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Heterosis for Biomass-Related Traits in Arabidopsis Investigated by Quantitative Trait Loci Analysis of the Triple Testcross Design With Recombinant Inbred Lines

Barbara Kusterer,* Hans-Peter Piepho,† H. Friedrich Utz,* Chris C. Schön,‡ Jasmina Muminovic,* Rhonda C. Meyer,§ Thomas Altmann§ and Albrecht E. Melchinger*,[1]

**Institute of Plant Breeding, Seed Science, and Population Genetics,* †*Bioinformatics Unit, Institute of Crop Production and Grassland Research and* ‡*State Plant Breeding Institute, University of Hohenheim, 70599 Stuttgart, Germany and* §*Department of Genetics, Institute of Biochemistry and Biology, University of Potsdam, 14476 Potsdam, Germany and Max-Planck-Institute of Molecular Plant Physiology, 14476 Potsdam-Golm, Germany*

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ABSTRACT

Arabidopsis thaliana has emerged as a leading model species in plant genetics and functional genomics including research on the genetic causes of heterosis. We applied a triple testcross (TTC) design and a novel biometrical approach to identify and characterize quantitative trait loci (QTL) for heterosis of five biomass-related traits by (i) estimating the number, genomic positions, and genetic effects of heterotic QTL, (ii) characterizing their mode of gene action, and (iii) testing for presence of epistatic effects by a genomewide scan and marker × marker interactions. In total, 234 recombinant inbred lines (RILs) of Arabidopsis hybrid C24 × Col-0 were crossed to both parental lines and their F_1 and analyzed with 110 single-nucleotide polymorphism (SNP) markers. QTL analyses were conducted using linear transformations Z_1, Z_2, and Z_3 calculated from the adjusted entry means of TTC progenies. With Z_1, we detected 12 QTL displaying augmented additive effects. With Z_2, we mapped six QTL for augmented dominance effects. A one-dimensional genome scan with Z_3 revealed two genomic regions with significantly negative dominance × additive epistatic effects. Two-way analyses of variance between marker pairs revealed nine digenic epistatic interactions: six reflecting dominance × dominance effects with variable sign and three reflecting additive × additive effects with positive sign. We conclude that heterosis for biomass-related traits in Arabidopsis has a polygenic basis with overdominance and/or epistasis being presumably the main types of gene action.

THE improved vigor of F_1 hybrids in comparison with their parental homozygous lines, defined as heterosis (Shull 1922), is a widely exploited phenomenon in plant breeding (Schnell 1982; Duvick 1999). In general, heterosis is largest in allogamous and smallest in strictly autogamous crops. Furthermore, its relative amount usually increases with the complexity of a trait and can exceed 100% for traits such as grain yield in maize (Becker 1993).

Ever since its discovery at the beginning of the 20th century (East 1908; Shull 1908), heterosis has attracted the attention of geneticists and breeders because of its poorly understood genetic nature. The first hypotheses on the genetic causes underlying heterosis are based on dominance and overdominance gene action. Regarding the former, superiority of hybrids results from the accumulation of dominant favorable alleles from both homozygous parents (Davenport 1908; Bruce 1910; Jones 1917). In contrast, the overdominance hypothesis suggests the superiority of the heterozygous state over either homozygote (Hull 1945; Crow 1948). A third hypothesis implies that heterosis results from epistatic interactions among alleles at different loci (Powers 1944; Williams 1959).

Quantitative trait loci (QTL) mapping approaches have proven to be powerful in dissecting the genetic basis of complex traits and heterosis in crops. In a pioneer QTL study with maize, Stuber *et al.* (1992) detected 11 QTL for grain yield, mostly with a strong tendency toward dominance and overdominance. A reanalysis of their data set with a different biometrical model (Cockerham and Zeng 1996) led to the conclusion that heterosis in the maize hybrid B73 × Mo17 was attributable not only to dominance of favorable alleles but also to epistatic effects between linked QTL. Contradictory results were reported in studies on heterosis in rice. Findings of several authors (Xiao *et al.* 1995; Li *et al.* 2001; Luo *et al.* 2001) indicated that heterozygotes were superior to both parental homozygotes at most QTL, suggesting the presence of overdominance

[1]*Corresponding author:* Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, Fruwirthstrasse 21, 70599 Stuttgart, Germany. E-mail: melchinger@uni-hohenheim.de

or pseudo-overdominance. In contrast, a study by Yu *et al.* (1997) as well as more recent investigations with an immortalized F_2 population (Hua *et al.* 2002, 2003) showed that heterozygosity was not necessarily advantageous for trait performance in genotypes derived from a highly heterotic hybrid.

To determine the contribution of different genetic effects to midparent heterosis (MPH) of quantitative traits, Melchinger *et al.* (2007, accompanying article in this issue) developed a novel theory based on classical quantitative genetic approaches utilizing design III (Comstock and Robinson 1952) and the triple testcross (TTC) design (Kearsey and Jinks 1968). They developed a generalized derivation of the relative contributions of different genetic effects to MPH for multiple QTL and all types of higher-order epistasis and derived genetic expectations of heterotic QTL identified by QTL mapping. Furthermore, they devised a joint likelihood-ratio test for detecting QTL involved in heterosis.

Arabidopsis has emerged as a leading model species in plant genetics and functional genomics. It possesses considerable advantages for studies on the causes underlying heterosis such as the ease with which appropriate large mapping populations can be established, genotyped, and phenotyped. However, only few investigations on heterosis for biomass-related traits have been published up to now (Barth *et al.* 2003; Kearsey *et al.* 2003; Meyer *et al.* 2004; Kroymann and Mitchell-Olds 2005). In a previous study (Kusterer *et al.* 2007), we used a TTC design to estimate the relative contribution of dominance and epistatic effects to heterosis by biometric analyses of first- and second-degree statistics.

The goals of this study were to apply the novel approach of Melchinger *et al.* (2007) to detect and characterize QTL for heterosis of biomass-related traits in Arabidopsis hybrid C24 × Col-0, using the TTC design. In particular, our objectives were to (i) estimate the number, genomic positions, and genetic effects of QTL contributing to heterosis, (ii) characterize their mode of gene action, and (iii) elucidate the role of epistasis in the manifestation of heterosis.

MATERIALS AND METHODS

Plant materials, experimental design, and traits measured: Plant materials and phenotypic data were described in detail in our previous article (Kusterer *et al.* 2007). Briefly, of 409 recombinant inbred lines (RILs) derived from the cross between Col-0 (parent P1) and C24 (parent P2), we studied a randomly chosen subset of 234 RILs together with their TTC progenies. Performance of testcross progenies of the nth RIL with testers P1, P2, and F_1 is denoted by H_{1n}, H_{2n}, and H_{3n} ($n = 1, 2, \ldots, 234$), respectively. Owing to the large number of entries to be tested, the entire set of 234 RILs and their 702 TTC progenies was subdivided into three experiments, each with 78 RILs plus their corresponding TTC progenies and six checks. Each experiment was arranged in a split-plot design with three replicates. Checks and RILs with their TTC progenies were grown in different main plots. Main plots were arranged in a 12×7 α-design. Each main plot comprised four entries: 1 RIL and its 3 TTC progenies. The main plots of checks also comprised four entries: parents P1 and P2, as well as the F_1 and F_2 generations from one of the two reciprocal forms P1 × P2 and P2 × P1. In all instances, the entries within each main plot were randomly assigned to the subplots. We recorded rosette diameter (in millimeters) of individual plants 22 days after sowing (RD22) and 29 days after sowing (RD29) and calculated the absolute growth rate per day (GR) (in millimeters per day) as $GR = (RD29 - RD22)/7$. Biomass yield above ground (BY) (in milligrams) was recorded for the bulk of 10 plants from each subplot after drying in an oven to practically 0% moisture content. Dry matter content (DMC) (in percentage) was calculated as the ratio between dry and fresh biomass, multiplied by 100.

Molecular markers and linkage maps: Single-nucleotide polymorphism (SNP) analyses were performed according to Törjék *et al.* (2003) for 110 SNP markers across the 409 RILs. A linkage map was constructed as described in detail by Törjék *et al.* (2006). Deviation of marker allele frequencies from 0.5 was tested with a χ^2-test statistic using a sequential Bonferroni correction of P-values (Holm 1979).

Data analysis: For each RIL, we calculated the linear transformations $Z_1 = (H_1 + H_2)/2$, $Z_2 = H_1 - H_2$, and $Z_3 = H_1 + H_2 - 2H_3$ at the main plot level. These transformations provided the basis for all further biometric and quantitative genetic analyses. The checks were not included in the analysis.

Entry means adjusted for incomplete blocks and experiments were calculated for each transformation Z_s ($s = 1, 2, 3$) by a mixed-model analysis across experiments. Following Kearsey and Jinks (1968), presence of additive × additive epistasis at the level of the entire genome was examined by testing the average of adjusted-entry means of Z_3 across RILs for deviation from zero using an appropriate χ^2-test. Genotypic (σ_g^2) and error variances (σ_e^2) as well as phenotypic and genotypic correlations between Z_s and Z_u ($s \neq u$) were estimated by established procedures (Mode and Robinson 1959; Searle 1971) from analyses of variance and covariance of the transformed observations. In addition, heritability (h^2) on an entry-mean basis was computed for each Z_s from variance components as $h^2 = 100 \times \sigma_g^2/(\sigma_g^2 + \sigma_e^2/r)$, where r corresponds to the number of replicates. Significance of variance components estimated by restricted maximum likelihood (REML) was tested by Wald statistics. This test is approximate and asymptotically equivalent to a likelihood-ratio test (Rao 1973). The Wald statistic was compared with a chi-square distribution with 1 d.f. and the P-value was halved to account for the fact that the null hypothesis places the parameter on the boundary of the parameter space (Stram and Lee 1994). All computations were performed with SAS PROC MIXED (Sas Institute 2004).

QTL analyses: QTL analyses were carried out by using adjusted entry means of Z_1, Z_2, Z_3, and H_3 of each RIL as well as their SNP data and the linkage map. Composite-interval mapping (CIM) (Jansen and Stam 1994; Zeng 1994) was used for the detection, mapping, and characterization of QTL. For all Z_s as well as H_3, a genetic model fitting only one genetic effect (corresponding to the additive effect in conventional QTL mapping) was chosen for QTL mapping, as described by Utz *et al.* (2000), because the SNP marker data referred to RILs. LOD threshold levels for QTL detection were determined by a permutation test (Churchill and Doerge 1994) using 2000 permutations. For Z_s and H_3 of each trait, the LOD threshold ranged between 1.7 and 1.9 for an experimentwise error rate of 30%. Therefore, a LOD threshold value of 1.8 was used to declare presence of a QTL for every Z_s and H_3. Estimates of QTL positions and effects for Z_s as well as H_3 were obtained at the position where the corresponding LOD

score reached a global maximum in the region under consideration. In addition, genetic effects of QTL for the other transformations Z_u ($u \neq s$) were determined at the position of Z_s to obtain estimates of the augmented dominance ratio (ratio of augmented dominance effect d_i* to augmented additive effect a_i* estimated by Z_2 and Z_1, respectively, see below for definitions) and also potential QTL × genetic background interactions revealed by Z_3 (Table 1). The proportion of the genotypic variance explained (p) was determined according to the procedure described by Utz *et al.* (2000) from the ratio $p = R^2_{adj}/h^2$, where h^2 is the heritability and R^2_{adj} is the adjusted partial correlation coefficient of a putative QTL or the multiple correlation coefficient of a set of QTL in the simultaneous fit. It must be noted that partial R^2 values for the detected QTL do not add up to the R^2 of the multiple-QTL model due to linkage disequilibrium between markers and corresponding multicollinearity of the regression problem.

In addition to one-dimensional genome scans for epistasis with Z_3, we also tested Z_3 and H_3 for presence of digenic epistatic effects by two-way analyses of variance between all pairs of marker loci. As a modification of the procedure described by Holland (1998), in this search for significant marker pairs we included the same set of markers as cofactors as used in CIM for QTL mapping of main effects, to eliminate their influence on the detection of epistatic QTL. Marker pairs were selected on the basis of the Bayesian information criterion (BIC) (Piepho and Gauch 2001), if the BIC value for the model with epistasis was at least 2 units below the BIC value for the model without epistasis, following Raftery (1995). Finally, all selected epistatic marker pairs as well as the positions of QTL from CIM for a given trait and Z_3 or H_3 were subjected to a further step of backward elimination in multiple regression based on the BIC. For those marker × marker interactions remaining in the final model, epistatic effects were estimated simultaneously with the QTL main effects.

In addition to separate QTL scans for each transformation Z_s, we followed for each trait the method of Jiang and Zeng (1995) to conduct a joint mapping for all three transformations Z_s, as proposed by Melchinger *et al.* (2007) for QTL mapping with design III. Using the permutation test (Churchill and Doerge 1994), we obtained the following LOD threshold values (corresponding to an experimentwise error rate of 30%) to declare presence of a QTL in the joint analysis: 3.1 for RD22, RD29, and GR; 3.3 for DMC; and 3.4 for BY.

All QTL computations were performed with the software package PLABQTL (Utz and Melchinger 1996), with an extension for calculation of the BIC according to the method of Burnham and Anderson (2004) to accommodate selection of cofactors and comparison of the models with and without digenic epistatic interactions.

Quantitative genetic expectations: Melchinger *et al.* (2007) provided general formulas for first- and second-degree statistics as well as QTL parameters for the transformations Z_s under the F_2-metric model with arbitrary linkage and digenic epistasis. Quantitative genetic expectations of the statistics most relevant to our further analyses, ignoring linkage, are given in Table 1, using the following notation: a_i and d_i denote the additive and the dominance effect at QTL i, respectively; and aa_{ij}, ad_{ij}, da_{ij}, and dd_{ij} denote the additive × additive, additive × dominance, dominance × additive, and dominance × dominance epistatic effects between loci i and j, respectively. QTL detected by genome scans with Z_1 reflect augmented additive effects $a_i^* = a_i - \frac{1}{2}[da_i]$ that, apart from the additive effect a_i, also include $[da_i]$, *i.e.*, the sum of dominance × additive effects of QTL i with all other QTL in the genetic background. QTL detected with Z_2 capture augmented dominance effects $d_i^* = d_i - \frac{1}{2}[aa_i]$, which include the dominance effect d_i and $[aa_i]$, *i.e.*, the sum of additive × additive effects of QTL i with all other QTL in the genetic background. Finally, QTL mapping with Z_3 provides a one-dimensional genome scan for QTL × genetic background interactions of type dominance × additive $[da_i]$. Interactions between marker pairs linked to QTL i and j depend for Z_3 only on dominance × dominance effects (dd_{ij}) and for H_3 only on additive × additive effects (aa_{ij}) (Table 1). In contrast, different types of epistatic effects are confounded in interactions between marker pairs for Z_1 and Z_2 and, hence, the latter were not considered in our study.

RESULTS

Linkage map construction: The SNP assays of the 409 RILs yielded an almost complete data set with <0.1% missing data points. The complete linkage map with 110 markers covered all five Arabidopsis chromosomes uniformly and spanned in total 425.7 cM, with an average interval length of 3.9 cM between markers (Figure 1). The maximum distance between markers was 13 cM. Altogether, the total length of our map was within the range of other crosses with Arabidopsis (Loudet *et al.* 2002; Malmberg *et al.* 2005). Allele frequencies on one chromosomal region of chromosomes 1, 4, and 5, as well as on two chromosomal regions of chromosome 3 deviated significantly ($P < 0.05$) from Mendelian expectations. On chromosomes 1, 3 (position 3/52–3/57), and 5, there were excesses of Col-0 alleles of 10, 20, and 10% over genomic regions of 8, 5, and 11 cM, respectively. On chromosomes 3 (position 3/0–3/2) and 4, predomination of C24 alleles reached ~10 and 20% over genomic regions of 2 and 5 cM, respectively. A detailed description of the segregation distortion was given by Törjék *et al.* (2006). Heterozygosity across the 110 SNP markers in each RIL averaged 1.8%, with a maximum of 8.2%.

Trait means, variances, and heritability: Means, genetic variances, and heritabilities of the original observations H_1, H_2, and H_3 for the TTC progenies were presented in our previous article (Kusterer *et al.* 2007). Means and genetic variances for the transformation Z_1 were significantly ($P < 0.01$) greater than zero for all traits (Table 2). Heritability of Z_1 ranged between 59% for DMC and 80% for RD29. For Z_2, the mean deviated significantly ($P < 0.01$) from zero for all traits except RD22. Estimates of $\sigma^2_g(Z_2)$ were always highly significant ($P < 0.01$) and almost twice as large as $\sigma^2_g(Z_1)$ for RD22 and BY. Heritability of Z_2 ranged between 45% for GR and 78% for RD22 and BY. For Z_3, the mean deviated significantly ($P < 0.05$) from zero only for RD29. Estimates of $\sigma^2_g(Z_3)$ were highly significant ($P < 0.01$) for all traits and approximately twice the corresponding values of $\sigma^2_g(Z_2)$ for RD22, RD29, and BY. For GR and DMC, estimates of $\sigma^2_g(Z_s)$ were of similar size for Z_1, Z_2, and Z_3. Estimates of $\sigma^2_e(Z_s)$ differed for Z_1, Z_2, and Z_3, because (i) Z_1 refers to the mean of H_1 and H_2, whereas Z_2 and Z_3 refer to contrasts of H_1, H_2 and H_3, and (ii) the error of main plots contributes to $\sigma^2_e(Z_1)$ but cancels in the model equation of Z_2 and Z_3. Heritability of Z_3

TABLE 1

Expectation ($\mathcal{E}(X)$), variance ($\sigma_g^2(X)$), and conditional expectation ($\mathcal{E}(X \mid \text{QTL } i)$) of the contrast between the two homozygous marker classes at the position of QTL i and conditional expectation ($\mathcal{E}(X \mid \text{QTL } i \times \text{QTL } j)$) of the interaction between the two homozygous marker classes at the positions i and j for $X = Z_1$, Z_2, Z_3, and H_3 in terms of genetic effects of the F_2-metric under digenic epistasis in the absence of linkage

	Statistic[a]			
Variable X	$\mathcal{E}(X)$	$\sigma_g^2(X)$	$\mathcal{E}(X \mid \text{QTL } i)$	$\mathcal{E}(X \mid \text{QTL } i \times \text{QTL } j)$
Z_1	$\mu + \frac{1}{8}\sum_{i\neq k}\sum aa_{ij}$	$\frac{1}{4}\sum_i\left(a_i - \frac{1}{2}\sum_{k\neq i} da_{ik}\right)^2 + \frac{1}{32}\sum_{i\neq k}\sum(aa_{ik} + dd_{ik})^2$	$a_i^* = a_i - \frac{1}{2}\sum_{k\neq i} da_{ik}$	$aa_{ij} + dd_{ij}$
Z_2	$\sum_i a_i$	$\sum_i\left(d_i - \frac{1}{2}\sum_{k\neq i} aa_{ik}\right)^2 + \frac{1}{8}\sum_{i\neq k}\sum(ad_{ik} + da_{ik})^2$	$2d_i^* = 2d_i - \sum_{k\neq i} aa_{ik}$	$ad_{ij} + da_{ij}$
Z_3	$\frac{1}{4}[aa] = \frac{1}{4}\sum_{i\neq k}\sum aa_{ik}$	$\frac{1}{8}\sum_{i\neq k}\sum(ad_{ik}^2 + da_{ik}^2 + dd_{ik}^2)$	$[da_i] = \sum_{k\neq i} da_{ik}$	$2dd_{ij}$
H_3	μ	$\frac{1}{4}\sum_i a_i^2 + \frac{1}{32}\sum_{i\neq k}\sum aa_{ik}^2$	a_i	aa_{ij}

For description of Z_1, Z_2, Z_3, and H_3 and for notation of genetic effects see MATERIALS AND METHODS.

[a] Summation is over the entire set of QTL involved in the trait expression.

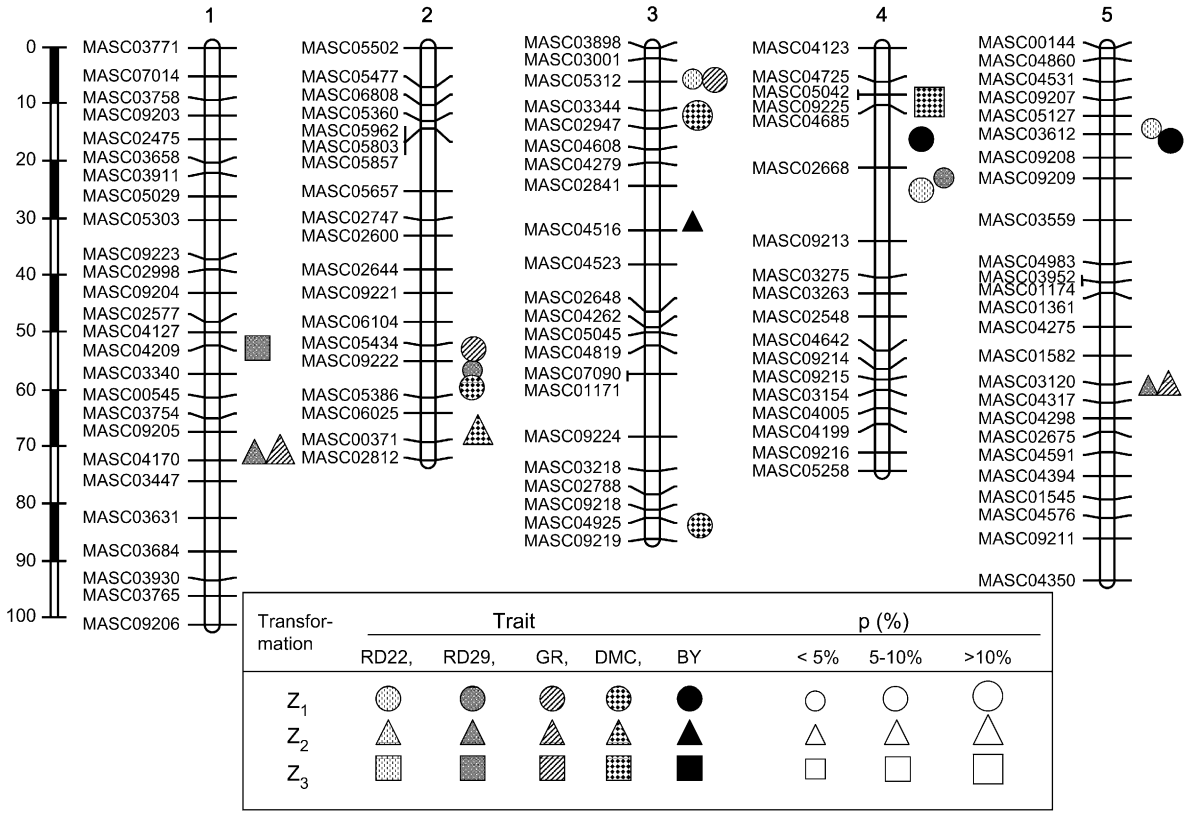

FIGURE 1.—Genomic locations and proportion (p) of the genetic variance ($\sigma^2_g(Z_s)$) explained by QTL detected for linear transformations Z_1, Z_2, Z_3 for five biomass-related traits in Arabidopsis hybrid C24 × Col-0. For description of linear transformations and traits see MATERIALS AND METHODS.

ranged from 30% for GR and DMC to 81% for BY. Genotypic correlations between Z_1 and Z_2 were mostly close to zero, as expected from theory (MELCHINGER *et al.* 2007) and the small differences between parents C24 and Col-0 in comparison with the range between RILs for *per se* performance of all traits reported in our previous study (KUSTERER *et al.* 2007). By comparison, moderately positive correlations were observed between Z_1 and Z_3.

Identification of QTL affecting biomass-related traits: We detected a total of 20 QTL for the five biomass-related traits in Arabidopsis hybrid C24 × Col-0 (Table 3 and Figure 1). Several of these QTL regions affected more than one trait. However, each QTL position was declared significant only for one of the three transformations Z_s, on the basis of the LOD thresholds determined by permutation tests. In the joint QTL analysis of all three transformations, 7 of the 20 QTL detected in the separate analyses of Z_1, Z_2, or Z_3 were also found, but no additional QTL could be detected.

For RD22, we revealed three QTL, all by QTL mapping with Z_1 (Table 3). They were located on chromosomes 3, 4, and 5 and explained individually from 3.9 to 6.3% and simultaneously 12.6% of $\sigma^2_g(Z_1)$. Effects of these QTL for Z_2 and Z_3 were practically zero.

For RD29, we detected five QTL (Table 3). Two of them were found for Z_1, one located on chromosome 2 and the other on chromosome 4 at the same marker interval as the QTL for RD22, each explaining ~5% of $\sigma^2_g(Z_1)$. Two QTL were detected for Z_2, one located on chromosome 1 and one on chromosome 5, explaining 6 and 5% of $\sigma^2_g(Z_2)$, respectively. For Z_3, we found one QTL on chromosome 1, explaining ~5% of $\sigma^2_g(Z_3)$.

For GR, we detected four QTL (Table 3). For Z_1, we found two QTL, one located on chromosome 2 in the same region as the Z_1 QTL for RD29 and DMC and the other on chromosome 3, explaining individually 5 and 6% of $\sigma^2_g(Z_1)$. For Z_2, two QTL, explaining 15 and 11% of $\sigma^2_g(Z_2)$, were detected on chromosomes 1 and 5, respectively, at exactly the same positions as Z_2 QTL for RD29. In a simultaneous fit, these QTL accounted for 28% of $\sigma^2_g(Z_2)$.

For DMC, we detected five QTL (Table 3). Three of them were found for Z_1, located on chromosomes 2 and 3, explaining between 7 and 11% of σ^2_g. The simultaneous fit of all five QTL explained 31.1% of $\sigma^2_g(Z_1)$. Only one QTL was detected for Z_2, located on chromosome 2 and explaining 11% of $\sigma^2_g(Z_2)$. Likewise, for Z_3 we detected only one QTL located on chromosome 4, which accounted for 16% of $\sigma^2_g(Z_3)$.

For BY, we detected three QTL (Table 3). Two of these were found for Z_1 on chromosomes 4 and 5 and explained ~9% of $\sigma^2_g(Z_1)$. One QTL was detected for Z_2 on chromosome 3, which explained only 3.2% of $\sigma^2_g(Z_2)$.

TABLE 2

Summary statistics (mean, genotypic variance σ_g^2, error variance σ_e^2, heritability h^2, phenotypic correlation r_p, genotypic correlation r_g) with associated standard errors for linear transformations Z_1, Z_2, and Z_3 of five biomass-related traits in Arabidopsis hybrid C24 × Col-0

Linear transformation	Statistic	Trait				
		RD22 (mm)	RD29 (mm)	GR (mm/day)	DMC (%)	BY (mg)
Z_1	Mean	30.3 ± 0.3**	77.5 ± 0.5**	6.78 ± 0.04**	7.79 ± 0.03**	98.6 ± 1.6**
	σ_g^2	34.1 ± 4.2**	99.0 ± 11.9**	0.42 ± 0.07**	0.24 ± 0.04**	776.3 ± 109.8**
	σ_e^2	27.1 ± 1.8**	75.0 ± 5.2**	0.71 ± 0.05**	0.49 ± 0.03**	1084.4 ± 72.6**
	h^2	79.1 ± 2.5	79.8 ± 2.4	64.00 ± 4.37	59.10 ± 5.06	68.2 ± 3.7
Z_2	Mean	0.7 ± 0.4	2.5 ± 0.7**	0.26 ± 0.06**	0.57 ± 0.04**	9.6 ± 2.0**
	σ_g^2	51.2 ± 6.3**	124.2 ± 17.5**	0.46 ± 0.11**	0.25 ± 0.06**	1513.5 ± 186.0**
	σ_e^2	44.6 ± 3.0**	163.6 ± 11.4**	1.73 ± 0.12**	0.73 ± 0.05**	1296.7 ± 87.0**
	h^2	77.5 ± 2.6	69.5 ± 3.6	44.56 ± 6.83	50.36 ± 6.69	77.8 ± 2.6
Z_3	Mean	−1.0 ± 0.6	−2.0 ± 1.0*	−0.13 ± 0.09	−0.05 ± 0.05	1.4 ± 3.3
	σ_g^2	124.6 ± 15.8**	211.9 ± 33.6**	0.61 ± 0.23**	0.25 ± 0.09**	4421.2 ± 523.1**
	σ_e^2	119.5 ± 8.0**	370.7 ± 26.0**	4.18 ± 0.29**	1.72 ± 0.12**	3058.7 ± 206.8**
	h^2	75.8 ± 2.8	63.2 ± 4.4	30.60 ± 8.60	29.77 ± 8.78	81.3 ± 2.2
	r_p (Z_1, Z_2)	−0.02	0.11	0.16*	−0.04	−0.09
	r_p (Z_1, Z_3)	0.37**	0.30**	0.23**	0.16*	0.41**
	r_p (Z_2, Z_3)	−0.05	0.03	−0.07	0.01**	−0.15*
	r_g (Z_1, Z_2)	−0.04	0.11*	0.24**	0.09	−0.12*
	r_g (Z_1, Z_3)	0.38**	0.27**	0.13	0.24*	0.42**
	r_g (Z_2, Z_3)	−0.06	0.00	−0.21*	−0.05	−0.14*

Phenotypic correlation was significant at $^*P < 0.05$ and $^{**}P < 0.01$, respectively, and the genotypic correlation exceeded twice and three times its standard error, respectively. For description of the linear transformations and traits see MATERIALS AND METHODS.

We detected a total of six marker pairs with significant epistatic effects for Z_3 across all traits (Table 4). Three of these marker pairs were found for RD22 with variable sign of the estimated effect dd_{ij}, explaining between 4.6 and 6.5% of $\sigma_g^2(Z_3)$. Likewise, three marker pairs displaying epistatic effects dd_{ij} with variable sign were detected with Z_3 for BY, explaining between 3.0 and 6.7% of $\sigma_g^2(Z_3)$; one of the markers involved in significant interactions was always located on the long arm of chromosome 5. For H_3, we detected consistently one epistatic marker pair with a positive sign of the estimated effect aa_{ij} for RD29, GR, and DMC, explaining between 5.6 and 10.7% of $\sigma_g^2(H_3)$. Each of these marker pairs involved the same marker on the long arm of chromosome 5; for RD29 and GR, the other marker was also identical.

DISCUSSION

Composition of heterosis under epistasis: MELCHINGER *et al.* (2007) provided a general formula for MPH under the F_2-metric model. According to this formula, with digenic epistasis, MPH depends on both dominance and additive × additive effects. Moreover, the contribution of individual QTL to MPH corresponds exactly to their augmented dominance effects d_i^*.

Usefulness of the TTC design for heterosis studies: HUA *et al.* (2003) pointed out that most of the previous molecular-marker-based genetic analyses of heterosis were based on performance measurements of traits rather than on heterosis itself and that the genetic basis of heterosis was inferred from the genetic components of trait performance. To obtain a better picture of the genetic components underlying heterosis, these authors advocated the use of direct measurements of heterosis in data analysis, as provided, for example, by the immortalized F_2 design. Even though design III and the TTC design do not fulfill this criterion, we believe they are ideal designs for unraveling the basis of heterosis because QTL mapping with Z_2 provides estimates of augmented dominance effects d_i^*, the contribution of a QTL to MPH. Since QTL mapping with Z_1 also provides estimates of the augmented additive effect a_i^*, which enters the expression for the parental difference in the presence of epistasis, design III and the TTC design allow us also to determine the augmented dominance ratio $d_i^*/|a_i^*|$ at each QTL. The TTC design for QTL mapping with Z_3 has a further advantage in that it permits a one-dimensional genome scan for epistatic effects $[da_i]$ contributing to a_i^*.

Interpretation of first- and second-degree statistics: As pointed out by KEARSEY and JINKS (1968), the transformation Z_3 provides a test for $[aa]$, the sum of aa_{ij} epistatic effects averaged over all pairs of QTL (Table 1). In our study, this test was significant only for RD29. By comparison, a generation means analysis that included

TABLE 3

QTL positions and effects for linear transformations Z_1, Z_2, and Z_3 as well as dominance ratio $d_i^*/|a_i^*|$ and joint mapping of five biomass-related traits in Arabidopsis hybrid C24 × Col-0

		Z_1			Z_2				Z_3			
Chromosome	Position (cM)[a]	LOD score[b]	Effect a_i^*	p (%)[c]	LOD score	Effect $2d_i^*$	p (%)	$d_i^*/\|a_i^*\|$	LOD score	Effect $[da_i]$	p (%)	Joint LOD score[d]
						RD22 (mm)						
3	6	*1.85*	*−2.03***	*3.9*	0.23	−0.55	0.5	−0.13	0.02	0.18	0.0	2.08
4	24	*3.10*	*2.87***	*6.3*	0.03	0.17	0.0	0.03	0.24	−0.78	0.4	*4.24*
5	14	*1.87*	*2.14***	*4.2*	0.08	−0.19	0.1	−0.04	0.78	1.43	1.4	2.87
Total				12.6			0.6				1.8	
						RD29 (mm)						
1	52	0.38	−3.13	1.2	0.00	−0.46	0.1	−0.07	*2.11*	*−3.92***	*5.4*	2.30
1	70	0.02	2.04	0.5	*2.12*	*3.20***	*5.8*	0.78	0.01	0.33	0.0	2.17
2	58	*2.02*	*−5.89***	*4.9*	0.28	0.99	0.7	0.08	0.32	1.50	0.9	2.85
4	20	*2.10*	*5.92***	*5.3*	0.34	1.13	1.0	0.09	0.00	0.19	0.0	2.53
5	60	0.54	−2.28	0.7	*1.82*	*2.55***	*4.6*	0.56	0.01	0.43	0.0	2.23
Total				10.4			12.5				7.1	
						GR (mm/day)						
1	70	0.0	−0.03	0.0	*3.95*	*0.31***	*15.3*	5.17	0.03	0.00	0.0	*3.99*
2	50	*2.02*	*−0.31***	*5.0*	0.12	0.14	3.6	0.22	0.02	0.04	0.0	0.40
3	6	*2.23*	*−0.34***	*6.4*	0.01	−0.01	0.0	−0.01	0.06	−0.13	0.3	0.22
5	60	0.10	−0.04	0.0	*2.38*	*0.26***	*11.4*	3.25	0.00	0.06	0.0	2.46
Total				10.5			28.0				0.6	
						DMC (%)						
2	60	*4.59*	*0.29***	*6.6*	0.23	0.13	1.1	0.22	0.51	−0.02	0.0	*5.79*
2	68	0.06	0.02	0.0	*2.92*	*−0.30***	*11.1*	−7.50	0.06	0.00	0.0	*4.68*
3	14	*3.58*	*0.23***	*10.5*	0.01	−0.01	0.0	−0.02	0.26	0.11	2.7	*6.55*
3	84	*2.17*	*0.20***	*6.6*	0.02	−0.01	0.0	−0.02	0.26	0.04	0.3	2.48
4	8	0.02	0.02	0.0	0.06	0.02	0.2	0.50	*3.41*	*−0.31***	*16.4*	*3.47*
Total				31.1			13.7				20.5	
						BY (mg)						
3	30	0.40	−3.25	1.0	*2.41*	*6.59**	*3.2*	1.01	0.02	0.96	0.0	2.64
4	16	*3.43*	*10.69***	*9.8*	0.01	−0.82	0.0	−0.04	0.03	2.32	0.2	*4.38*
5	16	*2.69*	*9.33***	*8.9*	0.21	−2.80	0.6	−0.15	0.50	5.87	1.5	3.24
Total				*17.4*			3.6				1.7	

For description of linear transformations and traits see MATERIALS AND METHODS.
[a] Position on chromosome according to the map published by TÖRJÉK *et al.* (2006).
[b] Estimates of QTL, for which the LOD score surpassed the threshold level, are given in italics.
[c] Proportion of genotypic variance explained by the QTL in a simultaneous fit.
[d] Joint mapping over all linear transformations Z_1, Z_2, and Z_3.

the checks (P1, P2, F_1, F_2) and RILs in addition to the TTC progenies yielded significantly ($P < 0.05$) positive estimates of $[aa]$ for BY (KUSTERER *et al.* 2007). Compared with Z_3, the latter approach has a higher statistical power to detect epistasis owing to a smaller coefficient in the error variance for the estimate of $[aa]$.

Table 1 also provides a quantitative genetic interpretation of $\sigma_g^2(Z_s)$. Using the F_2-metric, the genetic variance can be partitioned into independent components of the genetic effects with no genetic covariance among them (KAO and ZENG 2002). Thus, we obtain $\sigma_g^2(Z_1) = \frac{1}{2}\sigma_A^2 + \frac{1}{4}\sigma_{AA}^2 + \frac{1}{2}\sigma_{DA}^2 + \sigma_{DD}^2$, $\sigma_g^2(Z_2) = 4\sigma_D^2 + 2\sigma_{AA}^2 + 2\sigma_{AD}^2 + 2\sigma_{DA}^2$, and $\sigma_g^2(Z_3) = 2\sigma_{AD}^2 + 2\sigma_{DA}^2 + 4\sigma_{DD}^2$, where σ_A^2, σ_D^2, σ_{AA}^2, σ_{AD}^2, σ_{DA}^2, and σ_{DD}^2 denote the variances of a_i, d_i, aa_{ij}, ad_{ij}, da_{ij}, and dd_{ij} effects summed over all loci or loci pairs, respectively, as defined for the F_2-metric by YANG (2004). This demonstrates that in a TTC design with RILs, $\sigma_g^2(Z_s)$ for $s = 1$, 2, and 3 can be strongly influenced by epistatic effects. In particular, the significant estimates of $\sigma_g^2(Z_3)$ for all traits observed in our study reveal the presence of epistasis of type $a \times d$ and $d \times a$ and/or $d \times d$ or even higher-order epistasis. Estimates of σ_{AA}^2 and σ_{DD}^2 reported in our previous study (KUSTERER *et al.* 2007) on the analysis of variance of the original observations H_s ($s = 1$, 2, 3) support this conclusion.

Interpretation of QTL-mapping results: Until recently, QTL analyses of heterosis with design III (*e.g.*, STUBER

TABLE 4

Marker positions and estimated digenic epistatic effects dd_{ij} and aa_{ij} determined by two-way analyses of variance with variables Z_3 and H_3 of five biomass-related traits in Arabidopsis hybrid C24 × Col-0

	Marker *i*		Marker *j*		dd_{ij}		
Trait	Chromosome	Position[a]	Chromosome	Position[a]	LOD score	Effect	p (%)[b]
			Variable Z_3				
RD22	1	81	2	55	1.96	−2.68	5.06
RD22	2	24	5	37	1.77	2.53	4.57
RD22	4	24	5	14	2.54	−3.30	6.53
BY	2	10	5	53	1.96	10.48	5.1
BY	2	70	5	53	2.57	−12.16	6.7
BY	3	30	5	70	1.14	8.44	3.0
	Marker *i*		Marker *j*		aa_{ij}		
Trait	Chromosome	Position[a]	Chromosome	Position[a]	LOD score	Effect	p (%)[b]
			Variable H_3				
RD29	1	8	5	92	2.15	6.62	5.63
GR	1	8	5	92	2.56	0.57	9.1
DMC	2	29	5	92	2.30	0.23	10.7

For description of Z_3 and H_3 and traits see MATERIALS AND METHODS.
[a] Position on chromosome according to the map published by TÖRJÉK *et al.* (2006).
[b] Proportion of genotypic variance explained by the marker pair interaction.

et al. 1992) were performed with (i) marker data of the candidates (*e.g.*, F_3 lines or RILs) from a segregating population of cross P1 × P2 and (ii) phenotypic data (H_1 and H_2) of their testcross progenies. QTL effects determined with this approach have expectations $d_i^* + a_i^*$ and $d_i^* - a_i^*$ (MELCHINGER *et al.* 2007) and gene action at each QTL must be deduced from these estimates. By comparison, the novel QTL-mapping approach devised by MELCHINGER *et al.* (2007) also uses marker data of the candidates, but employs linear transformations Z_1, Z_2, and Z_3 of the performance of TTC progenies. The advantages of this approach are that (i) estimates of the detected QTL reflect directly a_i^* and d_i^* and (ii) the joint analysis of Z_1 and Z_2 enables testing of hypotheses on the type of gene action.

Across all five biomass-related traits, we detected a total of 20 QTL with main effects. This number compares favorably with the 38 QTL reported for 22 traits in a design III study of Arabidopsis hybrid Col × Ler (KEARSEY *et al.* 2003) but is lower than that in crops like maize (STUBER *et al.* 1992; COCKERHAM and ZENG 1996) and rice (LI *et al.* 2001; LUO *et al.* 2001; HUA *et al.* 2002, 2003). However, the studies with maize and rice investigated grain yield and yield components. These traits display much larger MPH than forage yield in maize (MELCHINGER *et al.* 1992) or biomass-related traits in Arabidopsis, where MPH was largest for BY but still <66% except under extremely high light intensities and at an earlier time of evaluation (MEYER *et al.* 2004). Altogether, the number of detected QTL was at the lower end of our expectations because the conditions for QTL mapping were benign in our study. First, we used replicated experiments with a total of 30 plants per entry and heritabilities for all Z_s were moderately high for all traits except GR and DMC for Z_3 (Table 2). Thus, experimental errors in the phenotypic measurements most likely did not hamper QTL detection. Second, we chose a sufficiently large population size ($N = 234$). On the basis of theoretical results (CHARCOSSET and GALLAIS 1996), the power of QTL detection with this population size is >90% for a QTL explaining 10% of σ_g^2 for a trait with $h^2 = 50\%$. Third, QTL mapping was performed with marker data from RILs, where the heterozygous marker class is empty. As a consequence, under a purely additive genetic model, as applies to testcross progenies, RILs have the highest power for detection of QTL in comparison with F_2, F_3, or backcross populations (MORENO-GONZALEZ 1993; CHARCOSSET and GALLAIS 1996). Fourth, we used a high-density linkage map with an average marker distance of 3.9 cM and a maximum interval length of 13 cM, which favors QTL detection and improves the resolution of separating closely linked QTL (LUO *et al.* 2001).

Segregation distortion observed for four genomic regions may have reduced the power of QTL detection in our study, because of unequal size of marker classes (CRANE and CRANE 2005). Nevertheless, according to the theoretical results of COCKERHAM and ZENG (1996), this effect on power of QTL detection is presumably of secondary importance with the degree of distortion observed in our RIL population. Likewise, errors in the assignment of marker genotypes of RILs to the phenotypic observations of their TTC progenies would reduce the power of QTL detection. To avoid such mistakes, we

used the same seed lot of each RIL for producing the TTC progenies, as well as propagating the RILs and sampling of plant materials for the SNP assays.

Given the large number of entries evaluated in our study ($N = 936$), we had to subdivide the entire set of 234 RILs and their TTC progenies into three separate experiments. Even though utmost care was exercised to warrant uniform temperature, light, and moisture conditions, a perfect control of the environmental conditions across the experiments was not possible. However, an analysis of variance of the checks that were included as multiple entries in each experiment revealed only minor genotype × experiment interactions. Nevertheless, if genotype × experiment interactions affected biomass-related traits in the TTC progenies, our mapping procedure would favor the detection of those QTL that displayed no or little QTL × environment interactions. Moreover, h^2 calculated from pooled values of individual experiments would tend to overestimate the heritabilities across experiments. Consequently, estimates of p given in Table 3 represent a lower limit for the proportion of σ_g^2 across experiments explained by the detected QTL.

Twelve of the 20 QTL were detected for Z_1 with significant augmented additive effects a_i* (Table 3). Eight of these 12 QTL colocalized with QTL detected for H_3 (data not shown), which reflect additive effects a_i without any confounding by digenic epistatic effects (Table 1). This is in agreement with the findings from our previous study (Kusterer *et al.* 2007) showing that σ_A^2 was the predominant variance component compared with σ_D^2 and the epistatic variances. Three QTL were detected for DMC, which explained altogether about one-third of $\sigma_g^2(Z_1)$. Since DMC is related to the developmental stage, we compared the QTL detected for this trait with the 12 QTL for flowering time in Arabidopsis revealed by El-Lithy *et al.* (2006). Two QTL for DMC, on chromosomes 3 (position 3/14) and 4 (position 4/8), mapped to genomic regions reported for flowering time, thus suggesting that the underlying genes have a pleiotropic effect on these two traits.

Five of six QTL detected for transformation Z_2 displayed a positive augmented dominance effect d_i*, which is in agreement with the positive MPH for the underlying traits. A QTL with negative d_i* effect was revealed only for DMC, as expected from the negative MPH for this trait. The two largest QTL for d_i*, explaining simultaneously 28% of $\sigma_g^2(Z_2)$, were found for GR, the trait with the highest average squared degree of dominance in our previous study (Kusterer *et al.* 2007).

Altogether, it is difficult to conclude whether the small number of QTL detected in our study was due to the low level of heterosis for all traits except BY or a lack of statistical power in detecting QTL or a combination of both.

Comparison of individual and joint QTL mapping: We found no common QTL positions between Z_1, Z_2, and Z_3. This implies that the QTL detected displayed either only significant augmented additive effects a_i* detected by Z_1, or significant augmented dominance effects d_i* detected by Z_2, or significant epistatic effects $[da_i]$ detected by Z_3. Consequently, the dominance ratio $d_i^*/|a_i^*|$ shown in Table 3 was either close to zero or extremely high. The only exception was the QTL for BY on chromosome 3, where a_i* and d_i* were of equal size. This result is in harmony with the findings of Cockerham and Zeng (1996) and Frascaroli *et al.* (2007), who reported only a small overlap between QTL for Z_1 and Z_2 in single-marker analyses of design III progenies of maize hybrid B73 × Mo17 and in CIM of TTC progenies of maize hybrid B73 × H99, respectively. Likewise, most QTL detected for heterosis of grain yield in rice displayed either a dominance ratio well above 1.0 or close to zero (Yu *et al.* 1997; Hua *et al.* 2002, 2003). Altogether, these findings imply that estimates of the average degree of dominance $D = \sqrt{\sigma_g^2(Z_2)/4\sigma_g^2(Z_1)}$ are not very informative for drawing conclusions on the primary mode of gene action involved in heterosis at individual QTL because a mixture of QTL with additive and overdominance would result in an estimate of D that would suggest partial dominance at most QTL.

The joint QTL analysis across all three transformations Z_s did not improve the power of QTL detection in our study (Table 3). In most instances, the LOD score of the joint analysis was approximately equal to the sum of the LOD scores for the individual transformations. This result is in accordance with theory (Jiang and Zeng 1995) and was expected on the basis of the low phenotypic and genotypic correlations between Z_1, Z_2, and Z_3 (Table 2). However, since the LOD threshold was much higher for the joint mapping (corresponding to a χ^2-distribution with 4 d.f. according to Jiang and Zeng 1995) than that of individual Z_s (corresponding to a χ^2-distribution with 2 d.f.), only 7 of the 20 QTL found with the latter approach could be confirmed by the joint analysis. Nevertheless, joint mapping was instrumental in dissecting the two closely linked QTL on chromosome 2 using the procedure described by Jiang and Zeng (1995).

Detection of epistatic QTL: We used two approaches to detect epistasis among QTL. First, a one-dimensional genome scan was performed by QTL mapping with Z_3 to detect QTL × genetic background interactions (Table 1). This test revealed two genomic regions with significantly negative $[da_i]$ effects, one for GR and one for DMC. Since $a_i^* = a_i - \frac{1}{2}[da_i]$, it follows that the additive effect a_i is smaller than the augmented additive effect a_i* estimated at these positions by QTL mapping with Z_1. This conclusion was confirmed by comparison with additive effects a_i determined by QTL mapping with H_3 (data not shown).

Second, we performed two-way ANOVAs with all marker pairs (using cofactors determined by CIM and subsequent backward elimination) for Z_3 and H_3. This yields statistical tests for epistatic effects of types dd_{ij} and

aa_{ij} (Table 1), respectively, but because Z_3 and H_3 are correlated, the statistical tests for Z_3 and H_3 are not stochastically independent. Very few significant digenic epistatic effects were found with this approach (Table 4), presumably because of its low statistical power as a consequence of the aggravated multiple-test problem associated with two-dimensional genome scans. Interestingly, none of the markers contributing to significant aa_{ij} effects, identified with H_3, colocalized with main-effect QTL detected with Z_2, despite the fact that augmented dominance effects d_i* reflect not only d_i but also minus half the sum of aa_{ik} effects. Two explanations can be given for this observation: (1) positive d_i effects counterbalanced the effects of the detected positive aa_{ij} effects and/or (2) $[aa_i]$, the sum of aa_{ik} interactions of QTL i with all other QTL k, contributing to d_i*, was smaller than the aa_{ij} effect estimated from H_3 due to other interactions aa_{ik} ($k \neq j$) with a negative sign that were too small to be detected by marker × marker contrasts with H_3 in our approach. Hence, if the goal is to determine the relative contribution of epistatic *vs.* dominant gene action to MPH, investigating only pairwise interactions aa_{ij} is not sufficient to obtain a realistic picture of the role of epistasis in heterosis. What is needed are new designs that for each QTL allow separate estimation of d_i and $[aa_i]$, as pointed out by Melchinger *et al.* (2007).

Comparison of QTL for different traits: In agreement with the significant correlations among biomass-related traits in the TTC progenies reported in our previous study (Kusterer *et al.* 2007), we also found common QTL positions for different traits (Figure 1). All QTL detected for GR were found either for RD22 (one QTL) or for RD29 (three QTL) with the same type of gene action. Likewise, two QTL regions for RD22 were also identified as QTL for BY with the same type of gene action. However, with the limited sample size (N= 234) and moderate size of the detected genetic effects, confidence intervals for QTL positions were still in the order of 10–15 cM (see Manichaikul *et al.* 2006) in spite of our high-density map. Thus, we were not able to distinguish pleiotropic or closely linked QTL regions. Hence, fine-mapping approaches with introgression libraries or near-isogenic lines and, subsequently, comparison of DNA sequences between the parents in regions of candidate genes are required to solve this problem.

Genetic basis of heterosis: In our previous article (Kusterer *et al.* 2007), MPH was highest (49%) for BY, medium (23–28%) for RD22, RD29, and GR, and negative (−2%) for DMC. By QTL mapping for augmented dominance effects d_i* with Z_2, we detected one QTL explaining 11% of MPH for BY and two QTL explaining 18% of MPH for GR. This suggests that heterosis for BY and GR has a truly polygenic mode-of-inheritance with a large number of underlying QTL, each with only a small contribution. The small number of QTL detected in our study in comparison with similar investigations in maize (Stuber *et al.* 1992; Lu *et al.* 2003) and rice (Hua *et al.* 2002, 2003) is most likely attributable to the moderate level of MPH for biomass-related traits in Arabidopsis hybrid C24 × Col-0. However, our analyses allow only inferences on augmented dominance effects d_i* and not on dominance effects d_i themselves. If positive effects d_i were counterbalanced by positive effects aa_{ij}, as suggested by positive estimates of $[aa]$ in our previous article (Kusterer *et al.* 2007) as well as positive estimates of aa_{ij} detected by marker × marker interactions for H_3 (Table 4), this has resulted in smaller values for d_i* in comparison with d_i and has prevented their detection by QTL mapping with Z_2. This hypothesis is supported by results from rice (Yu *et al.* 1997; Hua *et al.* 2002, 2003) showing that additive × additive epistasis is a major component of heterosis for yield and yield components in autogamous species. In the past, inferences on the primary mode of gene action at QTL involved in heterosis have been drawn from the average degree of dominance D, estimated from the ratio $D = \sqrt{(2\sigma_D^2/\sigma_A^2)}$. For the materials and traits investigated in this study, Kusterer *et al.* (2007) reported D values between 0.54 and 0.74, close to the ratio $\sqrt{\sigma_g^2(Z_2)/4\sigma_g^2(Z_1)}$. Following the arguments of previous studies (Comstock and Robinson 1952; Moll and Robinson 1967), one would conclude that most QTL contributing to heterosis display partial to complete dominance. However, our results on the augmented dominance ratio $d_i^*/|a_i^*|$ suggest that the majority of QTL display either additive gene action or overdominance, but owing to the limited power of QTL detection in our study and lack of a statistical test for testing $|a_i^*| = d_i^*$, further research is warranted to substantiate this conclusion. Nevertheless, our results demonstrate that considering only D can be rather misleading concerning the type of gene action at the majority of loci contributing to heterosis.

Our observation of overdominant gene action at one-quarter of the detected QTL is in harmony with recent findings in tomato (Semel *et al.* 2006). In a population of introgression lines, which carried defined chromosome segments of a wild relative, these authors reported prevalence of overdominant QTL for fitness-related traits. Likewise, most QTL detected for heterosis of grain yield in the maize hybrid B73 × H99 were in the overdominance range (Frascaroli *et al.* 2007). Nevertheless, pseudo-overdominance of closely linked loci with dominant genes in repulsion phase could be an alternative explanation. This hypothesis would also be consistent with DNA sequence comparisons of maize inbred lines, which differed in their gene content in a given genomic region as a result of transposon-induced gene shuffling (Fu and Dooner 2002). If the different sets of genes in each parent complement each other in their action, this would result in pseudo-overdominance. The Arabidopsis hybrid C24 × Col-0 represents an ideal

model system to study this hypothesis, because the DNA sequence of Col-0 is completely known (Lin *et al.* 1999; Mayer *et al.* 1999; Salanoubat *et al.* 2000; Tabata *et al.* 2000; Theologis *et al.* 2000) and very detailed knowledge of C24 *vs.* Col-0 polymorphisms will soon become available from resequencing by hybridization (Clark *et al.* 2007). Furthermore, introgression libraries of Col-0 genome segments in C24 genetic background and vice versa have been constructed (O. Törjek, R. C. Meyer, M. Zehnsdorf, M. Teltow, G. Strompen, H. Witucka-Wall, A. Blacha and T. Altmann, unpublished results), which allow mapping of heterosis to defined genomic regions, eliminating a major part of genome-wide epistasis.

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