Theor Appl Genet (2015) 128:657–666
DOI 10.1007/s00122-015-2461-9

ORIGINAL PAPER

Deciphering allelic variations for seed glucosinolate traits in oilseed mustard (*Brassica juncea*) using two bi-parental mapping populations

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Received: 5 November 2014 / Accepted: 10 January 2015 / Published online: 28 January 2015
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Abstract
Key message QTL mapping by two DH mapping populations deciphered allelic variations for five different seed glucosinolate traits in *B. juncea.*
Abstract Allelic variations for five different seed glucosinolate (GS) traits, namely % propyl, % butyl, % pentyl, aliphatics and total GS content were studied through QTL analysis using two doubled haploid (DH) mapping populations. While the high GS parents in two populations differed in their profiles of seed aliphatic GS, the low GS parents were similar. Phenotypic data of seed GS traits from three environments of the two populations were subjected to QTL analysis. The first population (referred to as DE population) detected a total of 60 QTL from three environments which upon intra-population meta-QTL analysis were merged to 17 *S-QTL* (Stable QTL) and 15 *E-QTL* (Environment QTL). The second population (referred to as VH population) detected 58 QTL from the three environments that were merged to 15*S-QTL* and 16*E-QTL*. In both the populations, majority of *S-QTL* were detected as major QTL. Inter-population meta-analysis identified three *C-QTL* (consensus QTL) formed by merging major QTL from the two populations. Candidate genes of GS pathway were co-localized to the QTL regions either through genetic mapping or through in silico comparative analysis. Parental allelic variants of QTL or of the co-mapped candidate gene(s) were determined on the basis of the significantly different R^2 values of the component QTL from the two populations which were merged to form *C-QTL*. The results of the study are significant for marker-assisted transfer of the low GS trait and also for developing lines with lower GS than are present in *Brassica juncea*.

Keywords *Brassica juncea* · Seed glucosinolates · QTL mapping · Allelic variations

Introduction

Glucosinolates (GS) are secondary metabolites mainly found in the family Brassicaceae including crop *Brassica* species. GS play an important role in the nutritional qualities of *Brassica* products. Seeds of rapeseed and mustard are a source of edible oil and have a protein-rich seed meal. High GS in the seed meal when fed to poultry and livestock pose health risks (Fenwick et al. 1983; Griffiths et al. 1998). Due to availability of low GS natural genetic variation in *B. napus*, low GS cultivars of *B. napus* have been successfully cultivated containing <20 μmol/g of seed and all the modern rapeseed cultivars grown in Europe, Canada and China are of canola quality containing <2.0 % erucic acid in the seed oil and <20 μmol/g of seed GS. It is also felt that there is, however, a significant residual content of GS in rapeseed/canola seed meal and further reduction of the total GS content would be nutritionally desirable

Communicated by Carlos F. Quiros.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-015-2461-9) contains supplementary material, which is available to authorized users.

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(McVetty et al. 2009). In *B. juncea*, although low GS lines have been reported in the East European type germ plasm (Love et al. 1990a), no productive and commercially viable low GS lines have yet been developed.

Aliphatic GS derived from amino acid methionine constitute the major type of GS in *Brassica* species including oilseed mustard *B. juncea*. Natural variability of *B. juncea* could be broadly classified into two diverse gene pools—the Indian and the east European types (Srivastava et al. 2001; Burton et al. 2004). The two gene pools of *B. juncea* apart from being genetically diverse can also be distinguished on the basis of their chemo-types for aliphatic GS. While east European types contain 2-propenyl GS (3C), the Indian types contain both 2-propenyl (3C) and 3-butenyl GS (4C) (Gland et al. 1981; Love et al. 1990b; Sodhi et al. 2002). Several QTL mapping studies have been undertaken to study the genetics of total seed GS in *B. napus* (Magrath et al. 1994; Toroser et al. 1995; Uzunova et al. 1995; Howell et al. 2003) as well as in *B. juncea* (Cheung et al. 1998; Mahmood et al. 2003; Lionneton et al. 2004; Ramchiary et al. 2007a), each study reporting variable number of QTL. Genetic complexity of the GS trait in *B. juncea* was first underscored by Ramchiary et al. (2007a) while transferring low phenotype from the east European line to Indian line and identified 'context dependent' QTL by analyzing advanced backcross populations. The study hypothesized involvement of a different genetic network for the GS trait in east European gene pool lines as these contain mostly 2-propenyl aliphatic GS.

Several structural and regulatory genes responsible for most biosynthetic steps in the GS pathway have been identified in Arabidopsis (Wittstock and Halkier 2002; Bak and Feyereisen 2001; Grubb et al. 2004; Halkier and Gershenzon 2006; Sønderby et al. 2010) till date. Recently, 102 GS orthologs and paralogs have been physically mapped in the 10 chromosomes of *B. rapa* derived from 52 orthologs of GS genes identified in Arabidopsis (Wang et al. 2011). Several of these GS genes have been mapped in *B. napus* (Liu et al. 2011, 2012; Feng et al. 2012), *B. oleracea* (Li and Quiros 2002, 2003; Gao et al. 2014), *B. rapa* (Zang et al. 2009) and *B. juncea* (Bisht et al. 2009; Yang et al. 2014), and in some cases functional validation of some of these genes in *Brassica* species have been achieved.

The present study focuses on the genetic dissection of aliphatic GS in *B. juncea* and on the identification of genes/QTL responsible for the trait variation in east European lines that primarily contain 3C GS. In this study, the seed GS QTL data of two bi-parental populations have been compared. Whereas in one population, the high GS parent contained 3C type aliphatic GS, in the other, the high GS parent predominantly contained 4C type aliphatic GS in the seed. The low GS parents in these two populations are similar and have comparable GS profiles. It is envisaged that the comparison might help identify specific loci that would be critical in marker-assisted breeding for the transfer of low GS trait to the east European parent containing high levels of 3C GS in the seeds. QTL analysis and subsequent inter-population meta-analysis led to the identification of 'consensus' and 'population-specific' QTL. Mapping of candidate genes of GS biosynthetic pathway and their co-mapping with the QTL were carried out and the data were used to decipher the allelic variations among the parents for the genes/QTL responsible for profile of seed aliphatic and total GS contents. The findings of the present study are significant for marker-assisted transfer of the low seed GS trait to the east European *B. juncea* with high GS background and also for developing low GS lines having lesser GS contents than the currently available low GS material in *B. juncea.*

Materials and methods

Plant materials and measurement of seed GS

Two bi-parental mapping populations of *B. juncea* were used in the study. The first mapping population was 184 microspore-derived doubled haploid (DH) lines derived from F_1 of cross between an east European high GS (containing ~93.8 μmol/g of seed GS) line Donskaja-IV and a low GS (containing ~13.4 μmol/g of seed GS) line EH-2 (henceforth referred to as DE population). The second mapping population was 123 DH lines derived from F_1 of cross between an Indian high GS variety Varuna (containing ~109.8 μmol/g of seed GS) and a low GS line Heera (containing ~12.1 μmol/g of seed GS). The second population will henceforth be referred to as VH population. VH population has earlier been used by Ramchiary et al. (2007a) for the QTL dissection of seed GS trait. Low GS parents in both the populations (EH-2 in DE and Heera in VH population) had similar seed aliphatic GS content and profiles because EH-2 is an EMS-induced early maturing variant of Heera. On the other hand, the high GS parents in both the populations differ with respect to the profiles of aliphatic GS. While the high GS parent Donskaja-IV in the DE population synthesizes primarily propyl type (3C) aliphatic GS, the high GS parent Varuna in VH population synthesizes both propyl (3C) (~15 % of the seed aliphatic GS) and butyl types (4C) (~82 % of the seed aliphatic GS) (Table 1).

DE mapping population was sown in lines in the field at one location in Delhi (normal winter growing season, shortday condition) over a period of three years. Open-pollinated mature seeds from five competitive plants were pooled for estimation of seed GS content and aliphatic profiles using HPLC (Shimadzu Prominence UFLC-20AD)

Table 1 Phenotypic variation of five GS traits [percent propyls (% Prpyl), percent butyls (% Btyl), percent pentyls (% Ptyl), aliphatics (Ali) and total GS content (ToGsl)] in parents and two bi-parental mapping populations, DE and VH

Trait	Parental mean ± SD and range of various GS traits in DE population					Parental mean ± SD and range of various GS traits in VH population				
	Donskaja-IV	EH-2	DE population			Varuna	Heera	VH population		
			DEL-1	DEL-2	DEL-3			DEL	GWL	LEH
% Prpyl	99.2 ± 0.05	5.0 ± 2.1	1.6–99.4 (44.7)	0.5–100.0 (42.2)	0.8–99.6 (41.2)	15.2 ± 3.50	1.9 ± 2.4	0.0–55.1 (18.6)	0.0–50.4 (17.3)	0.0–50.3 (16.7)
% Btyl	0.7 ± 0.10	79.0 ± 0.7	0.6–97.8 (52.3)	0.4–100.0 (58.0)	0.4–100.0 (56.0)	82.3 ± 3.27	90.9 ± 1.93	44.8–99.0 (74.0)	48.1–99.6 (76.1)	47.6–100.0 (77.4)
%Ptyl	0.1 ± 0.05	16.0 ± 2.1	0.1–21.7 (3.3)	0.2–20.8 (4.0)	0.3–22.4 (4.7)	2.7 ± 0.63	7.5 ± 3.42	0.0–35.7 (7.3)	0.0–37.9 (6.6)	0.0–36.6 (5.9)
Ali	93.1 ± 7.63	12.6 ± 0.3	11.4–122.0 (63.3)	3.9–102.5 (63.3)	8.2–118.6 (60.2)	108.6 ± 2.80	11.3 ± 0.59	5.5–119.4 (69.2)	12.6–118.0 (74.3)	13.4–109.2 (65.9)
ToGSL	93.8 ± 7.67	13.4 ± 0.2	12.4–122.7 (64.6)	7.3–105.4 (59.1)	8.2–119.7 (60.8)	109.8 ± 2.80	12.1 ± 0.72	7.5–121.7 (70.9)	14.0–119.3 (75.9)	14.6–110.3 (67.2)

Mean values are shown in parentheses

% Prpyl = 100 × (all propyl fraction GS/total aliphatic GS); % Btyl = 100 × (all butyl fraction GS/total aliphatic GS); % Ptyl = 100 × (all pentyl fraction GS/total aliphatic GS)

SD standard deviation

following Kräling et al. (1990). The percentages of propyls, butyls and pentyls were calculated following the procedure of Magrath et al. (1993).

Phenotyping data of VH mapping population used in the study was from three locations at Delhi, Gwalior and Leh that was earlier reported by Ramchiary et al. (2007a).

Statistical analyses and QTL mapping

Trait means, ANOVAs and correlations were determined using software SPAR 2.0 (2005). QTL mapping of the seed GS traits consisting of five parameters, namely percentage propyls (Prpyl), percentage butyls (Btyl), percentage pentyls (Ptyl), aliphatics (Ali) and total GS (ToGsl) was undertaken with composite interval mapping (CIM) using WinQTLCart 2.5 (Wang et al. 2006). The default genetic distance and walking speed were set to 10 and 1 cM, respectively. 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 2.5 was chosen for declaring the presence of a putative QTL. For the purpose, a DE linkage map with 653 DNA markers consisting of 344 IPs (intron polymorphic), 278 SSRs, 26 SNPs and 5 Indels covering a total genetic length of 1598.5 cM was used. A reconstructed VH linkage map (merging the VH maps developed earlier by Ramchiary et al. 2007b; Panjabi et al. 2008; Paritosh et al. 2014) with 1908 makers consisting of 305 AFLPs, 11 RFLPs, 543 IPs, 327 SSRs, 28 gene markers and 694 SNPs covering a genetic length of 1881.0 cM was used in the study (details of these two maps will be reported elsewhere).

The component QTL detected by WinQTLCart 2.5 was further subjected to QTL meta-analysis in two ways using Biomercator 2.1 (Arcade et al. 2004; Goffinet and Gerber 2000). First, QTL detected from different environments on a LG in a particular mapping population loaded into the project using the values detected by WinQTLCart 2.5 as source for positions of QTL confidence intervals and QTL for same trait were integrated through the '*Meta-analysis*' command of BioMercator 2.1 (called intra-population meta-analysis) and the merged QTL were designated as *S-QTL* (Stable QTL). The QTL that did not merge were designated as *E-QTL* (Environmental QTL). Second, QTL obtained from different bi-parental populations were combined in a single study for detecting a 'consensus' QTL (*C-QTL*). For this, a workspace was created harboring genetic maps and QTL data of two populations considering each of the multiple locations as independent experiments and was loaded into the project through Darvasi's formula (Arcade et al. 2004). Using the most informative bi-parental map (VH map in the present study) as reference map, the

markers and QTL information of DE map were projected on to it to develop a consensus map using the '*Map projection'* command of BioMercator 2.1 (called inter-population meta-analysis). QTL from the two different bi-parental maps which did not merge by inter-population meta-analysis were designated as 'population-specific' QTL. The QTL that fell beyond the length of the corresponding LGs upon projection were excluded from the analysis.

Mapping of candidate gene markers

Of the 52 reported candidate genes involved in GS biosynthesis in *A. thaliana* (Wang et al. 2011), primers for nine genes were earlier used by Bisht et al. (2009). For the remaining 43 genes, IP markers were developed in the present study from 21 genes showing suitability for the development of IP markers using the gene sequences of *A. thaliana* (Panjabi et al. 2008). Primer sequences for all the 30 genes including nine genes of Bisht et al. (2009) are shown in supplementary Table S1. Markers were mapped to DE and VH maps following the mapping procedure of Ramchiary et al. (2007a) and were co-localized with the detected QTL in case the candidate genes mapped to the QTL interval. Unmapped candidate genes were co-localized to QTL regions through *in-silico* mapping by aligning the candidate genes to collinear regions identified between *Arabidopsis* and *Brassica juncea* using comparative genomics (Paritosh et al. 2014), wherever possible. In case the candidate gene(s) mapped in one population but could not be mapped in the other due to lack of parental polymorphism, in silico mapping procedure was followed to determine the likely position in the genome. Candidate gene markers were named following the nomenclature proposed by Østergaard and King (2008).

Results

Phenotypic variation of seed GS in parents and two mapping populations

Parents

Aliphatic GS was observed to be the major contributor to the total seed GS contents in four parents of the two bi-parental populations used in the study. The high GS parent Donskaja-IV in DE population contained ~93.1 μmol/g of seed aliphatic GS against the total seed GS content of ~93.8 μmol/g of seed. Similarly, the high GS parent Varuna in VH population contained ~108.6 μmol/g of seed aliphatic GS against the total seed GS content of ~109.8 μmol/g of seed. The trend was also similar for the low GS parents in two populations (Table 1). However, the parental contrast of DE population differed from the parental contrast of VH population for their aliphatic GS profiles. Parents Donskaja-IV and EH-2 in DE showed absolute contrast for the three types of aliphatic GS wherein the parent Donskaja-IV had functional 3C pathway and the parent EH-2 had functional pathways for both 4C and 5C aliphatic GS. On the other hand, the parents Varuna and Heera of VH population showed contrast only for 3C and 5C aliphatic GS (Table 1) indicating that 4C pathway (butyl) in both Varuna and Heera are functional.

Mapping populations

The phenotypic distribution, mean and range for five traits namely, Prpyl, Btyl, Ptyl, Ali and ToGsl content under study were found to be stable in both the mapping populations in all the three environments indicating minimal influence of environment on the expression of seed GS traits. All the five traits showed continuous distribution among the DH lines indicating quantitative nature of the traits (data not shown). While transgressive segregation was observed for most of the traits, the direction of transgressive segregation differed in two populations. In DE population, transgressive segregants beyond the higher parental values were recorded for Btyl. On the other hand, transgressive segregants beyond the higher parental values were recorded for Prpyl and Ptyl in VH population. VH population also recorded transgressive segregants beyond the lower parental values for Btyl (Table 1). Estimates of correlation co-efficient were found to follow a reverse trend between the two populations. In VH population, Btyl was found to show positive correlation with Ali and ToGsl while in DE population, the Prpyl showed positive correlation with Ali and ToGsl (Supplementary Table S2).

QTL analysis

DE population

QTL analysis by composite interval mapping (CIM) for the five traits, namely Prpyl, Btyl, Ptyl, Ali and ToGsl identified a total number of 60 QTL from the three environments (Supplementary Table S3 and Figure S1). Overlapping QTL intervals detected QTL for more than one trait and it was more conspicuous among the three types of aliphatic GS traits (Prpyl, Btyl and Ptyl) indicating network biosynthetic pathway for the aliphatic GS. Subsequent intra-population meta-analysis merged these 60 component QTL to 17 *S-QTL* and 15 *E-QTL* (Supplementary Figure S1). Of the 17 *S-QTL*, 11 were detected as three-environment QTL and the remaining six were detected as two-environment QTL. Among the 11 three-environment *S-QTL*, six *S-QTL* were formed by merging major QTL explaining more than 10 % of the phenotypic variance (R^2) and remaining

Table 2 Details of the three *C-QTL* identified by merging the QTL data of VH and DE populations through inter-population meta-analysis

Details of *S/E-QTL*		Details of consensus QTL (*C-QTL*)			Co-mapped candidate gene(s)
S/E-QTL	Mapping populations	LG	Name	Flanking interval	
S-Prpyl-A3-1(3.1), *S-Btyl-A3-1*(2.5), *S-Ptyl-A3-1*(13.3)	DE	A3	*Bju.GSL.A3.1*	26.9–30.8	*BjuA.GSL-ELONG.c/d, BjuA.Myb28.a*
S-Btyl-A3-1(14.1), *S-Ptyl-A3-1*(49.2), *S-Ali-A3-1*(21.8), *S-Togsl-A3-1*(23.8)	VH				
S-Ali-A9-1(45.3), *S-ToGsl-A9-1*(45.2)	DE	A9	*Bju.GSL.A9.1*	17.8–23.0	*BjuA.Myb28.b*
S-Ali-A9-1(20.2), *S-ToGsl-A9-1*(20.5)	VH				
S-Prpyl-B4-1(44.4), *S-Btyl-B4-1*(42.9), *S- Ptyl-B4-1*(6.3)	DE	B4	*Bju.GSL.B4.1*	65.1–67.7	*BjuB.CYP79F1*
S-Prpyl-B4-(48.1)*1, S-Btyl-B4-1*(44.8)	VH				

Value in the parenthesis is the phenotypic variance (%) of the *S/E-QTL* estimated by averaging the phenotypic variance explained by each of the component QTL as indicated in Supplementary Table S3 and Table S4

[a] Co-mapping of candidate gene (s) established through in silico comparative mapping

five were formed by merging minor QTL. All the remaining two-environment *S-QTL* and *E-QTL* were detected as minor QTL (Supplementary Table S3).

Among the major *S-QTL*, QTL for propyl and butyl GS were detected in both LG A2 and B4 and mapped to one genetic interval in each linkage group. The average phenotypic variance (R^2) explained by propyl QTL in LG A2 (*S-Prpyl-A2-1*) and LG B4 (*S-Prpyl-B4-1*) were 18.5 and 44.4 %, respectively. On the other hand, the average phenotypic variance (R^2) explained by butyl QTL in LG A2 (*S-Btyl-A2-1*) and LG B4 (*S-Btyl-B4-1*) were 18.2 and 42.9 %, respectively. Aliphatics (*Ali*) and total GS content (*ToGsl*) each detecting one major *S-QTL* mapped to the same genetic interval in LG A9. The average phenotypic variance (R^2) explained by the QTL for *Ali* (*S-Ali-A9-1*) was 45.3 % and for *ToGsl* (*S-Ali-A9-1*) was 45.2 %.

VH population

QTL analysis for five GS traits using the reconstructed VH map detected a total of 58 QTL in three environments at LOD threshold of 2.5 (supplementary Table S4). The analysis detected 17 more QTL than the 41 QTL reported by Ramchiary et al. (2007a). This difference could primarily be attributed to the high threshold LOD of 2.8 followed by Ramchiary et al. (2007a). The 58-component QTL were subsequently converged to 15 *S-QTL* and 16 *E-QTL* through intra-population meta-analysis (supplementary Table S4; Figure S2). The 15 *S-QTL* included 12 three-environment and 3 two-environment QTL. All the three-environment *S-QTL* were detected in LG A3, A9, B4 and B8 and were formed by merging major QTL for different GS traits except *S-Prpyl-B8-2* and *S-ToGsl-B8-1*. No major QTL was detected in LG A2 in VH population. It was in contrast to the DE population that detected *S-QTL* for propyl, butyl and pentyl GS in this LG. All the two-environment *S-QTL* and all the 15 *E-QTL* except *E-Ali-B3-1* in LG B3 were formed with minor effect QTL.

The three-environment major *S-QTL* in LG A3 detected *S-QTL* for Btyl (*S-Btyl-A3-1*), Ptyl (*S-Ptyl-A3-1*), Ali (*S-Ali-A3-1*) and ToGsl (*S-ToGsl-A3-1*) and explained 14.1, 49.2, 21.8 and 23.8 % of the phenotypic variance, respectively. In the LG A9 the three-environment major effect *S-QTL* were detected only for aliphatics and total GS. Average phenotypic variance explained by these *S-QTL,* namely *S-Ali-A9-1* and *S-ToGsl-A9-1* were 20.2 and 20.5 %, respectively. LG B4 detected *S-QTL* for Prpyl (*S-Prpyl-B4-1*) and Btyl (*S-Btyl-B4-1*) and the average phenotypic variance explained by these QTL were 48.1 and 44.8 %, respectively. The major *S-QTL* detected in the LG B8 were for Prpyl (*S-Prpyl-B8-1*) and Ptyl (*S-Ptyl-B8-1*) and explained average of 12.8 and 15.4 % of the phenotypic variance, respectively. Nonetheless, these two *S-QTL* in LG B8 have been shown to be context-dependent QTL as their effect disappeared in advanced backcross generation when transferred to Varuna genetic background (Ramchiary et al. 2007a).

Inter-population meta-analysis

Inter-population meta-analysis was done by merging the component QTL of both DE and VH maps. Cumulatively a total of 118 component QTL detected for five GS traits that included 60 component QTL from DE (supplementary Table S3) and 58 component QTL from VH population (supplementary Table S4) were merged by meta-analysis for the identification of 'consensus' (*C-QTL*) and 'population-specific' QTL. The consensus QTL were named with initials '*Bju*' for *B. juncea* and the population-specific QTL were named either with initials 'DE' or 'VH' for the respective population.

A total of three *C-QTL,* namely *Bju.GSL.A3.1*, *Bju.GSL.A9.1* and *Bju.GSL.B4.1* distributed over LGs A3, A9 and B4 were detected and were formed by merging major QTL. *C-QTL* along with their co-mapping information with

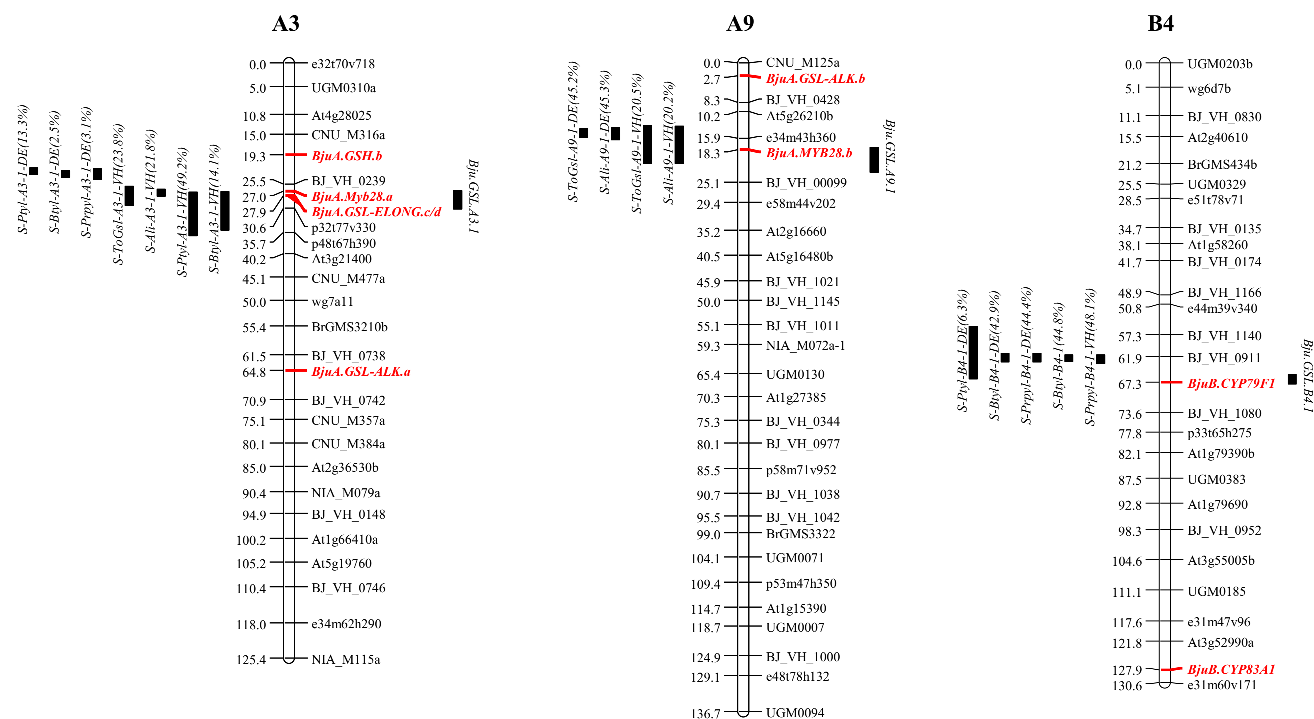

Fig. 1 Genomic positions of six *C-QTL*, two in the LG A3 and one each in LGs A7, A9, B3 and B4. *S/E-QTL* have been shown in the *left* and the corresponding *C-QTL* in the *right* side of the linkage map. *S/E-QTL* with VH or DE indicates the origin of the population from which the QTL is detected. Value in the parenthesis is the phenotypic variance of the *S/E-QTL* estimated by averaging the phenotypic variance explained by each of the component QTL as indicated in supplementary Table S3 and Table S4

GS pathway candidate genes has been shown in Table 2 and Fig. 1. Of the three major *C-QTL*, *Bju.GSL.A3.1* was formed by merging QTL for all the five GS traits and was observed to co-localize with two candidate genes *BjuA.GSL-ELONG.c/d* and *BjuA.Myb28.a* (Table S1). The *C-QTL, Bju.GSL.A9*.1 in the LG A9 was formed by merging QTL for aliphatic and total GS content and co-mapped with the candidate gene *BjuA.Myb28.b* (Table S1). The *C-QTL*, *Bju.GSL.B4*.1 in the LG B4 was formed by merging the QTL for C3, C4 and C5 aliphatic GS and co-mapped to the candidate gene *BjuB.CYP79F1* (Table S1).

A total of seven population-specific QTL were detected of which four were contributed by DE population and the remaining three were from VH population (Table 3). All these population-specific QTL were formed by merging only minor QTL except the QTL, *DE.GSL.A2.1* which was formed by merging major *S-QTL* for propyl and butyl GS from DE population and co-mapped with the candidate genes *BjuA.GSL-ELONG.a* (Fig. 2, Table S1).

Discussion

The two mapping populations (DE and VH) used in the present study for comparative QTL analysis of seed GS traits are chosen on the basis of the difference in the aliphatic GS profiles between the high GS parents. The analysis of these two populations, therefore, would provide a clearer picture on genetic architecture of the trait and enable identification of right QTL which will find application in marker-assisted transfer of low GS trait in two gene pool lines of *B. juncea*. It has been hypothesized in the present study that the low GS parents in two populations are common (EH-2 is the EMS-induced early mutant of Heera and possess similar seed GS profiles) and hence would possess same allele in a QTL for a seed GS trait. On the other hand, the two high GS parents in two populations might possess different alleles for a gene/QTL (*C-QTL*) in case the component QTL has been detected in their respective population with significantly different R^2 (phenotypic variance explained) values. It would, therefore, allow identification of allelic variations of a QTL among the parents used in the study. Moreover, co-mapping of several candidate gene markers involved in the GS biosynthesis would allow the elucidation of genetic mechanism of GS biosynthesis under east European and Indian chemo-type backgrounds and development of accurate and easy-to-use markers for the diversification of the trait.

Merging of QTL data from two bi-parental populations (DE and VH) for the five studied GS traits identified three

Table 3 Details of seven 'population-specific' QTL detected by inter-population meta-analysis through merging of QTL data of VH and DE populations

Details of *S/E-QTL*		Details of population-specific QTL			Co-mapped candidate gene(s)
S/E-QTL	Mapping population	LG	Name	Flanking interval	
E-Ptyl-A1-1(4.8), *E-Ali-A1-1*(4.1), *E-ToGsl-A1-1*(4.4)	VH	A1	*VH.GSL.A1.1*	26.9–30.8	*FMO*$_{GS\text{-}OX1\text{-}5}$[a*], *BCAT-4*[a*]
S-Prpyl-A2-1(18.5), *S-Btyl-A2-1*(18.2), *S-Ptyl-A2-1*(6.7)	DE	A2	*DE.GSL.A2.1*	83.4–91.4	*BjuA.GSL-ELONG.a,*
E-Ali-A3-1(3.4), *S-ToGsl-A3-1*(3.2), *E-Ptyl-A3-1*(6.1)	DE	A3	*DE.GSL.A3.1*	74.8–105.3	*IPMI SSU2*[a*]
E-Ali-B1-1(2.8), *S-ToGsl-B1-1*(2.8)	DE	B1	*DE.GSL.B1.1*	18.6–73.0	*BjuB.Myb28.a*
E-Ptyl-B6-1(4.4), *S-Ali-B6-1*(5.2), *S-ToGsl1-B6-1*(5.1)	DE	B6	*DE.GSL.B6.1*	57.0–78.9	*BjuB.UGT74A1*
S-Prpyl-B8-1(12.8), *S-Btyl-B8-1*(4.6), *S- Ptyl-B8-1*(12.7), *E-Ali-B8-1*(4.4), *E-ToGsl-B8-1*(4.5)	VH	*B8*	*VH.GSL.B8.1*	30.8–43.7	–
S-Prpyl-B8-2(5.4), *E- Btyl-B8-1*(3.2), *E-Ptyl-B8-1*	VH	B8	*VH.GSL.B8.2*	103.1–144.4	*BjuB.GSL-ELONG.b*

Value in the parenthesis is the phenotypic variance (%) of the *S/E-QTL* estimated by averaging the phenotypic variance explained by each of the component QTL as indicated in supplementary Table S3 and Table S4

* Candidate genes reported to be associated with aliphatic glucosinolate biosynthesis (Sønderby et al. 2010)

[a] Co-mapping of candidate gene (s) established through *in-silico* comparative mapping

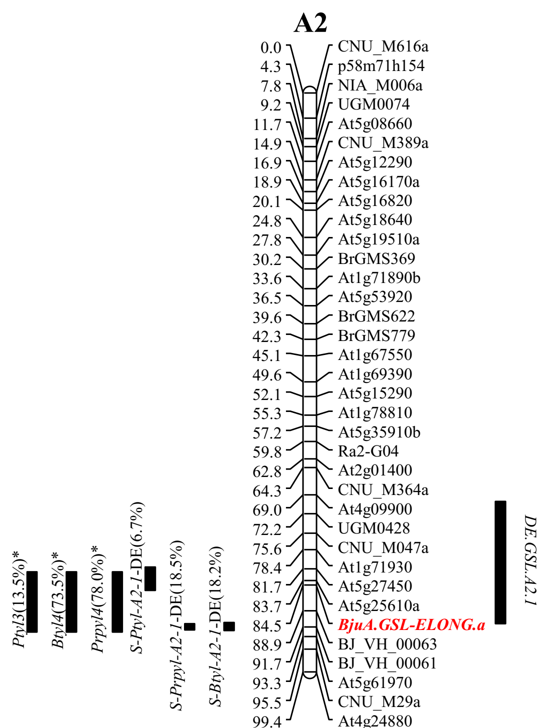

Fig. 2 Genomic position of a 'population-specific' QTL, *DE.GSL.A2.1* in the LG A2 of DE population. It mapped to the same position that was detected in the advanced backcross (BC_4DH) population derived from a cross between Varuan × Heera by Ramchiary et al. (2007a). *S/E-QTL* of DE population along with the QTL detected in the study by Ramchiary et al. (2007a) (marked in '*') have been shown in the *left* and the corresponding 'population-specific' QTL *DE.GSL.A2.1* in the *right* side of the linkage map. Value in the parenthesis is the phenotypic variance explained by the QTL

'consensus' QTL (*C-QTL*) all of which co-mapped with GS pathway candidate genes (Table 2). The *C-QTL, Bju. GSL.A9.1, was* observed to be exclusively contributing to the genetic variation of aliphatic and total seed GS. The QTL co-mapped with the candidate gene *Myb28*. Role of *Myb28* in regulating biosynthesis of methionine-derived GS biosynthesis has been well documented in the model species Arabidopsis and in *Brassica* species. Hirai et al. (2007), by analysing knockout and knockdown mutants in *A. thaliana* reported that *Myb28* is a positive regulator for basal level production of aliphatic GS. Recently, Augustine et al. (2013) reported reduction of seed aliphatic GS to 11.3 µmol/g DW through RNAi-based targeted suppression of *BjMYB28* in a high glucosinolate *B. juncea* line, Varuna, containing >100 µmol/g DW of seed GS. Hence, the co-mapping of aliphatic and total GS QTL with *Myb28* transcription factor indicates that *Myb28* contributes to the base level genetic variation of aliphatic GS.

The *C-QTL* (*Bju.GSL.A9.1*) in LG A9 was formed by merging of major QTL from two populations (supplementary Table S3 and Table S4). The QTL was detected with an average R^2 value explaining ~45 % of the phenotypic variance in DE population with Donskaja-IV holding trait enhancing allele (supplementary Table S3). On the other hand, the same QTL in the VH population explained ~20 % of the phenotypic variance with Varuna holding the trait-enhancing allele (supplementary Table S4). It suggests an allelic variation between the two high GS parents and predicts higher functionality for Donskaja-IV allele than for the Varuna allele. Hence, the allelic variation of the LG A9 QTL *Bju.GSL.A9*.1 contributing to aliphatic and total GS from the four parents was deciphered in the following order: Donskaja-IV > Varuna > EH-2 = Heera.

The other major *C-QTL* that contributed to the aliphatic and total GS was *Bju.GSL.A3.1* in the LG A3 (Table 2). This *C-QTL* was formed by merging the QTL for 3C, 4C, 5C GS from both the populations and additionally the QTL for aliphatic and total GS from VH population. The QTL

for aliphatic and total GS in this *C-QTL* explains ~24 % of phenotypic variance with Varuna holding the trait-enhancing allele. The region also co-mapped with candidate genes *BjuA.GSL-ELONG.c/d* and *BjuA.Myb28.a*. Previous study by Bisht et al. (2009) reported involvement of *BjuA.Myb28.a* gene for genetic variation of aliphatic GS in VH population through the analysis of recombinants between *BjuA.GSL-ELONG.c/d* and *BjuA.Myb28.a* and reported that Heera allele of *BjuA.Myb28.a* was responsible for reduction in the total aliphatic GS content. However, in the present study, although the candidate gene *BjuA.Myb28.a* co-mapped to the same QTL region in both the populations, no QTL for aliphatic and total GS could be detected in the DE population. It suggests no allelic variation between Donskaja-IV and EH-2 for *BjuA.Myb28.a*. The study, therefore, predicts the allelic variation of the gene *BjuA.Myb28.a* from the *C-QTLBju.GSL.A3.1* in the following order: Varuna > Heera = EH-2 = Donskaja-IV. The study also resolved the fact that two loci of *Myb28* in LG A9 and A3 equally (each contributing ~20 % of phenotypic variance) control the aliphatic GS content in VH population (supplementary Table S4) as against the single locus in LG A9 (explaining ~45 % of the phenotypic variance) in DE population (supplementary Table S3).

The same *C-QTLBju.GSL.A3.1* in the LG A3 that merged the QTL for 3C, 4C, 5C GS from both the populations was also observed to be co-mapped to the candidate genes *BjuA.GSL-ELONG.c/d*. It has been shown that *BjuA.GSL-ELONG.c/d* controls 5C elongation in VH population of *B. juncea* (Bisht et al. 2009) in which Heera allele increases pentyl GS (5C). In the present study, the mean R^2 explained by the corresponding *S-QTL* (*S-Ptyl-A3-1*) in the VH population was 49.2 % with Heera holding the trait-enhancing allele (supplementary Table S4) as against 13.3 % (*S-Ptyl-A3-1*) in the DE population with EH-2 holding the trait-enhancing allele for the 5C GS (supplementary Table S3). On the basis of the level of expression, three types of alleles for the gene *BjuA.GSL-ELONG.c/d* could be predicted, i.e. Heera = EH-2 > Donskaja-IV > Varuna.

Another significant observation from the comparative QTL analysis of seed GS trait was the co-mapping of *C-QTLBju.GSL.B4.1* with the candidate gene *BjuB.CYP79F1* in the LG B4 (Table 2). This *C-QTL* converged QTL for 3C, 4C and 5C from both the populations. In an earlier study in *B. juncea*, Bisht et al. (2009) showed that this QTL (*J16Gsl4*) in LG B4 is responsible for the variation of 3C GS and no candidate gene could be mapped to the QTL region. In the present study, candidate gene *BjuB.CYP79F1* was mapped to the region in both populations as dominant marker. The gene showed amplification from parents Varuna and Donskaja-IV and showed no amplification from parents Heera and EH-2. Earlier studies in *B. oleracea* (Li et al. 2003; Gao et al. 2006) reported involvement of *BoGSL-PRO* (ortholog of Arabidopsis gene At1g18500), a member of the gene family encoding the enzyme methylthioalkylmalate synthase (MAM) for the 3C GS. This has been further confirmed by Sotelo et al. (2014) through identification of metabolic QTL and candidate genes for GS synthesis in *B. oleracea* leaves, seeds and flower buds. Our observation in the present study in *B. ju*ncea is a departure from the studies in *B. oleracea*. de Kraker et al. (2007) observed that heterologous expression of the *A. thaliana IPMS1* (At1g18500) and IPMS2 (At1g74040) cDNA in *E.coli* resulted in synthesis of isopropylmalate synthases (IPMSs: EC 2.3.3.13) which are involved in leucine biosynthesis. We, therefore, believe that the 3C GS synthesis in *B. juncea* could be controlled by gene *CYP79F1,* and not by the ortholog of At1g18500. In the present study, it was also observed that the QTL *Bju.GSL.B4.1* explained a mean R^2 value of ~42.9 % (supplementary Table S3) in DE population and ~48.1 % (supplementary Table S4) in VH population for 3C GS, indicating no allelic difference between Donskaja-IV and Varuna for this QTL. Hence, the level of expression of four alleles used in the study would be Varuna = Donskaja-IV > Heera = EH-2 for the QTL *Bju.GSL.B4.1*.

Of the seven population-specific QTL identified from the study, majority of the QTL were formed by merging minor QTL. However, a mention may be made for a population specific QTL, *DE.GSL.A2.1* in the LG A2 of the DE population. The QTL was formed by merging major QTL for Prpyl and Btyl and co-mapped with candidate gene, *BjuA.GSL-ELONG.a*. In an earlier study, Ramchiary et al. (2007a) detected no QTL in this interval from VH population (F_1DH), but identified a major QTL (*J2Gsl1*) in BC_4DH population (Varuna was used as recurrent parent) explaining phenotypic variance of 78.0 % for 3C, 73.5 % for 4C and 13.5 % for 5C GS (Fig. 2). Subsequent study by Bisht et al. (2009) implicated this QTL for the 4C GS synthesis with Varuna holding the trait-enhancing allele. Placing together the earlier findings of this QTL from the advanced backcross VH population and the detection of the same QTL from the DE population from the present study, the allelic variation controlling the 4C GS at this locus in the LG A2 could be in the order of Varuna > Heera = EH-2 > Donskaja-IV.

QTL mapping of aliphatic GS has been undertaken in *B. juncea* (Cheung et al. 1998; Mahmood et al. 2003; Lionneton et al. 2004; Ramchiary et al. 2007a). However, QTL dissection per se may not provide correct information about the loci which could be utilized for marker-assisted transfer of the trait through backcross breeding. It is pertinent in case of breeding for low GS trait in *B. juncea* given the divergent composition of GS in the Indian and the east European gene pool lines. The study by Ramchiary et al. (2007a) in *B. juncea* provided an insight into the

complexity of the trait while attempting to transfer the low GS trait from an east European donor line to the Indian cultivar through backcross breeding. It led to the identification of 'true' QTL which could be used in marker-assisted introgression of low GS trait to Indian chemo-type high GS lines. The study identified three important QTL in the LG A2 (referred to as *DE.GSL.A2.1* in the present study), LG A3 (referred to as *C-QTLBju.GSL.A3.1* in the present study) and LG A9 (referred to as *C-QTLBju.GSL.A9.1* in the present study) that were the crucial loci for marker-assisted introgression of alleles from low GS parent Heera to high GS Indian gene pool line Varuna. It was also shown that the QTL in LG B4 referred to as *C-QTLBju.GSL.B4.1* in the present study) is not required in breeding low glucosinolate Indian gene pool lines of *B. juncea* (Ramchiary et al. 2007a, Bisht et al. 2009).

A parallel exercise of backcross transfer would be most ideal for the identification of 'true' loci which should find application for backcross transfer of low GS trait in the east European chemo-type germplasm. However, the results of the present study from two bi-parental populations involving parents with divergent composition of aliphatic GS offer information about prediction of 'true' QTL. We may not expect any context dependency leading to allelic heterogeneity that was observed in VH population for 4C GS in LG A2 and 3C and 5C GS in LG B8 (Ramchiary et al. 2007a) as the parental contrast between EH-2 and Donskaja-IV is unambiguous for both total GS and profiles of aliphatic GS. Hence, it is predicted that EH-2 allele of *C-QTLBju.GSL.A9.1* in LG A9 and *Bju.GSL,B4.1* in LG B4 would be crucial in marker-assisted backcross transfer of low GS trait to high 3C GS east European germplasm. Moreover, the information on allelic status detected in the present study could serve as a reference for formulating the breeding plans to develop low GS lines lower than the presently available low GS germplasm resources in *B. juncea*.

Author contribution statement KR carried out the phenotyping and mapping work. MS helped in candidate genes mapping. VG helped with genotyping and YSS helped in field experiments and seed glucosinolates estimation. AM developed microspore-derived DH mapping populations. DP and AKP conceived and supervised the overall study and along with KR wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgments The study was funded by the National Dairy Development Board (NDDB) and also partly supported by the Department of Biotechnology, Government of India, through the award of a Centre of Excellence on Brassica breeding. K. Rout and M. Sharma acknowledge the receipt of research fellowship from the NDDB and UGC, respectively.

Conflict of interest The authors declare that they have no competing interests.

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