QTL analysis of flowering time and photoperiod sensitivity in rapeseed (*Brassica napus* L.)

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Abstract

Flowering time and photoperiod sensitivity in rapeseed (*Brassica napus* L.) are two important agronomic traits that relate to developing cultivars with wide geographical adaptability. The objective of this study was to dissect the genetic control of the two traits into the various components such as the main-effect quantitative trait loci (QTLs), espistatic QTLs and QTL-by-environment interactions (QEs). Doubled haploid (DH) lines were produced from an F1 between two spring *B. napus* canola varieties Hyola401 and Q2 which displayed low and high sensitivity to photoperiod, respectively. The data on flowering time of the DH lines were collected from the field experiments conducted in two locations, one location with a short and the other with a long photoperiod regime over two years. A genetic linkage map was constructed that comprised 248 marker loci including 82 SSR, 94 SRAP and 72 AFLP markers. Further QTL analysis resolved the genetic components of flowering time and photoperiod sensitivity into the main-effect QTLs, epistatic QTLs and QEs. A total of 7 main-effect QTLs and 11 digenic interactions involving 21 loci located on 13 out of the 19 linkage groups were detected for the two traits. In addition, three main-effect QTLs and four pairs of epistatic QTLs were involved in QEs showing important effects on flowering time. Among the seven main-effect QTLs, the one on linkage group 18 was revealed to simultaneously affect days to flower (DTF) and photoperiod sensitivity (PS) and explain for the highest percentage of the phenotypic variation. The implications of the results for *B. napus* breeding have been discussed.

Key words: *Brassica napus* L. - flowering time - photoperiod sensitivity - quantitative trait loci (QTLs) - espistatic QTLs – QTL - by-environment interactions (QEs)

Introduction

Flowering is an important and complex adaptive trait for oilseed production conditioned by environmental factors (photoperiod and temperature), and internal genes and the interactions of genes (Teutonica & Osborn, 1995; Camargo & Osborn, 1996; Osborn et al., 1997; Robert et al., 1998). *Brassica napus* is a long-day plant, indicating that it can flower and be harvested earlier under long day than short day (Butruille et al., 1999; Ferreira et al., 1995). Besides temperature, photoperiod is another key factor which decides a plant flowering and setting seed or not (Clark et al., 1995; Juenger et al., 2005; Lagercrantz et al., 1996; Putterill et al., 1995; Yano et al., 2000). To our knowledge, most spring *B. napus* varieties from Canada and Europe are sensitive to photoperiod and flower early in the long-day condition but become very late under the short-day regime. Q2 is a Canadian spring canola cultivar with a high PS thereby limiting its cultivation in the traditional areas. But Hyola401, one spring canola cultivar, exhibiting a low PS, is grown not only in the traditional areas as a summer crop under the LD condition but also in the tropical areas as a winter crop under the SD condition (Kennard et al., 1994; Song et al., 1995). As a result, in order to ensure optimal pollination and seed production, it is essential that flowering takes place at an optimal time of the year.

Photoperiod sensitivity is controlled genetically and interacts with other flowering genes to condition flowering time, thus limiting geographic adaptation of plants. The genetic control of photoperiod sensitivity have been studied successfully in modern plants rice and *Arabidopsis thaliana* (Putterill et al., 1995; Yano et al., 2000), but as for the number of genes and type of their interactions, it is inconclusive so far.

The rapid development of molecular marker technology has facilitated the mapping of QTLs associated with DTF in *B. napus* and other *Brassica* species (Li & Quiros, 2001; Vos et al., 1995). Many main-effect QTLs controlling DTF in *B. napus* and other *Brassica* have been identified using DH and other populations (Axelsson et al., 2001; Bohuon et al., 1998; Butruille et al., 1999; Camargo & Osborn, 1996; Lan & Paterson, 2000; Osborn et al., 1997; Robert et al., 1998; Teutonica & Osborn, 1995).

The quantitative nature of DTF in *Brssica* species has been summarized by Osborn and Lukens (2003). But how to dissect the genetic components, especially to consider epistasis and QEs which play an important role in affecting DTF in rice and *Arabidopsis* was not described in detail for a lack of appropriate analysis tools (Li et al., 2003; Murfet, 1977; Yu et al., 2002; Yue & Xiong, 2005). Fortunately, the QTLMAPPER 1.6 developed by Wang *et al* (1999). succeeded in dividing the total effects into three effects: main, epistatic and QE effects, and quantifying these effects. The objectives of this study were to identify QTLs controlling DTF and PS in one *Brassica napus* DH population derived from Hyola401×Q2.

Materials and methods

plant materials and field experiments: The materials used in this study were two spring canola *B. napus* cultivars Hyola401 and Q2, exhibiting a low and high photoperiod sensitivity, respectively. Doubled haploid (DH) plants produced from F_1 plants of the cross Hyola401×Q2 using the microspore culture developed by Shi and Liu (1993). DH population and their parents, were grown in the summer-autumn growing season in Hezheng (35°20' N, 103°21'E, China, 2003 and 2004) with a 14.3 h day length and a 15.1°C average temperature and in the autumn-spring growing season in Zhaoqing (23°16'N, 112°56' E, China, 2003 and 2004) with a 10.1 h day length and a 15.5°C average temperature. At each location a randomized complete block design was used with two replications. Each DH line and parent comprising 40-45 individuals planted in two rows with 15 cm between plants and 30 cm between rows.

traits measurements: DTF was recorded as the number of days from the sowing date to the date when 50% of the plants in one DH line or parent had at least one open flower. The mean DTF of each DH line was used for QTL analysis. The degree of PS of each DH line or parent was calculated by the delay in DTF in Zhaoqing as compared to DTF in Hezheng.

DNA markers analysis: Total genomic DNA was isolated using a modified SDS method (Li et al. 1994).A total of 248 marker loci that comprised 82 SSR (simple sequence repeat), 72 AFLP (amplified fragment length polymorphism) and 94 SRAP (sequence-related amplified polymorphism) loci were detected. The SSR, AFLP and SRAP markers were designed according to http://ukcrop.net/perl/ace/search/BrassicaDB, Vos *et al.* and Li et al., respectively. The protocols of SSR and AFLP were followed as described by Piquemal et al. (2005) and Liu et al. (2005), respectively. The SRAP system was based on the procedure of AFLP selective amplification described by Vos et al (1995). with only one modification, 100ng of genomic DNA. Amplifications were carried out in a MJ Research PTC-225 thermocycler (MJ Research, Waltham, Mass.) using the cycling parameters described by Li et al..The PCR products were separated on a 6% denaturing polyacrylamide gel at 85W for about 2.5 h and visualized by the silver staining system (Promega, Madison, Wis.).

data analysis: A genetic linkage map comprising 248 marker loci was constructed using MAPMAKER 3.0 (Lincoln et al., 1992). QTLMAPPER 1.6 based on a mixed linear model approach (Zhu & Weir, 1998), which estimates QTL main effects, epistasis, as well as predicting QE interaction effects, treating the locations as two environments, was employed to assess QTLs controlling the PS and DTF. In the analysis, the likelihood ratio (LR) and *t*-test were combined to test the significance of the single-locus QTL additive effects, epistatic effects and the QTL by environment (QE) effects. The LR value corresponding to P=0.005 (equivalent to LOD=3.2 for df=4) was used as the threshold for claiming the putative main-effect, epistatic QTLs or QEs. The peak points of the LR in the linkage map were taken as the putative positions of the QTLs. When a QTL was involved in more than one epistasis, its position and additive effect were taken from the point showing the largest effect. The relative contribution of a genetic component was calculated as the proportion of phenotypic variance explained by that component in the selected model.

Table 1 Descriptive statistics of DTF and PS for the parents and the DH population observed in the two locations (Hezheng and Zhaoqing) (SD standard deviation)

Trait[a]	Location	Parent (mean ± SD)		DH population			
		Hyola401	Q2	mean ± SD	Range	Skewness	Kurtosis
DTF	Hezheng	47.5±0.5	54.5±0.7	54.3±5.4	44.0 – 70.0	0.49	-0.38
	Zhaoqing	89.5±2.1	105.5±0.7	98.9±14.7	55.0 – 121.0	-1.57	1.65
PS		42.0±1.4	51.0±1.4	44.6±12.2	-7.0 – 64.0	-1.63	1.86

[a]Abbreviations are described in the abstract

Results

measurements of DTF and PS: Table 1 shows a summary of the descriptive statistics of DTF and PS for the two parents and DH lines. Highly significant differences between the parents were detected using the least significant difference (LSD) test at the 0.01 probability level for the two traits. Q2 had always a greater DTF and PS than Hyola401 at both locations, implying that Q2 was more sensitive to photoperiod than Hyola401. Furthermore, the DTF difference between two parents was less in Hezheng (7 days) than that in Zhaoqing (16 days), indicating that photoperiod sensitivity difference of two parents can be displayed more significantly in the short day condition. Figure 1 shows the distribution of the two traits in parents and DH population. A certain number of DH lines showed transgressive segregations in both directions for the two traits at the two locations, showing flowering time and PS as typical quantitative traits. In addition, the LSD (least significance difference) test detected significantly different DTF of the DH lines (P<0.01) between Hezheng and Zhaoqing. A two-way ANOVA revealed that there were highly significant differences between the two locations (environments) and also highly significant genotype-by-environment interactions for DTF in addition to the major genotype effects (Table 2).

Table 2 A two-way ANOVA for DTF in the DH population evaluated in two locations

Variation[a]	df	MS	F	P	$F_{0.01}$
G	148	3342.51	947.28 **	0.00	1.38
E	1	13692.21	3880.44 **	0.00	6.72
G × E	148	147.62	41.83 **	0.00	1.38
Error	298	3.53			

** means significance at 0.01 level, [a]G, Genotype; E, environment; G × E, genotype-by-environment interaction

Table 3 Main effects, digenic epistatic effects and environmental interactions of QTLs detected by two-locus analysis using QTLMAPPER 1.6 for DTF at the likelihood ratio LR-threshold of 14.9 (equal to a chi-square value for *df*=4 at *P*=0.005) combining the field data from Hezheng and Zhaoqing.

Ch-Ini[a]	Flanking markers	QTL	Ch-Inj[a]	Flanking markers	QTL	LOD	a_i[b]	$h^2 a_i$[c]	a_j[b]	$h^2 a_j$[c]	aa_{ij}[c]	$h^2 a_{ij}$[c]	ae_i[d]	$h^2 ae_i$[c]	ae_j[d]	$h^2 ae_j$[c]	aae_{ij}[d]	$h^2 aae_{ij}$[c]
2-5	em16-me2c - em15-me9b		2-8	em15-me9c - EA5-MG6d		4.64					3.19	3.10			-3.06	0.97	7.09	0.73
2-8	em15-me9c - EA5-MG6d		6-8	Ol11-B05b - Na12-E02		6.46					-1.29	4.18					-2.45	1.33
4-5	EA6-MG11c - EA3-MG4d		10-22	CB10524 - em2-me2b		4.91									3.66	0.77	2.75	0.41
5-2	Na14-E11 - Na12-E03b		18-3	em6-me7b - EA9-MG12b		24.28									6.33	1.17		
5-32	em10-me5c - em10-me7d		5-38	em15-me9a - em4-me10b	*dtf5*	9.57			3.98	11.43	1.12	4.14			3.33	1.60		
6-9	Na12-E02 - CB10569		11-7	EA3-MG11d - EA3-MG16b		17.57									5.86	0.86		
11-8	EA3-MG16b - EA7-MG8b	*dtf11*	11-12	Ra2-E12 - em8-me8c		17.65	3.68	18.47			0.89	1.45	6.48	1.45				
18-4	EA9-MG12b - em5-me9		18-7	EA1-MC8d - em6-me7c		31.13					2.01	2.44	-6.21	0.67			3.30	0.59
18-5	em5-me9 - EA9-MG6	*dtf18*	18-8	em6-me7c - EA3-MG4c		27.19	-4.74	28.44					-6.35	1.23				

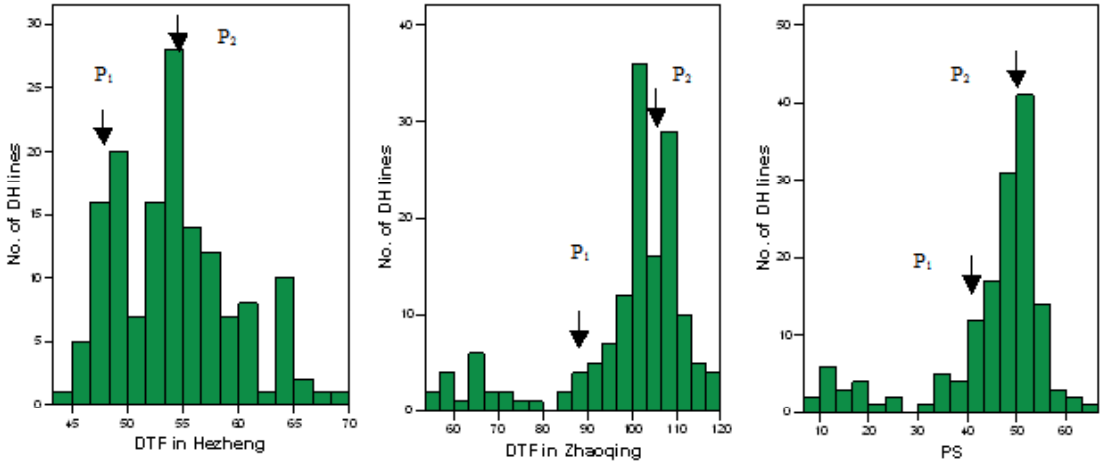
[a]Ch-Ini and Ch-Inj represent the chromosome number-interval of the points being tested in the analysis
[b]a_i and a_j are the additive effects of the test points *i* and *j*, respectively; a positive values of a_i and a_j implies the Hyola401 genotype having a positive effect on the trait
[c]aa_{ij} is the effect of additive-by-additive interaction between points *i* and *j*; a positive value implies the parental two-locus genotypes having a positive effect and the recombinants having a negative effect
[d]ae_i, $ae_j$and aae_{ij} are effects of the environmental interaction of locus *i*, *j* and epistasis, respectively; a positive value implies that the effect in Zhaoqing is larger than that in Hezheng
[e]$h^2 a_i$, $h^2 a_j$, $h^2 aa_{ij}$, $h^2 ae_i$, $h^2 ae_j$ and $h^2 aae_{ij}$ are the percentages of the phenotypic variations explained by a_i, a_j, aa_{ij}, ae_i, ae_j and aae_{ij}, respectively

Table 4 Main effects and digenic epistatic effects of QTLs detected by two-locus analysis using QTLMAPPER 1.6 for PS at the likelihood ratio LR-threshold of 14.9(equal to a chi-square value for *df*=4 at *P*=0.005)

Ch-Ini[a]	Flanking markers	QTL	Ch-Inj[a]	Flanking markers	QTL	LOD	a_i[b]	$h^2 a_i$[d]	a_j[b]	$h^2 a_j$[d]	aa_{ij}[c]	$h^2 a_{ij}$[d]
3-2	EA5-MG6c - EA3-MG16d	*ps3*	3-6	em12-me9c - Ol10-B06		5.25	-3.98	8.35			4.08	5.77
3-7	Ol10-B06 - em7-me10e		19-6	em2-me1a - em2-me1b		6.30					3.18	5.31
4-20	CB10330 - em6-me7a		8-4	Na12-A02a - EA1-MC8e		5.40					2.54	4.10
9-6	Na10-C01b - CB10103b		16-1	CB10034b - BRAS050b		4.10					3.22	4.30
10-1	Na12-H04 - em2-me2a		14-3	em10-me7c - CB10369		8.46					-3.90	2.02
14-1	em10-me10b - em10-me5b	*ps14*	14-5	em10-me10f - EA1-MC8a		8.75	-4.78	12.04				
18-1	EA4-MG5a - Na12-H09c		18-5	em5-me9 - EA9-MG6	*ps18*	15.52			-3.08	24.99	-3.12	6.11
10-6	EA5-MG6a - em15-me6c	*ps10*				8.20	-3.44	7.94				

[a]Ch-Ini and Ch-Inj represent the chromosome number-interval of the points being tested in the analysis
[b]a_i and a_j are the additive effects of the test points *i* and *j*, respectively; a positive values of a_i and a_j implies the Hyola401 genotype having a positive effect on the trait
[c]aa_{ij} is the effect of additive-by-additive interaction between points *i* and *j*; a positive value implies the parental two-locus genotypes having a positive effect and the recombinants having a negative effect
[d]$h^2 a_i$, $h^2 a_j$, $h^2 aa_{ij}$, are the percentages of the phenotypic variations explained by a_i, a_j, aa_{ij}, respectively

linkage map: A total of 248 loci covered all 19 chromosomes with a total genetic distance of 1634.7 cM and an average genetic distance of 6.6 cM between adjacent marker loci. The 82 SSR marker loci from Piquemal *et al.* corresponded well with his map in the order. (Fig. 2)



P1: Hyola401, P2: Q2

Figure 1 The frequency distribution for DTF and PS in the DH population derived from two spring B. napus cultivars, 'Hyola401' and 'Q2' with a low and high photoperiod sensitivity, respectively.

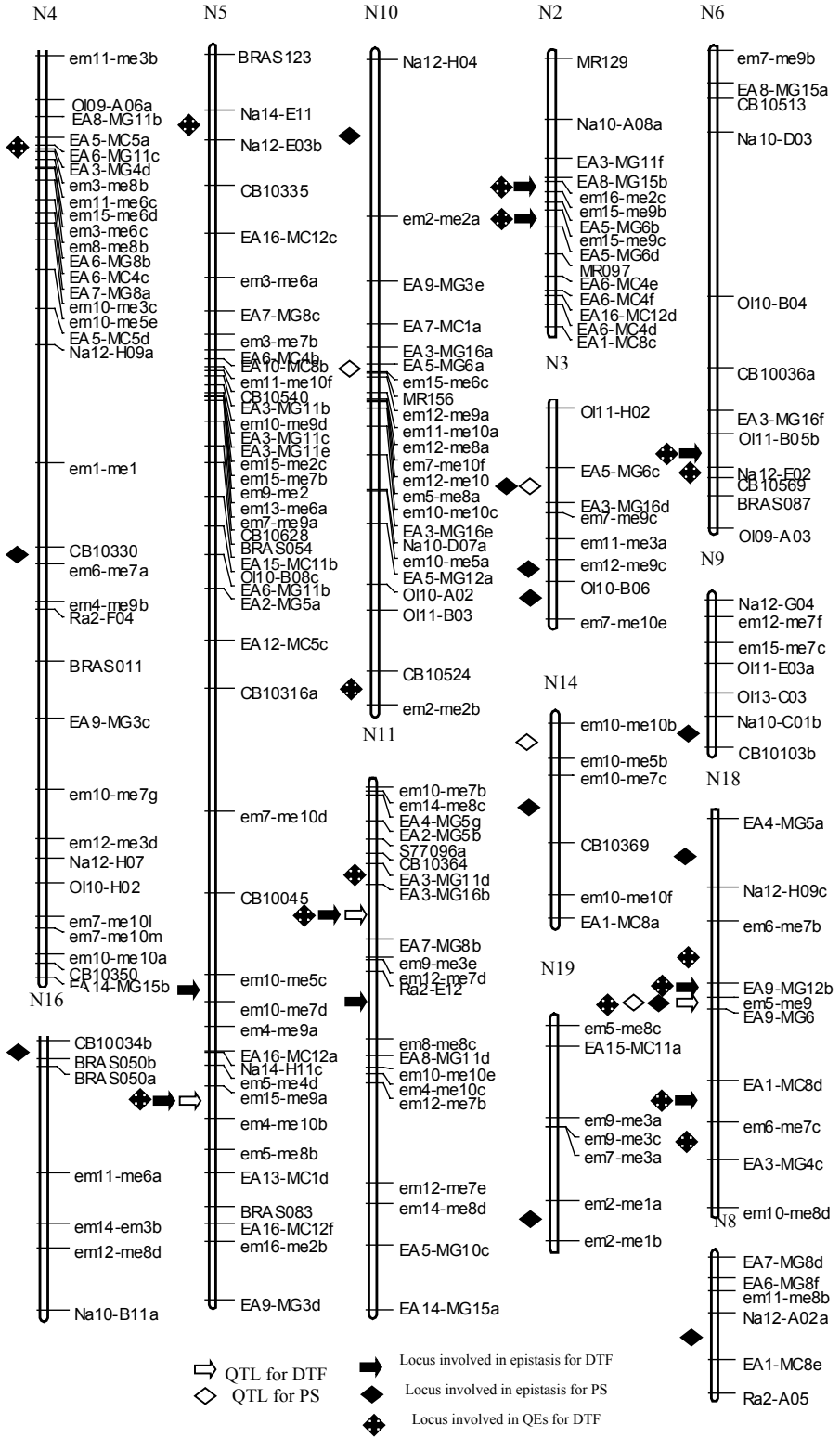


Figure 2 Distribution of main-effect QTLs, epistatic QTLs and QEs on the linkage map as detected by QTLMAPPER 1.6

QTL for days to flowering (DTF): Three main-effect QTLs controlling DTF, *dtf5*, *dtf11* and *dtf18*, located on LG5, LG11 and LG18, respectively, were identified in this DH population by combining the field data from Hezheng and Zhaoqing (Table 3, Fig. 2) and they together accounted for 58.3% of the phenotypic variation. The alleles from Hyola401 at *dtf5* and *dtf11* increased DTF by 4.0 and 3.7 days, respectively and explained for a total of 29.9% of the phenotypic variation. The allele from Q2 at *dtf18* increased DTF by 4.7 days and accounted for 28.4% of phenotypic variation which was the highest effect among 3 main-effect QTLs. There were 5 pairs of loci involved in epistatic effects. Furthermore, 2 pairs of the 5 loci involved 2 of the 3 main-effect QTLs, *dtf5* and *dtf11*. Among them, the parental two-locus genotypes seemed to increase DTF for 4

pairs while the recombinant two-locus combinations increased DTF only for the remaining 1 pair. The total epistatic effects explained for 15.3% of the phenotypic variation. All of 3 main-effect QTLs and 4 pairs of epistatic QTLs were involved in the environmental interactions. The overall QEs accounted for 11.8% of the phenotypic variation.

QTL for photoperiod sensitivity (PS): A total of 4 main-effect QTLs conditioning PS, *ps3*, *ps10*, *ps14* and *ps18* were revealed on LG3, LG10, LG14 and LG18, respectively (Table 4). Compared with Hyola401, the alleles from Q2 at all the 4 main-effect QTLs resulted in higher PS. In all, these QTLs explained for 53.3% of the phenotypic variation. Epistatic effects were detected for 6 pairs of loci, but only 2 of the 6 pairs involved 2 main-effect QTLs, *ps3* and *ps18*. The total epistatic interactions accounted for 27.6% of the phenotypic variation.

Discussion

In this study, we mapped QTLs for two important agronomic traits, DTF and PS in *Brassica napus*, by combining field data from two environments with different photoperiod regimes, 14.3 h and 10.1 h daylength over two years.

A two-ANOVA detected the large genotype-by-environment interactions in the DTF trait variation, which was reflected in the large number of QEs indentified in the QTL analysis. It was suggested that QEs might play important roles in conditioning flowering time of plants. However, for QEs and epistasis, they were roughly investigated by a two-ANOVA in the previous studies. As a result, the analytical tools need to be developed to dissect and more precisely quantify the QEs components.(additive-by-environment interactions; esptatic-by-environment interactions). Nonetheless, the QTLMAPPER 1.6 developed by Wang et al (1999). succeeds in partitioning the total effects into main effects, epistatic effects and QE effects, and quantifying their effects in this study. For the first time, epistatic interactions and QEs relating to flowering time were analyzed in detail and quantified in *Brassica napus* by using QTLMAPPER 1.6 developed by Wang *et al.*.

The present study revealed three main-effect QTLs explaining for 58.3% of the phenotypic variation of DTF. The QE interactions accounted for 11.8% of the variation including the 8.7% additive-by-environment and 3.1% epistatic-by-environment components. This result indicated that flowering process was sensitive to environmental changes. The previous studies reported about 3 to 7 main-effect QTLs accounting for 50-70% of the total variation of flowering time in *B. napus*. The four main-effect QTLs for PS together explained for 53.3% of the total phenotypic variation. It was interesting that the epistatic interactions for PS accounted for a high portion of the variation (27.6%), implying a complex genetic basis of the trait. One main-effect QTL, *dtf18* or *ps18*, was located between one co-dominant SRAP marker em5-me9 and one dominant AFLP marker EA9-MG6 on LG18 and accounted for the largest percentage of the phenotypic variation, 28.4% for DTF and 25.0% for PS (Table 3, 4). This co-localization may be explained very well by a high correlation between DTF and PS (r^2=0.59, p<0.01). In addition, the pleiotropic effects or tight linkage of genes could be another explanation of this co-localization. Furthermore, the SRAP marker, a simple and efficient marker system based on PCR amplification facilitates easily being used in practical manipulation. As a consequence, this QTL can serve for marker-assisted selection (MAS) in *Brassica napus* breeding program.

Sernyk et al (1983).and Schuler et al (1991). drew one conclusion by extensive comparison studies that flowering time in *Brassica napus* always exhibited obvious transgressive segregation, but not heterosis. In this study, it is also very clear that the transgressive segregation can be observed in both directions in the DH population for DTF and PS, thereby indicating that the two traits were conditioned by polygenes and also that the two parents contain genes controlling earliness, lateness and photoperiod sensitivity. It is expected that most of the DH lines and two parents flower later in short-day Zhaoqing than long-day Hezheng. But among 149 lines, there are 8 DH lines flowering earlier in Zhaoqing than in Hezheng, which is likely due to temperatures differences at the two locations. It was thus concluded that these 8 DH lines were not sensitive to photoperiod. In addition, some alleles from the early-flowering parent 'Hyola401' increase DTF, namely delay flowering (positive values in a_i and a_j in Table 3), but those from the late-flowering parent 'Q2' decrease DTF, namely hasten flowering (negative values in a_i and a_j in Table 3). This kind of effect has been detected in some QTL analysis and gives us another genetic explanation for transgressive segregation. As for application in breeding, it has been studied quit successful to select early-flowering individuals from predominantly late genotypes because numerous genes segregate always toward promoting flowering in higher plants. Out of 248 marker loci used to build genetic linkage map, 82 SSR marker loci from Piquemal *et al.* correspond well with his map in the order (Fig. 2), indicating a good collinearity of SSR markers in *Brassica napus* and the homology of different *Brassica napus* cultivars (Lombard & Delourme, 2001; Lagercrantz et al., 1996; Piquemal et al., 2005; Axelsson et al., 2001).

Conclusion

In this study we analyzed the QEs affecting flowering time in detail for the first time in *Brassica napus*. The results showed that QEs play important roles in the genetic control of flowering process. As a consequence, taking into account implications of QEs besides main-effect QTLs and digenic interactions in practical breeding program may help improve breeding efficiency.

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