (*Sql1*) on J3, test weight (*Tsw2* and *Tsw3*) on J4, plant height

(*Plht3*) and oil content (*Oil5*) on J9 and main shoot length (*Msl4*) and siliqua length (*Sql8*) on J18. We also observed a few regions in J11 (*Msl3*) and J16 (*Sqp4* and *Sqp5*) where the favorable QTL from Heera could possibly be transferred to Varuna without negative effects. A more ambitious breeding program to combine the best of the trait values of the two gene pools will require fine mapping of the LGs where QTL are mapping in a clustered manner.

We conclude that at the present level of observations, heterosis breeding coupled with selective improvement of the two gene pools through backcross based transfer of some useful free lying additive QTL will be a more realistic strategy for further advances in *B. juncea* breeding.

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