Australian Journal of Crop Science AJCS

AJCS 10(2):152-160 (2016) ISSN:1835-2707

QTL controlling glucosinolate content in seeds of *Brassica napus* L.

Jun Liu[1], Arvind H. Hirani[2], Zhe Li[1], Chunren Wu[1], Peter B.E. McVetty[2], Fouad Daayf[2] and Genyi Li[2]*

[1]Monsanto Canada Inc., 900 - One Research Road, Winnipeg, Manitoba R3T6E3 Canada
[2]Department of Plant Science, University of Manitoba, Winnipeg, Manitoba R3T 2N2 Canada

***Corresponding author: genyi.li@umanitoba.ca**

Abstract

Glucosinolates are a group of endogenous secondary metabolites commonly found in *Brassica* plants. Oilseed rape (*Brasscia napus* L.) contains toxic glucosinolates in its otherwise high quality meal. Molecular breeding has many advantages over traditional breeding and may provide the solution to further decrease glucosinolate content and/or optimize glucosinolate profiles in the meal. In the present study, the *Brassica* 60K SNP (single nucleotide polymorphism) Infinium® microarrays supplemented with sequence related amplified polymorphism (SRAP) markers were used to map the whole *B. napus* genome of a DH population derived from an F_1. Eight thousand eight hundred and thirty-nine SNP and 35 SRAP markers were organized into 1,220 bins covering 2,597.7 centiMorgans (cM) on 19 chromosomes from 88 DH lines. The average bin density was 0.47 bin/cM. Nine quantitative trait loci (QTLs) controlling total and major glucosinolate components were localized on chromosomes A1, A8, A9, C2, C3 and C9 with the most abundant and significant QTLs residing on A9. The total phenotypic variances explained by the QTLs ranged from 7.84% for progoitrin to 40.10% for 4C aliphatic glucosinolate content. It was worthwhile to use the co-segregating flanking markers of these QTLs for marker assisted selection in practical canola breeding.

Keywords: glucosinolate, quantitative trait loci (QTLs), *Brassica napus*, molecular marker, SRAP, SNP.
Abbreviations: QTL_Quantitative trait loci; SNP_single nucleotide polymorphism; DH_double haploid; SRAP_sequence related amplified polymorphism; SSR_simple sequence repeats; AFLP_amplified fragment length polymorphism; RFLP_restriction fragment length polymorphic; GSL_glucosinolates; PRO_progoitrin; GNP_gluconapin; GLS_glucoalyssin; BN_glucobrassicanapin; GBC_glucobrassicin; HPLC_high performance liquid chromatography; RIL_recombinant inbred lines; TAli_total aliphatic; LG_linkage group; TGC_total glucosinolate content

Introduction

Glucosinolates (β-thioglucoside-N-hydroxysulfates) are a group of endogenous allelochemicals, a subset of secondary metabolites mainly found in plants of order *Brassicales*. There are over 132 different glucosinolates found in nature (Baskar et al., 2012). Their enzyme-mediated hydrolytic products function as defense substances against natural enemies (Fahey et al., 2001; Uzunova et al., 1995) and abiotic stresses. Some glucosinolates may have detrimental effects on livestock palatability and health, while others are beneficial and may be used as anti-carcinogens (Fahey et al., 2001) or condiments (Lou et al., 2008), which has triggered tremendous interest in glucosinolate research in Brassica crops. Great success was achieved by converting rapeseed into low erucic acid and low glucosinolate canola cultivars in 1960-70s, which paved a foundation for quality improved rapeseed to become one of the most three important oilseed crops in the world (http://apps.fas.usda.gov/psdonline/circulars/oilseeds.pdf). However, further decreasing glucosinolate content and modifying glucosinolate profiles in *B. napus* seed meal has been a long term breeders' objective which has been hard to achieve using traditional breeding because of the complexity of the mechanisms involved in glucosinolate synthesis and regulation. Molecular breeding is well suited, on the other hand, to investigate intrinsic genetic structure, especially quantitative trait loci (QTLs) controlling the traits. In *B. napus*, Uzunova et al., (1995) and Toroser et al., (1995) used first-generation hybridization-based restriction fragment length polymorphism (RFLP) maps to identify 4 QTLs which explained 61.7% of phenotypic variation for total glucosinolate content and 5 QTLs which explained 71.0% of phenotypic variation for total aliphatic glucosinolate content in separate linkage groups (LGs) of the genomes from seeds of two DH (doubled haploid) populations, respectively. In another study, two of these previously detected 4 QTLs for total seed glucosinolate content were proposed to be the same as the two major QTLs in a set of spring and winter cultivars and lines described by de Quiroz and Mithen (1996). Zhao and Meng (2003) used mainly RFLP markers and identified 3 QTLs controlling total glucosinolate content, and 3 to 15 QTLs for different glucosinolates in seeds of an F_3 population. One locus associated with aliphatic glucosinolates and another with indole glucosinolates were linked to plant resistance to *Sclerotinia* stem rot. Howell et al., (2003) used traditional quantitative genetics and Harper et al., (2012) used associative transcriptomics to report 3 QTLs controlling total glucosinolate content on LG A9, C2 and C9 in seeds of *B. napus*. Li et al., (2014) used association mapping to screen a diverse panel of 472 accessions with the 60K Infinium® (Illumina Inc., San Diego, CA, USA) microarray and found SNP clusters associated with total seed glucosinolate content on chromosomes A9, C2, C7 and C9. The close relatives of *B. napus*, especially its proposed progenitors *B. rapa* and *B. oleracea*, also attracted interest for their glucosinolate traits.

In *B. rapa* Lou et al., (2008) used amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers to identify 6 QTLs for aliphatic, 3 QTLs for indolic and 3 QTLs for aromatic glucosinolate content in leaves of 2 DH populations. Hirani et al., (2013) illustrated a major QTL controlling 5C aliphatic glucosinolate content on chromosome A3 in a recombinant inbred line (RIL) population and validated through homeologous gene replacement through interspecific hybridization. In *B. oleracea*, Sotelo et al., (2014) identified 82 major QTLs controlling the synthesis of various glucosinolates in a 155-line DH population by using SSR and RFLP markers. Eighteen of these QTLs were consensus ones found in seeds, leaves and buds of the plants. Epistatic effects were observed for the consensus QTLs. Seven candidate loci, 3 for aliphatic and 4 for indolic glucosinolate content were proposed in *Brassicaceae* by the authors. There were also studies in *B. juncea* (Cheung et al., 1998; Mahmood et al., 2003; Ramchiary et al., 2007) and in *Arabidopsis thaliana* Kliebenstein et al., (2001) which reported QTLs functioning differently for either total glucosinolate content or for glucosinolate components. The genetic mechanisms regulating to the biosynthesis of glucosinolates and their components are still poorly understood in *B. napus*. In the present study, we used the newly developed *Brassica* 60K Infinium® SNP microarray and SRAP molecular markers for a bi-parental *B. napus* DH population and found 9 QTLs distributed on 6 chromosomes using 8,839 SNP and 35 SRAP markers. Two thousand five hundred and ninety-eight cM of genetic distance were covered. Chromosome A9 had more QTLs which were highly significant and played a major role in the biosynthesis and regulation of major glucosinolate components.

Results

Glucosinolate identification and quantification

Five major glucosinolates (Fig 1), aliphatic 2-hydroxy-3-butenyl (progoitrin, PRO), 3-butenyl (gluconapin, GNP), 5-methylsulfinylpentyl (glucoalyssin, GLS), 4-pentenyl (glucobrassicanapin, GBN) and indolic 3-indolylmethyl (glucobrassicin, GBC) were identified. The total glucosinolate content of the 2 parental lines were 7.35 µmol/g seed for the low glucosinolate line M77 and 95.10 µmol/g seed for the high glucosinolate line M23 (Table 1).
Total glucosinolate content (TGC) varied from 2.64 to 105.74 µmol/g seed and had a range of 103.10 µmol/g seed. There was only one line having TGC lower than M77. However, there were 14 lines having TGC higher than M23. The average TGC was 68.33 µmol/g seeds and the median 76.77 µmol/g from the 88 DH lines was skewed toward the high glucosinolate end (Table 1S). Aliphatic glucosinolates (4C, four carbon + 5C, five carbons, T-ALI, total aliphatic) were the dominant glucosinolates in the seed with an average 96.66% of the TGC. This is in agreement with the results from Velasco et al., (2008). In contrast, the detected indolic GBC only accounted for 3.34% of TGC. It was quite consistent that over 90% of TGC in almost all the seed samples was from T-ALI. On the other hand, four-carbon aliphatic glucosinolates predominated in T-ALI ranging from 73.22 to 100% of T-ALI. Similarly PRO accounted for the largest proportion of 4C with a mean of 67.54%. There were only four lines having more than 50% GNP among the 88 DH lines. There was a trend when TGC was less than 30 µmol/g seed, where the proportion of aliphatic glucosinolates decreased and indolic glucosinolates increased with the decrease of TGC. Correlation coefficients (r) reflected the relationships among the different GSL components. For example, PRO had strong positive relationship with 4C, T-ALI and TGC with r = 0.9541, 0.9551 and 0.9514, respectively. To a lesser extent GNP also had positive relationship with 4C, T-ALI and TGC by r = 0.8588, 0.8435 and 0.8472. GLS and GBN had similar patterns in that the relationships were all positive; each of their relationships with 5C was stronger than that with T-ALI and TGC such as r = 0.7739 and 0.9153 for both with 5C, respectively, and 0.3788, 0.3888 for GLS and 0.7516, 0.7476 for GBN with T-ALI and TGC. Four carbon aliphatic glucosinolates had r = 0.9945 and 0.9935 with T-ALI and TGC but the 5Cs' corresponding r = 0.7053 and 0.7069. T-ALI was a good representative for TGC with r = 0.9993 but GBC was not with r = -0.51.21 (Fig 2).

Construction of genetic map with SNP and SRAP markers

Eight thousand eight hundred and thirty-nine polymorphic SNP markers were generated and mapped onto 19 chromosomes. One thousand one hundred and eighty-five SNP bins were grouped with 35 SRAP markers to assembly a genetic map which covered genetic distance of 2597.7 cM in the 19 chromosomes (Table 2). The average genetic density was 0.47 bins/cM and 0.91 unique SNP positions per cM. Chromosome A3 had the most bin number of 108 and unique SNP positions of 280, while chromosome C6 had the fewest bins of 24 and fewest unique SNP positions of 37. There were 690 SNP bins, 3,855 SNP markers, 1,431 unique SNP positions and 1351.5 cM of genetic distance was covered in subgenome A. The corresponding values were 495 SNP bins, 4,984 SNP markers, 965 unique SNP postions and 1,246.2 cM in subgenome C. The average densities of bins and unique SNP positions were 0.53/cM and 1.09/cM in subgenome A and 0.40/cM and 0.74/cM in subgenome C.

QTL identification for glucosinolate related traits

There were 17 QTLs regulating the glucosinolate traits PRO, GNP, 4C, GLS, GBN, 5C, T-ALI, GBC and TGC individually on 6 chromosomes (Table 3). Among them, chromosome A9 had the most QTLs with 8. Chromosomes A1 and C3 anchored 3 QTLs each. There was only 1 QTL on each of chromosome A8, C2 and C9, respectively (Fig 3 and Fig 4). Chromosome A9 not only had more QTLs than other chromosomes, the QTLs on this chromosome also had significant effects on glucosinolate traits. For example, LOD values of the QTLs on chromosome A9 ranked the highest for 5 QTLs among all of the 17 QTLs and explained the highest phenotypic variances ranging from 23.87% to 40.10% with the average of 30.12%. Four of the 5 QTLs on chromosome A9 had positive additive effects and contributed to increases of 4C with 40.10%, PRO with 29.63%, T-Ali with 28.96% and TGC with 28.05% of their corresponding phenotypic variances. One QTL, on the other hand, had negative genetic effect and contributed to the decrease of GBC with 23.87% of phenotypic variance. The other three QTLs had positive genetic effects on 5C, GBN and GLS with LOD values ranging from 3.52 to 6.03 and explained 14.18 to 18.78% of phenotypic variances for their traits. Two QTLs for 5C and GLS on chromosome A1 were located at the same position, 102.0 cM from the left end and had positive genetic effects explaining 14.93% and 14.94% of phenotypic variances. One QTL controlling 4C was located at 33.1 cM

Table 1. Glucosinolate content (µmol/g seed) in seeds of a doubled haploid *Brassica napus* population M730 and their parents*.

Content	PRO	GNP	4C	PRO/ 4C%	GNP/ 4C%	GLS	GBN	5C	GLS/ 5C%	GBN/ 5C%	T-Ali	4C/ T-ALI%	5C/ T-ALI%	GBC	TGC	T-ALI/ TGC%	GBC/ TG%
Min	0.62	0.00	0.62	33.65	0.00	0.00	0.00	0.00	19.52	14.76	0.62	73.22	0.00	0.41	2.64	23.48	0.43
M77	2.00	0.90	2.90	68.97	31.03	0.41	0.22	0.63	65.08	34.92	3.53	82.15	17.85	3.82	7.35	48.03	51.97
Mean	39.85	19.15.	59.00	67.54	32.46	3.13	3.91	7.04	44.42	55.59	66.04	89.34	10.660	2.28	68.33	96.66	3.34
Median	46.10	18.16	67.24	69.46	30.41	2.75	3.28	6.59	49.36	51.09	73.81	89.31	10.68	2.08	76.77	96.75	3.25
M23	56.68	28.98	85.66	66.17	33.83	2.62	5.29	7.92	33.08	66.79	93.57	91.55	8.46	1.53	95.10	98.39	1.61
Range	64.86	45.66	93.71	66.35	66.35	8.78	13.51	18.45	65.84	65.72	104.26	26.78	26.78	5.03	103.10	76.09	75.71
Max	65.48	45.66	94.33	100.00	66.35	8.78	13.51	18.45	85.36	80.48	104.88	100.00	26.78	5.44	105.74	99.57	76.14

*: PRO, progoitron; GNP, gluconapin; 4C, four-carbon aliphatic glucosinolate; GLS, glucoalyssin; GBN, glucobrassicanapin; 5C, five-carbon aliphatic glucosinolate; T-ALI, total aliphatic glucosinolate; GBC, glucobrassicin; and TGC, total glucosinolate content.

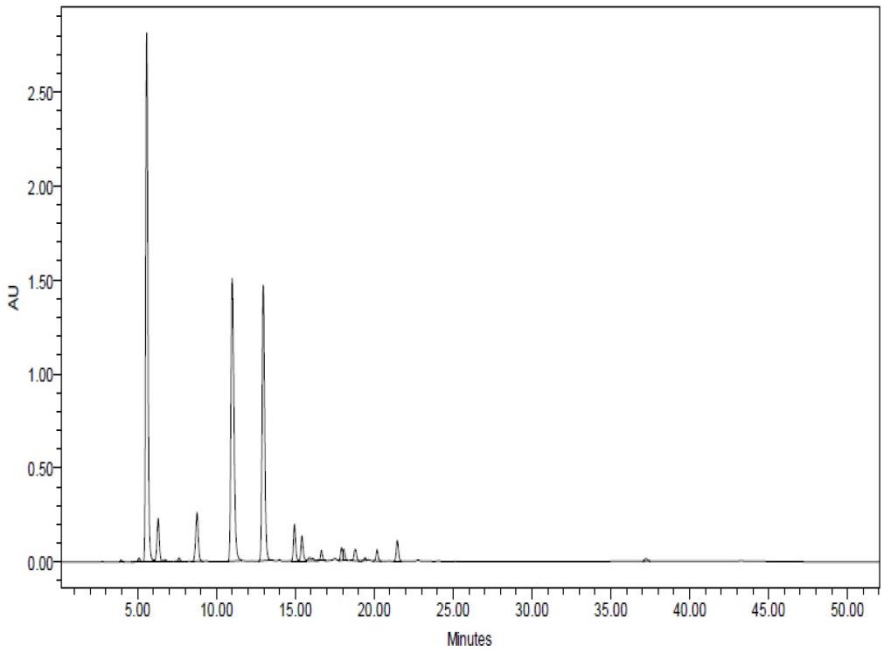

Fig 1. Five major glucosinolates in seeds of a doubled haploid *Brassica napus* population M730 identified by retention time (minutes) on HPLC, progoitrin (PRO), glucoalyssin (GLS), gluconapin (GNP), glucobrassicin (GBC) and glucobrassicanapin (GBN) .

Table 2. Bin assignments for the polymorphic SNP markers on the 19 chromosomes

Chromosome	Number of Bins	Number of SNP Bins	Number of SNP Markers	Unique SNP Positions (UP)	Number of SRAP Markers	Genetic Distance cM	Bin/cM	UP/cM
A1	71	71	374	135	0	131.2	0.54	1.03
A2	47	47	220	93	0	164.5	0.29	0.57
A3	108	108	752	280	0	163.1	0.66	1.72
A4	62	59	400	136	3	100.4	0.62	1.35
A5	76	76	418	142	0	141.1	0.54	1.01
A6	69	67	206	108	2	179.7	0.38	0.60
A7	82	81	507	214	1	118.8	0.69	1.80
A8	38	38	258	58	0	76.2	0.50	0.76
A9	90	85	360	141	5	177.8	0.51	0.79
A10	58	58	360	124	0	98.7	0.59	1.26
Subgenome A	701	690	3855	1431	11	1351.5	0.53	1.09
C1	50	45	395	103	5	135.8	0.37	0.76
C2	60	60	1823	149	0	141.5	0.42	1.05
C3	99	97	794	209	2	199.5	0.50	1.05
C4	87	80	633	128	7	166.7	0.52	0.77
C5	38	38	158	58	0	138.5	0.27	0.42
C6	24	24	198	37	0	75.0	0.32	0.49
C7	67	66	419	114	1	135.5	0.49	0.84
C8	59	55	409	119	4	134.7	0.44	0.88
C9	35	30	155	48	5	119.0	0.29	0.40
Subgenome C	519	495	4984	965	24	1246.2	0.40	0.74
Genome AC	1220	1185	8839	2396	35	2597.7	0.47	0.91

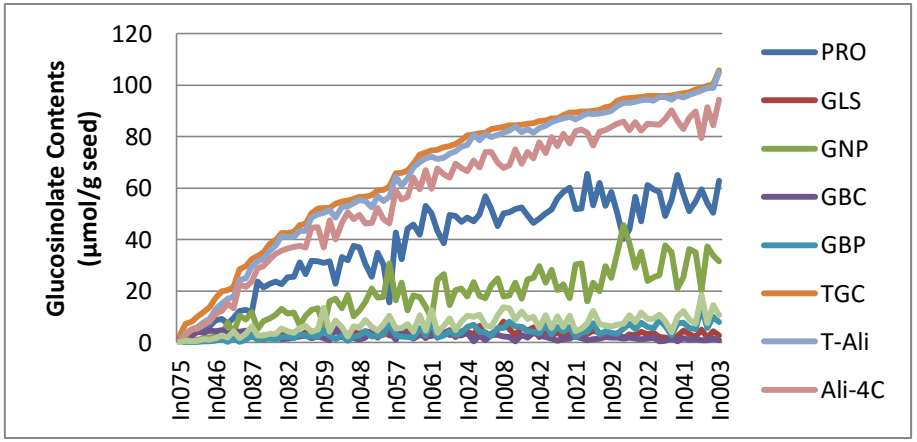

Fig 2. The distribution of content of glucosinolate components in seed of a *Brassica napus* doubled haploid population M730*, *: PRO, progoitron; GNP, gluconapin; 4C, four-carbon aliphatic glucosinolate; GLS, glucoalyssin; GBN, glucobrassicanapin; 5C, five-carbon aliphatic glucosinolate; T-ALI, total aliphatic glucosinolate; GBC, glucobrassicin; and TGC, total glucosinolate content.

Table 3. Quantitative trait loci regulating GSL (glucosinolate) traits detected by composite interval mapping using SNP (single nucleotide polymorphism) and SRAP (sequence related amplified polymorphism) markers from a *Brassica napus* doubled haploid population M730*.

Chr	QTL	Pos	Peak	C.I.	L-B	R-B	L-marker	L-cM	R-marker	R-cM	LOD	Additive	R^2	GSL
9	*BnQ5-1*	49.1	49.2	6.9	46.1	53.0	*SRAP105*	45.2	SNP0681	53.4	12.0	18.01	0.4010	4C
13	*BnQ8-1*	69.8	71.5	7.7	65.3	73.0	SNP0970	65.0	SNP0972	71.7	4.5	-8.97	0.1218	4C
1	*BnQ1*	33.1	33.1	9.8	26.6	36.4	SNP0022	32.1	SNP0024	34.5	4.3	-12.40	0.1145	4C
													0.1647	4C
9	*BnQ6-1*	53.5	53.5	11.4	47.2	58.6	SNP0680	49.1	*SRAP141*	56.3	6.0	2.44	0.1878	5C
1	*BnQ2-1*	102.0	102.0	11.6	94.7	106.3	SNP0076	100.2	SNP0079	107.6	4.9	1.52	0.1493	5C
													0.3371	5C
9	*BnQ4-2*	44.3	44.5	14.6	34.0	48.6	SNP0679	29.8	*SRAP105*	45.2	7.2	-0.63	0.2387	GBC
9	*BnQ5-2*	49.1	49.2	14.7	41.2	55.9	*SRAP105*	45.2	SNP0681	53.4	5.2	1.10	0.1629	GBN
1	*BnQ2-2*	102.0	102.0	11.3	94.7	106.0	SNP0076	100.2	SNP0079	107.6	4.0	0.69	0.1494	GLS
9	*BnQ6-2*	55.4	55.4	7.1	51.5	58.6	SNP0680	49.1	*SRAP141*	56.3	3.5	1.05	0.1418	GLS
													0.2912	GLS
12	*BnQ7*	122.7	123.0	17.6	119.5	137.1	SNP0936	121.5	SNP0938	123.7	4.7	3.95	0.1488	GNP
9	*BnQ4-1*	42.4	42.5	9.8	35.4	45.2	SNP0679	29.8	*SRAP105*	45.2	10.0	9.84	0.2963	PRO
8	*BnQ3*	62.8	62.8	9.4	55.4	64.8	SNP0647	61.8	SNP0648	64.8	6.3	9.93	0.1659	PRO
19	*QTL9*	53.9	-	-	-	-	-	-	SRAP271	56.5	3.2	4.86	0.0784	PRO
													0.5406	PRO
9	*BnQ5-3*	49.1	49.2	8.1	45.4	53.5	*SRAP105*	45.2	SNP0681	53.4	8.9	15.53	0.2896	T-Ali
13	*BnQ8-2*	70.6	71.5	9.2	63.6	72.8	SNP0970	65.0	SNP0972	71.7	3.5	-15.90	0.0970	T-Ali
													0.1926	T-Ali
9	*BnQ5-4*	49.1	49.2	8.1	45.4	53.5	*SRAP105*	45.2	SNP0681	53.4	8.6	14.93	0.2805	TGC
13	*BnQ8-3*	70.6	71.5	9.2	63.6	72.8	SNP0970	65.0	SNP0972	71.7	3.5	-15.75	0.0999	TGC
													0.1806	TGC

*: Chr, chromosome; Mk, marker; Pos, position in cM from left telomere; Peak, position in cM from left telomere; C.I., 95% confidence interval for the detected QTL; L-B, left border of the 95% confidence interval; R-B, right border of the 95% confidence interval; L-Mk, left marker; L-cM, left marker position in cM; R-Mk, right marker; R-cM; right marker position in cM; LOD, logarithm of odds; Additive, additive genetic effect; GSL, glucosinolate; PRO, progoitron; GNP, gluconapin; 4C, four-carbon aliphatic glucosinolate; GLS, glucoalyssin; GBN, glucobrassicanapin; 5C, five-carbon aliphatic glucosinolate; T-Ali, total aliphatic glucosinolate; GBC, glucobrassicin and TGC, total glucosinolate content.

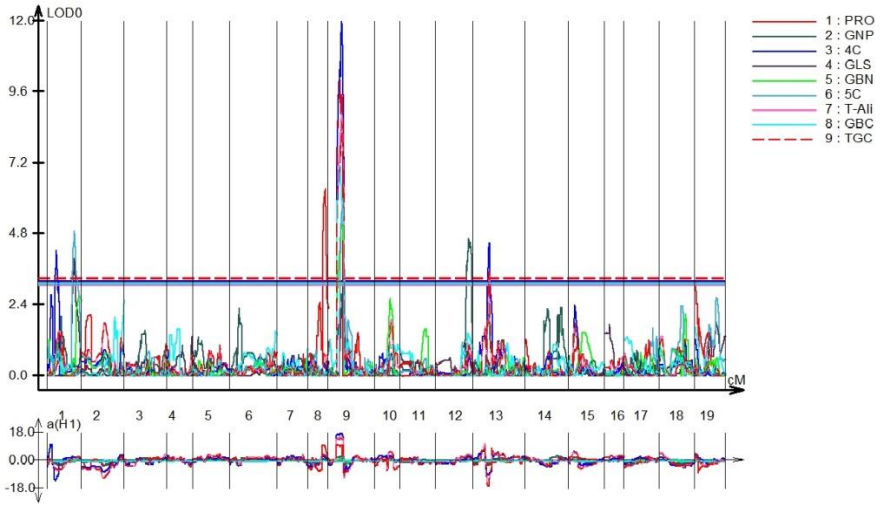

Fig 3. QTLs identification with composite interval mapping by SNP (single nucleotide polymorphism) and SRAP (sequence related amplified polymorphism) markers for 9 traits, PRO (progoitron), GNP (gluconapin), 4C (four-carbon aliphatic glucosinolates), GLS (glucoalyssin), GBN (glucobrassicanapin); 5C (five-carbon aliphatic glucosinolates), T-Ali (total aliphatic glucosinolates), GBC (glucobrassicin) and TGC (total glucosinolate content) from seeds of a doubled haploid *Brassica napus* population M730. Upper graph: QTLs identified by the peaks. Horizontal axis indicates chromosome number; Lower graph: additive effects of the QTLs on each chromosome.

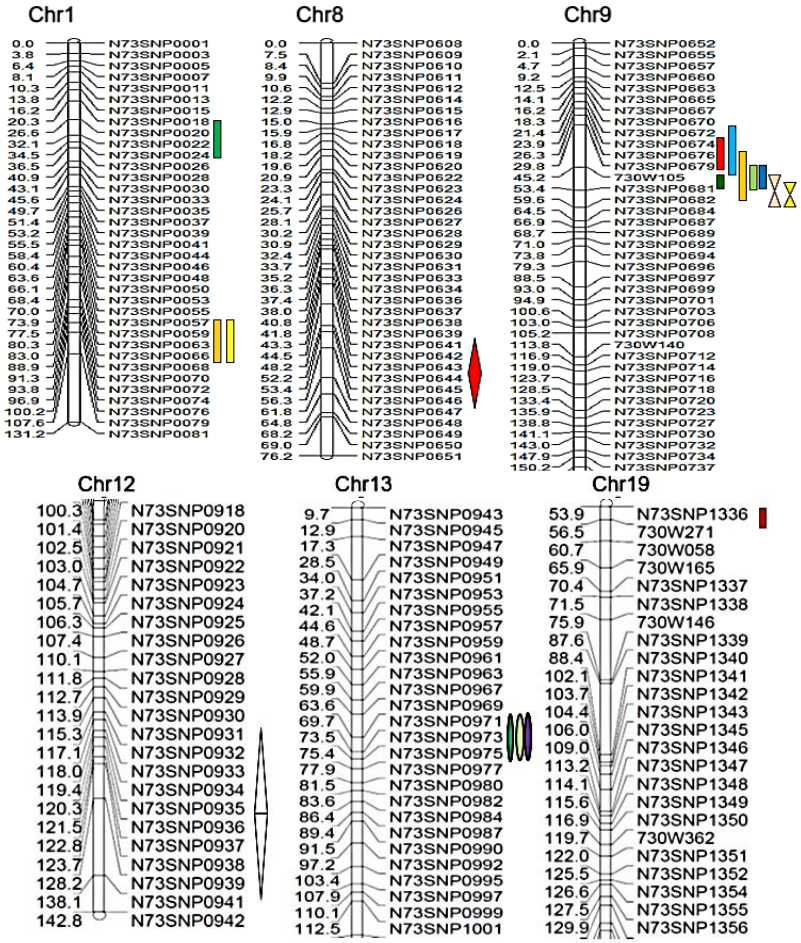

Fig 4. QTLs identification with composite interval mapping by SNP (single nucleotide polymorphism) and SRAP (sequence related amplified polymorphism) markers from seeds of a doubled haploid *Brassica napus* population M730. Red bars indicated peak locations of QTLs, rectangle bar, round-corner rectangle bar, diamond, parallelogram, double-sided border bar, two triangle, diamond with a cross, ellipse were QTL 1-8. Dark green, four carbon aliphatic glucosinoalte; orange, five carbon aliphatic glucosinolate; yellow, glucoalyssin; red, progoitrin; blue, glucobrassicin; dark orange, glucobrassicanapin; light green, total aliphatic glucosinolate; purple, total glucosinolate content; white, gluconapin and the lengths of various shapes specified their 95% of confidence intervals of QTLs.

and had negative genetic effect on 4C explaining 11.45% of phenotypic variance. The 3 QTLs on chromosome C3 were located at approximately the same position and controlled 3 different glucosinolate traits 4C, TGC and T-Ali with negatively additive effects represented by R^2 = 12.18%, 9.99% and 9.70% respectively. There was one QTLs located at 62.8 cM on chromosome A8 controlling PRO, one at 122.8 cM on C2 controlling GNP and one at 53.9 cM on C9 controlling PRO explaining 16.59%, 14.88% and 7.84% of phenotypic variances. Three QTLs on chromosome A8, A9 and C9 controlling PRO or another 3 QTLs on chromosome A1, A9 and C3 controlling 4C explained their compound phenotypic variances 54.06% or 16.47%. Two QTLs on chromosome A1 and A9 controlling GLS and 5C, another 2 QTLs on chromosome A9 and C3 controlling T-Ali or TGC explained their corresponding compound phenotypic variances 29.12%, 33.71%, 19.26% or 18.06%. There was only 1 QTL each controlling GNP, GBN and GBC and explained their phenotypic variances 14.88%, 16.29% and 23.87%.

Discussion

In *B. napus* germplasm, about 30 different glucosinolates were reported (Lou et al., 2008), however a few glucosinolates predominate total glucosinolate content in seeds such as progoitrin, gluconapin, glucobrassicanapin etc. Most glucosinolate compounds exit in small quantity which is difficult to quantify for QTL mapping to find meaningful QTL. In this study, five major glucosinolates including both aliphatic and indolic glucosinolates in the seed of a biparental *B. napus* DH population were assessed for QTL mapping. The difference of 87.75 µmol/g seed for total glucosinolate content between the spring-type canola parent line, M77 and the semi-winter-type rapeseed parent line, M23 was a good indicator to use their progenies for QTL mapping. There was only one DH line having lower total glucosinolate content than line, M77 while 14 DH lines had higher total glucosinolate content than line M23, indicating that over-expression patterns and multi-gene inheritance for glucosinolate content predominated. Line M77 carrying low-glucosinolate alleles, similarly Howell et al., (2003) had only 2.64 µmol/g seed total glucosinolate content. The low-glucosinolate line M77 also possessed alleles contributing to increased total glucosinolate content of the 14 DH lines which had higher total glucosinolate content than line M23. Epistatic effects of the QTLs were also involved in glucosinolate content over-expression. Total glucosinolate content was mainly determined by 4C aliphatic glucosinolates, predominately PRO. Indolic glucosinolates only accounted for a small proportion of total glucosinolate content especially when the total glucosinolate content was high. Interestingly, when total glucosinolate content was lower than a given threshold level there was a trend showing that indolic glucosinolate content increased with a decrease in TGC. This suggests that although the genetic effects of the QTL controlling GBC were minor, these QTL or QTLs were difficult to eliminate. It was apparent that 4C aliphatic glucosinolates were more important than 5C aliphatic glucosinolates in rapeseed seeds since they predominantly contributed to total aliphatic or total glucosinolate content. It was possible to develop dense genetic maps which more accurately located functional genes or QTLs and their co-segregating markers in this study using microarray chips. The 60K *Brassica* Infinium® chips were a powerful tool with dual-color imaging system for genetic studies and breeding since they targeted the whole genome. However, there were some gaps between neighboring SNP markers. Therefore, SRAP markers supplements helped pinpoint the tentative QTLs more accurately as was the case for chromosome A9.

The 17 QTLs regulating the 9 glucosinolate traits individually distributed over 6 chromosomes. The two QTLs on chromosome A1 with their peaks at exactly the same position 102.0 cM were specified by the same SNPs SNP0076, SNP0078 and SNP0079 and shared the 95% confidence interval (CI) 11.3 cM from left border (LB) 94.7 cM to right border (RB) 106.0 cM, so they might be the same QTL controlling two related traits GLS and 5C. The two QTLs on chromosome A9 with peaks at 42.5 cM and 44.5 cM were also specified by the same markers SNP0679, SRAP273 and SRAP105 and shared the 95% CI 9.8 cM from LB 35.4 cM to RB 45.2 cM, so they might be the same QTL controlling two traits PRO and GBC. The four QTLs on chromosome A9 with their peaks at the same position 49.2 cM were located by the same markers SRAP105, SNP0680 and SNP0681 and shared the 95% CI 6.9 cM from LB 46.1 cM to RB 53.0 cM and might be the same QTL regulating 4C, GBN, T-Ali and TGC. The QTLs on the same chromosome A9 with peaks at 53.5 cM and 55.4 cM were identified by the same markers SNP0680, SNP 0681 and SRAP141 and shared 95% CI 7.1 cM from LB 51.5 cM to RB 58.6 cM regulating two related traits GLS and 5C. Similarly the three QTLs on chromosome C3 with their peaks at exactly the same position 71.5 cM were indicated by the same SNPs SNP0970, SNP0971 and SNP0972 and shared 95% CI 7.5 cM from LB 65.3 cM to RB 72.8 cM regulating three related traits 4C, T-Ali and TGC might be the same QTL as well. It agreed with Sotelo et al., (2014) that one QTL could regulate more than one glucosinolates. Chromosome A9 harbored the largest number and the most significant QTLs controlling 8 out of the 9 glucosinolate traits in this study. Previous studies also detected significant QTLs for total glucosinolate content on chromosome A9 in *B. napus* (Harper et al., 2012; Howell et al., 2003; Li et al., 2014). Similarly, Rahman et al., (2014) reported common and significant QTLs for total glucosinolate content on A9 linkage group from *B. rapa* populations. Detection of a large number of QTL on chromosome A9 may be explained by the existence of several genes with multiple copies that are involved in glucosinolate biosynthesis pathway in *Brassica* species. Finding of many QTL on chromosome A9 is supported by whole genome sequence data and its comparative analysis with Arabidopsis, Wang et al., (2011) revealed 3 transcription factors, 7 side chain elongation and 5 side chain modification genes in comparative analysis of whole genome with Arabidopsis. Furthermore, a total 102 putative genes identified are involved in glucosinolate biosynthesis through genome wide comparative analysis with 52 ortholog of Arabidopsis (Wang et al., 2011). It suggests that the glucosinolate biosynthesis pathway is complex itself in Arabidopsis, which make it complex in *B. rapa* due to duplication or triplication events during evolutionally separation in diploid species, and even more complex in allotetraploid species such as *B. napus*. Epistatic effect existed in the interactions of the QTLs (Sotelo et al., 2014). Therefore, it was more desirable to identify each single component of glucosinolate content to find major QTLs for practical crop breeding. QTLs controlling total glucosinolate content in *B. napus* seeds were also identified on LG C2 and C9 (Harper et al., 2012; Howell et al., 2003), on C2, C7 and C9 (Li et al., 2014). Rahman et al., (2014) found QTLs on A2 and A7 from *B. rapa*. In this study, we located QTLs on A1, A8, C2, C3 and C9 in addition to those QTLs on A9. This indicated that QTLs for glucosinolate traits widely distributed

over both the A and C genomes. The QTL for GNP on C2 might be the same as those reported by Li et al., (2014). Future studies with more emphasis on individual glucosinolates using genetically diverse germplasm will be required to validate and fine map QTLs for glucosinolate traits.

Materials and Methods

Population and environments

The spring canola inbred line M77 was pollinated by the semi-winter rapeseed inbred line M23 to produce F_1 from which the doubled haploid (DH) lines were generated using microspore culture and subsequent chromosome doubling techniques described by Weber et al., (2005). Eighty-eight DH lines along with their parents were grown in a growth room under 22/16 °C and artificial lighting 16/8 h day/night. Liquid fertilizer (20-8-20) at 200 ppm mixed with magnesium sulfate 8.74 g/l was applied once a day with watering. The plants were grown in 10 cm pots, one plant per pot in Sunshine® #4 mixed soil (Sun Gro Horticulture Canada Ltd). Plant leaf tissues were sampled at 3-leaf stage for genotyping. After harvesting mature seeds from each DH line, seeds were dried up at 35°C overnight prior to glucosinolate extraction.

Detection of SNP and SRAP markers

Brassica 60K Infinium® SNP microarray BeadChips (Illumina Inc., USA) were used for the whole genome genotyping of the 88 DH lines along with their parents following the manufacture's protocols (Illumina Inc., USA) for library preparation and marker identification. To generate SRAP markers, DNA was extracted by CTAB (Cetyltrimethyl ammonium bromide) method according Li and Quiros (2003). Twenty-nine primer pairs, eight fluorescently labeled forward primers with FAM (blue), NED (yellow), PET (red) and VIC (green) fluorescent dyes and 24 unlabeled reverse primers were used. PCR (polymerase chain reaction) was programmed according to Sun et al., (2007) on a PCR machine (Eppendof®, ON, Canada). The PCR products were mixed and denatured in formamide (Hi-Di^{TM}, Life technologies, USA) with the size standard dye Liz-500 (Life technologies, USA). The denatured DNA was loaded onto the Genetic Analyzer (3130xl Genetic analyzer, Life technologies, USA) to separate PCR products. ABI GeneScan 3.7 (Life technologies, USA) was used to analyze the data. Genographer® v1.6.0 was used to score SRAP markers.

Genetic mapping and QTL identification

The JoinMap® 3.0 (Van-Ooijen and Voorrips 2001) was used to group and localize the SNP and SRAP markers which were translated into homozygous values acceptable by WinQTLCart v2.5_011 (Statistical Genetics, NCSU, USA, 2012). To facilitate QTL analysis, the markers at the same position or within 0.5 cM on the same chromosome were grouped into the same bin. The first marker was used to represent the bin for testing. The calculation environments were set up as follows: Composite Interval Mapping (CIM) with a significance level of 0.05 and 1,000 times of permutation , at walking speed 1 cM by Model 6, Kosambi function, five control markers, window size 10.0 cM, backward and forward regression method both with probability for into and out of 0.1. The threshold for QTL declaration was set up at LOD (logarithm of odds) 3.

Glucosinolate analysis

Two hundred mg of seed of each DH line were ground in liquid nitrogen. Glucosinolate extraction protocol was used according to Kliebenstein et al., (2001) and Liu et al., (2012) with minor modifications. Total glucosinolate was eluted by Sephadex DEAE and de-sulfonated by sulfatase before quantification. Eighty microliter of the glucosinolate samples from each line were analyzed using high performance liquid chromatography (HPLC) (Hewlett-Packard 1100) with a 5 mm column (Lichrocart 250-4 RP18e, Fisher Scientific, Canada) in which acetonitrile and water was used as solvents. The composition of each glucosinolate samples was determined at wavelength 229 nm. The running program was set up with acetonitrile gradient 1.5 -7% (v/v) 8 min, 7 – 15% 4 min, 15 – 55% 18 min, 55 – 92% 5 min, 92% 5 min, 92 – 1.5% 5 min, 1.5% 3 min and 0% 4 min. The peaks detected were identified by referring to (Liu et al., 2011). The areas under the major peaks were converted to $\mu molg^{-1}$ seed by using the response factors from (Vinjamoori et al., 2004).

Conclusion

Glucosinolate is one of the most important plant secondary metabolites found in the *Brassica* species, which play an important role in agriculture and animal industries. It is important to manipulation different glucosinolate compounds in canola/rapeseed and other vegetable crops for effective applications, such as reduction of detrimental GSL compounds in seed meal and increasing those GSL compounds which possess anticancer properties in Brassica vegetables. It is therefore important to identify genes/QTL involve in glucosinolate biosynthesis. In this study QTL mapping was carried out for individual glucosinolates and 9 QTL detected on different chromosomes. A large number of QTL identified on chromosome A9 which is known to hold several glucosinolate biosynthesis genes including transcription factors, side chain elongation and side chain modification. Flanking markers can be deployed in marker assisted selection in canola/rapeseed breeding to manipulate glucosinolate profile and content.

Acknowledgment

This research was financially supported by the Monsanto Canada Inc. and NSERC.

References

Baskar V, Gururani MA, Yu JW, Park SW (2012) Engineering glucosinolates in plants: current knowledge and potential uses. Appl Biochem Biotechnol. 168(6):1694-717.

Cheung WY, Landry BS, Raney P, Rakow GFW (1998) Molecular mapping of seed quality traits in *Brassica juncea* l. Czern. and Coss. Acta Hort. 459:139-148.

de Quiroz H, Mithen R (1996) Molecular markers for low-glucosinolate alleles in oilseed rape (*Brassica napus* L.). Mol Breed. 2(3):277-281.

Fahey JW, Zalcmann AT, Talalay P (2001) The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochem. 56(1):5-51.

Harper AL, Trick M, Higgins J, Fraser F, Clissold L, Wells R, Hattori C, Werner P, Bancroft I (2012) Associative

transcriptomics of traits in the polyploid crop species *Brassica napus*. Nat Biotechnol. 30(8):798-802.

Hirani AH, Zelmer CD, McVetty PBE, Daayf F, Li G (2013) Homoeologous *GSL-ELONG* gene replacement for manipulation of aliphatic glucosinolates in *Brassica rapa* L. by marker assisted selection. Front Pl Sci. 4:55.

Howell PM, Sharpe AG, Lydiate DJ (2003) Homoeologous loci control the accumulation of seed glucosinolates in oilseed rape (*Brassica napus*). Genome. 46(3):454-60.

Kliebenstein DJ, Gershenzon J, Mitchell-Olds T (2001) Comparative quantitative trait loci mapping of aliphatic, indolic and benzylic glucosinolate production in *Arabidopsis thaliana* leaves and seeds. Genetics. 159(1):359-70.

Li F, Chen B, Xu K, Wu J, Song W, Bancroft I, Harper AL, Trick M, Liu S, Gao G, Wang N, Yan G, Qiao J, Li J, Li H, Xiao X, Zhang T, Wu X (2014) Genome-wide association study dissects the genetic architecture of seed weight and seed quality in rapeseed (*Brassica napus* L.). DNA Res. 21(4):355-67.

Li G, Quiros CF (2003) In planta side-chain glucosinolate modification in Arabidopsis by introduction of dioxygenase Brassica homolog *BoGSL-ALK*. Theor Appl Genet. 106(6):1116-21.

Liu Z, Hirani AH, McVetty PB, Daayf F, Quiros CF, Li G (2012) Reducing progoitrin and enriching glucoraphanin in *Brassica napus* seeds through silencing of the *GSL-ALK* gene family. Plant Mol Biol. 79(1-2):179-89.

Liu Z, Hammerlindl J, Keller W, McVetty PE, Daayf F, Quiros C, Li G (2011) *MAM* gene silencing leads to the induction of C3 and reduction of C4 and C5 side-chain aliphatic glucosinolates in *Brassica napus*. Mol Breed. 27(4):467-478.

Lou P, Zhao J, He H, Hanhart C, Del Carpio DP, Verkerk R, Custers J, Koornneef M, Bonnema G (2008) Quantitative trait loci for glucosinolate accumulation in *Brassica rapa* leaves. New Phytol. 179(4):1017-32.

Mahmood T, Ekuere U, Yeh F, Good AG, Stringam GR (2003) Molecular mapping of seed aliphatic glucosinolates in *Brassica juncea*. Genome. 46(5):753-60.

Rahman H, Kebede B, Zimmerli C, Yang R-C (2014) Genetic study and QTL mapping of seed glucosinolate content in *Brassica rapa* L. Crop Sci. 54(2):537-543.

Ramchiary N, Bisht NC, Gupta V, Mukhopadhyay A, Arumugam N, Sodhi YS, Pental D, Pradhan AK (2007) 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. Theor Appl Genet. 116(1):77-85.

Sotelo T, Soengas P, Velasco P, Rodríguez VM, Cartea ME (2014) Identification of metabolic QTLs and candidate genes for glucosinolate synthesis in *Brassica oleracea* leaves, seeds and flower buds. PLoS ONE. 9(3):e91428.

Sun Z, Wang Z, Tu J, Zhang J, Yu F, McVetty PB, Li G (2007) An ultradense genetic recombination map for *Brassica napus*, consisting of 13551 SRAP markers. Theor Appl Genet. 114(8):1305-17.

Toroser D, Thormann CE, Osborn TC, Mithen R (1995) RFLP mapping of quantitative trait loci controlling seed aliphatic-glucosinolate content in oilseed rape (*Brassica napus* L). Theor Appl Genet. 91(5):802-8.

Uzunova M, Ecke W, Weissleder K, Robbelen G (1995) Mapping the genome of rapeseed *(Brassica napus* L.). I. Construction of an RFLP linkage map and localization of QTLs for seed glucosinolate content. Theor Appl Genet. 90(2):194-204.

Velasco P, Soengas P, Vilar M, Cartea ME, del Rio M (2008) Comparison of glucosinolate profiles in leaf and seed tissues of different *Brassica napus* crops. J Am Soc Hortic Sci. 133(4):551-558.

Vinjamoori DV, Byrum JR, Hayes T, Das PK (2004) Challenges and opportunities in the analysis of raffinose oligosaccharides, pentosans, phytate, and glucosinolates. J Anim Sci. 82(1):319-28.

W. VOJ, Voorrips RE (2001) JoinMap 3.0, Software for the calculation of genetic linkage maps. Plant research international, Wageningen, the Netherlands.

Wang H, Wu J, Sun S, Liu B, Cheng F, Sun R, Wang X (2011) Glucosinolate biosynthetic genes in *Brassica rapa*. Gene. 487(2):135-142.

Zhao J, Meng J (2003) Detection of loci controlling seed glucosinolate content and their association with Sclerotinia resistance in *Brassica napus*. Plant Breed. 122(1):19-23.