

Oil Content in a European × Chinese Rapeseed Population: QTL with Additive and Epistatic Effects and Their Genotype–Environment Interactions

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ABSTRACT

Rapeseed (*Brassica napus* L.) is one of the most important oilseed crops both in Europe and in China. The objective of this study was to investigate whether the European and the Chinese gene pools of winter oilseed rape contain different alleles for high seed oil content. A linkage map of rapeseed comprising 125 SSR markers and covering 1196-cM genome length was constructed from an F₁ derived doubled haploid (DH) population from a cross between the German cultivar Sollux and the Chinese cultivar Gaoyou, both selected for high oil content. In total, 282 DH lines were evaluated in replicated field experiments in four environments, two each in Germany and in China. QTL were mapped and their additive and epistatic effects as well as interactions with environments were estimated by a mixed model approach implemented in the mapping software QTLMapper. Eight QTL with additive effects and nine pairs of loci with additive × additive epistasis were detected, which together accounted for 80% of the phenotypic variation. The alleles increasing oil content were dispersed between the parents, which explained the transgressive segregation observed: seven DH lines surpassed the better parent by more than 3% oil content. Five of the eight QTL with additive effects showed significant genotype × environment interactions, and 10 additional QTL with genotype × environment interactions, but no significant additive main effect were observed. Epistatic interactions mainly occurred between QTL which also showed additive effects or additive × environment interactions. In conclusion, a marker assisted selection to recombine positive alleles from European and Chinese material is a powerful approach to further increase the oil content of rapeseed, but epistatic effects and genotype × environment interactions have to be considered.

THE MOST IMPORTANT traits in oilseed breeding are seed yield and seed oil content. Whereas seed yield is an extremely complex character, seed oil content might be a suitable trait for the application of marker-assisted selection. However, accumulation of seed oil is influenced by environmental conditions, which makes its genetic control complicated and difficult to understand. Previous studies in oilseed rape (*B. napus*) have indicated that additive gene action is the main genetic factor in the control of seed oil content with dominance and epistasis being not significant (Grami and Stefansson, 1977; Engqvist and Becker, 1991). Using molecular markers, Ecke et al. (1995) and Cheung and Landry (1998) detected three and two quantitative trait loci (QTL) for oil content in *B. napus* and *B. juncea* (L.) Czernj. & Cosson, respectively. However, in both studies two of these QTL showed a close linkage with the two

erucic acid genes of *B. napus* and *B. juncea*, indicating a positive effect of erucic acid synthesis on oil content. Gül et al. (2003) identified six QTL for oil content using the same population as already used by Ecke et al. (1995) but evaluated in multiple environments.

Chinese and European cultivars of *B. napus* were analyzed by isozyme markers and it was shown that they belong to different gene pools, especially if cultivars of non-canola quality are considered (Becker et al., 1995; Zhao and Becker, 1998). Germplasm with high oil content has been selected in both gene pools, and the positive alleles for oil content may represent distinct loci.

The objective of the present study is to analyze QTL for oil content in a population derived from a European × Chinese cross between two cultivars selected for high oil content and to develop genotypes with higher oil content than both parents by combining positive alleles from European and Chinese gene pools. A quantitative genetic analysis should determine the relative importance of epistatic effects and of genotype × environment interactions.

MATERIALS AND METHODS

Plant Material

The segregating doubled haploid (DH) population used for map construction and QTL mapping was developed from a cross between the German winter rapeseed cultivar Sollux and the Chinese variety ‘Gaoyou’. Sollux was released in 1973 by ZG Winterraps (German Democratic Republic). Gaoyou is an inbred line from a cross between the Chinese breeding line ‘695’ and the Japanese cultivar Nongling 18 developed by pedigree selection and registered in 1990 by Zhejiang Agricultural University. In China, this cultivar is sown in autumn, but under German growing conditions, it behaves more like spring rapeseed because it has poor winter hardiness and no vernalization requirement. Both cultivars have high erucic acid and glucosinolate contents and they were selected for this study because of their high seed oil contents (around 50% for Sollux and 48% for Gaoyou). In total, 380 DH lines were developed by microspore culture applied to F₁ plants, according to a modified procedure of Lichter (1982). After 6 wk vernalization F₁ seedlings were transferred to an environmentally controlled growth chamber maintained at a 16-h photoperiod, a day/night temperature of 12/8°C, and a relative humidity of 80%. Flower buds of 3 to 4 mm in length were collected from the terminal raceme and the two or three uppermost primary branches. Induction medium was prepared according to Lichter (1982). Ploidy level was determined by flow cytometry of cells from young leaves from plantlets. For chromosome doubling, roots of plantlets were washed and immersed into a 0.05% (w/v) solution of colchicine overnight.

Field trials

A subset of 282 lines randomly selected from the total of 380 lines of the DH population, the two parents and the F₁ generation were evaluated in the growing period 2000–2001

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at four locations, two near Göttingen in Germany (Reinshof and Weende), and two in China: Xian in West China and Hangzhou in East China. The mean daily temperature from sowing to flowering was about 1 and 4°C and from flowering to maturity 3 and 4°C lower in Göttingen than at Xian and Hangzhou, respectively. The average total growth period of parents and DH lines were 84 d longer in Göttingen than in China and 8 d longer at Xian than at Hangzhou, and the growth period from flowering to maturity were 72, 58, and 55 d at Göttingen, Xian, and Hangzhou respectively. The Chinese parent Gaoyou was 25 and 15 d earlier in flowering and maturity, respectively, than the Germany parent Sollux on average over all locations, and the DH lines showed continuous variation between 187 and 214 d for flowering time, and between 252 and 273 for days to maturity.

A randomized complete block design with two replications was used. The seeds were sown in double rows for each plot, with rows of 2.5-m length and a spacing of 0.33 m between rows and 0.12 m between plants within rows in Göttingen and 0.15 m in China. Seed samples of at least 10 g were bulk harvested from the terminal raceme and the two uppermost primary branches of five healthy plants in each plot. Seed oil content was determined by near-infrared reflectance spectroscopy (NIRS, Tillmann, 1997) based on 90 g kg⁻¹ seed moisture.

SSR Primer Pairs and PCR Analysis

Total genomic DNA was extracted from young leaves of greenhouse grown plants representing the 282 DH lines evaluated in the field trials, the parental lines, and F₁ plants by the procedure described by Uzunova et al. (1995). In total, about 500 specific primer pairs flanking microsatellite sequences were tested with the parents and F₁ plants. Of these primer pairs, 112 with the designation MR or MD had been developed at the Institute of Agronomy and Plant Breeding, University of Göttingen (Uzunova and Ecke, 1999; Rudolph, 2001). The remaining primer pairs, designated HMR, were analyzed by Saaten-Union Resistenzlabor GmbH, Hovedissen, Germany. The designations of the markers are derived from the primer pair name combined with a lower case letter indicating the specific marker locus. For example, HMR295b is the second locus that could be mapped with primer pair HMR295. PCR reactions were performed in a volume of 10 µL with 25 ng of template DNA, 0.5 µM of each primer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 2% DMSO, 1× reaction buffer (10 mM Tris-HCl, 1.5 mM MgCl₂, 50 mM KCl, pH 9.0) and 1 unit of *Taq* DNA polymerase (PiqLab). After an initial denaturing step of 2 min at 94°C a “touch down” amplification profile was used (Kresovich et al., 1995). This profile included a denaturing step of 60 s at 94°C and an extension step of 45 s at 72°C. The initial annealing step was 30 s at 65°C for two cycles. The annealing temperature was subsequently reduced by 1°C every two cycles until a final temperature of 55 or 50°C was reached, depending on the primer pair. The annealing temperature of 55 or 50°C was employed for the last 20 cycles of the amplification. PCR products were detected either using an Applied Biosystems 3100 capillary sequencer (Foster City, CA) or by separation on 4% (w/v) MethaPhor agarose gels (FMC BioProducts, Rockland, ME, USA) in 1× TBE buffer (8.9 mM Tris-borate, 0.2 mM EDTA, pH 8.4) followed by ethidium bromide staining.

Segregation Analysis and Map Construction

The fit of marker allele segregations to the expected 1:1 segregation ratio was tested for each SSR marker locus by a χ^2 test ($P \leq 0.05$). Linkage analysis and map construction were performed by MAPMAKER/EXP version 3.0 (Lincoln et al., 1993). Map construction was performed in three steps: (i)

selection of loci which segregation fit the expected ratio to construct a core map; markers were assigned to linkage groups by the “group” command with the minimum LOD score parameter set to 3.00 and the maximum distance parameter to 40 cM (Kosambi function); (ii) the most probable marker order within each group was determined by the commands “three point,” “order,” and “ripple”; and (iii) assigning markers with deviating segregation ratios to existing linkage groups by the functions “near” and “try”.

Data Analysis and QTL Mapping

Phenotypic data for oil content of the 282 DH lines over four environments were analyzed with the MINQUE method (Zhu, 1992) to test the difference among DH lines and estimate variance components. A data set consisting of marker and map information as well as mean values of oil content (averages of two replications) for the 282 DH lines in each location was prepared and used for mapping analysis.

QTLMapper version 1.0 (Wang et al., 1999) was used for the QTL mapping and the estimation of additive and additive × additive epistatic effects as well as interaction effects with environments (QE). The genetic model used can be expressed as:

$$y_{hk} = \mu + a_i x_{A_{ik}} + a_j x_{A_{jk}} + aa_{ij} x_{AA_{ijk}} + u_{E_{hk}} e_{E_{hk}} + u_{A_i E_{hk}} e_{A_i E_{hk}} + u_{A_j E_{hk}} e_{A_j E_{hk}} + u_{AA_{ij} E_{hk}} e_{AA_{ij} E_{hk}} + \sum_{f(h)} u_{M_{f(h)}} e_{M_{f(h)}} + \sum_{l(h)} u_{MM_{l(h)}} e_{MM_{l(h)}} + \epsilon_{hk}$$

The meaning of each parameter was as described in Wang et al. (1999) and Luo et al. (2001), where y_{hk} is the phenotypic value of a quantitative trait measured on the k th DH line in environment h ; μ is the population mean; a_i and a_j are the additive main effects (fixed effects) of the two putative QTL Q_i and Q_j , respectively; aa_{ij} is the additive × additive epistatic effect (fixed effect) between Q_i and Q_j ; $x_{A_{ik}}$, $x_{A_{jk}}$ and $x_{AA_{ijk}}$ are coefficients of QTL effects derived according to the observed genotypes of the markers M_{i-} , M_{i+} and M_{j-} , M_{j+} flanking the QTL, $e_{E_{hk}}$ is the random effect of environment h with the coefficient $u_{E_{hk}}$; $e_{A_i E_{hk}}$ and $e_{A_j E_{hk}}$ are the random additive × environment interaction effects with coefficients $u_{A_i E_{hk}}$ and $u_{A_j E_{hk}}$ for Q_i and Q_j , respectively; $e_{AA_{ij} E_{hk}}$ is the random epistasis × environment interaction effect with the coefficient $u_{AA_{ij} E_{hk}}$; $e_{M_{f(h)}}$ is the effect of marker f nested within the h th environment with coefficient $u_{M_{f(h)}}$; $e_{MM_{l(h)}}$ is the effect of marker × marker interaction nested within the h th environment with coefficient $u_{MM_{l(h)}}$; and ϵ_{hk} is the residual effect. The marker factors $e_{M_{f(h)}}$ and $e_{MM_{l(h)}}$ in the model are used to absorb additive and epistatic effects of background QTL.

QTL mapping was performed in three steps (Luo et al., 2001). First, markers with significant effects on the trait analyzed were identified by stepwise regression analysis based on single marker genotypes for putative main-effect QTL and based on all possible pairwise marker combinations for epistatic interactions to identify putative QTL regions. The stepwise regression was performed separately for each environment with a significance threshold of $P = 0.01$. The second step was to identify all putative main-effect QTL and epistatic interactions in putative QTL regions detected in the first step, using marker main and interaction effects to control the background genetic variation. The third step was to estimate genetic effects associated with significant additive QTL and epistatic QTL pairs at the positions of the respective LOD peaks in individual putative QTL regions using the restricted maximum likelihood estimation method (Wang et al., 1999). QTL with additive and epistatic effects were filtered using a significance threshold of $P = 0.005$. Finally, the main effects and the genotype environment interaction effects were tested by

a *t* test with the jackknifing resampling procedure. QTL were presented when genetic main effects (*a* and *aa*) or QE interaction effect (*ae* and *aee*) were significantly different from zero ($P \leq 0.005$) in this test.

RESULTS

Development of an SSR map

Linkage analysis was performed on the basis of 139 marker loci derived from amplification with 102 primer pairs that had shown polymorphisms in the marker screening. Of these markers, 125 formed 21 linkage groups. On the basis of a number of markers with known linkage group assignments, all of the 21 groups could be associated with the 19 linkage groups of the rapeseed RFLP map published by Parkin et al. (1995), indicating that some of the 21 groups of the SSR map represent different, unlinked parts of the same chromosome. The SSR map of *B. napus* (Fig. 1) spans 1196 cM of the rapeseed genome (Kosambi function) with an average interval of 9.6 cM between markers. Forty-four (35.2%) of the 125 mapped markers showed significant deviations from the expected 1:1 segregation ratio ($P \leq 0.05$) with 32 and 12 being skewed toward Sollux and Gaoyou alleles, respectively. These markers are largely clustered on linkage groups 2, 5, 9, 10, and 11-1. About 70% (86 of 125) of the mapped markers showed a codominant inheritance.

Phenotypic Variation among DH Lines

Table 1 shows the phenotypic variation of seed oil content at the four locations. The seed oil content of the parents differed among locations. Oil content of Sollux at Reinshof and Weende was about 3% higher than that of Gaoyou, while the opposite was found at Hangzhou. At Xian both parental cultivars had nearly the same oil content. Large transgressive segregations among the DH lines were observed in each environment with differences between the lines with the highest and lowest oil contents of up to 19 and 11% in Germany and in China, respectively. The DH line with highest oil content was about 10% higher in oil content than the canola check cultivars both in China and Germany and about 5% higher than the high erucic check cultivar in China. Variance component estimates indicated that the genetic effects ($V_G = 8.89$, $P \leq 0.01$) were larger than the genetic × environment interaction effects ($V_{GE} = 5.53$, $P \leq 0.05$).

The frequency distributions of the 282 DH lines for oil content were continuous at all four test locations (Fig. 2). More than 95% of the lines showed oil contents between 40 and 48% at Hangzhou and Xian and between 48 and 56% at Reinshof and Weende, demonstrating the better growing conditions and much longer v egetation period in the two German environments.

Additive Effects and Additive × Environment Interactions

In total, 18 QTL were detected showing additive main effects (*a*) and/or additive × environment interaction

effects (*ae*; Table 2 and Fig. 1). Of these, three QTL showed only additive effects and 10 showed only significant additive by environment effects while the remaining five exhibited both *a* and *ae* effects. Positive alleles for oil content were dispersed between the two parents. Among the eight QTL with additive main effects, Gaoyou alleles increased oil content at three genomic loci on linkage groups 7, 11-1 and 18 while Sollux alleles increased oil content at the remaining five loci. Together, the additive main effects of the eight QTL sum up to 5.4% of oil content for homozygous genotypes and explain about 40% of the phenotypic variation observed in the DH population as expressed by the difference between lines with highest and lowest mean values (Table 1). Additive by environment interactions of QTL were detected in 15 genomic regions, with a prevalence of significant interaction effects at Hangzhou, China, and Reinshof, Germany. Furthermore, the results indicate that Chinese and European alleles are often more positive at locations in China and Germany, respectively, as seen with the seven QTL on linkage groups 1, 2, 3, 9, 11-1, 12, and 16. However, for the four loci on linkage groups 10, 14-2, 15, and 17, the alleles from the Chinese parent were more positive under European growing conditions and European alleles were more positive at Chinese locations.

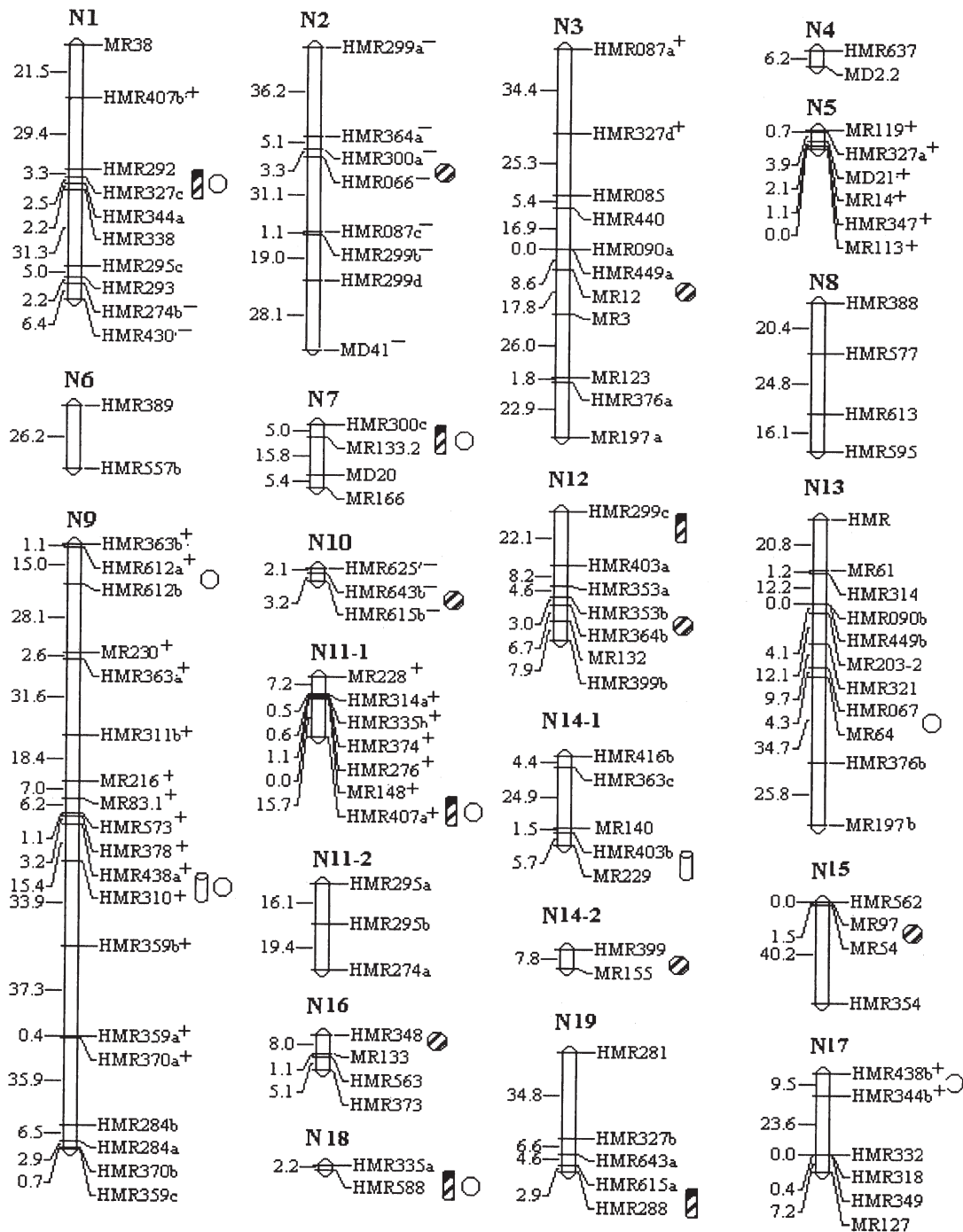
Epistatic Effects and Epistasis × Environment Interactions

In total, 17 loci were mapped that were involved in epistatic interactions in 11 digenic combinations (Table 3). Thirteen of these 17 loci coincide with the QTL showing additive main or additive × environment interaction effects (Table 2). Only four loci on linkage groups 1, 4, 13, and 17 did not show additive effects but showed epistatic interactions in combination with one of the QTL with additive effects.

From the 11 pairwise combinations, seven showed only epistatic main effects (*aa*) and two showed only epistasis by environment effects (*aee*), while two displayed both *aa* and *aee* effects in one or two locations. The epistatic effects of the nine pairs of loci with epistatic main effects sum up to 5.0% maximum difference in oil content between homozygous genotypes, which is almost the same value as the total effect of all QTL showing additive main effects. Epistatic main effects were negative at four pairs of loci, indicating that recombination of the parental alleles increased oil content, while at the other five pairs of loci the parental allele combinations were positive for oil content. Only four pairs of loci displayed epistasis by environment interaction effects, which is a much lower frequency than that of additive × environment interactions.

QTL Genotypes of Lines with Extreme Phenotypes

To confirm the mapped QTL, the 20 lines with the highest and lowest oil content were phenotypically selected. Their average oil content over four locations was at least 2% higher than that of both parents. For these



▮: QTLs with α and $\alpha\alpha$ effects, ⊗: QTLs with $\alpha\epsilon$ and $\alpha\alpha$ effects

○: QTLs with α effect, ⊙: QTLs with $\alpha\epsilon$ effect

Fig. 1. SSR linkage map based on the Sollux/Gaoyou (F₁) DH population. Distances between markers are given in centimorgans, calculated from recombination frequencies according to the Kosambi mapping function. Map positions of putative QTL controlling oil content are presented on the right side of the linkage groups by circles or vertical bars. α : additive main effect, $\alpha\alpha$: Additive \times additive epistatic main effect of two loci, $\alpha\epsilon$: Additive by environment interaction effect. '+' and '-' indicate markers showing significant deviations ($P \leq 0.05$) from the expected 1:1 segregation ratio in favor of Sollux or Gaoyou alleles, respectively.

Table 1. Oil content (%) in the Sollux/Gaoyou F₁ DH population and check cultivars at four locations.

Location	Parents		F ₁	DH Population (n = 282)				Check cultivars†		
	Sollux	Gaoyou		Max	Min	Mean	SD	CK ₁	CK ₂	CK ₃
Xian	45.3	45.4	45.6	49.2	40.4	44.7	1.60	39.6	40.0	44.1
Hangzhou	41.6	44.7	42.5	49.5	38.3	44.2	1.80	38.1	38.4	43.4
Reinshof	53.5	50.6	55.2	57.0	37.8	51.9	2.33	47.1	48.7	46.0
Weende	51.6	48.2	53.1	56.0	40.7	51.1	1.95	47.0	47.2	44.8
Mean	48.0	47.2	49.1	52.9	39.3	48.0	1.92			

† Three check cultivars in China (CK₁, CK₂, and CK₃) were Zheshuang 72, Zheyou 758, and Gaoyou 605 respectively. CK₁, CK₂, and CK₃ at Reinshof and Weende were varieties Mohican, Express, and Lirajet. Except CK₃ in China, which is high in erucic acid and glucosinolate contents, all the other checks are canola quality.

lines, the genotypes of 16 markers were compared (Table 4). The first six markers were linked with QTL displaying additive main effects followed by a pair of markers linked with two QTL showing both additive and epistatic effects. The remaining four pairs of markers were linked with loci associated with the largest epistatic effects. The pair of loci on linkage groups 1 and 12 that also showed a high epistatic effect (Table 3) was not included because one of the markers could not be scored in eight of the 20 lines. For the markers on linkage groups 7 and 18, linked with the QTL with the highest additive main effects, the positive Gaoyou alleles were observed in 18 (90%) and 19 (95%) of the 20 selected lines, respectively. For the pair of markers on linkage groups 11-1 and 12, 80% positive allele combinations (considering both *a* and *aa* effects) were found. With 60 to 65% the fit of positive alleles for the markers on linkage groups 1, 14-1, and 19 and the pair of marker loci on linkage groups 1 and 2 was not high, however. When all 16 SSR loci were considered, the fit of positive alleles ranged from 55 to 95% with an average of 71% for lines with high oil content.

DISCUSSION

In this study, 18 QTL associated with oil content in *B. napus* with additive main and/or additive × environment interaction effects were mapped. This number of QTL is much higher than the three and six QTL that could be mapped in a doubled haploid population derived from two European winter rapeseed genotypes in two previous studies by Ecke et al. (1995) and Gül et

al. (2003), respectively. The higher number of mapped QTL might be partly due to the larger size of the mapping population with about twice as many DH lines as the population used by Ecke et al. (1995) and Gül et al. (2003), resulting in a higher power of detection for QTL. The main reason, however, is probably that two genetically very distinct lines were crossed, which have been selected independently in China and Germany for high oil content. Both parents are high in erucic acid and glucosinolate content, which is a disadvantage for direct use of the material in canola breeding. However, the divergence between European and Chinese gene-pools is larger in such material compared with newer canola cultivars (Becker et al., 1995; Zhao and Becker, 1998), and in a parallel experiment with two canola parents, no transgressive DH lines were observed (data not shown).

In the studies by Ecke et al. (1995) and Gül et al. (2003), the two QTL with the largest effects on oil content corresponded to the two erucic acid genes. The alleles increasing oil content were associated and possibly identical to the alleles for high erucic acid content, which could be explained by the increase in molecular mass during the elongation of oleic acid to erucic acid (Ecke et al., 1995). These two QTL were located on linkage groups 6 and 12 of the RFLP map used (Uzunova et al., 1995). No QTL for oil content were mapped on the corresponding linkage groups N8 and N13 of the map used in the present study. This was not unexpected since the mapping population is not segregating for erucic acid content. Of the remaining QTL mapped by

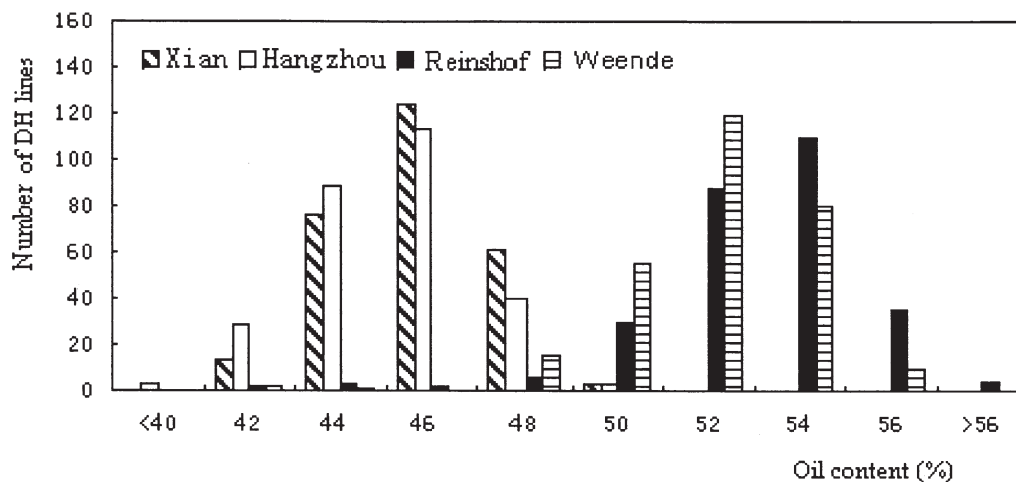


Fig. 2. Frequency distributions of oil content in the Sollux/Gaoyou F₁ DH Population at four locations.

Table 2. Estimated additive (*a*) and additive × environment interaction (*ae*) effects of QTL for oil content (%).

Linkage group	Marker interval	QTL Position† (cM)	<i>a</i> effect‡	<i>ae</i> at Xian	<i>ae</i> at Hangzhou	<i>ae</i> at Reinshof	<i>ae</i> at Weende
1	HMR292/HMR327	0.0	0.37**	-0.29**		0.24*	
2	HMR300a/HMR066	0.0			-0.29**	0.32**	
3	HMR449a/MR12	8.0			-0.34**	0.37**	
7	HMR300c/MR133.2	2.0	-0.55**		-0.27*		
9	HMR612a/HMR612b	0.0		-0.41**	-0.56**	0.59**	0.31**
9	HMR438a/HMR310	14.0	0.34**		-0.48**		
10	HMR643b/HMR615b	2.0		0.31**		-0.35**	
11-1	MR148/HMR407a	4.0	-0.26*		-0.65**	0.40**	
12	HMR299c/HMR403a	2.0	0.27*				
12	HMR353b/HMR364b	2.0		-0.75**	-0.34**	0.66**	0.41**
13	HMR067/MR64	0.0		0.23**	-0.29**		
14-1	HMR403b/MR229	0.0	0.22**				
14-2	HMR399a/MR155	6.0		0.24**	0.41**	-0.49**	-0.19*
15	MR97/MR54	0.0			0.27**	-0.31**	
16	HMR348/MR133	0.0			-0.33**	0.34**	
17	HMR438b/HMR344b	0.0			0.30**	-0.27**	
18	HMR335a/HMR588	0.0	-0.52**		0.42**	-0.23*	-0.21**
19	HMR615a/HMR288	0.0	0.22**				

* Indicates the significance level at 0.005 to declare the putative QTL positions and genetic effects.

** Indicates the significance level at 0.001 to declare the putative QTL positions and genetic effects.

† Distance of the QTL from the first marker of the indicated interval.

‡ The QTL effect is the phenotypic effect due to the substitution of a Gaoyou allele by an allele of Sollux.

Ecke et al. (1995) and Gül (2002), two on linkage groups 5 and 14 may be identical to the QTL mapped on the corresponding linkage groups N1 and N3 since they are at similar positions. The other six QTL with additive effects in this study represent new loci for oil content that have not been mapped and characterized before.

In the field trials, a high genotype × environment interaction variance was observed that was nearly as high as the variance due to genetic effects. This was reflected by the high frequency of QTL showing additive × environment interaction effects. Actually, the majority of QTL did not show significant additive main effects but only interaction effects. Furthermore, the interaction effects were of the same order of magnitude as the main effects. The high importance of environment interactions is probably a consequence of the population studied, which was derived from parents adapted to the very different growing conditions in China and Germany and which was tested in both environments. In almost all cases where significant interaction effects occurred, the positive alleles for oil content were different between China and Germany. In the majority of these cases, the alleles of the Chinese variety Gaoyou were positive in China and the alleles of the German variety

Sollux were positive in Germany. However, four QTL were found where the allele from the German variety increased oil content in China and the Chinese alleles were positive in Germany. In addition, at five loci on linkage groups 1, 7, 12, 14-1, 18, and 19 one of the alleles showed positive effects in both China and Germany.

The results from the QTL mapping indicate that additive effects and additive × environment interactions are main factors contributing to variation in oil content in *Brassica napus*, which is in agreement with earlier studies using quantitative genetic analysis (Grami and Stefansson, 1977; Grami et al., 1977; Röbbelen and Thies, 1980; Engqvist and Becker, 1991). However, the QTL mapping also demonstrated a substantial contribution to the variation in oil content by additive × additive epistatic effects. The importance of epistatic effects had not been recognized in the earlier studies but has been widely reported in several other crop species after QTL mapping, for example in soybean [*Glycine max* (L.) Merr.; Lark et al., 1995], tomato (*Lycopersicon esculentum* Mill.; Eshed and Zamir, 1996), and rice (*Oryza sativa* L.; Li et al., 2001; Luo et al., 2001; Zhuang et al., 2002; Mei et al., 2003). In rice, a large number of epistatic interactions explaining a high percentage of the pheno-

Table 3. Estimated epistatic (*aa*) and epistasis × environment interaction (*aae*) effects of QTL for oil content (%).

N†	Marker interval	N	Marker interval	<i>aa</i> ‡ effect	<i>aae</i> at Xian	<i>aae</i> at Hangzhou	<i>aae</i> at Reinshof	<i>aae</i> at Weende
1	HMR407b/HMR292	2	HMR300a/HMR066	-0.29**				
1	HMR407b/HMR292	17	HMR318/HMR439		-0.28**		0.20*	
1	HMR295c/HMR293	12	HMR353b/HMR364b	0.38**				
2	HMR300a/HMR066	10	HMR625/HMR643b	0.32**				
3	HMR449a/MR12	7	HMR300c/MR133.2	-0.20**	-0.26**		0.22*	
3	HMR449a/MR12	18	HMR335a/HMR588	-0.20**				
4	HMR637/MD2.2	12	HMR353b/HMR364b	-0.29**				
11-1	MR148/HMR407a	12	HMR299c/HMR403a	0.32**				-0.25*
11-1	MR148/HMR407a	16	HMR348/MR133	0.21*				
13	HMR314b/HMR090b	15	MR97/MR54	0.26**				
14-2	HMR399a/MR155	19	HMR615a/HMR288			-0.34**	0.18*	

* Significant at the 0.5 probability level.

† Significant at the 0.01 probability level.

‡ Linkage group.

‡ A positive sign of the epistatic effect indicates that parental allele combinations and a negative sign that recombinant allele combinations increase phenotypic values.

Table 4. Marker genotypes of the 20 DH lines with the highest oil content at four locations at 16 marker loci linked with QTL or pairs of QTL showing additive main (*a*) or epistatic main (*aa*) effects.

Parents and DH lines	Locations and oil content (%) [†]										<i>a</i> effect										<i>a</i> and <i>aa</i> effects										<i>aa</i> effect									
	X		H		R		W		mean		HMR 300c (7) [‡]		HMR 335a (18)		HMR 310 (9)		HMR 615a (19)		HMR 292 (1)		HMR 403b (14-1)		MR HMR 148 299c (11) (12)		HMR HMR 300a 625 (2) (10)		MD HMR 2.2 353b (4) (12)		HMR MR 314b 97 (13) (15)		HMR HMR 292 300a (1) (2)		Fit (%)							
	X	H	R	W	mean	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G					
Sollux	45.3	41.6	53.5	51.6	48.0	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G					
Gaoyou 7	45.4	44.7	50.6	48.2	47.2	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G				
Unfavorable marker alleles [§]	48.1	44.9	55.8	52.6	50.4	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G				
14	47.1	47.9	56.9	56.0	52.0	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G				
15	46.9	49.5	55.4	54.3	51.5	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G				
17	46.3	45.8	54.1	54.4	50.1	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G				
66	46.8	47.8	55.2	55.1	51.2	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G				
68	49.2	46.4	52.9	51.9	50.1	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G				
70	45.9	45.4	55.5	53.7	50.1	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G				
106	46.4	45.0	55.3	53.4	50.0	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G				
125	46.5	46.6	55.0	52.9	50.3	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G				
129	47.6	47.3	54.0	52.2	50.3	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G				
148	47.9	49.1	53.5	54.2	51.2	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G				
152	46.7	47.4	53.5	52.5	50.0	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G				
198	48.5	47.8	57.0	54.9	52.1	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G				
201	46.4	45.7	55.0	53.3	50.1	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G				
203	46.6	45.2	55.7	53.3	50.2	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G				
207	47.5	49.1	55.3	53.9	51.4	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S				
214	46.6	44.4	56.5	54.2	50.4	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G				
218	47.5	46.6	55.7	54.7	51.1	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G				
255	46.4	47.1	53.5	53.7	50.2	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S				
267	46.0	46.2	55.0	54.5	50.4	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S				
Fit (%) [¶]	90	95	70	65	63	63	65	65	63	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60				

[†] X = Xian, H = Hangzhou, R = Reinshof and W = Weende.

[‡] Linkage group.

[§] Marker genotypes that deviate from the positive genotype are displayed in italics.

[¶] Fit was calculated according to the positive marker genotypes.

typic variation was detected for a number of traits including grain and biomass yield and yield components by the same mixed model approach as implemented in QTLMapper. Three types of epistatic interactions can be distinguished: interactions between two QTL with additive effects (type I), interactions between a QTL with additive effect and a “background” locus without additive effect (type II), and interactions between two loci showing epistatic effects only (type III). In rice, some experiments have identified the most frequent interactions as type III (Li et al., 2001, Luo et al., 2001), whereas in other studies, mainly epistasis of type I was observed (Zhuang et al., 2002). In rapeseed, the majority of loci involved in epistatic interactions coincide with QTL also showing additive main or additive \times environment interaction effects (type I). Only four interactions of type II were found and no significant interaction of type III. Loci without additive effects involved in interactions of type II may represent genes that modify the action of other QTL, maybe even in a regulatory manner. On the other hand, they may also represent genes with additive effects that were too small to be significant. The latter hypothesis may also be applicable to loci involved in type III interactions.

The contribution of epistasis to the phenotypic variation in oil content may still be underestimated in the present study. Some of the loci involved in epistatic interactions interacted with more than one locus. For example each of the loci on linkage groups 1 (HMR407/HMR292), 2, 3, 11-1, and 12 (HMR353b/HMR364b) were involved in two digenic interactions with different loci. The involvement of loci in multiple digenic interactions may be a reflection of higher order epistatic interactions. Currently, the contributions of higher order interactions cannot be estimated since no method for QTL mapping is including such complex interactions. Also, a population of only 282 DH lines may not be large enough to resolve higher order epistatic interactions.

The large transgressive segregation for oil content observed in the mapping population at all locations indicated a dispersion of alleles with positive and negative effects between the parents. This was confirmed by the QTL mapping. Of eight QTL with additive effects, the Sollux allele increased oil content at five loci and the Gaoyou allele at three loci. Furthermore, the four loci with only additive \times environment interactions, where the Sollux allele increased oil content in China and the Gaoyou allele in Germany, will also have contributed to the transgressive segregations. In a marker-assisted selection using markers linked to the QTL, different strategies have to be followed in the two regions because of the high importance of QTL \times environment interactions. Since in nearly all cases the sign of the additive \times environment interaction effects is opposite in China and Germany different alleles will have to be selected for all QTL with interaction effects larger than the additive main effects. Furthermore, even QTL with strong main effects may be of quite different importance in China and Europe. For example, the QTL on linkage group 18 has a strong main additive effect with -0.5% on oil content. However, including interactions, the local

effects of the QTL are quite different between the regions with only -0.1% at Hangzhou, China, but more than -0.7% at the two locations in Germany. In addition, epistatic effects have to be taken into account in a marker assisted breeding program since these effects make a large contribution to the variation in oil content in rapeseed.

The results clearly indicate that an integration of positive alleles from Chinese and European materials into European and Chinese elite breeding lines, respectively, is a promising prospect for the breeding programs in both regions. Such integration may be facilitated by marker-assisted selection. The high breeding potential of the material developed was confirmed by an independent field experiment in Göttingen in 2002 (data not shown). The best DH lines selected from the QTL experiment had an oil content of 55% compared with 48% of the better parent Sollux.

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