

Genetic dissection of seed weight and seeds per silique by conditional QTL mapping in *Brassica napus*

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Abstract

Seed weight (SW) and seeds per silique (SP) are important components of yield in rapeseed. To analyze the genetic basis for this two complex quantitative traits and evaluate their genetic interrelationships and with respect to oil content, unconditional and conditional QTL mapping were conducted using SG-DH population over seven environments. The results showed that twelve and seven putative QTL for SW and SP were identified and together explained 74.75% and 40.79% of the total phenotypic variation in population, respectively. Comparison between unconditional and conditional mapping study revealed that *SPA1* and *SPA2* were independent from SW and three (*SPA1*, *SPA7* and *SPC1*) positively related to oil content, while another two QTL for SP (*SPA3* and *SPA6*) showed no interference by SW and OIL and six loci for SW (*SWA1*, *SWA4*, *SWA7*, *SWA9*, *SWC1-1* and *SWC8*) were independent from SP and OIL. So the possibilities for increasing seeds per silique without influencing seed weight, combining the oil content and seeds per silique or even jointly improve three of them in rapeseed simultaneously by marker assistant selection potentially exist

Key words: conditional QTL mapping, seed weight, seeds per silique, oil content, rapeseed.

Introduction

Developing high yield varieties is a major goal in rapeseed breeding. Of three yield components in rapeseed, seed size/weight (SW) and the number of seeds per silique (SP) are considered as the more suitable traits for genetic improvement than the number of siliques per plant because of their relatively high heritability. However, a significant negative correlation between these two traits was frequently observed and to a certain extent related with oil content. Therefore, a better understanding for their genetic control system and with respect to oil content is of important. To date, a number of QTL for SW and SP have been identified, but the genetic correlativity between two traits and with oil content at the QTL level have not yet been reported. In this research, by integrating the statistical procedures for analyzing conditional genetic effects into a QTL mapping program, a conditional mapping method was performed to dissect the complex relationships among SW, SP and oil content (OIL) at individual QTL, and to estimate the potentials of combining seed size, seeds number per silique as well as oil content at a maximum level simultaneously in breeding programs.

Materials and Methods

Mapping population and phenotypic data: Phenotypic data of seed weight, seeds per silique as well as oil content of the SG population (Zhao *et al.* 2005) were collected from seven experiments with two replications, 4 of them was tested in the year of 2000-2001 (Zhao *et al.* 2005) (coded 01X, 01H, 01R and 01We) and the other three were conducted in the years of 2004-2005, 2006-2007 and 2008-2009, respectively, in Hangzhou of China (coded 05H, 07H and 09H).

Data analysis and QTL mapping: The broad sense heritability of SW and SP was estimated as $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2 / n + \sigma_e^2 / nr)$. Phenotypic correlations analysis among SW, SP and OIL were performed using SPSS Version 11.5. Conditional phenotypic values were obtained by the method described by Zhu (1995), where y (Trait1|Trait2) indicates the phenotypic value of Trait1 was based on excluding the phenotypic influence of Trait2. With a newly updated SG map comprising 477 markers (map not shown), unconditional and conditional QTL analysis were conducted by composite interval mapping (Zeng 1993,1994) procedure of QTL Cartographer Version 2.5. A LOD threshold of 2.5 was used as the significance level for QTL detection.

Results

Phenotypic variation within DH population and correlation among traits: the phenotypic performance of SW and SP for the two parents and DH lines were summarized in Table 1. Two parents differed significantly for the two investigated traits. Sollux showed higher SW value at all 7 environments than Gaoyou, while except two locations in Germany, Gaoyou exhibited more SP than Sollux at all five Chinese environments. Large variation and transgressive segregation were observed for both traits in each environment. The broad-sense heritability for SW and SP were estimated up to 0.86 and 0.84, respectively, denoting high genetic contributions to these two yield components.

Table 1 Phenotypic values and correlation coefficients among seed weight, seeds per silique and oil content in SG-DH population

Traits	Trials	Parents		DH population		h^2_B	Correlation	
		Sollux	Gaoyou	Mean±SD	Range		SP	OIL
SW	01X	3.37	3.19	3.28 ± 0.51	2.27-5.60	0.86	-0.16**	0.13**
	01H	3.33	3.08	2.71 ± 0.39	1.86-4.21		-0.20**	0.15**
	01R	5.62	4.40	4.78 ± 0.60	3.17-6.61		-0.27**	0.03
	01We	5.63	4.36	4.79 ± 0.58	3.53-6.50		-0.24**	0.04
	05H	3.04	2.46	2.89 ± 0.39	2.17-4.47		-0.12**	-0.09
	07H	3.01	2.72	2.80 ± 0.31	2.16-3.93		-0.20**	-0.06
	09H	3.23	2.74	3.05 ± 0.32	2.47-4.12		-0.17**	-0.09
SP	01X	14.00	24.30	21.10 ± 3.35	12.70-30.90	0.84		0.23**
	01H	16.00	20.20	20.00 ± 2.30	12.00-25.30			0.12**
	01R	24.50	20.60	21.60 ± 3.17	10.00-28.30			0.30**
	01We	22.00	21.80	21.00 ± 3.14	9.90-28.80			0.23**
	05H	18.10	24.53	20.71 ± 2.68	10.00-27.55			0.14**
	07H	18.75	26.70	23.47 ± 2.41	15.75-29.10			0.30**
	09H	18.80	27.33	23.77 ± 2.82	14.55-32.05			0.23**

** indicate significant at $P=0.01$

The phenotypic correlations among SW, SP and OIL were listed in Table 1. SW and SP were significantly correlated in negative way ($r=-0.12$ to -0.27 , $P<0.01$), while significant positive correlation was observed between SP and oil content ($r=0.12$ to 0.30 , $P<0.01$) across all 7 environments. However, no significantly correlations between SW and OIL in five of seven environments were identified. These findings illustrated a strong genetic association between SP and other two traits, but a less association between SW and OIL.

Unconditional mapping: A total of 12 and 7 putative QTL (detected in at least two environments) for SW and SP were identified, which together explained 74.75% and 40.79% of the total phenotypic variation, respectively (Table 2). Positive alleles for SW and SP were both dispersed between two parents. Of them, Sollux alleles increased SW and SP at five and three genomic loci, while Gaoyou alleles favored to two traits at the remaining seven and four loci, respectively. For seed weight, three QTL (*SWA1*, *SWA7* and *SWC8*) were stably identified in more than five environments, in which, *SWA7* and *SWC8* showed most significant additive effects (0.14 and -0.16) and explained in average of 10.04% and 13.88% phenotypic variation within population. The rest of nine QTL were detected in two to four environments and contributed to the trait from 2.98 to 8.50%. Of seven QTL for seeds per silique, two major QTL *SPA6* and *SPA7* were both found in five environments and demonstrated strong additive effect of -0.86 and -0.77 and the remaining five were detected in 2 to 4 environments. Four putative QTL for SW were co-localized with SP on A2, A7, A9 and C1, except one on A2, the other three showed the opposite direction of additive effects.

Table 2 Putative QTLs for seed weight and seeds per silique in the SG-DH population based on unconditional and conditional mapping

QTL	SW						SW SP			SW Oil		
	Interval ^a	Peak ^b	LOD ^b	A ^c	R ² (%) ^d	E ^e	A ^c	R ² (%) ^d	E ^e	A ^c	R ² (%) ^d	E ^e
SWA1	HMR293-HMR295c	27.4-41.6	3.54-4.90	0.11	5.74	5	0.12	7.59	6	0.12	6.57	5
SWA2	RF32-ZAAS819a	2.0-10.0	2.59-7.70	-0.11	6.49	2	-0.13	8.33	2	-	-	-
SWA3	EM4ME8d-ZAAS474	44.9-53.0	2.59-2.94	0.08	2.98	2	0.08	2.98	2	0.09	3.14	2
SWA4	SHB-ZAAS1050	14.3-24.7	3.16-5.41	-0.13	5.13	3	-0.11	4.85	4	-0.15	6.10	2
SWA7	ZAAS864-BNmRNA	2.4-22.5	3.35-11.53	0.14	10.04	6	0.12	7.41	6	0.14	9.31	6
SWA9	MR230.1-ZAAS435b	107.3-140.3	2.90-9.16	-0.12	8.50	3	-0.14	7.32	3	-0.13	8.74	4
SWC1-1	ZAAS315-CB10369	6.2-8.2	2.70-6.53	-0.10	4.76	4	-0.10	5.48	4	-0.10	4.34	4
SWC1-2	ZAAS337/336-ZAAS733a	94.9	2.82-4.70	0.09	4.78	2	-	-	-	-	-	-
SWC2	ATH-B-EM7M5f	26.4-36.7	2.92-11.66	-0.11	4.19	2	-0.09	4.18	3	-	-	-
SWC4	Na12E06A-CB10092	31.9-39.9	3.12-3.67	-0.07	4.65	3	-0.08	6.19	3	-0.08	4.96	3
SWC7	Au39-ZAAS376	88.1-96.0	2.73-4.34	0.08	3.62	4	0.08	5.32	3	0.08	3.93	4
SWC8	ZAAS433-ZAAS406	8.2-18.3	7.21-13.43	-0.16	13.88	6	-0.15	11.31	7	-0.16	13.56	6

Table 3 Putative QTLs for seed weight and seeds per silique in the SG-DH population based on unconditional and conditional mapping

QTL	SP						SP SW			SP Oil		
	Interval ^a	Peak ^b	LOD ^b	A ^c	R ² (%) ^d	E ^e	A ^c	R ² (%) ^d	E ^e	A ^c	R ² (%) ^d	E ^e
SPA			2.93-									
1	NIP3-ZAAS165	72.1-80.2	6.18	0.81	6.59	4	0.88	8.39	4	0.71	5.53	2
SPA			3.08-									
2	ZAASRF32-ZAAS819a	0.0-4.0	6.20	0.93	6.70	3	0.98	7.75	3	-	-	-
SPA			124.4-									
3	CAS1-Ra2G11	140.9	3.34	0.67	4.48	2	0.65	4.68	2	0.68	4.90	2
SPA			-									
5	-	-	-	-	-	-	-	-	-	0.70	4.72	2
SPA			104.6-									
6	ZAAS556-PTPTP	110.6	6.32	0.86	8.27	5	0.94	9.39	5	0.78	7.48	5
SPA			2.65-									
7	APOC1-ZAAS1020	18.2-40.0	8.52	0.77	6.29	5	0.86	7.17	2	0.77	6.92	2
SPA			111.3-									
9	MR230.1-HMR612b	134.3	3.39	0.67	4.69	2	-	-	-	0.61	4.18	2
SPC			2.84-									
1	ZAAS337/336-ZAAS733a	94.9	2.88	0.62	3.78	2	-	-	-	-	-	-
SPC			-									
8	-	-	-	-	-	-	0.51	0.04	2	-	-	-

QTL nomenclature was designated using the trait name initials followed by linkage group number.

^a, closest marker interval of peak position at different growth environments. ^b, peak position or peak LOD score from the smallest to biggest value of the QTL in different specific environment. ^c, mean additive effect of the QTL detected at different environments; positive effects indicate the increasing allele originated from "Sollux"; negative effects indicate the increasing allele originated from "Gaoyou". ^d, mean proportion for the phenotypic variation explained by the QTL at different environments. ^e, number of growth environments where the QTL could be detected

Conditional mapping: Comparison between unconditional and conditional QTL mapping results, it was shown that for seed weight, one (*SWC1-2*) and two (*SWA2* and *SWC2*) of 12 QTL failed to be detected when conditioned both on SP (*SWC1-2|SP*) and OIL (*SWC1-2|OIL*), and on OIL (*SWA2|OIL* and *SWC2|OIL*), respectively. All the rest of 9 QTL exhibited only small changes in additive effects or in number of environments detected (Table 2). For seeds per silique, *SPC1*, which lied in the same position as *SWC1-2*, failed to be detected after conditional both on SW and OIL, *SPA9* and *SPA2* were no more significant when conditioned on SW and OIL, respectively. The number of environments detected for *SPA7* was reduced from five to two when conditioned on SW and OIL, and for *SPA1* was from four to two under conditional on OIL. However, the rest of two QTL (*SPA3* and *SPA6*) exhibited only slightly increased additive effects in the conditional analysis of *SP|SW* and three QTL (*SPA3*, *SPA6* and *SPA9*) showed independent from OIL. Two additional QTL in A5 and C8 were detected in two environments when respectively conditioned on OIL and SW. These results indicated that the QTL linked to the marker ZAAS733a showed pleiotropic effect for SW and SP with opposite effect directions and influenced by oil content, another two QTL for SW (*SWA2* and *SWC2*) were also

significantly affected by OIL. Seeds per silique could be significantly interfered by SW and OIL at the loci of *SPC1* and *SPA7*, by SW on *SPA9* and by OIL for *SPA1*. On the other hand it was shown that QTL of SW on linkage groups A1, A4, A7, A9, C1-1 and C8 were relatively independent from SP and OIL and exhibited strong additive, while *SPA3* and *SPA6* were not influenced both by SW and OIL, *SPA1* and *SPA2* showed less interference by SW, and *SPA9* was independent from OIL.

Discussion

Thousand Seeds weight and seeds per silique are two of three most important yield components and oil content in seeds is definitely the critical breeding objective. However, the interference among these traits makes difficulty to improve them in practical breeding simultaneously. The present research partly disclosed their complicated relationships through conditional and unconditional QTL mapping study.

Comparison between unconditional and conditional analysis revealed that the significant negative correlation (Table 1) between thousand seeds weight and seeds per silique might be resulted by one QTL for SW (*SWC1-2*) and three for SP (*SPC1*, *SPA7* and *SPA9*), which showed significantly interference each other. Marker assisted selection (MAS) for improving either trait based on these loci might lead to negative effect for another. However, *SPA1* and *SPA2* showed independent from SW, which might be used for MAS to increase seeds per silique without influencing seed weight. The positive correlation between oil content and seeds per silique was supported by the comparative results from three genomic loci (*SPC1*, *SPA7* and *SPA1*), which failed to show significant additive effects when conditioned on oil content (corresponding to three QTL for oil content with the same effect directions, see Zhao et al., 2005). It might suggest the possibility of combining the oil content and seeds per silique by marker aided selection through related genomic loci.

Regardless the complex relationships among SW, SP and OIL, it seems the potentials for jointly improve three of them in rapeseed simultaneously still exist, since six loci for SW (*SWA1*, *SWA4*, *SWA7*, *SWA9*, *SWC1-1* and *SWC8*) were independent from SP and OIL, and two QTL for SP (*SPA3* and *SPA6*) showed no interference by SW and OIL.

References

- Zeng ZB. 1994. Precision mapping of quantitative trait loci. *Genetics* 136: 1457-1468.
- Zhao JY, Becker HC, Zhang DQ, Zhang YF and Ecker W. (2006). Conditional QTL mapping of oil content in rapeseed with respect to protein content and traits related to plant development and grain yield. *Theor Appl Genet* 113: 33-38
- Zhao JY, Becker HC, Zhang DQ, Zhang YF and Ecker W. (2005). Oil content in a European-Chinese rapeseed population: QTL with additive and epistatic effects and their genotype-environment interactions. *Crop Sci.* 45: 51-59.
- Zhu J. (2010) Analysis of conditional genetic effects and variance components in developmental genetics. *Genetics* 141:1633-16