Genetic analysis of heterosis in rapeseed (*B. napus* L.)

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Abstract

In an attempt to elucidate the genetic basis of heterosis in rapeseed, QTL associated with heterosis for grain yield as well as early and late plant biomass were studied in a doubled haploid mapping population and its corresponding test crosses with the tester 'MSL-Express'. The mapping population consisted of 250 doubled haploid lines derived from a highly heterotic cross between the winter rapeseed cultivar 'Express' and a resynthesized line, 'R53'. A genetic map was constructed using SSR and AFLP markers and a framework map comprising 188 markers was used for composite interval mapping based on multiple regression and mixed model approaches. The heterosis in early plant biomass of rapeseed was studied in a greenhouse trial with four replicates while plant height and total grain yield were analysed in a field experiment carried out at four locations. The QTL mapping resulted in the localization of heterosis-relevant loci and in an assessment of their genetic effects as well as the relative contribution of different gene actions, e.g. dominance, overdominance and epistasis, to the expression of heterosis in rapeseed.

Key words: *Brassica napus*, rapeseed, heterosis, QTL, dominance, overdominance

Introduction

The term heterosis was coined by Shull (1922) as a descriptive synonym for hybrid vigor. This is a phenomenon in which the performance of an F_1 hybrids produced from a cross between genetically distant parents is superior to their midparent value or even to the better parent. Although this phenomenon has been extensively studied, its genetic basis is still not resolved. There exist three main hypotheses to explain the basis of heterosis: the dominance hypothesis (Davenport 1908), the overdominance hypothesis (Shull 1908) and the epistasis hypothesis (Schnell & Geiger 1970). In our study we developed a new genetic map, and carried out QTL mapping for grain yield, early and late plant biomass in an attempt to elucidate the genetic basis of heterosis in rapeseed.

Materials and methods

Plant material: The plant material consisted of a doubled haploid population of 250 lines developed from a cross between an inbred line of the commercial cultivar 'Express' and a resynthesized rapeseed line 'R53'. The doubled haploid lines were crossed with a male sterile line of 'Express' for the production of F_1 hybrids. In addition, in the greenhouse and field experiments the parents 'Express' and 'R53', the corresponding F_1 hybrid, and the commercial cultivar 'Elektra' were included as controls.

Genetic markers: SSR and AFLP marker techniques were applied for the development of the genetic map. The marker analyzes were carried out on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster city, USA). GeneScan and Genotyper software (Applied Biosystems, Foster city, USA) were used for raw data analysis and determination of fragment sizes.

Linkage analysis: Linkage analysis was performed using MapMaker/EXP 3.0 (Lander et al. 1993) with a minimum LOD score of 4.0 and a maximum recombination frequency of 0.4. The recombination frequencies were transformed to (cM) using the Kosambi mapping function.

Phenotypic experiments: The doubled haploid mapping population and its corresponding hybrids with the tester 'MSL-Express' were grown in replicated green house and field experiments. The field experiments took place at four locations in Northern and Central Germany following an alpha lattice design (26×10) with no replications. The heterotic traits of interest were plant height at maturity and total grain yield. The green house trial was focused on fresh shoot plant biomass, measured four weeks after sowing. It was carried out in multipot arrays with 9 pots per plot (9 plants per genotype). The experiment followed the same design used in the field trials with the difference that instead of four locations it was carried out with four replications.

Data analysis and QTL mapping: For the statistical analysis of the phenotypic data PLABSTAT Version 3A's LATTICE statement (Utz 2003) was used.

The phenotypic data from the doubled haploid lines and from the corresponding F_1 hybrids as well as the midparent heterosis of the doubled haploid lines and the corresponding hybrids were used separately as input for the QTL mapping. The QTL mapping and the estimation of the effects of the mapped QTL were performed with the software PLABQTL (Utz and Melchinger 1996), employing composite interval mapping by a regression approach (Haley and Knott 1992) in combination with the use of cofactores. The selection of coffactores followed Melchinger et al. (1998). The genetic effects estimated differ according to the nature of the mapping population and the input data. For the DH population the estimated main effect of a

putative QTL is its additive effect (a). For the testcrosses the estimated effect is a combination of both dominance (d) and additive effect - (a + d)/2 and (a – d)/2 if the donor or the recurrent parent carries the beneficial allele, respectively. When the midparent heterosis is used in the QTL mapping, the estimated genetic effect represents the dominance effect (– d/2). Therefore the effects presented in Table 3 were multiplied by 2.

Results

Genetic map: A primary genetic map was developed in a subset of 96 doubled haploid lines. It includes 364 markers distributed across 21 linkage groups and covers 1969 cM of the rapeseed genome. The framework map for QTL mapping was constructed by mapping 188 of the most evenly distributed markers in the remaining 154 doubled haploid lines. It covers 1863 cM of the genome.

Heterosis: The F_1 and parental means as well as the average doubled haploid line performance and the average hybrid performance together with the corresponding relative heterosis are shown in Table 1 and 2. For early plant biomass a highly significant midparent heterosis (MPH) of 33% was observed in the F_1 hybrid of the parents. The average heterosis for early plant biomass in the test crosses of the doubled haploid lines reached 12%. This decrease in comparison with the parental F_1 heterosis was expected since the tester 'MSL-Express' is genetically equivalent to the parent 'Express' used in the cross for the development of the doubled haploid population. This leads to 50% less heterozygous loci in the test hybrids than in the parental F_1 hybrid, explaining the twice-lower heterosis level. The plant height showed lower levels of heterosis: 16% and 14% for the F_1 hybrid of the parents and the average midparent heterosis of the test crosses, respectively. Significant midparent heterosis for grain yield of 30% was observed in the parental F_1 hybrid. The average testcross heterosis was 13%.

Table 1 F_1 and parental means, midparent value and relative heterosis

Trait[a]	Express ♀	R53 ♂	MPV	F_1	Heterosis (%)[b]	
					MPH	BPH
EFB	18.04	13.09	15.56	20.70	33.0**	15.0**
PH	143.72	150.22	146.97	170.64	16.0**	14.0**
GY	47.61	23.53	35.57	46.19	30.0**	-3.0

[a]EFB: Early fresh biomass [g/plant]; PH: Plant height [cm]; GY: Grain yield [dt/ha]
[b]MPH and BPH: midparent heterosis and better parent heterosis. **Significant at $P = 0.01$

Table 2 Means of 'Express', the doubled haploid population and the corresponding F_1 hybrids as well as the relative midparent and better parent heterosis

Trait[a]	Express ♀	Mean of				
		DH-Lines ♂	MPV	F_1	Heterosis (%)[b]	
					MPH	BPH
EFB	18.04	17.79	17.91	20.03	12.0**	5.0**
PH	143.72	155.90	149.81	169.99	14.0**	9.0**
GY	47.61	32.16	39.88	45.04	13.0**	-5.0**

[a]EFB: Early fresh biomass [g/plant]; PH: Plant height [cm]; GY: Grain yield [dt/ha]
[b]MPH and BPH: midparent heterosis and better parent heterosis. **Significant at $P = 0.01$

QTL analysis: The results of the QTL analysis using the three different datasets are presented in Table 3.

Table 3 QTL and their effects detected with doubled haploid lines (DH-lines), midparent heterosis values (MPH), and test cross hybrid values (F_1)

Trait[a]	LG	Interval	DH-lines			MPH			F_1		
			LOD	Effect[b]	Vp[c]	LOD	Effect	Vp	LOD	Effect	Vp
EFB	N3	E32M47ak-E32M51t	4.07	0.700	6.7	6.56	1.210	7.7			
EFB	N11	Ol10E12-E32M49n	12.29	1.221	22.9	3.41	0.694	3.8			
EFB	N13	BRAS065-E35M62g	7.45	0.850	12.3						
EFB	N19	CB10288-CB10575b	5.47	0.713	8.4						
EFB	N9	CB10373b-CB10022b							4.04	0.966	6.5
PH	N8	CB10003-E35M62h	3.47	-1.989	3.9						
PH	N9	E32M48e-CB10533a	3.48	-1.653	3.0						
PH	N12	E35M62f-Na12E04b	3.24	1.766	3.0	4.32	2.796	6.3			
PH	N16	BRAS048-CB10211b	6.19	-2.733	7.7						
PH	N16	CB10632-CB10213							6.21	-3.514	10.5
GY	N5	E32M47al-E35M62l	5.72	-1.449	8.7						
GY	N7	CB10439-MR153b	5.17	1.581	8.5						
GY	N12	E35M62f-Na12E04b	8.98	2.024	14.6	3.43	1.248	4.9			
GY	N13	CB10329b-CB10427	4.46	1.402	8.1	3.83	1.422	6.9			

[a,b]EFB: Early fresh biomass [g/plant]; PH: Plant height [cm]; GY: Grain yield [dt/ha];
[c] Vp: Explained phenotypic variation [%]

Early plant biomass: Four QTL were detected in the doubled haploid population, which explain 34.5% of the phenotypic variation. In all cases 'Express' contributed the beneficial allele. The midparent heterosis resulted in the localization of two QTL, which explain 9.7% of the phenotypic variation. The QTL were positioned on chromosome N3 and N11 in the same marker intervals where QTL from the doubled haploid population had been detected. Only one QTL on chromosome N9 was mapped with the test cross data, which explain 6.8% of the phenotypic variation. The dominance effect of the QTL on linkage group N3 was 1.7 times higher than the additive effect, showing overdominance, while on N11 only partial dominance was observed.

Plant height: Using the data from the doubled haploid population 4 QTL were detected, which explain 14 % of the phenotypic variation. On N12 the allele responsible for increased plant height came from 'Express', while in the rest of the loci the resynthesized parent contributed the superior allele. Only single QTL were detected with the midparent heterosis and testcross hybrid data which were located on linkage groups N12 and N16 respectively. The dominance effect of the QTL on N12 was larger than the additive effect indicating overdominance.

Grain yield: Four QTL were located in the doubled haploid population. They explain 30% of the phenotypic variation. In all cases with the exception of the locus on linkage group N5, the beneficial alleles were inherited from 'Express'. The two QTL detected on linkage group N12 and N13 with the midparen heterosis data explained 11% of the phenotypic variation. No QTL were detected with the test cross hybrid data. On linkage group N12 the QTL exhibited partial dominance, while the QTL on N13 showed full dominance.

Discussion

Genetic basis of heterosis: The plant material used and the crosses made were chosen to optimize the ability to detect QTL contributing to heterosis. Using one of the parents of the segregating doubled haploid population as a tester for hybrid production provided the opportunity for a straightforward determination of the effects. Under the assumption that the same QTL were observed in different datasets when their confidence intervals overlapped strongly, the degree of dominance could be determined. Different dominance levels were observed not only for different traits but also between different loci contributing to the same trait. The number of QTL detected with the testcross and the midparent htererosis data was considerably smaller and explained lower percentage of the phenotypic variation than the number of QTL detected in the doubled haploid population. A possible explanation for this result is that a number of loci with positive additive effects where no corresponding QTL was detected with the midparent heterosis or the test cross hybrid data, exhibit full or partial dominance with a magnitude lower than the power of detection in the QTL mapping. The failure to detect such QTL with the testcross hybrid data comes from the fact that in that case the effect represents a difference between the additive and dominance effects at this locus and the two effects are canceling each other in case of full dominance or the resulting effect is too low to be detected in case of partial dominance. The opposite situation explains why in some cases QTL were observed with the test cross population data and not with the other two data sets (the QTL on N9 for biomass, N16 for plant height). In case of a beneficial allele coming from the donor parent the genetic effect for that locus in the test cross population represents the sum of the additive and dominance effects, which are too low to be detected separately in the other data sets.

The observed overdominance at single loci in our study can not be distinguished from the pseoudo-overdominance generated from the linkage in repulsion phase of genes with partial or full dominance (Crow 1952). Fine mapping at these loci can help to break a possible linkage and to determine whether the overdominance observed was due to pseudooverdominance or not.

Preliminary studies of epistasis in our experiment (data not shown) demonstrate that digenic interactions could play a significant role in the expression of heterosis in rapeseed, since the epistasis explains as large or larger portion of the phenotypic variation than the main effect QTL. These preliminary results are in agreement with the results of Li et al. (2001) and Luo et al. (2001) who concluded that overdominance and epistatis are the primery genetic basis for heterosis in rice. Their study is in discrepancy with Xiao et al. (1995) whose experiment shows that dominance is the major genetic basis of heterosis in rice. With respect to the main effect QTL our study is in closer agreement with the results of Xiao (1995) in rice, than with these of Li and Luo (2001) in rice and Stuber (1992) in maize who observed pronounced overdominance.

Additional support for our result that dominance and partial dominance rather than overdominance are responsible for the heterosis in rapeseed is that for all analyzed traits doubled haploid lines with phenotypic performances higher than the performance of the F_1 hybrid were observed (data not shown). This result met one of the predictions of the dominance hypothesis that true inbreeding individuals with trait expressions similar to or exceeding the F_1, can be obtained from its selfed progeny.

From our experimental data we could conclude that dominance and partial dominance, together with epistasis play a significant role in the expression of heterosis in rapeseed.

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