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ORIGINAL PAPER

Genetic and correlation analysis of silique-traits in *Brassica napus* L. by quantitative trait locus mapping

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Abstract Rapeseed yield is directly and indirectly influenced by the silique-traits, such as silique length (*SL*), seeds per silique (*SS*), seed weight (*SW*), because the silique is an organ which produced yield and a major photosynthesis organ as well. In this study, a linkage map comprising 150 simple sequence repeat and 195 amplified fragment length polymorphism markers covering 1,759.6 cM was constructed in a doubled haploid population from a cross between two genotypes of 'HZ396' and 'Y106'. In field experiments across three seasons and two locations in China 140 doubled haploid lines and their corresponding parents were evaluated for silique-traits. In total, 26 quantitative trait loci (QTL) were detected, of which 15 were clustered and integrated into 5 pleiotropic unique QTL by meta-analysis. These unique QTL, which in a certain sense reflected the significant positive correlation between *SS* and *SL* and the significant negative correlation between *SW* and *SS* by the genomic location and effects of QTL detected, were mapped on linkage groups N7, N8 and N13. A trait-by-trait meta-analysis revealed 5, 2 and 3 consensus QTL for *SL, SS* and *SW*, respectively. Epistatic effects varied according to the specific traits performed. All the epistatic interactions showed significant additive by additive effects while no significant epistasis by environment effect was identified. These findings provided a better understanding of the genetic factors controlling silique-traits and gained insights into the gene networks affecting silique-traits at QTL level in rapeseed.

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Introduction

Rapeseed, *Brassica napus*, is cultivated as an oilseed crop worldwide. Exploitation of high-yield cultivars has been a major breeding target. According to Liu (1987), the silique is an organ which produced yield and a major photosynthesis organ as well. Since photosynthesis occurs in the silique, the silique-traits, such as silique length (*SL*), seeds per silique (*SS*), seed weight (*SW*), will directly and indirectly affect rapeseed yield. So these silique-traits have long been thought to be potent factors for improving rapeseed yield. Wei (2000) found that these silique-traits were controlled by nuclear genes instead of cytoplasmic genes. Li and Gu (1992) reported that additive effects were important for these silique-traits. Wang and Niu (2006) revealed that the additive effects and the dominant effects of *SL* were both significant. Zhang et al. (2010) utilized the joint segregation analysis of multiple populations in diverse genotypes and found that *SS* was controlled by a combination of two major genes plus polygenes. Chay and Thurling (1989) analyzed 112 full-sib families grouped on *SL* and found that the correlation between *SS* and *SL* is significant positive, suggesting that *SL* can serve as a trait for indirect selection of *SS* for breeding purposes. Liu (1987) reported that *SW* and *SS* were negatively correlated. In recent years, quantitative trait loci (QTL) analysis of *SS* and *SL* as well as *SW* was measured by using different populations in rapeseed (Chen et al. 2007; Quijada et al. 2006; Udall et al. 2006). Analysis of quantitative data by

Radoev et al. (2008) revealed 3 QTL for *SS* and 9 QTL for *SW*. Shi et al. (2009) detected a number of QTL for *SS* and *SW* and found that more than half of the QTL were expressed principally in response to the specific environments in which the population was grown. Although these investigations on these traits are available, the genetic basis for these traits in rapeseed is not well elucidated, and only few studies on analysis for epistasis have been carried out, and correlation analysis of the silique-traits at QTL level has not been investigated so far in rapeseed.

In this article, our goal was an analysis of the genetic basis and correlation of the silique-traits by QTL mapping. We identified the effects of QTL for *SL* and *SS* as well as *SW* in a doubled haploid (DH) population, and demonstrated the complexity of the genetic architecture by QTL meta-analysis. Moreover, we estimated the relative importance of epistatic effects and of epistasis × environment interactions for the silique-traits.

Materials and methods

Plant materials

The *B. napus* lines, 'Y106' exhibiting high *SS* and 'HZ396' with low *SS*, which were provided by Rapeseed Laboratory of Huazhong Agricultural University, had different genetic backgrounds and were used as parents (Zhang et al. 2010). Before development of the mapping population, DH lines had been developed from both of the parental genotypes. One F_1 plant of the cross of 'HZ396' × 'Y106' were used to develop DH lines by microspore culture using the method described by Shi and Liu (1993). The DH population, consisted of 140 lines, was used for map construction and QTL analysis.

Field experiment

The field trials were carried out over two seasons (2007, 2008) in Wuhan, a semi-winter-type rapeseed growing area in central China, and one season (2009) in Gansu, a spring-type rapeseed growing area in Northwestern China. On the trial in Wuhan in 2007, all 140 DH lines, together with their parents 'HZ396' and 'Y106', were grown in the design of completely random block with two replicates, two rows per plot consisting of 24 plants; length and width of the rows were 1.2 and 0.3 m, respectively. On the trial in Wuhan in 2008, a planting date trial was conducted. The planting date trial included two planting date treatments (21st September and 6th October, designed as 2008-I and 2008-II). Usually, the date of around 21st September is the optimum date as the farmer's field in Wuhan (Liu 1987). At each planting date trial, all the plant materials were planted in the design of completely random block with two replicates, one row per plot consisting of 12 plants. Thus, there were two different data sets on this planting date trial. On the trial in Gansu in 2009, all the plant materials were grown in the design of completely random block with two replicates, one row per plot consisting of 16 plants. The seeds were sown by hand and the field management followed standard agricultural practice. So, there were four data sets in the statistical analysis.

Data collection and statistical analysis

All the DH lines were open pollinated in all environments in order to keep a full record of fertilization rates. Samples for *SS* and *SL* as well as *SW* were collected according to Radoev et al. (2008). At the mature stage, ten plants from each DH line and parental line were selected randomly from each plot for analysis of the silique-traits. Then ten siliques, which were the first ten well-developed siliques of the main raceme immediately above the first side branch, were harvested from each plant. The trait of *SS* was estimated as a mean from the ten siliques after these siliques were air-dried, and so was *SL*. The trait of *SW* was measured in grams estimated from the average of three measurements of the weight of 1,000 well-filled seeds from the mixed seeds of the ten plants in a plot.

We measured *SS* and *SL* in the four environments while assessed *SW* only in 2007 and in the first planting date in 2008 in Wuhan. Since we were faced with high-temperature weather causing *SW* disaster during the silique maturity stage when the plant materials planted on 6th October in Wuhan in the planting date trial, we abandoned the measurement of *SW*. Similarly, we abandoned the evaluation of *SW* in Gansu, a spring-type rapeseed growing area in Northwestern China, as the plant materials, which belonged to semi-winter-type rapeseed, were extremely sensitive to damage from frost damage during the silique maturity stage.

The standard statistical procedures of Kong (2005) for genetic correlation were adopted. Estimates of components of variance and covariance were obtained using the SAS general linear model (GLM) procedure (SAS Institute 2000). The various genetic symbols have the following connotations: The genetic correlation was calculated by $r_G = cov_{Gxy}/(\sigma^2_{Gx} \times \sigma^2_{Gy})^{1/2}$, where cov_{Gxy}, σ^2_{Gx}, and σ^2_{Gy} were the genetic covariance and variance of the pair-wise traits, respectively; the broad-sense heritability was calculated by $h^2 = \sigma^2_g/(\sigma^2_g + \sigma^2_{ge}/n + \sigma^2_e/nr)$, where σ^2_g is the genotypic variance, σ^2_{ge} is the interaction variance of genotype with environment, σ^2_e was the error variance, n was the number of environments, and r was the number of replicates. The estimates of σ^2_g, σ^2_{ge}, σ^2_e, were obtained from an analysis of variance with environment considered as a random effect.

DNA extraction and linkage map construction

Leaf tissue was collected from seedlings of the parental and DH lines. Genomic DNA was extracted according to the CTAB method (Doyle and Doyle 1987) and preserved at −20°C. Before polymerase chain reaction (PCR), the DNA was diluted to the concentration of 50 ng/μL with double distilled H_2O. Simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) were used to survey DNA polymorphism among the parental lines and the DH population. Sequences of all SSR markers were obtained from public sources: http://ukcrop.net/perl/ace/search/BrassicaDB (Lowe et al. 2004), http://www.brassica.info/ssr/SSRinfo.htm (prefixes: Ra, Ol, Na, BN, MB and BRMS-) and http://www.osbornlab.agronomy.wisc.edu/research/maps/ssrs.html (prefix: FITO). Primer pairs with prefixes 'BRAS' and 'CB' were from the electronic supplementary material of Piquemal et al. (2005). Primer pairs with prefixes 'BnGMS' were from the electronic supplementary material of Cheng et al. (2009). For SSR markers that detected more than one polymorphic locus, the marker name is followed by a letter to distinguish the loci. For instance, the two loci detected by BnGMS315 were designated as BnGMS315a and BnGMS315b. Moreover, the estimated molecular weight of the fragments was arranged in alphabetical order. The PCR procedure, electrophoresis and silver staining were as described by Lu et al. (2004). For surveying with AFLP markers, DNA samples were digested with two restriction enzyme combinations, i.e., *Eco*RI/*Mse*I (E/M) and *Sac*I/*Taq*I (S/T). Sequences of all AFLP primers were obtained from Vos et al. (1995). AFLP markers in the mapping population were named according to the primer combination and the estimated molecular weight of the fragment. For example, EA02MC05-160 is a specific AFLP marker of length 160 bp amplified from primer pairs EA02/MC05. A linkage map was constructed using MAPMAKER/EXP V3.0 (Lincoln et al. 1992). A minimum LOD score of 3.0 and a maximum distance of 40 cM were used to group loci into the linkage groups. In order to construct the framework map and compare with published linkage maps, some public SSR markers were used as anchor markers. The values of recombination fractions were converted into genetic map distances (cM) by means of the Kosambi mapping function (Kosambi 1944).

Data analysis and QTL mapping

QTL analysis was performed by composite interval mapping (Zeng 1994) using WinQTL cartographer 2.5 software (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm). The experiment-wise LOD threshold was determined by permutation analysis (Churchill and Doerge 1994) with 1,000 repetitions. LOD scores corresponding to $P = 0.05$ (3.1 for DH) were used for identifying significant QTL. This threshold rejects some QTL, but as these QTL has been identified in a number of other independent studies (Chen et al. 2007; Shi et al. 2009), a less-stringent LOD threshold of 2.0 was adopted. The additive effect and the percentage of variance (R^2) explained by individual QTL were estimated. For the designation of QTL, we followed the recommendation by McCouch et al. (1997).

To avoid false-positive QTL due to less-stringent LOD threshold, meta-analysis of QTL (Goffinet and Gerber 2000) was conducted. Computations were conducted using the BioMercator 2.1 software (Arcade et al. 2004). This approach provides a modified Akaike criterion that can be used to determine the number of meta-QTL that best fitted the results on a given linkage group. Usually, a two-round strategy of QTL meta-analysis was adopted. The QTL identified in different experiments were first integrated into consensus QTL, trait by trait. The most likely model is the one minimizing the Akaike criterion (the lower the AIC value, the more likely the model). For each model, the most likely position(s) of consensus QTL(s) and confidence intervals are given. According to Price (2006) and Shi et al. (2009), two types of consensus QTL were defined: major QTL (those occurring at least once with $R^2 \geq 20\%$ or at least twice with $R^2 \geq 10\%$), and minor QTL (the remainder with relatively small effect). In the second round of QTL meta-analysis, the putative QTL for the different traits were integrated into unique QTL.

In the case of epistasis the estimated effect in the DH population is the additive × additive genetic interaction. We conducted digenic epistatic analysis using QTLNetwork 2.0 (http://www.cab.zju.edu.cn/ics/faculty/zhujun.htm) based on a mixed-model approach (Wang et al. 1999). The critical F value was calculated by permutation analysis with 1,000 repetitions and QTL effects were estimated by Monte Carlo Markov Chain. The significance threshold was chosen at $\alpha = 0.05$ for declaring a candidate intervals and its associated genetic effects.

Results

Phenotypic variation for silique length and seeds per silique as well as seed weight in the DH population of 'HZ396' × 'Y106'

The phenotypic performances for the parental lines and the DH population were carried out over 3 years and two locations (Table 1). The two parents (HZ396 and Y106) differed significantly for *SS* and *SL*. Y106 had higher-*SS* and longer silique than HZ396 in all four environments. However, the *SW* of the two parents in the two environments (2007 and

Table 1 Statistical analysis of seeds per silique and seed weight as well as silique length for the parental lines and the doubled haploid population

Years	Locations[a]	Traits	Parents			DH population				
			HZ396 (mean ± SD)	Y106 (mean ± SD)	*T* test for parents	Range	Mean ± SD	CV (%)	Skew	Kurt
2007	Wuhan	*SS*	11.0 ± 2.6	27.3 ± 3.7	19.14*	4.5–31.5	21.7 ± 5.9	27.26	−0.76	−0.25
		SL	3.64 ± 0.60	5.78 ± 0.39	13.63*	2.99–6.30	4.79 ± 1.11	23.17	−0.19	−0.25
		SW	4.46 ± 0.41	4.31 ± 0.15	5.63	1.52–5.20	3.32 ± 0.71	21.39	0.41	−0.37
2008	Wuhan-I	*SS*	11.5 ± 2.3	26.7 ± 2.0	28.59*	4.4–32.6	21.28 ± 7.29	34.26	−0.61	−0.87
		SL	3.39 ± 0.28	6.12 ± 0.60	13.01*	2.57–6.00	4.63 ± 0.74	15.98	−0.31	−0.6
		SW	4.11 ± 0.22	3.89 ± 0.17	5.79	2.09–5.1	3.23 ± 0.72	22.29	0.74	−0.28
	Wuhan-II	*SS*	7.7 ± 3.1	25.90 ± 2.1	21.28*	4.0–31.8	20.46 ± 6.44	31.48	−0.67	−0.32
		SL	3.38 ± 0.43	5.31 ± 0.45	13.92*	2.78–6.60	4.7 ± 0.73	15.53	−0.22	−0.11
2009	Gansu	*SS*	13.2 ± 2.4	29.0 ± 1.7	14.95*	3.7–35.3	23.44 ± 8.11	34.60	−0.70	−0.73
		SL	3.69 ± 0.14	6.84 ± 0.45	16.73*	2.71–7.40	5.07 ± 0.86	16.96	−0.30	0.09

SS seeds per silique, *SL* silique length, *SW* seed weight, *DH* double haploid, *CV* coefficient of variation

* Represents the significance in 0.05 level, $T_{(1,\ 0.05)} = 12.71$

[a] Wuhan-I, the plant materials planted on 21st September in Wuhan; Wuhan-II, the plant materials planted on 6th October in Wuhan

2008-I) was not significant. Among the DH lines, individual DH line differed significantly in *SS* in all 3 years. Many DH lines had *SS* values between the two parents, some DH lines exhibited values outside the lower or higher-*SS* parental lines, suggesting that alleles with positive effects for *SS* are distributed among the parents. These transgressive lines, *SS* of which over the four environments differed little in degree [see Table S1 of the Electronic Supplementary Material (ESM)], may provide useful germplasm for breeding. Similar results were found in the traits of *SL* and *SW* in the DH population. Because both skewness and kurtosis values of *SS* and *SL* as well as *SW* were less than 1.0 in the four data sets, the segregation pattern of the three traits appeared to fit a normal distribution model suitable for QTL identification.

A variance analysis of genotype × year interaction is presented in Table S2 (ESM). Significant differences among the DH lines were detected, but the year effects and the genotype × year interaction effects were not significant. This demonstrated that genetic influence on silique-traits was pervasive in the 3 years.

Correlation analysis of silique length and seeds per silique as well as seed weight

The genetic correlations and heritability of *SL, SW* and *SS* are summarized in Table 2. Significant phenotypic and genetic correlations were observed for all the traits in the four data sets. The evaluation of the genetic and phenotypic correlations between *SS* and the different traits showed highly significant positive correlations with a high correlation coefficient to *SL* (0.76 and 0.73, respectively) and significant negative correlations to *SW* (−0.57 and −0.62, respectively). The significant negative correlation between *SS* and *SW* suggested competition for assimilates at the silique development stage. For *SL* and *SW*, the genetic and phenotypic correlation was significant but low (−0.44 and −0.37, respectively). *SS,* among all the silique-traits, notably showed the high coefficients of determination with the other two traits (0.56 and 0.29, respectively). The broad-sense heritability of the three silique-traits in the DH population was about 0.80, indicating that the genotype × environment interaction was limited and the silique-traits of *SS* and *SL* more or less unaffected by planting date in a planting date trial.

Marker screening and linkage map construction

Of the 866 SSR primers, 153 (17.7%) were found polymorphic between 'HZ396' and 'Y106', resulting in 171 markers. AFLP were directly used to survey DNA polymorphism in the DH population. The screening of two different restriction enzyme combinations, i.e., *Eco*RI/*Mse*I (E/M) and *Sac*I/*Taq*I (S/T) resulted in the detection of 220 markers. In total, 391 markers were obtained. Then the locus genotypic frequency was calculated. The locus genotypic frequency distribution in the DH population is shown in Fig. 1. The locus genotypic frequency originated from the parental line 'HZ396' varied from 0.27 to 0.85 while the frequency of that derived from 'Y106' ranged from 0.18 to 0.79. Furthermore, the locus genotypic frequency majority fell between 0.41 and 0.60. In total, the locus genotypic frequency from the parental line 'HZ396' in the DH population was 0.51 while that from 'Y106' was 0.49. The fit of the parental allele segregations to the

Table 2 Genetic and phenotypic correlations (below diagonal) and determination coefficient (above diagonal) among silique length and seeds per silique as well as seed weight, and the broad-sense heritability of the three silique-traits in the doubled haploid population

Trait	*SS*	*SL*	*SW*
SS		0.56[b]	0.29
SL	0.76** (0.73)**[a]		0.19
SW	−0.57** (−0.62)**	−0.44** (−0.37)**	
[c]h^2(%)	82.5	76.9	87.5

For abbreviation, see Table 1

[c] h^2, broad-sense heritability

** Significant at $P = 0.01$

[a] Genetic and phenotypic correlations between two silique-related traits, values in the parentheses are phenotypic correlations

[b] Determination coefficient of the trait with another trait

expected 1:1 segregation ratio was tested by a χ^2 test ($\chi^2 = 0.2 < \chi^2_{(1,0.05)} = 3.84$), indicating that the DH population was a random population suitable for QTL mapping.

Then the 391 markers were used to construct a linkage map and 345 markers, which included 150 SSR and 195 AFLP markers, respectively, were assigned to 19 linkage groups (Fig. 2). The linkage map had a total length of 1,759.6 cM with an average interval of 5.1 cM between adjacent markers. The number of markers in different linkage group varied from 5 to 32 and the length of different linkage group ranged between 25.27 and 175.07 cM. The interval between adjacent markers in each linkage group ranged from 2.41 to 9.32 cM. Three big gaps located on N2, N13 and N14 were 28.8, 31.5 and 29.4 cM, respectively, due to limited marker polymorphisms in those regions. The 19 linkage groups were assigned to the public linkage maps by anchor SSR markers in bold italics (Fig. 2) when at least two SSR markers in each linkage group were consistent with the published linkage maps. The order of most markers is consistent to the maps of Piquemal et al. (2005) and Cheng et al. (2009).

Linkage group regions for allelic frequency skewed from the ratio 1:1 was analyzed by a chi-square test in the doubled haploid population (Table S3). A proportion of loci showed segregation distortion in the DH populations: 66 loci (19.1%) showed distorted segregation ratio ($P < 0.05$). Some loci with skewed segregation to 'HZ396' (female parent) tended to cluster on N2, N6, N9 and N15. And some loci with skewed segregation to 'Y106' tended to cluster on N11 and N13. Though there were markers deviating significantly from the expected 1:1 segregation ratio, there was adequate distribution of markers in different linkage group.

Mapping QTL for silique length and seeds per silique as well as seed weight

A total of 26 putative QTL that influenced *SS* and *SL* as well as *SW* at different environments were identified using WinQTL cartographer software (Table 3; Fig. 2). These QTL were mapped onto 10 linkage groups (N2, N3, N4, N7, N8, N12, N13, N14, N16, N19). Of them, the QTL *qSL*.N8-1 was consistently detected in the four data sets. The 3 QTL (*qSL*.N3-1, *qSS*.N8-1 and *qSS*.N8-2) were detected in three of the four data sets. Some of them, i.e. *qSL*.N12, *qSL*.N13-1, *qSL*.N16, *qSS*.N8-4, *qSS*.N13-1, *qSS*.N13-2, *qSW*.N7-2, were detected in two of the four data sets.

Due to the confidence intervals of the putative QTL for each trait in different experiments overlapped, meta-analysis integrated these putative QTL into consensus QTL trait by trait (Table 4). As a result, the 26 putative QTL were integrated into 10 reproducible consensus QTL. Of them, 5, 2 and 3 consensus QTL were detected for *SL* and

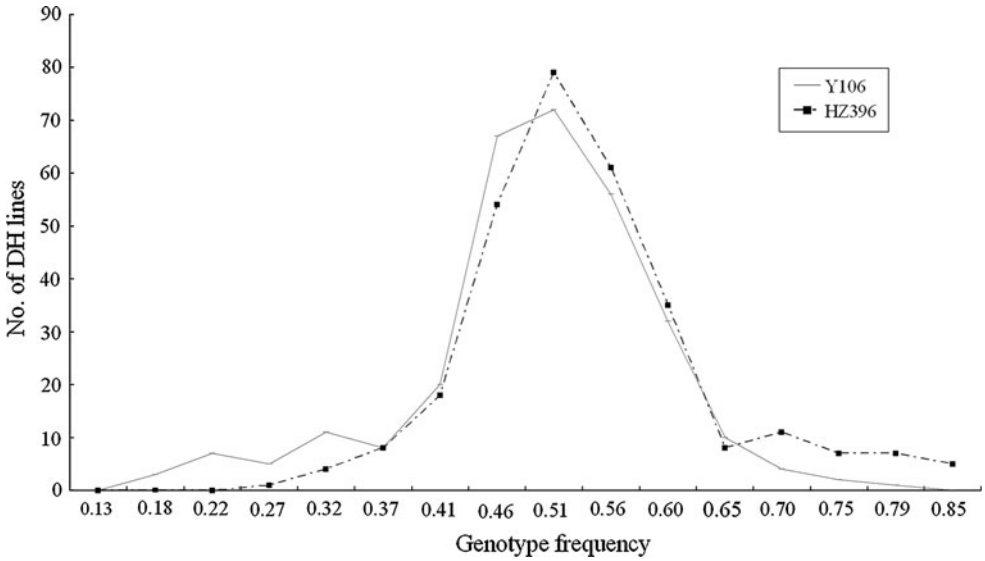

Fig. 1 The locus genotypic frequency distribution in the doubled haploid population developed from the cross of 'HZ396' × 'Y106'

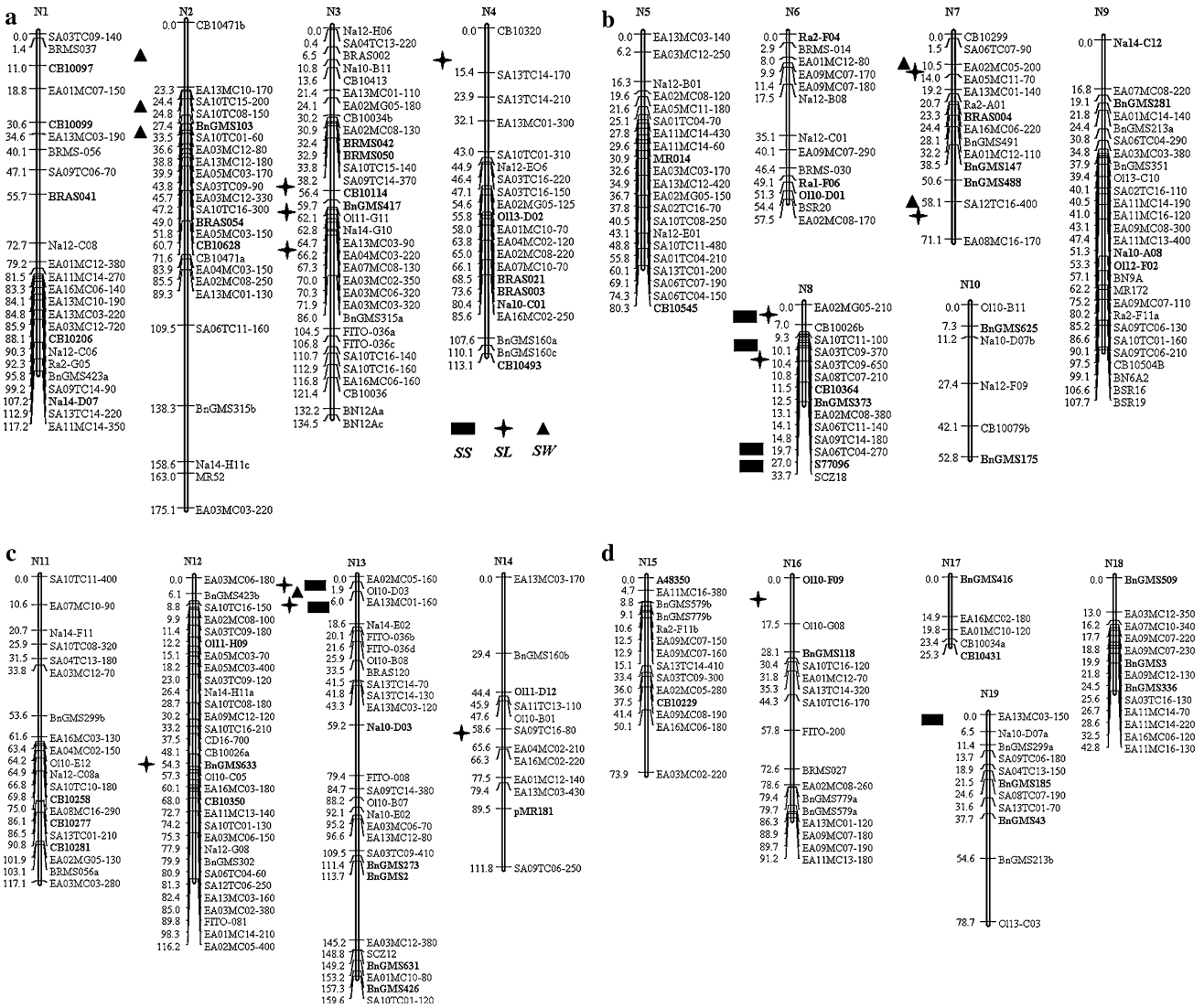

Fig. 2 Genetic linkage map of *Brassica napus* from the cross 'HZ396' × 'Y106'. The linkage groups were constructed from 140 doubled haploid lines using SSR and AFLP markers with public SSR markers from Piquemal et al. (2005) and Cheng et al. (2009) as anchors (*bold italics*). The framework map positions are estimated from recombination frequencies according to Kosambi's mapping function. The *squares*, *stars* and *triangles* indicate QTL identified for seeds per silique (*SS*), silique length (*SL*) and seed weight (*SW*), respectively

SS as well as *SW*, respectively. Some consensus QTL, i.e. *cqSL*.N3 and *cqSL*.N8, *cqSS*.N8 and *cqSS*.N13, *cqSW*.N2 and *cqSW*.N7, showed main effects and the others exhibited minor effects. Positive alleles for *SS* and *SL* were dispersed between the two parents while positive alleles encoding *SW* were originated from the parental line Y106 with the exception of *qSW*.N7-1.

Silique length

Thirteen putative QTL that influenced *SL* at different environments were identified using WinQTL cartographer software. The accumulated contribution of these putative QTL at different data sets explained 55.82, 45.84, 35.72 and 46.48% of the phenotypic variance, respectively. 5 consensus QTL were mapped in the DH population using meta-analysis. Two of these loci, on linkage group N3 and N8 had been identified as main effect QTL. For the QTL of *cqSL*.N8 and *cqSL*.N13, the female parent 'HZ396' contributed the increasing alleles. For the QTL of *cqSL*.N3, *cqSL*.N12 and *cqSL*.N16, the positive allele for silique length was inherited from 'Y106'.

Seeds per silique

In total, seven putative QTL that influenced *SS* were identified using WinQTL cartographer software. The phenotypic variance explained by these putative QTL at different data

Table 3 Putative QTL for silique length, seeds per silique and seed weight in the doubled haploid population

Putative QTL	Linkage group[a]	Position (cM)	2007			2008						2009		
			Wuhan			Wuhan-I			Wuhan-II			Gansu		
			LOD	A[b]	R^2 (%)[c]	LOD	A	R^2 (%)	LOD	A	R^2 (%)	LOD	A	R^2 (%)
qSL.N3-1	N3	42.2–56.4				2.84	0.25	11.16	2.82	0.28	9.16	2.79	0.29	10.70
qSL.N3-2	N3	56.5–61.7				3.54	0.23	8.85						
qSL.N3-3	N3	62.1–67.2										2.63	0.22	6.48
qSL.N4	N4	0.6–18.7	2.19	−0.19	7.08									
qSL.N7-1	N7	11.1–18.0							2.08	0.18	5.71			
qSL.N7-2	N7	57.9–68.1	2.81	0.19	7.90									
qSL.N8-1	N8	0.0–3.8	8.15	−0.30	18.80	4.11	−0.24	10.41	4.56	−0.37	13.14	3.52	−0.27	8.93
qSL.N8-2	N8	16.7–26.6	2.16	0.28	7.71									
qSL.N12	N12	43.1–55.3	3.06	0.18	7.09	3.48	0.24	9.6						
qSL.N13-1	N13	0.0–5.5	3.31	−0.18	7.02							3.14	−0.25	7.83
qSL.N13-2	N13	0.0–15.8				2.24	−0.18	5.86						
qSL.N14	N14	53.8–65.6										2.31	0.21	5.67
qSL.N16	N16	0.0–8.6	3.72	0.20	7.92				2.79	0.21	7.71	2.77	0.24	6.87
Total					55.82			45.84			35.72			46.48
qSS.N8-1	N8	0.0–2.7				2.11	−1.82	6.04	4.38	−3.10	12.23	2.14	−2.05	6.16
qSS.N8-2	N8	9.3–10.8	2.36	2.71	10.60	2.37	3.40	10.15				2.92	4.07	7.28
qSS.N8-3	N8	17.5–25.9							2.90	2.73	9.63			
qSS.N8-4	N8	19.7–26.3	2.52	2.35	8.35	2.41	2.83	8.27						
qSS.N13-1	N13	0.0–5.2	3.59	−1.77	10.72							5.79	−3.21	15.21
qSS.N13-2	N13	0.0–11.4				3.42	−2.31	9.70	3.33	−2.06	9.83			
qSS.N19	N19	0.0–5.8										2.47	2.07	6.11
Total					29.66			34.16			31.68			34.75
qSW.N2-1	N2	0.0–19.0				2.25	0.22	8.34						
qSW.N2-2	N2	24.0–24.8				3.67	0.42	10.98						
qSW.N2-3	N2	27.4–32.3	3.23	0.46	13.59									
qSW.N7-1	N7	4.1–17.8				2.06	−0.20	6.28						
qSW.N7-2	N7	47.5–66.7	3.11	0.23	10.38	3.20	0.27	11.70						
qSW.N13	N13	0.0–10.4	2.01	0.39	7.30	2.13	0.19	7.10						
Total					31.27			44.41						

For abbreviation, see Table 1

[a] N followed by a number designates the linkage group where the QTL was detected

[b] Additive effect: positive additivity indicate that the QTL allele originated from the parental line Y106; negative additivity means that the QTL allele originated from the parental line HZ396

[c] Percentage of the phenotypic variance explained by each QTL

sets were 29.66, 34.16, 31.68 and 34.75%, respectively. And 2 consensus QTL *cqSS*.N8, *cqSS*.N13 were detected in the DH population and showed main effects. The effect of *cqSS*.N13 was negative, meaning that the allele of the parent 'HZ396' increased *SS*. The QTL *cqSS*.N8 and *qSS*.N19 showed positive additive effects, indicating that the parent 'Y106' contributed the favorable alleles.

Seed weight

Six putative QTL, which explained 31.27 and 44.41% of the total phenotypic variance, respectively, were mapped in the DH population in 2007 and 2008. Most of the putative QTL showed positive additive effects, indicating the parent 'Y106' increased *SW*, with the exception of the QTL *qSW*.N7-1. 3 consensus QTL, *cqSW*.N2, *cqSW*.N7 and *cqSW*.N13, were detected in the DH population. Of them, *cqSW*.N2 and *cqSW*.N7 showed main effects.

Dissection of pleiotropic unique QTL

All pairs of the silique-traits showed significant genetic correlation in Table 2, which is also reflected by the genomic location and effects of QTL detected. In the

Table 4 The list of ten consensus QTL obtained after meta-analysis of 26 putative QTL for seeds per silique and seed weight as well as silique length separately

Consensus QTL	Linkage group	Peak position (cM)	LOD2_L[a]	LOD2_R	Confidence interval	A[b]	QTL type[c]
cqSL.N3	N3	53.75	43.70	63.81	20.11	0.29	Major QTL
cqSL.N8	N8	1.68	0.00	8.53	8.53	−0.30	Major QTL
cqSL.N12	N12	50.11	43.10	55.31	12.21	0.24	Minor QTL
cqSL.N13	N13	2.38	0.00	18.22	18.22	−0.25	Minor QTL
cqSL.N16	N16	0.01	0.00	18.19	18.19	0.24	Minor QTL
cqSS.N8	N8	11.31	3.37	19.25	15.88	4.07	Major QTL
cqSS.N13	N13	2.68	0.00	11.22	11.22	−3.21	Major QTL
cqSW.N2	N2	27.44	16.52	38.35	21.83	0.46	Major QTL
cqSW.N7	N7	60.11	47.92	72.30	24.38	0.27	Major QTL
cqSW.N13	N13	2.88	0.00	21.60	21.60	0.39	Minor QTL

[a] LOD2_L and LOD2_R represent the left and right position of the 95% confidence interval of the consensus QTL position in the analysis

[b] Additive effect: positive additivity indicate that the QTL allele originated from the parental line 'Y106'; negative additivity means that the QTL allele originated from the parental line 'HZ396'

[c] Major QTL: those occurring at least once with $R^2 \geq 20\%$ or at least twice with $R^2 \geq 10\%$; minor QTL: the remainder with relatively small effect

Table 5 The list of 5 pleiotropic unique QTL obtained after meta-analysis of 26 putative QTL for each linkage group separately

Unique QTL	Linkage group	Peak position (cM)	LOD2_L[a]	LOD2_R	Confidence interval (cM)
uq.N7-1	N7	12.1	0.0	18.0	18.0
uq.N7-2	N7	61.3	50.5	72.2	21.7
uq.N8-1	N8	1.2	0.0	7.0	7.0
uq.N8-2	N8	22.7	11.5	33.9	22.4
uq.N13	N13	2.6	0.0	10.1	10.1

[a] LOD2_L and LOD2_R represent the left and right position of the 95% confidence interval of the consensus QTL position in the analysis

second round of QTL meta-analysis, five unique QTL are found and located on linkage groups N7, N8 and N13 (Table 5). These unique QTL all showed pleiotropic effects. For the unique QTL on N13, the additive effect of *cqSW*.N13 showed a positive effect while the additive effects of *cqSS*.N13 and *cqSL*.N13 were negative at the same locus in the linkage group N13, which is a hint for the reason of positive correlation coefficient between *SS* and *SL* while negative correlation coefficient between *SS* and *SW*. For the unique QTL on N8, the increasing allele of *uq*.N8-1 for *SS* and *SL* was inherited from 'HZ396' and the increasing allele of *uq*.N8-2 for *SS* and *SL* was derived from 'Y106', a result that reflected the significant positive correlation between *SS* and *SL*. For the unique QTL on N7, *SL* and *SW* exhibited the opposite direction of parental contribution for *uq*.N7-1 while *SL* and *SW* exhibited the same direction of parental contribution for *uq*.N7-2. This result might explain the reason of the significant, but low negative correlation between *SL* and *SW*.

Analysis of epistatic effects and epistasis × environment interactions

The results of epistasis analysis that influenced *SS* and *SL* as well as *SW* are listed in Table 6. 13 loci involved in 7 digenic epistatic interactions were identified in the DH population. These loci covered 7 linkage groups (N3, N4, N5, N9, N11, N12, N15). All the epistatic interactions showed significant additive by additive effects, but no significant epistasis by environment effect was identified. No loci with significant main effects were included in epistatic interactions for these silique-traits.

Silique length

Three digenic interactions among six loci were detected in the DH population. The phenotypic variance explained by the three digenic interactions was statistically significant, but low. Together, they explained 8.94% of the phenotypic

Table 6 Estimated epistatic (aa) and epistasis × environment interaction (aae) effects of QTL for silique length, seeds per silique and seed weight

Traits	Ch-Ini[a]	Marker interval	Ch-Inj	Marker interval	aa_{ij}^{b}	R^2 (%)[c]	R^2 (%) (aae)[d]
SL	4–6	Na12-EO6/ SA03TC16-220	9–19	EA09MC07-110/ Ra2-F11a	−0.25**	2.80	0.00
	5–17	SA01TC04-210/ SA13TC01-200	12–3	SA10TC16-150/ EA02MC08-100	0.16**	3.23	0.15
	12–22	EA03MC06-150/ Na12-G08	15–8	SA13TC14-410/ SA03TC09-300	−0.14**	2.91	0.00
Total						8.94	0.15
SS	3–18	EA13MC03-90/ EA04MC03-220	5–2	EA03MC12-250/ EA13MC03-140	−2.91**	18.91	0.16
	5–17	SA01TC04-210/ SA13TC01-200	12–1	EA03MC06-180/ BnGMS423b	2.25**	4.90	0.14
Total						23.81	0.30
SW	11–17	CB10281/ EA02MG05-130	12–19	CB10350/ EA11MC13-140	0.22**	13.40	0.00
	11–19	BRMS056a/ EA03MC03-280	12–19	CB10350/ EA11MC13-140	−0.24**	1.81	0.00
Total						15.21	0.00

** Significant at the 0.01 probability level

[a] Ch-Ini and Ch-Inj represent the linkage group number-interval of the points being tested in the analysis

[b] aa_{ij} is the effect of the interaction between the points i and j. Positive values of the aaij effect indicate that the two-locus genotypes are the same as those in the female or the male parent and will increase the phenotypic traits values, while the recombinants have negative effects

[c] Percentage phenotypic variance explained by additive × additive epistasis

[d] No significant epistasis by environment effect was identified at Wuhan in 2007, Wuhan-I and Wuhan-II in 2008, Gansu in 2009

variation for *SL*. Two epistatic effects were negative, indicating that recombinant allele combinations could increase *SL* values. Another epistatic effect was positive, meaning that parental allele combinations contributed for longer silique.

Seeds per silique

There were two pairs of digenic epistatic interactions among 4 loci for *SS,* which explained 23.81% of the total phenotypic variance. One significant epistatic interaction between intervals EA13MC03-90/EA04MC03-220 and EA03MC12-250/EA13MC03-140 accounted for 18.91% of the phenotypic variance, which explained a considerably high portion of total variance. This epistatic effect was negative, which indicated that recombinant allele combinations instead of parental allele combinations could increase *SS* values.

Seed weight

Two pairs of digenic epistatic interactions among 3 loci for *SW* explained 15.21% of the total phenotypic variance. Of them, the epistatic interaction between intervals BRMS056a/EA03MC03-280 and EA03MC12-250/EA13MC03-140 accounted for 13.40% of the phenotypic variance, which was positive, demonstrating that parental allele combinations could increase *SW*. The other digenic interaction was statistically significant, but low.

Discussion

Double haploids, which have inherent advantages of providing a constant DNA supply and phenotyping opportunities for many different studies, have been used most often in rapeseed (Chen et al. 2007; Quijada et al. 2006; Radoev et al. 2008; Udall et al. 2006). In this study, we used a DH population, derived from the cross between two genotypes of contrasting *SS* and *SL*, to identify the genetic basis of the silique-traits at QTL level. From the results of QTL mapping (Tables 3, 6), it was found that for *SL* and *SS* as well as *SW* the mean phenotypic variance explained by additive effects was 45.97, 32.56 and 37.84%, respectively, while the phenotypic variance explained by epistasis was 8.94, 23.81 and 15.21%, respectively. It demonstrated that additive effects were more important than epistatic effects for *SL* while additive effects together with epistasis were

responsible for genetic variations of *SS* and *SW* in rapeseed. Similar results were found in previous studies. Wei (2000) found that additive effects were important in the genetics of the silique-traits, as similarly reported by Li and Gu (1992) and Zhang et al. (2008). Radoev et al. (2008) revealed that epistasis for *SS* and *SW* played an important role in the variation of the performance of the DH lines in rapeseed.

QTL meta-analysis revealed that ten reproducible consensus QTL with additive effects for the three silique-traits. Some of them had been detected by previous studies. The consensus QTL *cqSL*.N8, which was consistently detected in the four data sets, was similar as the one reported by Shi et al. (2009). Chen et al. (2007) used DH and immortalized F_2 populations to identify QTL of *SL* and found that there was one QTL in the linkage group N12, which was similar to *cqSL*.N12 in our paper. Udall et al. (2006) identified QTL associated with *SW* in linkage group N7, which was similar to *cqSW*.N7 in our study. The comparisons of different QTL mappings for the silique-traits would facilitate an assessment of intraspecific variation for these QTL.

All consensus QTL were repeatedly found at least two times in the different experiments. There is one or combination of the following two factors for the existence of stable QTL. The first explanation is that the stable QTL were responsible for major genetic effects and were associated with high LOD scores. As suggested by Tanksley (1993) and Zhuang et al. (1997), QTL with major effects are more likely to be stable across multiple environments. As mentioned above, *cqSS*.N8 and *cqSW*.N2 as well as *cqSW*.N7 are major QTL. The other explanation is that highly heritable traits tend to be more repeatable and stable across multiple environments (Paterson et al. 1991). From Table 2, the broad-sense heritability of the three silique-traits in the DH population was all high. Fine-mapping these stable QTL and validating the potential candidate genes is a reliable and feasible strategy for QTL cloning (Burgess-Herbert et al. 2008; Hu et al. 2008; Norton et al. 2008).

Though the gene networks affecting the silique-traits in rapeseed remained unknown, correlation analysis of QTL detected in the same DH population might provide insights. Five unique QTL were located on linkage groups N7, N8 and N13 and all showed pleiotropic effects. These pleiotropic unique QTL, in a certain sense, reflected the result of Chay and Thurling (1989) for the significant positive correlation between *SS* and *SL* and of Liu (1987) for the significant negative correlation between *SW* and *SS*. The high genetic and phenotypic correlation coefficients among the silique-traits further supported this conclusion.

High yield has been a major breeding target in rapeseed. However, a significant negative correlation for *SS* and *SW*, which are two important components of yield, make difficult simultaneous improving the two traits by conventional breeding. In other word, it is normally difficult to increase *SS* without decreasing *SW*. QTL detected in the present study may provide useful information for improving these traits by marker-assisted selection (MAS). For *SS*, there were *cqSS*.N8 and *qSS*.N19 whose effects were independent of *SW*. For *SW*, there were *cqSW*.N2 and *cqSW*.N7 whose effects were independent of *SS*. Moreover, most of these QTL were stable in most environments. Thus, we believe that MAS with markers located on the two sides of QTL, such as *cqSS*.N8 and *cqSW*.N7 and so on, would have good potential to increase the efficiency of breeding programs seeking high yield of cultivars or improved parents of hybrids.

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