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Mapping of quantitative trait loci for the bolting trait in *Brassica rapa* under vernalizing conditions

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ABSTRACT. Premature bolting can occur occasionally during spring cultivation of heading Chinese cabbage in East Asia when the plants encounter low temperatures (vernalization), leading to economic loss. Breeding bolting-resistant cultivars is the best choice for solving this problem. We looked for QTLs responsible for varietal differences in the bolting trait in *Brassica rapa* under environmental conditions that promote vernalization. To achieve this goal, we constructed a linkage map with 107 simple sequence repeats and 54 insertion/deletion markers based on a segregating population of 186 F_2 individuals. The resulting map consisted of 10 linkage groups and covered a total length of 947.1 cM, with an average genetic distance of 5.84 cM between adjacent markers. QTL analysis of the bolting trait was performed by two phenotypic evaluations (bolting index and flowering time) based on the scores in an F_2 population in the spring of 2010, and scores in $F_{2:3}$ families in autumn 2010 and spring 2011, respectively. Twenty-six QTLs that controlled bolting were detected, accounting for 2.6 to 31.2% of the phenotypic variance. The detected QTLs with large effects co-localized

mainly on linkage groups A02, A06, and A07. These QTLs may provide useful information for marker-assisted selection in a breeding program for late bolting or bolting-resistant cultivars in *B. rapa* crops.

Key words: *Brassica rapa*; Quantitative trait loci; Bolting trait; Linkage mapping

INTRODUCTION

Premature bolting is the most limiting factor for spring production of heading Chinese cabbage in East Asia. It is well known that heading Chinese cabbage is vernalization-dependent; both germinating seeds and the whole plant can perceive low temperature signals (Kakizaki et al., 2011). Encountering low temperatures (vernalization) is almost inevitable in Chinese cabbage spring cultivation, which leads to premature bolting. This results in a loose and unmarketable head, reducing the commercial value of the crop. Breeding bolting-resistant cultivars is urgently needed for spring production, and it will be a powerful, economical, and efficient way to solve the problem.

Classical genetic analysis has shown that the bolting trait in *Brassica rapa* is regulated by multiple genes (Mero and Honma, 1984, 1985), and that late bolting (in contrast to early bolting) is recessive. Conventional breeding is labor-intensive and requires many years of selection. Identifying and utilizing powerful and effective molecular markers that are tightly linked to the bolting-resistant gene will accelerate the breeding programs significantly.

Several studies related to bolting trait QTL mapping have been published within the last 20 years. Teutonico and Osborn (1995) identified two QTLs on the basis of scores in an F_2 population, that were strongly associated with variation in flowering time in unvernalized cultivars, but the two major QTLs were not detected when the plants were given 6 weeks of vernalization. Ajisaka et al. (2001), using an F_2 population by bulked segregant analysis, identified a major QTL that explained 77% of the phenotypic variance. Due to the fact that the bolting trait in *Brassica rapa* is sensitive to environmental conditions, particularly to temperature and day-length, Nishioka et al. (2005) performed QTL analysis using a double haploid (DH) population under five environmental conditions differing in day-length and/or temperature. Out of a total of ten QTLs detected, the most effective QTL, *BT1*, was detected in all five treatments and found to be the main locus that controlled the vernalization response. Zhang et al. (2006) and Yang et al. (2007) also using DH populations identified four QTLs on four linkage maps and eight QTLs on three linkage maps, respectively. Because there were either no or few anchored markers, the above-mentioned linkage groups were not assigned to the reference linkage groups, and therefore, it is impossible to compare these QTLs, which limit their application for use in marker-assisted selection in a breeding program.

Recently, several bolting-related QTLs have been detected using anchored markers (Lou et al., 2007, 2011; Li et al., 2009; Zhao et al., 2010; Kakizaki et al., 2011), which distributed on all the linkage groups, except A04 and A05. Lou et al. (2007) identified eight QTLs that were distributed on all ten linkage groups, except A04, A05, and A09, on the basis of four populations under five environmental conditions. At the top of linkage group A02, in which linked QTLs were detected in all the segregating populations and environmental conditions and that played a vital role in regulating bolting and flowering time in *Brassica rapa*. Similar results were reported by Zhao et al. (2010) and all the QTLs with large effect detected by two phenotypic measurements co-localized at the top of linkage group A02. Li et al. (2009) detected eight QTLs on the basis of

two sub-populations of F_2 with four phenotypic measurement under two environmental conditions, of which, three, two, and three QTLs were detected on the same regions in A02, A07, and A10, respectively. Under three conditions, ten QTLs were identified on A02, A03, A07, A09 and A10 (Lou et al., 2011), using a RIL population with two phenotypic scores, of which, the major QTL, *BtoFA10*, explaining from 13 to 18% of the phenotypic variation, was mapped at the higher-middle position on linkage group A10. On the basis of two environmental-phenotypic scores in an F_2 population, five QTLs were identified on A02, A03, A07, and A10 (Kakizaki et al., 2011), and there were no QTLs mapped to the same loci.

The aim of this study was to identify QTLs for bolting-resistant genes under vernalizating conditions. These QTLs and the flanking markers will be useful tools to incorporate into a bolting breeding program.

MATERIAL AND METHODS

Plant material

A mapping population of 186 F_2 individuals was derived by single-seed decent from an F_1 made by crossing two *B. rapa* inbred lines 08A061 (P_1) and 09A001 (P_2). The maternal parent (09A001) was a standard rapid cycling inbred line of *Brassica rapa* L. ssp. *Dichotoma*, which bolts readily and is vernalization-independent. The male parent (08A061) was an inbred line of heading Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) that was selfed for nine generations and was resistant to bolting. The $F_{2:3}$ population of 186 families was derived from each F_2 individual by self pollination.

Phenotypic evaluation

Phenotypic experiments were conducted at the Experiment Station of Shenyang Agricultural University, Shenyang, China. Two parents and their 186 F_2/$F_{2:3}$ materials were used to evaluate flowering time (FT) and bolting index (BI), which served as phenotypic scores in the QTL mapping. FT was measured as days from sowing the seeds to appearance of the first fully opened flower. BI was measured in a way similar to the method described by Yang et al. (2007) with minor modifications. BI was evaluated when the first fully opened flower was visible in F_2/$F_{2:3}$ populations. The bolting scale of each plant was established according to the following standard: 0 = no bud emergence; 1 = bud emergence, stalk length <2 cm; 3 = bud emergence, stalk length 2-5 cm; 5 = bolting, stalk length 5-10 cm; 7 = bolting, stalk length >10 cm; 9 = flowering, stalk length >10 cm. The evaluation of the F_2 186 individuals was carried out in the spring of 2010. The two parents, and F_2s were sown in pots (ø = 10 cm) in a greenhouse at a minimum temperature of 5°C under natural day length. In the case of the $F_{2:3}$ lines, three replications per line, with five plants for each replication were grown in the greenhouse for phenotypic analysis using a random block design. Evaluation of the $F_{2:3}$ families was carried out both in autumn 2010 and spring 2011. In autumn 2010, germinated seeds were vernalized at 2°-3°C in the dark for 30 days before being sown in pots. The temperature in the greenhouse was maintained at 10°-25°C and natural lighting was supplemented by metal halide lamps with a 14-h day length. In spring 2011, the above-mentioned $F_{2:3}$ seeds were sown in pots in the greenhouse. In the $F_{2:3}$ families, the average score of three replications for each line was used for QTL mapping. The coefficients of phenotypic correlation were calculated using mean values.

Phenotypic data analysis

Statistical analysis was performed using the SPSS17.0 software for windows. The Duncan multiple range test of one-way ANOVA (Duncan, 1955) was employed to detect possible differences between marker genotype means. Broad-sense heritabilities (h^2%) were calculated by dividing the genotypic variance by the phenotypic variance.

Linkage map construction and QTL analysis

Genomic DNA was extracted from young leaves of the two parental lines and 186 F_2 individuals following the procedure described by Murray and Thompson (1980) with minor modifications. On the basis of a previously constructed linkage map (Schranz et al., 2002; Suwabe et al., 2002; Choi et al., 2007; Kim et al., 2009; Ramchiary et al., 2011; Ge et al., 2011; Wang et al., 2011) and the public database available at the *Brassica* Genome Gateway (http://brassica.bbsrc.ac.uk), a total of 393 markers, which included 269 simple sequence repeat (SSR) and 124 insertion/deletion (InDel), were used in this study to screen polymorphisms between parents.

Linkage analysis and genetic map construction was carried out with JoinMap 3.0 (Van Ooijen and Voorrips, 2001). The linkage groups were generated at a minimum logarithm of odds (LOD) threshold of 3.0. Recombination distances in cM were calculated by the Kosambi function (Kosambi, 1943). The PCR profile was as follows: 95°C for 5 min, followed by 30-35 cycles of 94°C for 45 s, appropriate annealing temperature for 45 s, and 72°C for 60 s, with a final extension at 72°C for 7 min. Amplifications were carried out in a Bio-Rad iCycler thermocyler, and the products separated on 6% denaturing polyacrylamide gels.

QTL analysis was performed using a composite interval-mapping (CIM) analysis with Windows QTL Cartographer v2.5 (Wang et al., 2007). A permutation test was applied to each data set of 1000 repetitions to determine the LOD thresholds (P = 0.05). LOD values of 2.4 for both F_2 and $F_{2:3}$ families were used as significance thresholds for the presence of a candidate QTL. The final QTL results are graphically displayed using MapChart 2.1 (Voorrips, 2002) and QTLs considered to be co-localized when their LOD supports intervals overlapped.

RESULTS

Trait variations and correlations

The two parents, the F_2 population and each line of the $F_{2:3}$ families were all measured for their phenotypic scores. The parental lines showed statistically significant differences for each trait under all three environmental conditions, and transgressive segregations were observed both in the F_2 and $F_{2:3}$ (Table 1; Figures 1 and 2). The phenotypic scores of the two parents in spring and autumn environmental conditions indicated that long days promoted bolting or flowering. The phenotypic correlation coefficients between BI and FT in spring 2010 and autumn 2010 were -0.8502 and -0.8252, respectively. Significant negative correlation coefficients (data not shown) were observed between BI and FT for both the spring 2010 and the autumn 2010 growth conditions. The broad-sense heritabilities (h^2%) for the bolting trait under all three environmental conditions ranged from 84 to 94%, which indicated that genetic factors played a key role in phenotypic variation.

Table 1. Phenotypic values of each trait among parents and population under three growth conditions.

Growth conditions	Traits	Parents[a]			$F_2/F_{2:3}$	
		P_1	P_2	Diff.	Mean	Range
Spring 2010	BI	1.00 ± 0.00	8.6 ± 0.84	**	5.63 ± 2.64	0-9
	FT	79.81 ± 2.18	62.2 ± 1.39	**	68.89 ± 7.56	52-89
Autumn 2010	BI	0.90 ± 0.32	7.67 ± 0.94	**	3.48 ± 2.33	0-8.69
	FT	98.7 ± 5.27	66.0 ± 0.91	**	83.80 ± 15.23	61-143
Spring 2011	BI	1.17 ± 0.71	8.57 ± 0.85	**	4.80 ± 2.07	0-9.00

BI = bolting index; FT= flowering time; P_1 = 08A061; P_2 = 09A001. [a]Means ± SD (standard deviation). *,**Significance at the level of 0.05 and 0.01, respectively, according to the Student *t*-test (N = 15).

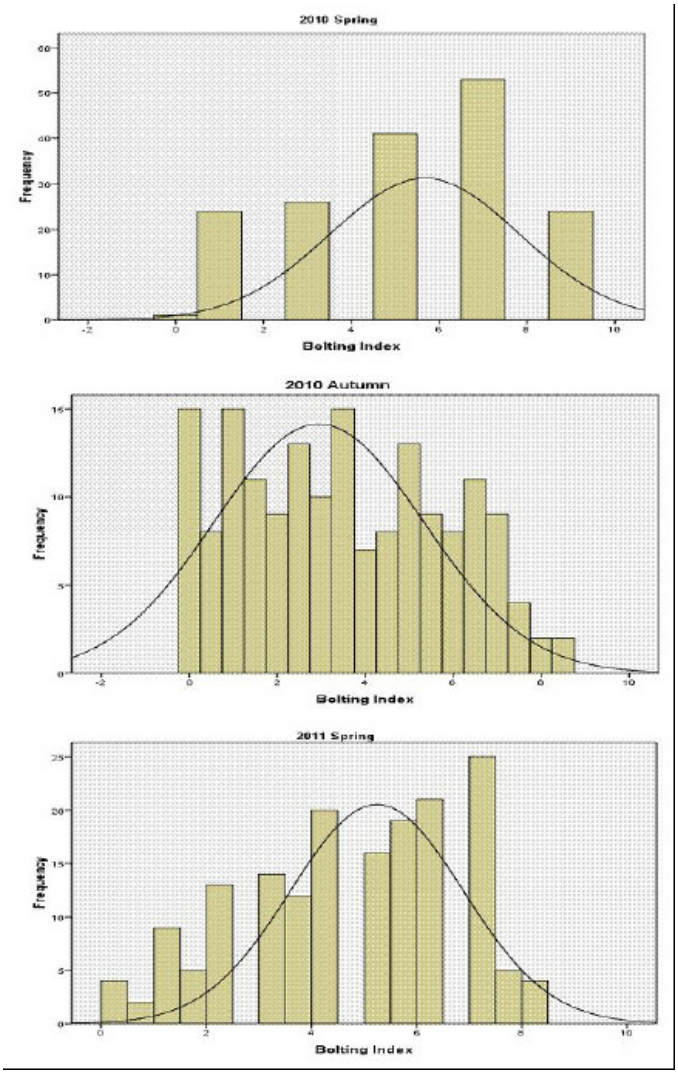

Figure 1. Frequency distribution of bolting index under three growth conditions.

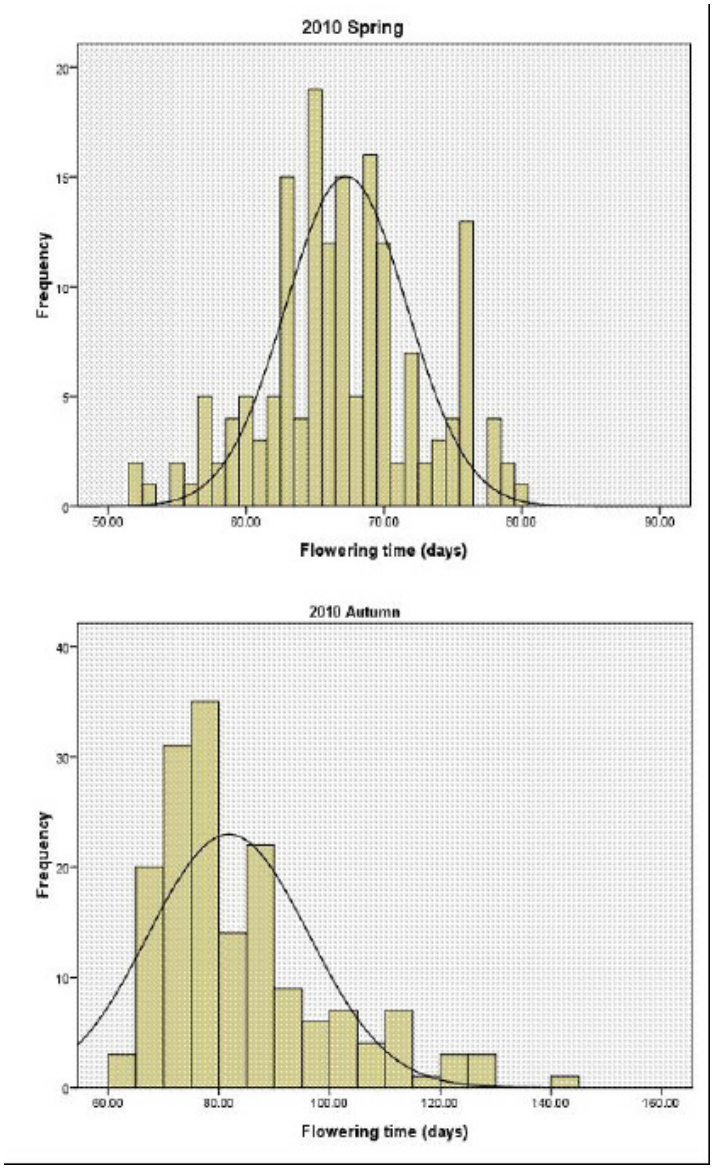

Figure 2. Frequency distribution of flowering time under two growth conditions.

Linkage map construction

A total of 393 markers, including 269 simple sequence repeat (SSR) and 124 insertion/deletion (InDel), were used in this study to screen polymorphisms between parents. Ulitimately, 113 (42.07%) SSRs and 57 (45.96%) InDel polymorphism markers were selected, which clearly showed co-dominance in the population. Excluding six SSRs and

three InDel markers which failed to be assigned to any linkage in the population, a total of 161 markers including 107 SSR and 54 InDel markers were mapped in a population of 186 F_2 individuals (Figure 3). The 10 linkage groups, designated A01-A10, corresponded to those on previously published linkage maps (Choi et al., 2007; Kim et al., 2009; Ramchiary et al., 2011; Wang et al., 2011). The total length of the map was 941.18 cM with an average distance of 5.84 cM between adjacent loci. The length of the linkage groups ranged from 58.3 cM (A10) to 129.04 cM (A1), and the number of markers on each of the 10 linkage groups ranged from 11 (A04) to 26 (A09). The average distance between adjacent markers ranged from 4.13 cM (A09) to 9.92 cM (A01). The 107 SSR markers were distributed across all 10 linkage groups, and the number of markers ranged from 4 in A02 to 17 in A03. The InDel markers were distributed across all 10 linkage groups, except in A03 and A05, and the number of markers ranged from 1 on A01 to 14 on A02. In the final linkage map, five gaps greater than 15 cM, but no gap greater than 20 cM, were present on A01 (3), A03 (1), and A08 (1), respectively.

QTL analysis

Under the three growth conditions and two phenotypic measurements, a total of 26 QTLs (Table 2; Figure 3) were detected on the five linkage groups distributed on A02, A03, A06, A07, A08 with eleven, two, five, six, and two QTLs, respectively, explaining from 2.60 to 31.38% of the phenotypic variation.

Table 2. QTL analyses of the bolting trait in *Brassica rapa* under three growth conditions.

Growth conditions	Trait	h^2 (%)	QTL	Linkage group	Confidence interval	Peak position	LOD	Exp%	Additive effect
2010 Spring	BI	92.71	10sp-BIQTL1	A02	2.0-14.8	2.01	8.05	16.13	-1.9187
			10sp-BIQTL2	A06	90.7-99.6	96.80	6.68	7.07	1.1311
			10sp-BIQTL3	A07	71.6-85.9	83.80	2.45	8.74	-1.1268
			10sp-BIQTL4	A08	21.1-47.4	35.10	2.53	3.47	-0.07784
	FT	95.18	10sp-FTQTL1	A02	0.0-7.8	0.01	3.87	2.94	2.2908
			10sp-FTQTL2	A02	9.2-29.8	16.46	2.54	7.89	4.084
			10sp-FTQTL3	A02	22.0-38.7	30.77	6.11	16.07	5.0764
			10sp-FTQTL4	A02	37.5-53.8	45.77	2.96	20.61	5.2812
			10sp-FTQTL5	A07	70.6-98.9	83.80	4.79	11.00	3.6348
2010 Autumn	BI	90.20	10au-BIQTL1	A02	1.1-18.9	5.01	21.88	31.38	-2.0348
			10au-BIQTL2	A02	10.3-23.4	16.46	4.32	5.68	-1.1285
			10au-BIQTL3	A02	60.1-75.8	73.80	4.78	5.27	-0.7785
			10au-BIQTL4	A02	65.6-82.2	77.60	6.12	6.21	-0.8392
			10au-BIQTL5	A06	82.2-99.8	93.96	2.63	3.83	0.6702
			10au-BIQTL6	A06	90.7-101.2	100.40	2.76	2.60	0.5392
			10au-BIQTL7	A07	78.5-93.6	88.89	2.79	5.76	-0.8655
			10au-BIQTL8	A08	71.2-87.5	84.87	2.68	2.79	-0.6086
	FT	94.80	10au-FTQTL1	A02	75.8-86.1	83.73	3.29	11.52	14.0842
			10au-FTQTL3	A07	37.1-55.9	51.83	2.58	6.56	-10.531
			10au-FTQTL4	A07	48.0-68.9	58.34	3.26	8.39	-12.0936
2011 Spring	BI	84.41	11sp-BIQTL1	A02	0.0-10.1	0.01	13.20	25.07	-1.7691
			11sp-BIQTL2	A03	2.6-20.5	11.99	3.48	4.62	-0.7296
			11sp-BIQTL3	A03	12.8-28.3	19.70	3.43	3.47	-0.6179
			11sp-BIQTL4	A06	83.5-96.4	92.96	5.01	11.33	1.1669
			11sp-BIQTL5	A06	92.9-99.1	96.80	5.08	11.36	1.1351
			11sp-BIQTL6	A07	76.0-90.9	83.8	3.88	12.95	-1.2221

h^2 (%) = broad-sense heritability; Exp% = percent phenotypic variation explained. BI = bolting index; FT= flowering time; LOD = logarithm of odds.

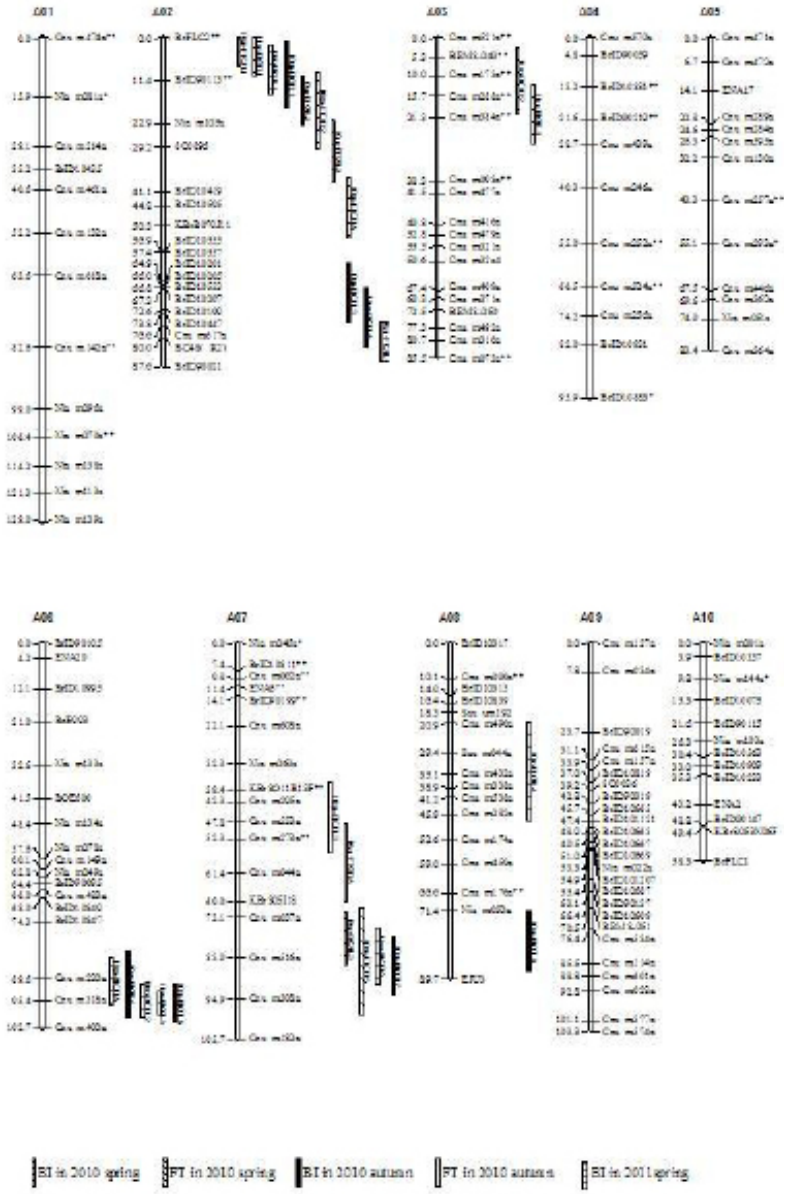

Figure 3. Genetic linkage map of *Brassica rapa* L. F_2 population with 186 individuals derived from a cross between 08A061 (P_1) x 09A001 (P_2) and positions of QTLs for the bolting trait under three growth conditions according to two phenotypic evaluations (bolting index and flowering time). The linkage map was produced that consisted of 107 SSR and 54 InDel markers and ten linkage groups. Linkage group numbers corresponded to those on previously published linkage maps (Choi et al., 2007; Kim et al., 2009; Ramchiary et al., 2011; Wang et al., 2011). Recombination distances are indicated on the left hand side of each linkage group in centimorgans (cM), and the locus names are depicted on the right side of each linkage group. The markers showing segregation distortion are indicated by asterisks ("*" and "**" indicates segregation distortion at $P < 0.01$ and $P < 0.001$, respectively). The QTLs detected in this study are represented by various type of bars placed at the right of each LG.

On linkage group A02, 2 of 11 QTLs, 10sp-FTQTL1 with LOD of 3.87 and 11sp-BIQTL1 with LOD of 13.20, mapped to the same position (0.01 cM), accounting for 2.94 and 25.07% of phenotypic variation, respectively. The QTL 10au-BIQTL1 (LOD 21.88), which had the greatest effect on bolting, was detected at position 5.01 cM on linkage group A02 accounting for 31.7% of the phenotypic variation observed. Another significant QTL, 10sp-BIQTL1 with LOD of 8.04, was also detected in the region, at position 2.01 cM, and accounted for 16.13% of the phenotypic variation. All of the above co-localized QTLs suggested that nearby the marker *BrFLC*$_2$ should be a 'hotspot' region for regulating the bolting trait.

Other significant QTLs were also detected on linkage group A02 at position 16.46 cM; two QTLs, 10au-BIQTL2 with LOD of 4.32 and 10sp-FTQTL2 with LOD of 2.54, mapped to the same position, accounting for 5.68 and 7.89% of the phenotypic variation, respectively. Other QTLs, such as 10sp-FTQTL3 with LOD of 6.11 at position 30.77 cM, 10au-BIQTL3 with LOD of 4.78 at position 73.80 cM, and 10au-BIQTL4 with LOD of 6.12 at position 77.60 cM, accounted for 16.07, 5.27 and 6.21% of the phenotypic variation, respectively.

The eleven QTLs detected on linkage group A02 were all detected by BI evaluation with a negative effect, in contrast to the QTLs detected by FT evaluation, which had a positive effect. All of additive (negative or positive) effects of the above-mentioned QTLs showed that alleles that contributed to late bolting were derived from the late bolting parent, 08A061 (P1). The two phenotypic assessments provided consistent, but complementary results. Other QTLs, such as 10sp-FTQTL3 with LOD of 6.11 at position 30.77 cM, 10au-BIQTL3 with LOD of 4.78 at position 73.80 cM, and 10au-BIQTL4 with LOD of 6.12 at position 77.60 cM, accounted for 16.07, 5.27 and 6.21% of the phenotypic variation, respectively.

Five QTLs were detected on linkage group A06, all of which were detected by BI measurement; no QTL was detected by FT measurement. Two QTLs, 11sp-BIQTL4 with LOD of 5.01 and 10au-BIQTL5 with LOD of 2.63, mapped to an interval of 1 cM, accounting for 11.33 and 3.83% of the phenotypic variation, respectively. Two QTLs, 10sp-BIQTL2 with LOD of 6.68 and 11sp-BIQTL5 with LOD of 5.08, mapped to the same loci on linkage group A06 at position 96.80 cM, accounting for 7.07 and 11.36% of the phenotypic variation, respectively. Another QTL with minor effect, 10au-BIQTL6, was detected near position 96.80 cM. All five QTLs identified by BI measurement had a positive effect, which showed that alleles that contributed to late bolting were derived from the early bolting parent 09A001(P2), unlike the QTLs detected by BI on linkage group A02, which had negative effects.

Three of six QTLs detected on linkage group A07, 10sp-BIQTL3 (LOD 2.46), 10sp-FTQTL5 (LOD 4.80) and 11sp-BIQTL6 (LOD 3.88), mapped to the same locus (83.80 cM), accounting for 8.74, 11.00 and 12.95% of the phenotypic variation, respectively. The three QTLs that were detected by BI evaluation with negative effect, and the QTLs detected by FT evaluation with positive effect, were similar to QTLs detected on linkage group A02. Three other QTLs with minor effect were detected on linkage group A07, and all had negative effect by both BI and FT phenotypic assessment.

Two minor QTLs were detected on both the A03 and A08 linkage groups. In the A03 linkage group, two QTLs, 11sp-BIQTL2 with LOD of 3.48 at position 11.99 cM, and 11sp-BIQTL3 with LOD of 3.43 at position 19.70 cM, were detected in spring 2011. Two QTLs were detected on the A08 linkage group: 10sp-BIQTL4 with LOD of 2.53 at position 35.10 cM, and 10au-BIQTL8 with LOD of 2.69 at position 84.87 cM. Unfortunately, due to the FT data from spring 2011 missing due to disease, we could not detect more QTLs. Unfortunately, due to disease, most of plants during 2011 spring died before flowering. We could not detect more QTLs for bolting.

It is well known that *BrFLC*$_2$, located at the top of linkage group A02, played a vital role in regulating bolting and flowering time in *Brassica rapa*. In the linkage groupA02, there were two other co-localized QTLs, 10au-BIQTL2 and 10sp-FTQTL2, which were located close to the BrID90113 marker. Based on BLAST screening and gene annotation (http://brassicadb.org/brad/index.php), a *FLC*-like gene *Bra023439* that is orthologous to AT5G13860 and a *CONSTANS*-like gene *Bra023541* that is orthologous to AT5G15850 were found nearby the BrID90113 marker. The 10sp-FTQTL3 locus on A02 was found to be close to a single copy of the flowering gene (Bra022475), *FT*. A flowering time control protein-related gene (Bra000446), *FCA*, which was orthologous to AT2G47310, was found located at 11sp-BIQTL2 in the linkage group A03. At 10au-BIQTL8 in the linkage group A08, a dwarf and delayed flowering gene (Bra016763), *DDF1*, orthologous to AT1G12610, was found.

To confirm the effects to QTLs correlated on the bolting trait, we classified the F_2 individuals and $F_{2:3}$ families on the basis of marker genotype of the 186 F_2 individuals (Table 3). Markers were selected according to distance between markers and QTL peak position following graphical genotypes (Young and Tanksley, 1989). The F_2 progeny with the *BrFLC*$_2$ allele derived from the male parent (08A061) showed significant late bolting under all growth conditions and by all phenotypic assessment methods. The genetic marker BrID90113 showed the same effect as the *BrFLC*$_2$ for the bolting trait. Conversely, the allele of marker Cnu-m318a on linkage group A06 which was derived from the male parent 08A061 showed significant association with early bolting under all growth conditions. The allele of marker Cnu-m516a on linkage group A07 derived from the male parent 08A061 also showed significant association with late bolting, except in autumn 2010, with FT serving as phenotypic assessment. From the above, we can postulate that QTLs on linkage groups A02 and A07 derived from the late bolting parent (P_1) will contribute to late bolting, thus QTLs on linkage group A06 contribute to late bolting derived from the early bolting parent 09A001(P_2). Alleles that contribute to late/early bolting were not completely derived from late/early parents, which was consistent with transgressive segregations observed in F_2 and $F_{2:3}$ populations. Pyramiding QTLs/alleles related to late bolting from different parents using marker-assisted selection will facilitate the breeding of new bolting resistant varieties.

Table 3. Relationship between marker genotype and phenotypic measurement (means ± SD).

Linkage group	Marker	Genotype	2010 Spring		2010 Autumn		2011 Spring
			BI	FT	BI	FT	BI
A02	BrFLC2	AA	3.36 ± 2.39^c	73.96 ± 6.38^a	1.36 ± 1.55^c	95.59 ± 18.30^a	3.06 ± 1.89^c
		BB	7.40 ± 1.94^a	63.09 ± 5.91^b	5.48 ± 1.92^a	74.29 ± 8.21^c	6.16 ± 1.78^a
		AB	5.33 ± 2.42^b	70.40 ± 6.83^a	2.94 ± 1.88^b	86.20 ± 14.30^b	4.45 ± 1.81^b
A02	BrID90113	AA	3.64 ± 2.17^c	73.60 ± 5.10^a	1.41 ± 1.47^c	96.43 ± 13.54^a	4.85 ± 1.64^c
		BB	7.56 ± 1.65^a	62.58 ± 5.29^b	5.73 ± 1.67^a	72.72 ± 6.51^c	7.47 ± 1.47^a
		AB	5.13 ± 2.53^b	70.57 ± 7.03^a	2.77 ± 1.97^b	87.29 ± 15.33^b	5.57 ± 2.01^b
A06	Cnu-m318a	AA	7.05 ± 1.58^a	65.38 ± 6.09^b	4.50 ± 2.46^a	78.79 ± 13.40^b	5.94 ± 1.76^a
		BB	4.87 ± 2.93^b	69.50 ± 7.53^a	3.34 ± 2.16^b	84.14 ± 14.65^a	4.23 ± 2.08^b
		AB	5.61 ± 2.55^b	69.03 ± 7.69^a	3.40 ± 2.09^b	83.79 ± 13.09^a	4.70 ± 1.87^b
A07	Cnu-m516a	AA	4.46 ± 2.70^b	71.65 ± 6.96^a	3.11 ± 2.09^b	84.61 ± 14.86^a	3.91 ± 2.13^b
		BB	6.06 ± 2.77^a	67.25 ± 8.43^b	3.66 ± 2.80^a	84.63 ± 17.01^a	5.27 ± 2.16^a
		AB	6.17 ± 2.33^a	67.61 ± 6.98^b	3.69 ± 2.21^a	82.99 ± 14.98^a	5.01 ± 1.88^a

AA = genotype of 08A061 (P_1, late bolting); BB = genotype of 09A001 (P_2, early bolting); BI = bolting index; FT = flowering time. Values of marker genotype mean not followed by same letter are significantly different ($P < 0.05$) to each other based on Duncan's multiple range test.

DISCUSSION

Phenotypic measurement and growth conditions

The most crucial step of QTL mapping for bolting is the evaluation of phenotypic scores. In the present study, two phenotypic assessments, bolting index (BI) and flowering time (FT), served as bolting trait measurements and were used for QTL detection. Flowering time has been more frequently used for QTL mapping because of the relative ease of the measurement (Nishioka et al., 2005). QTL mapping of the flowering time trait in *Brassica rapa* has been previously reported in several populations (Teutonico and Osborn, 1995; Osborn et al., 1997; Lou et al., 2007; Li et al., 2009; Zhao et al., 2010; Lou et al., 2011). For vegetables, bolting time is more important than flowering time because it is an accurate measurment of the transition from the vegetative to the reproductive phase. Until now, measuring the bolting trait in *Brassica rapa* has been a persistent and unresolved problem. Previous studies concerned with QTL mapping of the bolting trait used different measurements, such as the time of appearance of visible flower buds or the floral stalk (Ajisaka et al., 2001; Nishioka et al., 2005; Li et al., 2009), the time of flower stalk appearance or the height of the inflorescence or elongated stem (Li et al., 2009; Kakizaki et al., 2011), the scales of bolting measured at a single timepoint (Zhang et al., 2006; Yang et al., 2007), which depend on materials used in the various studies, growth conditions, and personal perference. Li et al. (2009) used three measurements for bolting in the same population, and some of the QTLs detected mapped to a single position. In the present study, we found that BI, FT and the number of days from sowing to reach a 5-cm-high elongated stalk (data not shown) gave the above-mentioned results, especially for the QTLs with major effects. However, we also found that using different phenotypic measurements inevitably identified different QTL. In the present study, all QTLs detected on linkage group A06 controled the bolting trait using BI as phenotypic assessment. Conversely, when FT was employed as the phenotypic measurement, no QTL was detected.

Bolting in *Brassica rapa* is a complex character trait influenced by environmental conditions, especially temperature (vernalization) and day-length. Under natural conditions in autumn, the late bolting parent (P_1) used in the present study showed no significant difference compared to their F_1, which is the opposite result observed when the plants were given low temperature treatment (data not shown). Under different conditions for a given population, QTLs detected with different positions and effects were reported in several studies (Nishiokda et al., 2005; Lou et al., 2007; Li et al., 2009; Zhao et al., 2010; Lou et al., 2011; Kakizaki et al., 2011). In general, the detection of QTLs under different environmental conditions using different phenotypic measurements is an effective method to identify the function of each candidate locus.

QTL analysis

To date, many QTLs for the bolting trait in *Brassica rapa* have been identified in different segregating populations, distributed on all linkage groups except A04 and A05. Some QTLs detected for the bolting trait in previous studies (Ajisaka et al., 2001; Nishioka et al., 2005; Zhang et al., 2006; Yang et al., 2007) cannot be compared to others, because the linkage groups cannot be aligned to the reference linkage groups. Of these, only a small number of

the QTL mapped to the same loci, which indicates that the bolting trait appears to be affected by the environment. In the present study, a total of 26 QTLs were detected for the bolting trait in *Brassica rapa* using two phenotypic measurements under three environmental conditions, distributed on linkage groups A02, A03, A06, A07, and A08. At the top of linkage group A02, co-localized with the well-known gene $BrFLC_2$, a QTL candidate region reported in several studies (Eric Schranz et al., 2002; Lou et al., 2007; Li et al., 2009; Zhao et al., 2010; Lou et al., 2011), was also identified in the present study. The other QTLs controlling the bolting trait on linkage group A02 were also reported, and alleles controlling late bolting that were derived from the bolting resistant parent were detected in the above-mentioned research. Most of the QTLs detected that control the bolting trait from previous studies (Lou et al., 2007; Li et al., 2009; Kakizaki et al., 2011) were found on linkage group A10, co-localized with the $BrFLC_1$ gene. Interestingly, all of the above-mentioned QTLs with $BrFLC_1$ were only detected under non-vernalized conditions. In the present study, no QTL was found to be associated with $BrFLC_1$ in three vernalizating conditions, and that of $BrFLC_1$, in contrast to the $BrFLC_2$ gene, maybe sensitive to the environment. As is well known, the *Brassica rapa* genome contains four *FLC* homologs, $BrFLC_1$, $BrFLC_2$, $BrFLC_3$ (in the A03 linkage group) and $BrFLC_5$ (in the A03 linkage group). To date, only few QTLs have been associated with $BrFLC_5$ (Eric Schranz et al., 2002, Kakizaki et al., 2011) and $BrFLC_3$ (Lou et al., 2011). With the exception of the *FLCs*, other candidate genes that underlie the flowering-time QTL (Lou et al., 2007; Li et al., 2009; Kakizaki et al., 2011) have also been detected, including *CLV1*, *FLM* and *LFY*, and mapped to the linkage group A06. *BrFT* was mapped to the linkage group A07 and *VNR2* was mapped to the linkage group A08.

On linkage groups A01, A06, and A08, QTLs controlling the bolting trait were also identified in different populations and under different growth conditions. Thus, we cannot confirm whether the QTLs detected on a given linkage group mapped to the same loci. Thus, developing common markers near detected loci on comparable linkage groups in different populations will serve as bridge markers to integrate the different maps, and will contribute to determining whether the detected QTLs belong to the same loci. To validate the QTLs detected in different populations and with different phenotypic scoring strategies, and to identify new loci controlling bolting, it will be necessary to develop secondary populations such as chromosome segment substitution lines or near isogenic lines. These will serve as powerful tools for validating the QTLs detected and for fine mapping the candidate genomic regions.

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REFERENCES

Ajisaka H, Kuginuki Y, Yui S, Enomoto S, et al. (2001). Identification and mapping of a quantitative trait locus controlling extreme late bolting in Chinese cabbage (*Brassica campestris* L. ssp. *pekinensis* syn. *Rapa* L.) using bulked segregant analysis. *Euphytica* 118: 75-81.

Choi SR, Teakle GR, Plaha P, Kim JH, et al. (2007). The reference genetic linkage map for the multinational *Brassica rapa* genome sequencing project. *Theor. Appl. Genet.* 115: 777-792.

Duncan DB (1955). Multiple-range and multiple F tests. *Biometrics* 11: 1-42.
Ge Y, Nirala R, Wang T, Liang C, et al. (2011). Development and linkage mapping of unigene-derived microsatellite markers in *Brassica rapa* L. *Breed. Sci.* 61: 160-167.
Kakizaki T, Kato T, Fukino N, Ishida M, et al. (2011). Identification of quantitative trait loci controlling late bolting in Chinese cabbage (*Brassica rapa*) parental line Nou 6 gou. *Breed. Sci.* 61: 151-159.
Kim H, Choi SR, Bae J, Hong CP, et al. (2009). Sequenced BAC anchored reference genetic map that reconciles the ten individual chromosomes of *Brassica rapa*. *BMC Genomics* 10: 432.
Kosambi DD (1943). The estimation of map distance from recombination values. *Ann. Eugenics* 12: 172-175.
Li F, Kitashiba H, Inaba K and Nishio T (2009). A *Brassica rapa* linkage map of EST-based SNP markers for identification of candidate genes controlling flowering time and leaf morphological traits. *DNA Res.* 16: 311-323.
Lou P, Zhao J, Kim JS, Shen S, et al. (2007). Quantitative trait loci for flowering time and morphological traits in multiple populations of *Brassica rapa*. *J. Exp. Bot.* 58: 4005-4016.
Lou P, Xie Q, Xu X, Edwards CE, et al. (2011). Genetic architecture of the circadian clock and flowering time in *Brassica rapa*. *Theor. Appl. Genet.* 123: 397-409.
Mero E and Honma S (1984). Inheritance of bolt resistance in an interspecific cross of *Brassica* species: *Brassica napus* L. X *Brassica campestris* L. ssp. *pekinensis*. *J. Hered.* 75: 407-410.
Mero E and Honma S (1985). Inheritance of bolt resistance in an inter specific Chinese cabbage X. turnip cross. *Hort. Sci.* 20: 881-882.
Murray MG and Thompson WF (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* 8: 4321-4325.
Nishioka M, Tamura K, Hayashi M, Fujimori Y, et al. (2005). Mapping of QTLs for bolting time in *Brassica rapa* (syn. campestris) under different environment conditions. *Breed. Sci.* 55: 127-133.
Obsorn TC, Kole C, Parkin IAP, Sharpe AG, et al. (1997). Comparison of flowering time genes in *Brassica rapa*, *B. napus* and *Arabidopsis thaliana*. *Genetics* 146: 1123-1129.
Ramchiary N, Nguyen VD, Li X, Hong CP, et al. (2011). Genic microsatellite markers in *Brassica rapa*: development, characterization, mapping, and their utility in other cultivated and wild *Brassica* relatives. *DNA Res.* 18: 305-320.
Schranz ME, Quijada P, Sung S-B, Lukens L, et al. (2002). Characterization and effects of the replicated flowering time gene *FLC* in *Brassica rapa*. *Genetics* 162: 1457-1468.
Suwabe K, Iketani H, Nunome T, Kage T, et al. (2002). Isolation and characterization of microsatellites in *Brassica rapa* L. *Theor. Appl. Genet.* 104: 1092-1098.
Teutonico RA and Osborn TC (1995). Mapping loci controlling vernalization requirement in *Brassica rapa*. *Theor. Appl. Genet.* 91: 1279-1283.
Van Ooijen JW and Voorrips RE (2001). JoinMap® 3.0, Software for the Calculation of Genetic Linkage Maps. Plant Research International, Wageningen, The Netherlands.
Voorrips RE (2002). MapChart: software for the graphical presentation of linkage maps and QTLs. *J. Hered.* 93: 77-78.
Wang S, Basten CJ and Zeng ZB (2007). Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleith, NC.
Wang Y, Sun SL, Liu B, Wang H, et al. (2011). A sequence-based genetic linkage map as a reference for *Brasscia rapa* pseudochromosome assembly. *BMC Genomics* 12: 239.
Yang X, Yu YJ, Zhang FL, Zou ZR, et al. (2007). Linkage map construction and quantitative trait loci analysis for bolting based on a double haploid population of *Brassica rapa*. *J. Integr. Plant Biol.* 49: 664-671.
Young ND and Tanksley SD (1989). Restriction fragment length polymorphism maps and the concept of graphical genotypes. *Theor. Appl. Genet.* 77: 95-101.
Zhang XW, Wu J, Zhao JJ, Song XF, et al. (2006). Identification of QTLs related to bolting in *Brassica rapa* ssp. *pekinensis* (syn. *Brassica campestris* ssp. *pekinensis*). *Agric. Sci. China* 5: 265-271.
Zhao JJ, Kulkarni V, Liu N, Carpio DPD, et al. (2010). *BrFLC2* (*Flowering Locus C*) as a candidate gene for a vernalization response QTL in *Brassica rapa*. *J. Exp. Bot.* 61: 1817-1825.