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Genetic Analysis of Heterosis for Yield and Yield Components in Rapeseed (*Brassica napus* L.) by Quantitative Trait Locus Mapping

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ABSTRACT

The main objective in this research was the genetic analysis of heterosis in rapeseed at the QTL level. A linkage map comprising 235 SSR and 144 AFLP markers covering 2045 cM was constructed in a doubled-haploid population from a cross between the cultivar "Express" and the resynthesized line "R53." In field experiments at four locations in Germany 250 doubled-haploid (DH) lines and their corresponding testcrosses with Express were evaluated for grain yield and three yield components. The heterosis ranged from 30% for grain yield to 0.7% for kernel weight. QTL were mapped using three different data sets, allowing the estimation of additive and dominance effects as well as digenic epistatic interactions. In total, 33 QTL were detected, of which 10 showed significant dominance effects. For grain yield, mainly complete dominance or overdominance was observed, whereas the other traits showed mainly partial dominance. A large number of epistatic interactions were detected. It was concluded that epistasis together with all levels of dominance from partial to overdominance is responsible for the expression of heterosis in rapeseed.

HETEROSIS is the superior performance of F_1 hybrids relative to the midparent value (MPV) or to the better parent. While the practical application of heterosis in plant breeding is quite successful in many crops through the development of hybrid varieties, the basic understanding of the phenomenon is not very advanced. Three main hypotheses exist to explain the genetic basis of heterosis: the dominance, overdominance, and epistasis hypotheses (Crow 1999; Goodnight 1999). The dominance hypothesis supposes that deleterious recessive alleles of one of the parents are complemented in the F_1 hybrid by the dominant alleles of the other parent. The overdominance hypothesis states that the heterozygous combination of the alleles at a locus is superior to either of the two possible homozygous combinations. Epistasis assumes that epistatic interactions between different loci are the reason for heterosis.

Currently, results from quantitative genetic experiments favor the dominance hypothesis (Crow 1999). On the other hand, theoretical considerations and some observations indicate that epistasis plays a significant role in the expression of heterosis (Goodnight 1999). In addition, results of multimeric enzyme studies are apparent examples of true overdominance (Stuber 1999).

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The extent of heterosis in rapeseed has been analyzed in a number of studies with widely varying results, depending on the materials used. In spring rapeseed hybrids an average high parent heterosis of 30% with a range of 20–50% was observed, while for winter rapeseed hybrids an average high parent heterosis of 50% was reported, ranging from 20 to 80% as reviewed by McVetty (1995). In a literature review Becker (1987) reported midparent heterosis values for yield in the range of 4–63% with average heterosis of 30 and 27% for winter and spring rapeseed, respectively.

QTL mapping has been increasingly used in recent years for studying heterosis. In maize Stuber *et al.* (1992) identified QTL for seven agronomic traits, including grain yield. The prevailing mode of action was overdominance. Testing all possible pairwise combinations of markers linked to the mapped QTL, no epistasis was found. A number of other studies (Graham *et al.* 1997; Lu *et al.* 2003; Frascaroli *et al.* 2007) showed that a variety of effects ranging from partial to overdominance, including pseudo-overdominance, play a role in the determination of heterosis in maize, while epistasis showed no significant influence.

In rice Xiao *et al.* (1995) concluded that dominance is the major causal factor of heterosis. No overdominance and epistasis was detected. These results are in disagreement with a series of studies on heterosis in rice by Yu *et al.* (1997), Li *et al.* (2001), Luo *et al.* (2001), and Mei *et al.* (2003, 2005). Plant height, grain yield, and yield components were analyzed by QTL mapping in recombinant inbred line populations, in the correspond-

ing testcross populations with an independent tester, and in backcross populations. In all studies most of the QTL contributing to heterosis showed overdominance and a large number of loci were involved in epistatic interactions associated with heterosis.

All studies mentioned above were carried out in maize, which is an outcrossing crop, or in rice, which is self-pollinated. The molecular basis of heterosis in rapeseed, an allopolyploid and partially allogamous crop, has not been investigated so far.

The main objective of this study was a genetic analysis of heterosis in rapeseed at the QTL level, including (i) identification of the levels of heterosis for grain yield and yield components; (ii) identification, localization, and estimation of the effects of QTL for grain yield and yield components; and (iii) assessment of the contributions of different genetic effects, *e.g.*, dominance, overdominance, and epistasis, to the expression of heterosis in rapeseed.

MATERIALS AND METHODS

Plant materials: The plant materials consisted of a population of 250 doubled-haploid lines (DHL) produced from a cross between "Express 617," an inbred line of the winter rapeseed cultivar "Express," and the resynthesized line "R53," as well as the 250 corresponding testcross hybrids between the doubled-haploid lines and the male-sterile tester "MSL-Express" (MSL 007). The doubled-haploid population was developed from one F_1 plant of the cross Express 617 × R53 as a commission by Saaten Union Resistenzlabor (Leopoldshöhe, Germany).

The winter rapeseed cultivar Express is of "canola" quality while R53, with an intermediate level of erucic acid and glucosinolate content, is a resynthesized line developed from an interspecific cross between *Brassica oleracea* var. *sabellica* and *B. rapa* ssp. *pekinensis*. The male-sterile version of Express, MSL 007, was provided by NPZ-Lembke. All lines of the doubled-haploid population restored pollen fertility in the crosses with MSL-Express.

Field experiment: The experiment was carried out following standard agronomic procedures in the growing season 2005/2006 at four locations in Germany with different agroecological conditions (Reinshof, Deitersen, Rauischholzhausen, and Grund-Schwalheim). The experimental design was a 26 × 10 α-lattice (Patterson and Williams 1976). At each location the material was grown with one replication and the four locations were treated as four replications in the statistical analysis. Each genotype was grown in a six-row plot of 11.25 m^2 with a 0.25-m row distance and a sowing density of 80 seeds/m^2. The parents Express 617 and R53, the F_1 hybrid (Ex × R53), and the commercial hybrid cultivar "Elektra" were used as checks, replicated five times within the lattice at each location. The doubled-haploid lines and the hybrids were grown in parallel beds, where each hybrid was placed at the same plot position in the second bed as the corresponding doubled-haploid line in the first bed. Thus each line and its corresponding hybrid were grown as near together as possible, while excluding the competition between the lines and the more vigorous hybrids.

Phenotypic data were collected for (1) total grain yield (GY), measured in metric tons per hectare (t/ha) adjusted to 91% dry matter; (2) thousand-kernel weight (TKW), measured in grams estimated from the average of three measurements of the weight of 100 seeds; (3) seeds per silique (*S*/Sil), estimated as a mean from nine siliques (the first three siliques of the main raceme immediately above the first side branch were harvested from three randomly chosen plants per genotype); and (4) siliques per square decimeter (Sil/dm^2), calculated from grain yield and the yield components by the formula Sil/dm^2 = GY/dm^2/(*S*/Sil × single-seed weight).

Phenotypic data analysis and heterosis estimation: For statistical analysis of phenotypic data the LATTICE procedure in PLABSTAT version 3A (Utz 2003) was used. The statistical model is

$$Y_{ijk} = \mu + r_i + b_{ij} + g_k + e_{ijk},$$

where Y_{ijk} is an observation of genotype k in block j of replication i, μ is the general mean, r_i is the effect of replication i, b_{ij} is the effect of block j in replication i, g_k is the effect of genotype k, and e_{ijk} is the residual effect of observation Y_{ijk}. The residual variance is a combination of genotype × location interaction variance and the within-location error variance. The broad-sense heritability (h^2) was estimated as $\hat{h}^2 = \hat{\sigma}_g^2/[(\hat{\sigma}_e^2/r) + \hat{\sigma}_g^2]$, where $\hat{\sigma}_g^2$ designates the genotypic variance, $\hat{\sigma}_e^2$ the residual variance, and r is the number of replications.

The levels of midparent and high parent heterosis of the F_1 hybrid of the parents Express and R53 are referred to as "F_1 heterosis." The mean of the heterosis of the 250 testcross hybrids is referred to as "average testcross heterosis." For testing the significance of heterosis values *t*-tests were applied.

Marker analysis and genetic map construction: A genetic map was constructed using SSR and AFLP markers in the doubled-haploid (DH) population. The DNA extraction was carried out with NucleonPhytoPure extraction kits (RPN8511; GE Healthcare Bio-Sciences, Uppsala, Sweden), according to the manufacturer's instructions.

Genetic markers: A total of 621 SSR primer pairs were used. Ninety-eight public SSR primer pairs that had been predominantly developed at IACR Long Ashton and John Innes Centre (Lowe *et al.* 2004) were obtained at http://brassica.bbsrc.ac.uk/cgi-bin/ace/searches/browser/BrassicaDB#results. The prefixes Ra, Ol, Na, and Ni in the names of these primer pairs and the derived markers indicate the species of origin: *B. rapa*, *B. oleracea*, *B. napus*, and *B. nigra*, respectively.

The primer pairs designated "BRAS" and "CB" were developed by Celera AgGen, sponsored by an international consortium of private breeding companies. The primer pairs with prefixes "MR" and "MD" were developed by the Institute of Agronomy and Plant Breeding of the University of Göttingen. Of the BRAS, CB, MR, and MD primer pairs 131 were published by Piquemal *et al.* (2005). The full list of the 621 SSR primer pairs used in this study is provided in supplemental Table 1.

SSR analyses were carried out according to the M13-tailing PCR technique (Schuelke 2000) with a modified tail and M13-universal primer (M13-tail 5′-TTT CCC AGT CAC GAC GTT-3′, M13-universal primer 5′-AG GGT TTT CCC AGT CAC GAC GTT-3′). The PCR reaction was carried out in a total volume of 20 μl under the following conditions: 0.05 units/μl FIREPol Taq polymerase (Solis Biodyne, Tartu, Estonia), 1× FIREPol PCR buffer, 2.5 mm $MgCl_2$, 0.2 mm dNTPs (Qbiogene, Heidelberg, Germany), 0.05 μm M13-universal primer, 0.05 μm tailed forward primer, 0.05 μm reverse primer, and 25 ng of template DNA. A two-step touchdown PCR program was used on a Biometra T1 Thermocycler (Biometra, Göttingen, Germany): 95° for 3 min; 5 cycles of 95° for 45 sec, 68° (−2°/cycle) for 5 min, 72° for 1 min; 5 cycles of 95° for 45 sec, 58° (−2°/cycle) for 1 min, 72° for 1 min; 27 cycles of 95° for

45 sec, 47° for 30 sec, 72° for 1 min; and 72° for 10 min. After the last cycle the samples were cooled to 4°.

For AFLP analysis 23 AFLP primer combinations were used, following the protocol of Vos *et al.* (1995), modified according to B. Kebede and F. Kopisch-Obuch (personal communication). The M13-universal primer used in SSR analyses and the *Eco*RI primers used in AFLP reactions were labeled with one of three different fluorescent dyes: FAM, HEX, and NED (Applied Biosystems, Darmstadt, Germany). The amplification products were separated on an ABI PRISM 3100 genetic analyzer (Applied Biosystems) using 36-cm capillary arrays and the GeneScan-500 ROX size standard (Applied Biosystems). GeneScan software version 3.7 (Applied Biosystems) was applied for the raw data analysis. The markers were scored using Genotyper software version 3.7 NT (Applied Biosystems). The same procedure was applied for SSR and AFLP analyses.

Marker names: In the case of a primer pair amplifying more than one polymorphic locus the names of the corresponding SSR markers consist of the primer pair name and a suffix a, b, c, etc. AFLP marker names consist of the names of the *Eco*RI and *Mse*I primers and a suffix showing the allele size and the parent that contributed the visible allele, where E and R designate Express 617 and R53, respectively.

Map construction: All primer pairs that showed polymorphisms in a screening with the two parents were applied to a subset of 96 lines of the doubled-haploid population to construct a primary map. Subsequently, 191 evenly distributed markers were selected and analyzed in the rest of the 250 lines of the doubled-haploid population for the development of a framework map suitable for QTL mapping.

The fit of marker-allele segregations to the expected 1:1 segregation ratio was tested by a χ^2-test ($P = 0.05$). Linkage analyses were performed using MAPMAKER/EXP 3.0 (Lincoln *et al.* 1993). The markers were grouped in linkage groups with a minimum LOD score of 4.0 and a maximum recombination frequency of 0.4. To determine the correct marker order within the linkage groups multipoint analysis was performed by "compare" and "try" commands. Double-crossover events were examined and the original scores rechecked for potential scoring errors. The order of the loci within the linkage groups was additionally verified by the "ripple" command with a sliding window of five loci and a LOD score threshold of 2.0.

Data sets for QTL mapping: The phenotypic data derived from the field experiments were organized in three different data sets, used separately for QTL mapping. The first data set included the adjusted means across the four locations of the doubled-haploid lines, the second data set consisted of the adjusted means of the testcross hybrids (DH lines × MSL-Express), and the third set included the midparent heterosis (MPH) of the testcross hybrids (MPH data set).

QTL mapping: The software QTLMAPPER version 1.0 (Wang *et al.* 1999) was used for QTL mapping. The program allows simultaneous interval mapping of both main-effect QTL and digenic epistatic interactions in recombinant inbred line, DH, or backcross populations. It is based on a mixed linear model and performs composite-interval mapping (Jansen and Stam 1994; Zeng 1994). The genetic model used can be expressed as

$$y_k = \mu + a_i x_{A_{ik}} + a_j x_{A_{jk}} + aa_{ij} x_{AA_{ijk}} + \sum_f u_{M_{fk}} e_{M_f} + \sum_l u_{MM_{lk}} e_{MM_l} + \varepsilon_k,$$

where y_k is the phenotypic value of a quantitative trait measured on the kth individual; μ is the population mean; a_i and a_j are the main effects (fixed) of the two putative QTL (Q_i and Q_j), respectively; aa_{ij} is the epistatic effect (fixed) between Q_i and Q_j; $x_{A_{ik}}$, $x_{A_{jk}}$, and $x_{AA_{ijk}}$ are coefficients of QTL effects with a sign according to the observed genotypes of the markers (M_{i-}, M_{i+} and M_{j-}, M_{j+}) and values determined by the test positions ($r_{M_i-Q_i}$ and $r_{M_j-Q_j}$); $e_{M_f} \approx N(0, \sigma^2_M)$ is the random effect of marker f with indicator coefficient $u_{M_{fk}}$ (1 for M_fM_f and -1 for m_fm_f); $e_{MM_l} \approx N(0, \sigma^2_{MM})$ is the random effect of the lth marker interaction (between marker K_l and marker L_l) with indicator coefficient $u_{MM_{lk}}$ (1 for $M_KM_KM_LM_L$ or $m_Km_Km_Lm_L$ and -1 for $M_KM_Km_Lm_L$ or $m_Km_KM_LM_L$); and $\varepsilon_k \approx N(0, \sigma^2_\varepsilon)$ is the random residual effect. The inclusion of e_{M_f} and e_{MM_l} is intended to absorb additive and epistatic effects of background QTL to control any bias in the estimation of QTL effects (Wang *et al.* 1999; Li *et al.* 2001).

The QTL mapping included four main steps. First, markers with a significant effect on the trait (cofactors) were identified by screening all available markers by stepwise regression. The regression analyses were based on single-marker genotypes for putative main-effect QTL and on all possible pairwise marker combinations for epistatic effects. The significance threshold was $P = 0.005$ (Wang *et al.* 1999). In the second step composite-interval mapping was performed in the genomic regions surrounding the markers selected in the first step. Detected putative main-effect QTL and epistatic interactions were kept fixed in the model to control the background variation by the random effects of the cofactors. In this step a significance threshold of $P = 0.002$ was applied, which has been shown by simulation analysis (Wang *et al.* 1999) and empirical studies (Li *et al.* 2001) to provide a consistent high power in detecting QTL of moderate main/epistatic effects with a very low probability of false positives. In the third step genetic effects and test statistics were estimated for the putative main-effect and epistatic QTL in the regions with LOD score peaks exceeding the applied significance threshold at $P = 0.002$. Finally, the percentage of the explained phenotypic variation was calculated for each detected QTL.

Confidence intervals for QTL were estimated by the 1-unit-down method (Lander and Botstein, 1989). QTL detected in the different data sets were considered to be the same QTL if more than two-thirds of their confidence intervals overlapped.

The genetic expectations of the parameters estimated with the above model differ according to the data set. The doubled-haploid lines provide an estimate for the additive effects a. Genetic effects detected with the heterosis data set represent dominance effects d, while for the testcrosses the estimated effects are a combination of both dominance and additive effects, $-(a + d)$ and $(a - d)$, if the donor or the recurrent parent carries a dominant allele increasing the trait, respectively (Table 1). An additional assumption is that the average of the testcross performance is higher than the MPV (positive heterosis); otherwise the estimated effects will have the opposite sign.

In the case of epistasis the estimated effect in the doubled-haploid population is the additive × additive genetic interaction. The effects calculated in the other two data sets are complex mixtures of all possible epistatic interactions: additive × additive (aa), additive × dominance (ad), and dominance × dominance (dd) interactions. If two loci A and B are considered, then the genetic effect in the testcross population represents $aa_{AB} + dd_{AB} - ad_{AB} - ad_{BA}$, while the effects estimated with MPH data are $dd_{AB} - aa_{AB} - ad_{AB} - ad_{BA}$.

RESULTS

Marker screening and genetic map construction: From 621 SSR primer pairs 501 (80.7%) gave clearly defined banding patterns. Of these, 199 (39.7%) showed

TABLE 1

QTL genotypes and genotypic values of populations and data sets

Population and data set[a]	Increasing allele contributed by	DH genotype[b]	Genotype of the population/ data set	Genotypic value[c]	QTL effect[d] $[(Q_EQ_E - q_Rq_R)/2$ or $(q_Eq_E - Q_RQ_R)/2]$
DH	Express	Q_EQ_E	Q_EQ_E	MPV + a	$2a/2 = a$
		q_Rq_R	q_Rq_R	MPV − a	
	R53	q_Eq_E	q_Eq_E	MPV − a	$-(2a)/2 = -a$
		Q_RQ_R	Q_RQ_R	MPV + a	
TC	Express	Q_EQ_E	Q_EQ_E	MPV + a	$(a - d)/2$
		q_Rq_R	Q_Eq_R	MPV + d	
	R53	q_Eq_E	q_Eq_E	MPV − a	$-(a + d)/2$
		Q_RQ_R	q_EQ_R	MPV + d	
MPH	Express	Q_EQ_E		MPV + $a - \frac{1}{2}$ (MPV + a + MPV + a)	$(0 - d)/2 = -d/2$
		q_Rq_R		MPV + $d - \frac{1}{2}$ (MPV + a + MPV − a)	
	R53	q_Eq_E		MPV − $a - \frac{1}{2}$ (MPV − a + MPV − a)	$(0 - d)/2 = -d/2$
		$Q_R Q_R$		MPV + $d - \frac{1}{2}$ (MPV − a + MPV + a)	

[a] Doubled-haploid (DH) and testcross (TC) populations and midparent heterosis (MPH) data set.
[b] Q_E/q_E, QTL allele of "Express"; Q_R/q_R, QTL allele of "R53"; Q and q, an allele increasing and decreasing the trait, respectively.
[c] MPV, mean of the two *EE* and *rr* or *ee* and *RR* homozygotes; a, additive effect; d, dominance effect.
[d] QTL effect as calculated by QTLMapper.

polymorphisms between Express 617 and R53, resulting in 235 markers. The screening of 23 AFLP primer combinations resulted in the detection of 144 markers.

Accordingly, the primary map (Figure 1) included 377 markers that were distributed across 19 extended linkage groups and one cosegregating AFLP marker pair, together covering 2045 cM of the rapeseed genome. On the basis of shared SSR markers the map was aligned to three previously established SSR linkage maps (Lowe *et al.* 2004; Piquemal *et al.* 2005; A. Sharpe and D. Lydiate, unpublished data) and 18 of the linkage groups could be designated according to the N nomenclature (Parkin *et al.* 1995). The linkage group LG10 included no markers with known linkage group assignments. It most probably represents linkage group N8 as all remaining linkage groups were unambiguously assigned to linkage groups N1–N7 and N9–N19.

The framework map (Figure 1) used for QTL mapping comprised 191 (127 SSR and 64 AFLP markers) of the most evenly distributed markers from the primary map, forming 19 extended linkage groups with an average marker interval of 9.6 cM.

Analysis of variance and heritability: The genetic variance and the heritability of grain yield and the yield components thousand-kernel weight, seeds per silique, and siliques per square decimeter are summarized in Table 2. Significant genetic variation was observed for all traits in all three data sets. The heritabilities of grain yield and thousand-kernel weight of 0.83 and 0.91, respectively, in the doubled-haploid population were high, while the heritabilities of seeds per silique and siliques per square decimeter were lower with only 0.67 and 0.66, respectively. Genetic variance and heritability in the testcross population were lower than in the doubled haploid population because all testcross hybrids shared a common parent and differential heterozygosity that could have increased variance was low. Heterozygosity in the testcross population showed a narrow distribution with a mean of 50% and a standard deviation of only 8.6%. Accordingly, correlations between testcross performance and heterozygosity were not significant. Only yield showed a significant correlation between heterozygosity and midparent heterosis, but with a coefficient of determination of 0.078 heterozygosity did not explain much of the variance in heterosis observed in the testcross hybrids.

The considerably higher heritability of grain yield compared to other studies (Diepenbrock and Becker 1995) can be attributed to the very high genetic variation of the doubled-haploid population. The heritabilities calculated with the midparent heterosis values were similar to the heritabilities estimated in the doubled-haploid population.

Levels of heterosis: The results on the F_1 and the average testcross heterosis are presented in Tables 3 and 4. The most complex trait, grain yield, showed the highest level of heterosis with 30.0% F_1 heterosis and 13.0% average testcross heterosis. No significant high parent heterosis for yield was observed. The average testcross high parent heterosis was negative and statistically significant, but low. Thousand-kernel weight did not show significant midparent F_1 heterosis; the average testcross midparent heterosis reached −1.2%, which was significant but very low. Seeds per silique exhibited a positive midparent heterosis of 11.2% in the F_1 hybrid and reached 12.7% in the testcross hybrids. This was the only trait showing a positive average high parent

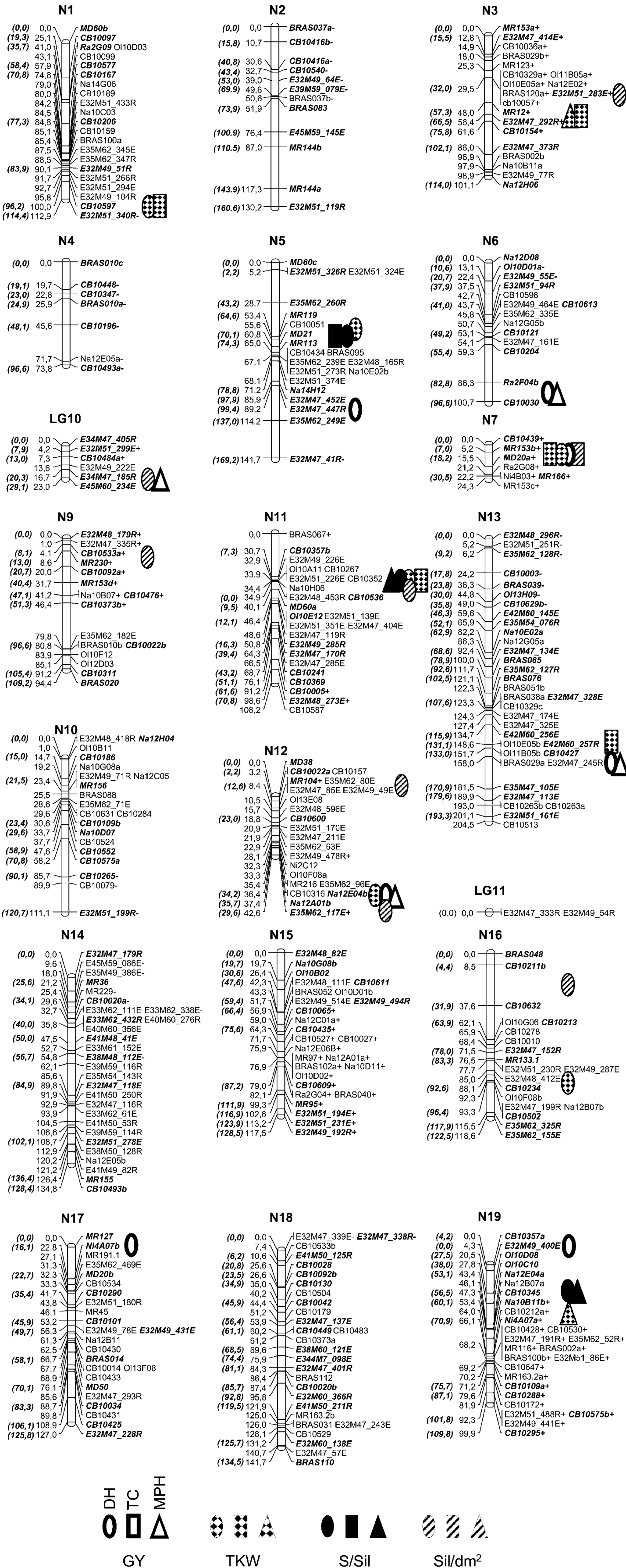

Figure 1.—Genetic linkage map of *B. napus* from the cross "Express" × "R53." The positions of the marker loci represent the distance from the first marker of the respective linkage group in centimorgans, estimated from the recombination frequencies between consecutive markers determined in 96 doubled-haploid lines. The recombination frequencies were transformed to centimorgans according to the Kosambi mapping function. The markers in bold italics were chosen for the construction of the framework map. The framework map positions, presented in parentheses, were estimated from recombination frequencies according to Haldane's mapping function on the basis of 250 DH lines. Markers deviating significantly from the expected 1:1 segregation ratio are labeled with "+" if skewed toward Express alleles and with "−" if the R53 allele is the more frequent one. The ovals, rectangles, and triangles indicate QTL identified in the doubled-haploid (DH) population and in the testcross (TC) population, and the midparent heterosis data, respectively. The different patterns illustrate QTL for grain yield (GY), thousand-kernel weight (TKW), seeds per silique (*S*/Sil), and siliques per square decimeter (Sil/dm²).

TABLE 2

Genetic variance, effective error mean, and heritability of the different traits in the doubled-haploid lines, the testcross hybrids, and the midparent heterosis data

Trait[a]	$\hat{\sigma}^2_g$	$\hat{\sigma}^2_e$	$\hat{h}^2$
	DH lines[b]		
GY	0.30**	0.24	0.83
TKW	0.09**	0.03	0.91
S/Sil	8.20**	16.37	0.67
Sil/dm^2	40.10**	83.10	0.66
	TC hybrids[b]		
GY	0.04**	0.11	0.61
TKW	0.02**	0.02	0.72
S/Sil	1.83**	12.07	0.38
Sil/dm^2	7.17**	45.14	0.39
	MPH data[b]		
GY	0.33**	0.23	0.85
TKW	0.06**	0.05	0.80
S/Sil	5.83**	27.79	0.46
Sil/dm^2	41.01**	122.73	0.57

Significance is shown at *P = 0.05, **P = 0.01, ***P = 0.001, respectively.

[a] GY, TKW, *S*/Sil, Sil/dm^2: grain yield, thousand-kernel weight, seeds per silique, and siliques per square decimeter, respectively; $\hat{\sigma}^2_g$, genetic variance; $\hat{\sigma}^2_e$, residual variance; $\hat{h}^2$, heritability.

[b] Population or data set.

heterosis. The highest F_1 midparent heterosis of yield-related traits was observed for siliques per square decimeter with 19.0%, but the average testcross midparent heterosis was not significant. Negative better parent heterosis was observed for F_1 and testcross hybrids but it was significant only in the case of the latter. For the F_1 hybrid the yield heterosis was largely explained by the heterosis levels of seeds per silique and siliques per square decimeter, with the latter contributing stronger to heterosis. Seeds per silique was the only yield component contributing to the average testcross midparent heterosis.

Analysis of main-effect QTL: The results of the main-effect QTL analyses for yield and yield-determining traits are summarized in Table 5 and Figure 1.

Grain yield: Six QTL with additive effects significant at $P = 0.001$ and one additional putative QTL ($P = 0.05$) were detected in the doubled-haploid population, which together explained 25.7% of the phenotypic and 31.0% of the genotypic variance. Six QTL showed positive additive effects, indicating that the parent Express contributed the favorable alleles. The effect of *GyN5* was negative, meaning that the allele of the resynthesized parent increased yield. *GyN12* exhibited the highest additive effect and alone explained 9.1% of the phenotypic variance.

Four QTL with significant dominance effects at $P = 0.001$ were detected with the midparent heterosis data. Together they explained 18.3% of the phenotypic and 21.5% of the genotypic variation. No QTL were detected in the testcross population.

The QTL detected simultaneously in the different data sets allowed an assessment of the degree of dominance (Table 5). *GyN12* exhibited partial dominance with a dominance ratio (d/a) of 0.7, while *GyN13* and *GyN6* showed overdominance. *GyLg10* was detected only in the midparent heterosis data, with a dominance effect of 0.10 t/ha. This effect was higher than the smallest additive effect detected in the doubled-haploid population. The failure to detect this QTL in the doubled-haploid population indicates that the additive effect of the QTL is smaller than the dominance effect, adding this QTL to the list of QTL showing overdominance.

Thousand-kernel weight: Six QTL with significant additive effects were mapped in the doubled-haploid population, which explained 28.5% of the phenotypic and 31.3% of the genotypic variance. Three QTL showed negative effects, while the remaining three QTL showed positive additive effects.

Five QTL detected in the testcross hybrids explained 21.7% of the phenotypic and 30.1% of the genotypic variance. *TkwN11* showed an effect as large as the additive effect detected at this locus in the doubled-

TABLE 3

F_1 and parental performance, midparent value, and F_1 heterosis

Trait[a]	Express ♀	R53 ♂	MPV	F_1	Heterosis (%)[b] MPH	HPH
GY	4.76	2.35	3.56	4.62	30.0**	−3.0 NS
TKW	4.44	4.21	4.32	4.29	−0.7 NS	−3.2**
S/Sil	25.41	23.97	24.69	27.46	11.2*	8.1 NS
Sil/dm^2	43.40	24.13	33.76	40.18	19.0**	−7.4 NS

Significance is shown at *P = 0.05 and **P = 0.01, respectively. NS, not significant.

[a] GY, TKW, *S*/Sil, Sil/dm^2: grain yield (tons per hectare), thousand-kernel weight (grams), seeds per silique, and siliques per square decimeter, respectively.

[b] MPH and HPH: midparent heterosis and high parent heterosis.

TABLE 4

Performance of "Express," the doubled-haploid population, and the corresponding testcross hybrids as well as the average testcross midparent and high parent heterosis

		Mean of				
					Heterosis (%)[b]	
Trait[a]	Express ♀	DH lines ♂	MPV	TC	MPH	HPH
GY	4.76	3.22	3.99	4.50	13.0**	−5.0**
TKW	4.44	4.19	4.31	4.26	−1.2**	−5.0**
S/Sil	25.41	21.43	23.42	26.28	12.7**	2.6**
Sil/dm^2	43.40	37.57	40.48	40.98	1.8 NS	−7.9**

Significance is shown at $^{*}P = 0.05$ and $^{**}P = 0.01$, respectively. NS, not significant.

[a] GY, TKW, *S*/Sil, Sil/dm^2: grain yield (tons per hectare), thousand-kernel weight (grams), seeds per silique, and siliques per square decimeter, respectively.

[b] MPH and HPH: midparent heterosis and high parent heterosis.

haploid population, which is a hint for absence of dominance.

Two QTL with dominance effects were mapped with the midparent heterosis data. They explained 17.4 and 21.8% of the phenotypic and genotypic variance, respectively. One of them showed a positive dominance effect but the largest dominance effect on linkage group N19 was negative. The dominance effects of QTL *TkwN1*, *TkwN7*, and *TkwN11* were calculated from the QTL effects identified in the DH and testcross (TC) populations. Most of the detected QTL for thousand-kernel weight showed only additive effects or low partial dominance and the two QTL *TkwN3* and *TkwN19* most probably exhibiting overdominance were with dominance effects with opposite signs, which explains the very low heterosis level observed for thousand-kernel weight.

Seeds per silique: Three QTL were mapped in the doubled-haploid population, which explained 18.6% of the phenotypic and 27.8% of the genotypic variance. The QTL *S/SilN5* and *S/SilN11* showed negative effects, meaning that the resynthesized parent contributed the increasing alleles. For *S/SilN19* the allele for more seeds per silique was inherited from Express. Two QTL were detected with the midparent heterosis data, which explained 5.2 and 11.3% of the phenotypic and genotypic variance, respectively.

One QTL was detected in the testcross population, which explained 11.8% of the phenotypic and 31.1% of the genotypic variation. The calculated dominance effect at this position was close to zero. The dominance effects of the QTL mapped with the midparent heterosis data were at lower levels than the additive effects at these positions, which indicated partial dominance with dominance ratios of 0.8 and 0.7 for *S/SilN11* and *S/SilN19*, respectively.

Siliques per square decimeter: Seven QTL were detected in the doubled-haploid population, which explained 48.0% of the phenotypic and 72.7% of the genotypic variance. In five cases the additive effect was positive. No QTL with dominance effects were identified, which was congruent with the insignificant level of heterosis observed for this trait (Table 4). Only one QTL was detected in the testcross population, explaining 8.8 and 22.6% of the phenotypic and genotypic variance, respectively.

Analysis of epistatic interactions: The results of the QTL analyses for epistasis are listed in supplemental Table 2 and have been summarized in Table 6.

Grain yield: Sixteen loci involved in nine digenic interactions were detected in the doubled-haploid population (Table 6). The epistatic interactions explained 15.8% of the phenotypic variation for grain yield in the doubled-haploid population. Two of these loci, on linkage group N6 and N19 had already been identified as main-effect QTL (Table 5). One epistatic effect was negative, indicating that a recombinant allele combination increased grain yield. The rest of the effects were positive, meaning that parental allele combinations contributed for higher grain yield. Eleven loci involved in six epistatic interactions were identified in the testcross hybrids, which explained 35.5% of the phenotypic variation. None of them exhibited a significant main effect. With midparent heterosis data 17 loci were detected in nine pairwise interactions, explaining 39.5% of the phenotypic variance. One locus on N13, interacting with a locus on N16, had already shown a significant main effect (Table 5).

Thousand-kernel weight: Eleven loci involved in seven digenic interactions were detected in the doubled-haploid population. They explained 19.0% of the phenotypic variance. Three of the epistatic interactions included loci that had shown significant main effects. In the testcross hybrids 17 loci in nine combinations were detected, which explained 36.5% of the phenotypic variance. Two of these loci had also shown significant main effects. Five significant epistatic interactions were identified with midparent heterosis data. They involved 10 loci and explained 25.6% of the phenotypic variance.

TABLE 5

QTL and their main effects detected in the doubled-haploid population (DH lines), the midparent heterosis values (MPH), and the testcross hybrid population (TC hybrids)

			DH lines				MPH				TC hybrids				
QTL[a]	LG	Interval	Pos[b]	LOD	Effect	V_P[c]	Pos	LOD	Effect	V_P	Pos	LOD	Effect	V_P	$\|d/a\|$[d]
					Grain yield (GY)										
GyN5	N5	E32M47_447R–E35M62_249E	99.4	9.1	−0.14***	4.3									
GyN6	N6	Ra2F04b–CB10030	94.8	4.2	0.05*	NE	94.8	3.4	0.09***	2.5					1.9
GyN7	N7	CB10439–MR153b	6.0	17.7	0.13***	3.4									
GyLg10	LG10	E34M47_185R–E45M60_234E					28.3	3.5	0.10***	2.7					
GyN12	N12	Na12E04b–Na12A01b	34.2	17.6	0.20***	9.1	31.6	5.5	0.13***	5.2					0.7
GyN13	N13	CB10427–E35M47_105E	139.0	7.3	0.14***	4.4	141.0	7.2	0.16***	7.9					1.2
GyN17	N17	MR127–Ni4A07b	16.0	11.0	0.09***	1.8									
GyN19	N19	E32M49_400E–CB10357a	0.0	7.0	0.11***	2.7									
					Total:	25.7				18.3					
					Thousand-kernel weight (TKW)										
TkwN1	N1	CB10597–E32M51_340R	108.2	5.9	−0.11***	8.4			(0.06)		114.2	4.2	−0.05***	3.0	0.5
TkwN3	N3	MR12–E32M47_292R					59.3	3.5	0.06***	5.4	66.5	5.4	−0.06***	5.0	
TkwN5	N5	MR119–MD21	68.6	3.5	0.06***	2.4									
TkwN7	N7	MR153b–MD20a	17.0	8.7	−0.10***	7.0			(0.03)		17.0	10.0	−0.07***	5.8	0.3
TkwN11	N11	CB10536–CB10357b	0.0	4.3	0.06***	2.7			(0.00)		0.0	10.1	0.06***	4.6	0.0
TkwN12	N12	E35M62_117E–Na12E04b	31.6	5.4	−0.08***	4.1									
TkwN13	N13	E32M47_328E–E42M60_256E									115.6	4.3	−0.05***	3.3	
TkwN16	N16	MR133.1–CB10234	85.3	5.9	0.08***	3.9									
TkwN19	N19	Ni4A07a–CB10109a					70.9	7.3	−0.09***	12.0					
					Total:	28.5				17.4				21.7	
					Seeds per silique (*S*/Sil)										
S/SilN5	N5	MD21–MR113	70.1	14.1	−1.36***	6.8			(0.19)		66.6	9.9	−1.55***	11.8	0.1
S/SilN11	N11	CB10536–CB10357b	4.0	15.6	−1.49***	8.2	0.0	4.9	−1.20***	5.2					0.8
S/Sil0N19	N19	CB10345–Na10B11b	56.5	6.1	0.99***	3.6	52.0	4.0	0.66*	NE					0.7
					Total:	18.6				5.2				11.8	
					Siliques per square decimeter (Sil/dm^2)										
Sil/dm^{2}N3	N3	E32M51_283E–MR12	35.5	10.7	2.88***	12.0									
Sil/dm^{2}Lg10	LG10	E34M47_185R–E45M60_234E	28.3	7.7	1.75***	4.4									
Sil/dm^{2}N7	N7	CB10439–MR153b									0.0	7.3	2.64***	8.8	
Sil/dm^{2}N9	N9	CB10533a–MR230	10.1	6.4	−1.15***	1.9									
Sil/dm^{2}N11	N11	MD60a–Ol10E12	9.5	4.7	1.40***	2.9									
Sil/dm^{2}N12a	N12	CB10022a–MR104	10.2	7.7	−1.79***	6.1									
Sil/dm^{2}N12b	N12	E35M62_117E–Na12E04b	33.6	14.3	3.22***	15.0									
Sil/dm^{2}N16	N16	CB10211b–CB10632	18.4	6.6	1.98***	5.7									
					Total:	48.0								8.8	

Significance is shown at $^{*}P = 0.05$ and $^{***}P = 0.001$. Values in parentheses were calculated from the effects of this locus detected with the other data sets.

[a] *Gy*, *Tkw*, *S/sil*, and *Sil/dm^2* indicate QTL for grain yield (tons per hectare), thousand-kernel weight (grams), seeds per silique, and siliques per square decimeter, respectively. N or LG followed by a number designates the linkage group where the QTL was detected.

[b] Positions are measured from the beginning of the linkage group in centimorgans (Haldane).

[c] V_P, percentage of explained phenotypic variance; NE, not estimated, in the case of putative QTL.

[d] $|d/a|$, dominance ratio.

TABLE 6

Number and type of epistatic interactions identified in the doubled-haploid (DH) and testcross (TC) populations and the midparent heterosis (MPH) data set

Trait[a]	Data set	No. of loci[b]	Type of epistasis[c] I	II	III	epQTL[d]	Range of the effects Min	Max	$V_p(e)$[e]	$V_p(m)$	$V_p(t)$
GY	DH	16	0	2	7	9	−0.108	0.116	15.8	25.7	41.5
GY	MPH	17	0	1	8	9	−0.081	0.058	39.5	18.3	57.8
GY	TC	11	0	0	6	6	−0.049	0.059	35.5	0.0	35.5
TKW	DH	11	0	3	4	7	−0.060	0.087	19.0	28.5	47.5
TKW	MPH	10	0	0	5	5	−0.030	0.035	25.6	17.4	43.0
TKW	TC	17	0	2	7	9	−0.030	0.029	36.5	21.7	58.2
S/Sil	DH	15	0	1	8	9	−0.550	2.029	30.2	18.6	48.8
S/Sil	MPH	19	0	0	10	10	−0.730	0.594	49.1	5.2	54.3
S/Sil	TC	14	0	0	8	8	−0.520	0.498	36.2	11.8	48.0
Sil/dm^2	DH	23	1	2	9	12	−1.420	1.682	31.2	48.0	79.2
Sil/dm^2	MPH	18	0	0	9	9	−1.440	1.171	39.0	0.00	39.0
Sil/dm^2	TC	21	0	0	12	12	−1.120	0.954	45.6	8.8	54.4

[a] GY, TKW, *S*/Sil, Sil/dm^2: Grain yield (tons per hectare), thousand-kernel weight (grams), seeds per silique, and siliques per square decimeter, respectively.

[b] Number of loci involved in digenic epistatic interactions.

[c] Epistatic interaction between (I) two loci with main effects, (II) a locus with main effect and a locus without significant main effect, and (III) two loci without significant main effects.

[d] Total number of epistatic interactions.

[e] $V_p(e)$, $V_p(m)$, $V_p(t)$: percentage of phenotypic variance explained by epistasis, main-effect QTL, and the sum of both, respectively.

Seeds per silique: In total 15 loci involved in nine digenic epistatic interactions, which explained 30.2% of the phenotypic variance, were detected in the doubled-haploid population. Eight showed positive effects, while one was negative. *S/SilN11* involved in an epistatic interaction exhibited a significant additive effect as well (Table 5). Eight digenic interactions between 14 loci were identified in the testcross population. Together they explained 36.2% of the phenotypic variance. None of these loci was identical to a locus with main effect. Analyses with midparent heterosis data resulted in the identification of 19 loci involved in 10 epistatic interactions. With 49.1% the epistasis for seeds per silique explained a considerably higher portion of the phenotypic variance than the 5.2% explained by the main-effect QTL. No loci with significant main effect were included in epistatic interactions.

Siliques per square decimeter: Twenty-three loci in 12 combinations were mapped in the doubled-haploid population, explaining 31.2% of the phenotypic variance. Four interactions were with negative and 8 with positive effects. Three of the mapped interactions included 4 loci on linkage groups N9, N12, and LG10, which had also shown significant main effects (Table 5). Twenty-one loci involved in 12 digenic interactions were identified in the testcross hybrids, explaining 45.6% of the phenotypic variance. The analyses with midparent heterosis data led to the detection of 18 loci, involved in 9 epistatic interactions, explaining 39.0% of the phenotypic variance.

DISCUSSION

Mapping populations: The plant material used in this study and the specific crossing scheme were chosen to optimize the ability to detect QTL contributing to heterosis, to estimate their effects as well as the degree of dominance and to determine whether they are involved in digenic epistatic interactions. The QTL mapping in the doubled-haploid and testcross populations allowed the identification of additive and non-additive gene actions. This was facilitated by the choice of MSL-Express as tester, a male-sterile version of the cultivar Express that was one parent of the doubled-haploid population. Accordingly, the testcross population was genetically equivalent to a BC_1 population. The use of one of the parents as a tester for hybrid production provided the opportunity for a straightforward determination of the genetic effects. With an independent tester that may have introduced additional alleles the effects of the QTL in the MPH data set and the testcross population would not necessarily represent the dominance effects and the sum or difference of additive and dominance effects, respectively (Table 1). The genotypes of backcross hybrids, on the other hand, can be unambiguously deduced from the marker information of the parental doubled-haploid lines (Mei *et al.* 2005). The disadvantage of using a backcross population is that only 50% of the possible heterosis is realized.

Mapping QTL with additive and dominance effects: In the QTL mapping for grain yield and three yield

components 23 QTL with additive effects were identified in the doubled-haploid population. Nine of them were congruent with QTL identified with the other data sets, allowing the assessment of the degree of dominance. In total 8 QTL showing dominance effects were mapped with the MPH data and the dominance effects at 4 loci could be calculated from the effects estimated in the DH and testcross populations. Of the 12 dominance effects estimated 5 showed overdominance and the remaining 7 exhibited partial dominance. In heterosis studies in maize Frascaroli *et al.* (2007) observed that QTL for traits with low heterosis were prevailingly in the additive to dominance range, while QTL for highly heterotic traits had effects in the dominance to overdominance range. Similarly in our study grain yield, which showed the highest level of heterosis, was the trait with the largest number of loci showing overdominance. Surprisingly, for thousand-kernel weight, which exhibited no heterosis, 2 loci showed overdominance. Only partial dominance was observed at QTL for seeds per silique and no QTL with dominance effects were identified for siliques per square decimeter.

Our results indicate that all levels of dominance play a role in the expression of heterosis in the rapeseed population studied. Considering all traits together, overdominance was observed at 41.7% of the loci showing dominance, while the remaining 58.3% exhibited partial dominance. On the other hand, the five QTL showing overdominance explained 30.5% of the phenotypic variance, a much larger portion than the 10.4% explained by the seven QTL exhibiting partial dominance.

In maize, Stuber *et al.* (1992) mapped QTL for seven major traits and suggested that overdominance plays a significant role in heterosis. The largest QTL for yield in that experiment was further dissected by Graham *et al.* (1997), who by fine mapping revealed that the seemingly overdominant action of the original QTL is actually pseudo-overdominance. In maize, Frascaroli *et al.* (2007) observed partial to full dominance for seedling emergence, days to pollen shedding, anthesis silking interval, and kernel weight, whose heterosis levels ranged from 5 to 34%. For highly heterotic traits as seedling weight, plant height, grain yield, and number of kernels per plant, whose heterosis levels ranged from 52 to 239%, prevailingly overdominance was observed.

Rapeseed is a partially allogamous crop with considerably lower levels of heterosis than maize. The highest level of heterosis was observed with 30% for grain yield compared to heterosis levels of >100% frequently observed in maize. Nevertheless in rapeseed 3 of 4 loci for grain yield showed overdominance. High levels of overdominance for reproductive traits were also reported in rice (Li *et al.* 2001; Luo *et al.* 2001; Mei *et al.* 2003, 2005) and tomato (Semel *et al.* 2006), leading Semel *et al.* to the hypothesis that an association of overdominant QTL for traits determining higher reproductive fitness was selected for in evolution. On the other hand, analyzing five biomass-related traits Kusterer *et al.* (2007b) mapped 6 QTL with significant dominance effects. Three of these showed overdominance although the traits, with heterosis levels ranging from −1.83 to 49.4%, were nonreproductive. In a second study in Arabidopsis with two reciprocal libraries of near-isogenic lines (NILs) (Melchinger *et al.* 2007b) 56 QTL for seven growth-related traits were mapped using the method described in Semel *et al.* (2006). The majority of the QTL showed overdominance but by a novel approach on generation means analysis using triple testcrosses of the NILs the authors were able to show that the seemingly overdominant gene action at many of the QTL was due to a combination of partial to full dominance effects and additive × additive epistatic interactions.

The number of QTL for all traits detected with the testcross and the midparent heterosis data was considerably smaller and explained lower percentages of the phenotypic variance than the number of QTL detected in the doubled-haploid population. One reason for this is likely to be QTL with an intermediate mode of inheritance. Lacking dominance such QTL cannot be detected in the midparent heterosis data. Furthermore, QTL with partial dominance effects, that is, dominance effects smaller than the additive effects, are less likely to be detected in the midparent heterosis data than in the doubled-haploid population. This may also cause a certain bias in the QTL detected in the midparent heterosis data in favor of QTL showing overdominance, since small partial dominance effects would more often remain under the detection threshold.

An impediment in detecting QTL in the testcross hybrid population is the so-called "masking effect of the tester" (Gallais and Rives 1993). If the tester carries an increasing allele that is dominant, no QTL can be detected in the testcrosses.

Evidences for epistasis on population level: Under the assumption of regular meiosis and no gametic selection the mean of a DH population should be equal to the midparent value of the parents if no epistasis is involved. In the case of seeds per silique and siliques per square decimeter a significant difference ($P = 0.05$) was observed between the midparent value of Express and R53 and the mean of the doubled-haploid lines, which indicates the presence of epistatic interactions. For siliques per square decimeter the lower midparent value in comparison to the doubled-haploid population mean could be a result of negative epistatic gene complexes occurring in the parental genotypes, which are broken due to recombinations in the doubled-haploid lines. In contrast, the reduced doubled-haploid line mean of seeds per silique compared to the midparent value of Express and R53 could be due to a loss of positive epistatic interactions occurring in the two parents. Furthermore, the testcross hybrids should on average

have about 2 times fewer heterozygous loci than the parental F_1 hybrid. This provides an explanation for the 2 times lower average testcross midparent heterosis than the F_1 midparent heterosis observed for grain yield. However, the heterosis of siliques per square decimeter was reduced not 2 times but 10 times. In contrast, the average testcross midparent heterosis for seeds per silique increased to 12.7% compared to the F_1 midparent heterosis of 11.2%. These unexpected results might be explained by the fact that epistasis is reducing the midparent values of the F_1 for siliques per square decimeter and increasing this value for seeds per silique.

Mapping QTL with epistatic effects: A large number of epistatic interactions were detected with the three different data sets (Table 6), indicating that epistasis plays an important role not only in the variation of the performance of the doubled-haploid lines but also in the expression of heterosis in rapeseed. Epistasis explains as large or even larger portions of the phenotypic variance than the main effects (Table 6). For grain yield and thousand-kernel weight the phenotypic variance in heterosis explained by dominance effects was 18.3 and 17.4%, respectively, while epistasis explained 39.5 and 25.6% of the phenotypic variance. The difference between the phenotypic variances explained by dominance and by epistasis was even more pronounced for seeds per silique and siliques per square decimeter, where 5.2 and 0.0%, respectively, were explained by dominance, while the epistatic interactions accounted for 49.1 and 39.0% of the phenotypic variance, respectively.

In general, larger numbers of digenic epistatic interactions were identified than main-effect QTL. For example, only four QTL with main effects were detected in the MPH data for grain yield, but 9 digenic epistatic interactions. In the case of siliques per square decimeter no main-effect QTL was mapped but the 9 epistatic interactions detected explained 39.0% of the phenotypic variance. According to Li *et al.* (2001) the epistatic interactions can be classified in three groups. Epistatic interactions between two loci with significant main effects represent type I, interactions between a main-effect QTL and a locus without significant main effects are of type II, and interactions between two loci with no significant main effects are of type III. Our results confirm those of Li *et al.* (2001), Luo *et al.* (2001), and Yu *et al.* (1997) in rice, that epistasis occurs not only between main-effect QTL. Li *et al.* (2001) and Luo *et al.* (2001) detected predominantly type III epistatic interactions in rice. We observed just a single epistatic interaction of type I, 11 of 105 (10.5%) were of type II, and the remaining 93 (88.5%) were of type III. Our results are in discrepancy with Zhao *et al.* (2005), who identified 11 digenic interactions for oil content in a doubled-haploid population developed from a cross between a European and a Chinese cultivar. Seven of these epistatic interactions were of type I and 4 of type II. No type III interactions were detected.

The large number of epistatic interactions identified in our study differed from the studies of Stuber *et al.* (1992), Lu *et al.* (2003), and Mihaljevic *et al.* (2005), where no significant epistasis was detected in maize by testing all possible pairwise combinations of markers linked to the mapped QTL. Using the same approach no epistasis was detected by Xiao *et al.* (1995) in rice as well. On the other hand, Yu *et al.* (1997), applying two-way analyses of variance using all possible pairwise combinations of marker genotypes to test epistasis, and Luo *et al.* (2001) and Li *et al.* (2001), using a mixed linear model with background variation control to map simultaneously main and epistatic effects, reported that epistasis is a common feature of most loci associated with inbreeding depression and heterosis in rice. In Arabidopsis Kusterer *et al.* (2007a) found a significant role of epistasis in the expression of heterosis while analyzing generation means and variance components for biomass-related traits in a triple-testcross design with a recombinant inbred line population. Furthermore, in a study on QTL mapping in Arabidopsis using NIL libraries Melchinger *et al.* (2007b) found about three times more loci with significant additive $\times$ additive epistatic interactions with the genetic background than with dominance effects. Since according to Melchinger *et al.* (2007a) heterosis is determined by the dominance effects of QTL and the sum of their additive $\times$ additive epistatic interactions, this study again indicates a strong role of epistasis in the expression of heterosis in Arabidopsis. The results of our study in rapeseed, together with the available data in rice and Arabidopsis, support the hypothesis of Li *et al.* (2001) that epistasis for complex traits is more pronounced in self-pollinated than in cross-pollinated species because coadapted gene complexes with favorable epistasis can be more easily maintained.

Some of the loci involved in epistasis in this study interacted with more than one locus; for example, the locus at position 78.9 cM on linkage group N13 was involved in *GyN13/N14* and *GyN13/N18* (supplemental Table 2). The participation of loci in multiple digenic epistatic interactions could be a reflection of the existence of higher-order epistatic interactions, meaning that the number of epistatic interactions may still be underestimated in this study.

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