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ORIGINAL PAPER

QTL analysis reveals context-dependent loci for seed glucosinolate trait in the oilseed *Brassica juncea*: importance of recurrent selection backcross scheme for the identification of 'true' QTL

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Abstract Seed glucosinolate content in *Brassica juncea* is a complex quantitative trait. A recurrent selection backcross (RSB) method with a doubled haploid (DH) generation interspersing backcross generations was used for the introgression of low glucosinolate alleles from an east European gene pool *B. juncea* line, Heera into an Indian gene pool variety, Varuna. Phenotypic comparisons among the DH populations derived from early to advanced backcrosses revealed a shift in the mean values for various glucosinolates with the advancement of backcrossing, indicating a change in the selective values of the alleles with change in the genetic background due to the existence of epistasis and context dependencies. QTL mapping for various seed glucosinolates from early (F_1DH) and advanced generation (BC_4DH) populations confirmed the presence of epistasis and context dependency. The common QTL detected in both F_1DH and BC_4DH changed their R^2 values from the former to the later generation. Some of the QTL detected in the F_1DH became irrelevant in the BC_4DH population. Further, new QTL were detected in the BC_4DH population for various glucosinolates. A validation study on a population of low glucosinolate DH lines derived from all the backcross generations of the RSB breeding programme revealed that the QTL detected in BC_4DH were the 'true' QTL. Using glucosinolate as an example, the study provides strong evidence for the importance of the RSB method for the identification of the 'true' QTL which would be significant for marker assisted introgression of a complex quantitative trait whose expression is influenced by epistatic interactions.

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Introduction

QTL mapping has been carried out in a large number of crops and in many cases attempts have been made to introgress QTL from otherwise ill-adapted donor sources to elite lines and varieties. In many cases either the transfers were unsuccessful or ultimately were of much less consequence than expected from the initial mapping of QTL (reviewed by Hospital 2005). In extreme situations new QTL have been shown to appear on backcrossing, and the old ones, discerned in a balanced population, disappear due to epistatic interactions (*in senso stricto*) and/or due to the overall 'context' of the recipient genome (*in senso lato*). Methods have been proposed to accommodate the effects of 'context' (Boer et al. 2002; van Eeuwijk et al. 2002; Jansen et al.

2003). However, the applications of such methods have not proved to be as effective in actual breeding programmes. In some perceptive theoretical studies, Hill (1998) and more recently Luo et al. (2002) and Luo and Ma (2004) have proposed the 'Recurrent Selection Backcross (RSB)' method of QTL analysis for the dissection of complex quantitative traits. In the RSB scheme, selection for the quantitative trait allows maintenance of the donor genome region(s) that contain the QTL in the increasing 'context' of the recipient genome. The Near Isogenic Line (NIL) obtained through this method could be used for accurately mapping the 'true' QTL effecting the trait under consideration.

This study relates to the QTL mapping of seed glucosinolate content in *B. juncea*, a major oilseed crop of dryland areas of India. Glucosinolate content is a complex trait and its expression is controlled by a gene network in the family Brassicaceae (Halkier and Gershenzon 2006). In *B. juncea*, aliphatic glucosinolates, 2-propenyl and 3-butenyl are the main types. Genetic studies on aliphatic glucosinolates reported 2 (Love et al. 1990b) to 6–8 genes (Stringam and Thiagarajah 1995; Sodhi et al. 2002) depending upon the parents selected for the cross. The later two studies were conducted using crosses between the low glucosinolate lines of an east European gene pool affinity and the high glucosinolate lines belonging to the Indian gene pool. The two gene pools are highly divergent for both agronomic traits and DNA level polymorphism (Pradhan et al. 1993; Srivastava et al. 2000) as well as for glucosinolates. The *B. juncea* belonging to the east European gene pool predominantly contains 2-propenyl (approximately 99%) whereas the germplasm of the Indian gene pool contains mostly 3-butenyl with some amount of 2-propenyl and 4-pentenyl types (Gland et al. 1981; Love et al. 1990a, b; Sodhi et al. 2002).

Although QTL mapping of aliphatic glucosinolates has been undertaken in *B. juncea* (Cheung et al. 1998; Mahmood et al. 2003; Lionneton et al. 2004), no systematic efforts have been made to identify the loci which could be utilized for marker assisted transfer of low glucosinolate through backcross breeding. We report in this study the transfer of low glucosinolate through RSB method and demonstrate the existence of epistatic and context dependent interactions among the genes involved in aliphatic glucosinolate biosynthesis. We observed that due to the prevalence of epistasis, QTL analysis of aliphatic glucosinolates in a balanced F_1DH population was not fully informative in dissecting all the 'true' QTL for the germplasm of the Indian gene pool. In this study we, therefore, provide strong evidence for the relevance of the RSB method for the identification of the 'true' QTL which would be significant in marker assisted introgression of a complex trait in situations where context dependencies are present.

Materials and methods

The breeding scheme and the plant material

Two parents, Varuna, a high glucosinolate Indian variety and Heera, a low glucosinolate east European line were crossed and the low glucosinolate trait was transferred to the Indian parent Varuna (recipient parent) through recurrent selection and backcrossing. The RSB breeding scheme for the introgression of the low glucosinolate trait is given in Fig. 1. To identify low glucosinolate individuals (low glucosinolate is recessive in expression), a cycle of microspore derived doubled haploid (DH) production (Mukhopadhyay et al. 2007) was introduced between each generation of backcrossing. A two-step selection process

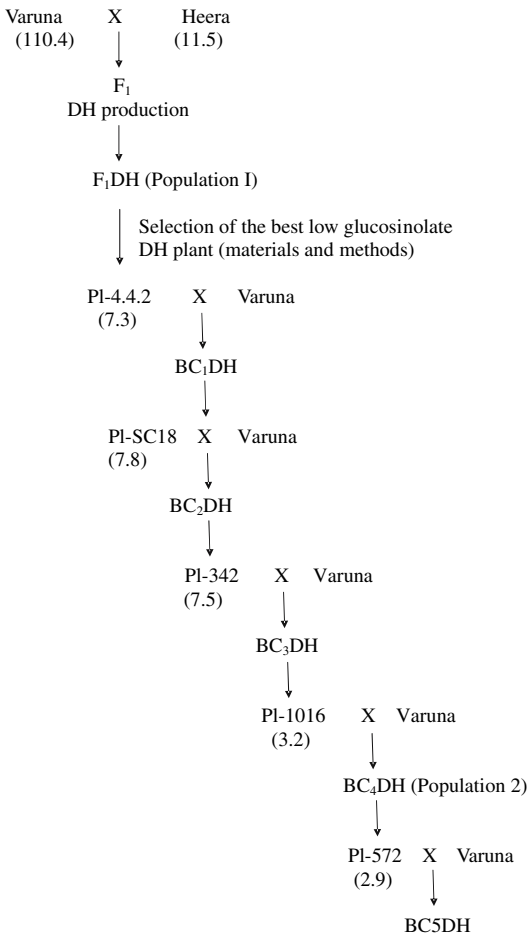

Fig. 1 The diagram illustrates the details of the recurrent selection and backcross (RSB) breeding scheme used in the present study. As low glucosinolate trait is recessive in nature, a generation of DH was introduced between each backcrossing to produce homozygous individuals for the identification of low glucosinolate individuals in the segregating progeny. Two populations (Population 1 and Population 2) used for genetic analysis and mapping of aliphatic glucosinolates have been shown in the diagram. The *number in the parenthesis* is the seed glucosinolate content (μmol/g seed) of selected low glucosinolate lines used as parents in the RSB programme

was applied to identify the best low glucosinolate DH plant that was used as the donor parent for the next cycle of backcrossing. In the first step, phenotypic selection was employed among low glucosinolate DH segregants to identify plants which had a glucosinolate content either at par or lower than the low glucosinolate parent. In the second step, these selected plants were subjected to whole genome background selection using AFLP markers (Pradhan et al. 2003). The plant with the maximum recurrent genome content was used as a donor parent for the next backcross cycle. This process of backcrossing, DH production and selection was adopted till the end of the RSB programme (BC_5DH, Fig. 1).

Three segregating populations originating from the RSB breeding programme were used in the present study. Population 1 consisting of 1,137 F_1DH lines and Population 2 consisting of 1,160 BC_4DH lines were taken directly from the RSB breeding scheme (Fig. 1). Population 3 consisting of 567 F_2 individuals, was derived from a cross between two low glucosinolate BC_2DH lines Pl-60 and Pl-145. The glucosinolate profiles of the parental lines used in the RSB breeding programme and for the parents of the three segregating populations have been shown in Table 1. The study also included analysis of 107 low glucosinolate DH lines derived from F_1DH to BC_5DH including namely, 4 lines from F_1DH, 8 from BC_1DH, 24 from BC_2DH, 25 from BC_3DH, 24 from BC_4DH and 22 from BC_5DH [Fig. 1S of Electronic supplementary material (ESM)].

An experiment consisting of 123 lines of F_1DH mapping population under three environmental conditions (for details of the experimental design see Ramchiary et al. 2007) revealed little variation for the various types of aliphatic glucosinolates (Table 1S of ESM). Hence, the phenotypic data of Delhi environment was used for the genetic analysis of aliphatic glucosinolates. The lines were sown as single 2 m long rows at a spacing of 45 cm between the rows. Open pollinated seeds harvested from five randomly selected plants were bulked and used for glucosinolate analysis.

Seed glucosinolates profiles were estimated by high pressure liquid chromatography (HPLC) (Shimadzu HPLC model LC-10) following Kraling et al. (1990). The percentages of propyls, butyls and pentyls were calculated following the procedure given by Magrath et al. (1993).

Genotyping and QTL analysis

DNA was isolated from well-expanded leaves of field grown plants following the methodology of Rogers and Bendich (1994). A framework map earlier used for the QTL dissection of yield components (Ramchiary et al. 2007) was used for the QTL analysis of seed glucosinolates. Genotyping of BC_4DH mapping population was done using those markers that showed the presence of donor alleles (Heera) in BC_3DH parental line Pl-1016 (Fig. 1). The loci representing the remaining genomic regions were scored as having recipient parent alleles (Varuna) as these regions became homozygous during recurrent backcrossing. The genotyping of low glucosinolate segregants used in the validation study was performed using 26 AFLP primer pairs recommended for whole genome background selection of *B. juncea* genome (Pradhan et al. 2003) and graphical genotype was constructed using GGT software (http://www.dpw.wau.nl/pv/pub/ggt/).

QTL mapping was done by both single marker analysis and 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. For declaring the presence of a QTL, genome-wide threshold values ($P = 0.05$) were estimated from 1,000 permutations of trait data across all genetic intervals (Churchill and Doerge 1994; Doerge and Churchill 1996). A threshold LOD score of 2.8 was chosen for declaring the presence of a putative QTL.

Table 1 Glucosinolate (GSL) profiles (μmol/g seed) of parents used in the present study

Line/genotype	Propyls	Butyls	Pentyls	Aliphatic GSL	Other GSL	Total GSL
Varuna	21.0 ± 2.3	85.8 ± 3.9	2.5 ± 0.0	109.2 ± 4.0	1.2 ± 0.2	110.4 ± 4.2
Heera	0.5 ± 0.0	10.0 ± 1.0	0.5 ± 0.0	10.9 ± 1.0	0.6 ± 0.0	11.5 ± 1.1
Pl-4.4.2 (F_1DH)	0.0 ± 0.0	5.0 ± 0.3	0.7 ± 0.0	5.7 ± 0.4	1.6 ± 0.4	7.3 ± 0.7
SC-18 (BC_1DH)	0.0 ± 0.0	4.4 ± 0.3	1.3 ± 0.0	5.7 ± 0.4	2.1 ± 0.2	7.8 ± 1.3
Pl-60 (BC_2DH)	1.3 ± 0.0	0.0 ± 0.0	0.4 ± 0.0	1.7 ± 0.3	2.2 ± 1.0	3.9 ± 0.8
Pl-145 (BC_2DH)	0.0 ± 0.0	8.1 ± 0.5	1.5 ± 0.2	9.6 ± 0.8	1.5 ± 0.3	11.1 ± 1.3
Pl-342 (BC_2DH)	0.0 ± 0.0	4.2 ± 1.0	1.8 ± 0.3	6.0 ± 0.7	1.5 ± 0.2	7.5 ± 0.9
Pl-1016 (BC_3DH)	0.0 ± 0.0	1.5 ± 0.2	0.0 ± 0.0	1.5 ± 0.2	1.7 ± 0.2	3.2 ± 0.3

Results

Transfer of low glucosinolate trait through RSB breeding

One generation of DH interspersing backcross generations (Fig. 1) resulted in the production of a large number of low glucosinolate (containing less than 20 µmol/g seed) DH individuals (data not shown) at each cycle as described by Sodhi et al. (2002). This facilitated the application of stringent selection pressure for identifying low glucosinolate DH segregants with glucosinolate content lower than the low glucosinolate parent (Heera) in every DH generation. The pedigree and other details of the selected low glucosinolate DH individuals that were subjected to whole genome background selection, are shown in Fig. 1S of ESM. The maximum recurrent genome content of 86% was achieved in BC_4DH and no substantial increase in recurrent genome content was achieved in BC_5DH as revealed by whole genome AFLP analysis. The two most desirable low glucosinolate DH plants Pl-267 and Pl-1055 in the BC_5DH generation had total glucosinolate contents of 1.7 and 2.4 µmol/g seed, respectively (Fig. 1S of ESM).

Phenotypic variations in glucosinolates contents in different segregating populations

Phenotypic variations for total and various types of aliphatic glucosinolates, namely propyls, butyls and pentyls, were studied by comparing the phenotypic data obtained from the F_1DH (Population 1) and the BC_4DH (Population 2) populations. The mean and range of values for total and various types of aliphatic glucosinolates are given in Table 2 and their frequency distribution in Figs. 2S, 3S and 4S of ESM. The frequency distribution for total glucosinolates showed a normal distribution in both the populations (Fig. 2S of ESM), but the mean value showed an increase from 65.8 µmol/g seed in F_1DH to 80.4 µmol/g seed in BC_4DH (Table 2). This shift in the mean was primarily due to the increase in the frequency of transgressive segregants with the advancement of backcross generations (Table 3, Fig. 2S of ESM).

The frequency distribution for the percentage of propyls and butyls showed complex patterns and did not segregate into distinct phenotypic classes. The mean percentage propyls increased from 15.3% in F_1DH to 35.9% in BC_4DH (Table 2). This was due to a decrease in the frequency of transgressive segregants having a lower percentage of propyls with concomitant increase in the frequency of transgressive segregants having a higher percentage of propyls with the advancement of backcross generations (Table 3, Fig. 3S of ESM). On the other hand, a decreasing trend was observed for butyl glucosinolates where the F_1DH showed a mean butyl percentage of 76.9% as compared to 56.5% for the BC_4DH (Table 2). This was due to a decrease in the frequency of transgressive segregants having a higher percentage of butyls with concomitant increase in the frequency of transgressive segregants having a lower per-

Table 2 Mean and range of various glucosinolates estimated from F_1DH and BC_4DH segregating populations used in the present study

Parents/segregating population	Population size[a]	Total glucosinolates (µmol/g seed)		Percentage of propyls		Percentage of butyls		Percentage of pentyls	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
Varuna		110.4	–	19.2	–	78.6	–	2.3	–
Heera		11.5	–	4.6	–	91.7	–	4.6	–
F_1DH (Population 1)	1,137	65.8	3.6–126.2	15.3	0.0–97.0	76.9	12.0–99.6	7.4	0.0–35.7
Pl-1016		3.2	–	0.0	–	100.0	–	–	–
Varuna (recurrent parent)		110.4	–	19.2	–	78.6	–	2.3	–
BC_4DH (Population 2)	1,160	80.4	0.6–141.3	35.9	0.0–100.0	56.5	0.0–100.0	7.6	0.0–40.5

[a] For the estimation of percentage of propyls, butyls and pentyls, population size was 610 and 1,128 in F_1DH and BC_4DH, respectively

Table 3 Percentage of transgressive segregants in F_1DH and BC_4DH derived through RSB breeding

Glucosinolate traits studied	F_1DH			BC_4DH		
	Lower than the low parent	Higher than the high parent	Population size	Lower than the low parent	Higher than the high parent	Population size
Total glucosinolates	0.6 (7)	2.2 (26)	1,137	7 (80)	13 (146)	1,160
Percentage of propyls	46 (286)	35 (211)	610	27 (300)	42 (476)	1,128
Percentage of butyls	50 (303)	25 (153)	610	67 (761)	13 (151)	1,128

Figures in the parentheses indicate the number of individuals in the respective categories. Low and high parents for percentage of propyls were Heera and Varuna, respectively, and the reverse was the case for percentage of butyls

centage of butyls with the advancement of backcross generations (Table 3, Fig. 4S of ESM). This trend was further confirmed by the mean values for the percentage of propyls (27.7%) and the percentage of butyls (62.6%) being observed to be intermediate in Population 3, a F_2 derivative of BC_2DH (data not shown).

Phenotypic variation for total glucosinolates was also studied in Population 3, derived from two low glucosinolate BC_2DH lines. The total glucosinolate content in the F_2 progeny ranged from 1.7 to 112.0 µmol/g seed with a mean value of 30.4 µmol/g seed (Table 4). Of the 567 plants analyzed for glucosinolate content, 147 were found to be low glucosinolate type (less than 20 µmol/g seed) indicating the presence of gene interactions (Table 4).

QTL analysis of seed glucosinolates in F_1DH

The mapping population of 123 F_1DH individuals used earlier for developing a molecular map in *B. juncea* was used for the QTL dissection of seed glucosinolates by CIM (Ramchiary et al. 2007). A total of 17 QTL were detected for various glucosinolates (Fig. 2, Table 2S of ESM). In a molecular network model like the biosynthetic pathway of aliphatic glucosinolates, one gene may show functional association with more than one type of glucosinolates and detect QTL for various types. Hence, each genetic interval detecting QTL for various glucosinolates was assigned to one locus and all the 17 QTL were assigned to nine loci (Fig. 2). Among the nine loci, those in linkage groups J3 (genetic interval 21.3–36.8 cM), J9 (genetic interval 5.6–26.5 cM), J12 (genetic interval 27.8–47.3 cM) and J16 (genetic interval 56.3–73.6 cM) were considered as major QTL in the glucosinolate biosynthetic pathway. Other detected loci, in linkage groups J1 (genetic interval 52.0–70.1 cM), J7 (genetic interval 10.8–33.0 cM) and J18 (genetic interval 0.0–6.3 cM) and one additional locus each in J12 (genetic interval 116.8–138.4 cM) and J16 (genetic interval 0.0–12.5 cM) were considered as minor QTL as these explained less than 5% of the total phenotypic variance with regard to various types of glucosinolates for which these QTL were detected (Fig. 2, Table 2S of ESM).

QTL analysis of seed glucosinolates in BC_4DH

A BC_4DH mapping population consisting of 167 lines was randomly selected from a total number of 1,128 lines. QTL analysis by CIM detected a total of 17 QTL for various glucosinolates which were mapped to five genetic intervals, i.e., 82.2–92.6 cM in J2, 21.3–36.8 cM in J3, 5.6–26.5 cM in J9, 56.3–73.6 cM in J16 and 45.4–58.0 cM in J17 (Fig. 2, Table 1S of ESM). All these QTL were detected with LOD values of more than 8.0 (Table 2S of ESM).

Comparison of QTL data derived from F_1DH and BC_4DH

Comparison of QTL analysis data of F_1DH and BC_4DH revealed that out of the four major QTL detected in F_1DH only three could be detected in BC_4DH. These three consistent QTL were in the linkage groups J3 (genetic interval 21.3–36.8 cM), J9 (genetic interval 5.6–56.5 cM) and J16 (genetic interval 56.3–73.6 cM) and showed changes in the R^2 values between F_1DH and BC_4DH (Fig. 2, Table 2S of ESM). The major QTL in J12 (genetic interval 27.8–47.3 cM) and all the five minor QTL detected in F_1DH population could not be detected in the BC_4DH population (Fig. 2, Table 2S of ESM). Two new QTL one in J2 (genetic interval 82.2–92.6 cM) and the other in J17 (genetic interval 45.4–58.0 cM) were detected in BC_4DH, that were not identified in the QTL analysis of F_1DH. The QTL detected in J2 in the BC_4DH population was observed to be a major QTL as it explained more than 70% of the total phenotypic variation for the propyl and the butyl glucosinolates (Table 2S of ESM).

Validation of the five QTL of the BC_4DH population through tracking the donor alleles in low glucosinolate segregants

The QTL selected in BC_4DH were validated by tracking the retention of donor alleles in 107 low glucosinolate DH lines spanning the five backcross generations of the RSB breeding programme (list of these lines is shown in Fig. 1S of ESM). Graphical genotype based on whole genome background selection markers is shown in Fig. 5S of ESM.

Table 4 Mean, range and segregation ratios for the glucosinolate trait in F_2 of a cross between two low glucosinolate parents

Parent/cross	Generation	Number of plants analysed	Total glucosinolates (µmol/g seed)		Observed frequency		No. of genes segregating	χ^2
			Mean	Range	Low	High		
Pl-60	BC_2DH	–	3.9	–	–	–	–	–
Pl-145	BC_2DH	–	11.1	–	–	–	–	–
Pl-60 × Pl-145	F_2	567	30.4	1.7–112.0	147	420	1	0.26

A Chi square test (χ^2) was performed at the 95% confidence limit ($P < 0.05$) to determine the goodness of fit

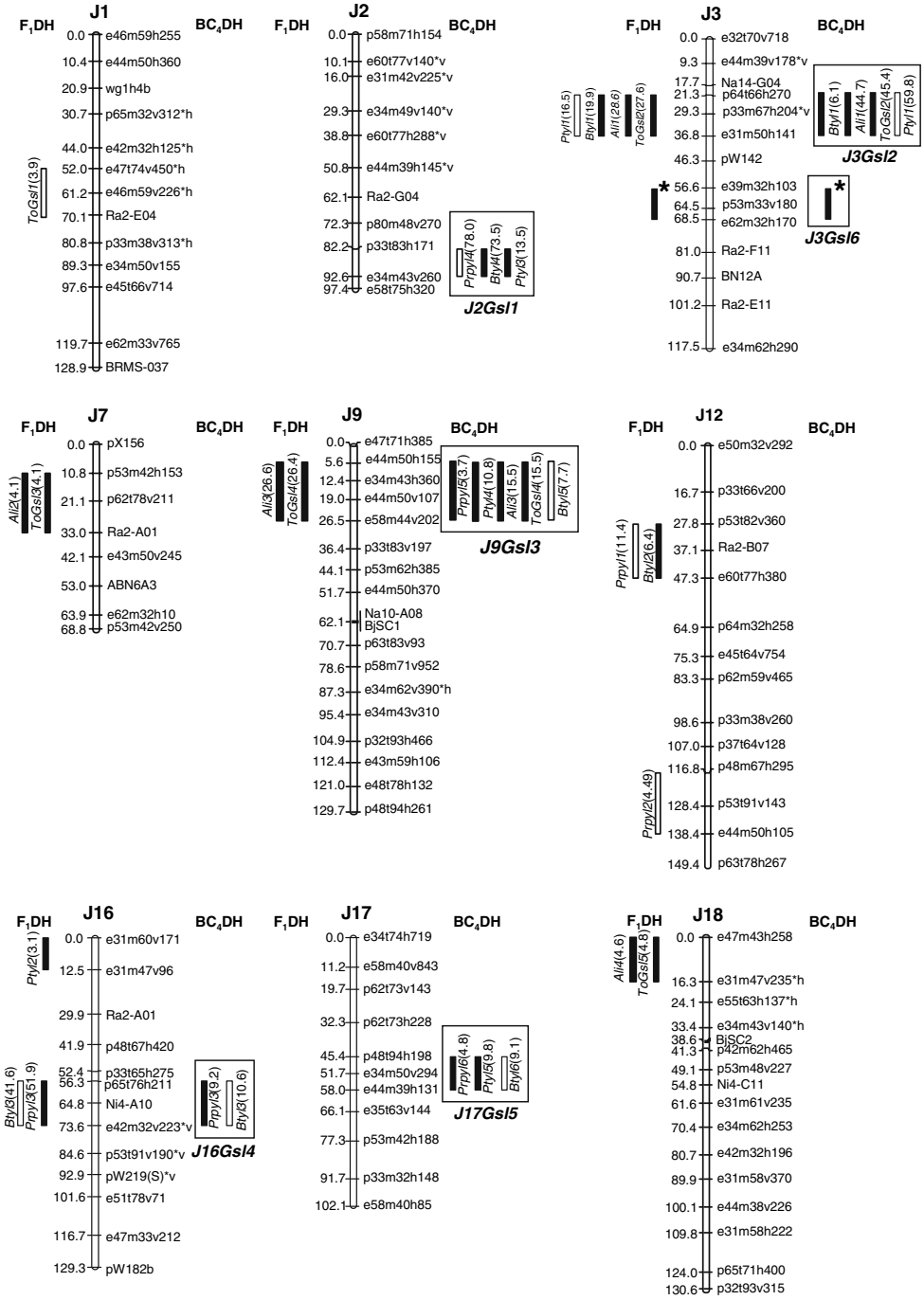

Fig. 2 A framework map of *B. juncea* showing quantitative trait loci (QTL) for various glucosinolates. Linkage groups are marked following the nomenclature of Ramchiary et al. (2007). QTL detected in the F_1DH and the BC_4DH mapping populations are shown on the *left* and the *right* hand side of each linkage group, respectively. The traits marked in the map are percentage of propyls (*Prpyl*), percentage of butyls (*Btyl*), percentage of pentyls (*Ptyl*), aliphatic glucosinolate (*Ali*) and total glucosinolates (*ToGsl*). QTL were designated using the trait name initials followed by the QTL number for a trait. The *number in the parenthesis* is the R^2 value of the QTL. For various types of glucosinolates, the trait enhancing allele from Heera is indicated by *empty* and from Varuna by *solid QTL bars*. QTL for various glucosinolates mapping to the same genetic interval were considered as one locus and were designated as *J2Gsl1* in J2, *J3Gsl2* and *J3Gsl6* in J3, *J9Gsl3* in J9, *J16Gsl4* in J16 and *J17Gsl5* in J17 as revealed by BC_4DH QTL analysis. *Asterisk* QTL detected by single marker analysis

Graphical genotyping indicated complete recovery of recipient parent genomes in the linkage groups J1, J4, J5, J6, J7, J8, J10, J11, J12, J13, J14, J15 and J18 by BC_4 or earlier. One chromosomal fragment each, in J2, J3, J9 and J17, was found to be consistently inherited from the donor parent while a fragment in J16 showed segregation among the low glucosinolate individuals (Fig. 5S of ESM, panel D). We observed co-localization of inherited donor fragments with all the five QTL of BC_4DH in J2, J3, J9, J16 and J17. In J3, retention of almost the entire linkage group of the donor parent was observed even after five backcrosses, suggesting the presence of more than one locus for glucosinolates in this linkage group (Fig. 5S of ESM, panel B). We undertook single marker analysis of J3 linkage group specific markers and detected a sixth QTL in the region 58.6–69.5 cM in both F_1DH and BC_4DH populations.

The six QTL detected in BC_4DH were designated *J2Gsl1* in J2 (genetic interval 82.2–92.6 cM), *J3Gsl2* in J3 (genetic interval 21.3–36.8 cM), *J9Gsl3* in J9 (genetic interval 5.6–26.5 cM), *J16Gsl4* in J16 (genetic interval

56.3–73.6 cM), *J17Gsl5* in J17 (genetic interval 45.5–58.1 cM) and *J3Gsl6* in J3 (genetic interval 58.6–69.5 cM) (Fig. 2). However, the segregation of *J16Gsl4* in J16 among low glucosinolate individuals revealed that this locus may not be meaningful for the transfer of the low glucosinolate trait from the east European line Heera to the Indian *B. juncea* types.

Discussion

In the present study we demonstrate the existence of epistatic and context dependent interactions of loci involved in the glucosinolate biosynthetic pathways in *B. juncea*, by comparing the QTL data obtained at the beginning (F_1DH) and near the end (BC_4DH) of the RSB breeding programme. Our results affirm the importance of the RSB approach for the identification of 'true' QTL for the low glucosinolate trait from the donor parent Heera and their values and effects in the context of *B. juncea* germplasm belonging to the Indian gene pool.

Comparison of QTL data between F_1DH and BC_4DH helped identify three consistent QTL (Fig. 2) over recurrent backcrossings. Although there was no change in the status of favorable alleles for all these common QTL, there were significant changes in additive effects and R^2 values for these common QTL detected over the backcross generations (Fig. 2, Table 2S of ESM). These observations are in accordance with our observations on phenotypic variations where we observed shifting of the mean values for various glucosinolates due to a gradual change in the frequency of transgressive segregants with the advancement of backcrosses. These shifts in all probability are due to a change in the selective values of the alleles with the progressive change in the genetic background towards the recurrent parent.

The most important observation in the comparative QTL analysis was the disappearance (QTL detected in the genetic interval 27.9–47.2 cM of J12 in the F_1DH QTL analysis) and the appearance of the major QTL (*J2Gsl1* of J2 in BC_4DH QTL analysis) during recurrent backcrossing. These results are highly significant from the point of view of marker assisted transfer of low glucosinolate loci from east European type germplasm to Indian gene pool varieties since we would not have achieved success in marker assisted transfer of a low glucosinolate trait using the QTL data of F_1DH.

Some earlier studies have also reported disappearance of QTL. Sebolt et al. (2000) performed marker-assisted backcrossing of two QTL for seed protein concentration in soybean. Only one QTL was confirmed in the $BC_3F_{4:5}$ progeny. When this QTL was introgressed in three different genetic backgrounds, it had no effect in one case. Shen et al. (2001) introgressed four QTL for drought resistance detected in a DH population. Among the four QTL, one exhibited the expected effect, one was finally revealed as false positive, one segment was shown to contain two QTL in repulsion phase (+/−) that reduced its expression and one segment did not exhibit the expected effects in the BC_3F_3 progeny. In another study, Lecomte et al. (2004) while transferring the five QTL strongly involved in tomato fruit quality through MAS, observed significant interaction between the QTL and the genetic background and also reported the appearance of new QTL.

Validation of all the QTL detected in the BC_4DH mapping population with the retained donor genomic regions in low glucosinolate segregants obtained from RSB breeding scheme indicates that the BC_4DH mapping population is more informative in identifying 'true' QTL in the presence of epistasis and context dependency. Incorporation of DH generations between backcrossing generations in the RSB breeding scheme and stringent phenotypic selection helped in the retention of QTL with small effects (*J3Gsl6* in J3). Hill (1998) suggested that interspersing a generation of inter se mating between each generation of backcrossing makes it possible to apply stronger selection and hence, to fix the QTL of smaller effects. Since the RSB method of introgression is based entirely on phenotypic selection, retention of QTL with small effects in a RSB breeding programme would be possible for traits with high heritability like glucosinolates in the present study.

In breeding parlance, backcross breeding has been taken as an obvious and simple method for transferring a highly heritable simple trait from exotic to productive lines. It was proposed by many authors starting from Wright (1952) that RSB schemes could be very useful in discerning 'true' QTL and their values in divergent genetic contexts. As evident from the present study, the RSB strategy shown earlier through simulation studies (Hill 1998; Luo et al. 2002; Luo and Ma 2004), can now be practiced in discovery of QTL for applications at a much higher level of certainty using convenient molecular markers.

With the identification of the 'true' QTL for the glucosinolate trait in the present study, it would now be safe to perform marker-assisted introgression of these QTL without confirming the estimated effect by phenotypic evaluation during the introgression process. The linkage groups J2 and J9 correspond to N2 and N9, respectively, of *B. napus* (Quijada et al. 2006) and J3 corresponds to A2 of *B. juncea* (Mahmood et al. 2003) where major QTL for glucosinolates have been mapped. We observed serious linkage related problem in J2 and J3 when QTL data of yield components (Ramchiary et al. 2007) was compared with glucosinolate QTL data of the present study. Similar negative association of the low glucosinolate QTL with seed yield QTL was also observed in the linkage group N2 of *B. napus*

(Quijada et al. 2006). Hence, for the development of productive low glucosinolate *B. juncea*, the marker-assisted introgression should be undertaken through disruption of linkage related deleterious association after fine mapping of the target QTL. This could probably be achieved by candidate gene approach using gene sequences of *GSL-ELONG*, *GSL-ALK*, *GSL-PRO* and others involved in glucosinolate biosynthetic pathway which have been characterized in *Arabidopsis* and other *Brassica* species (Halkier and Gershenzon 2006; Gao et al. 2007) or by developing some tightly linked PCR based markers. We are currently marking the loci involved with the low glucosinolate trait using candidate gene-specific markers and through comparative genomics of *Arabidopsis* and *B. juncea* to develop accurate and simple to use markers for diversification of the low glucosinolate trait into the Indian germplasm of *B. juncea*. Given the divergent composition of glucosinolates in the Indian and the east European gene pool, it is expected that the 'true' QTL for the low glucosinolate trait in the east European germplasm will be different from the corresponding QTL that are identified for the Indian germplasm.

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