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ORIGINAL PAPER

Dissection of the genetic basis of heterosis in an elite maize hybrid by QTL mapping in an immortalized F_2 population

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Abstract The genetic basis of heterosis for grain yield and its components was investigated at the single- and two-locus levels using molecular markers with an immortalized F_2 (IF_2) population, which was developed by pair crosses among recombinant inbred lines (RILs) derived from the elite maize hybrid Yuyu22. Mid-parent heterosis of each cross in the IF_2 population was used to map heterotic quantitative trait loci. A total of 13 heterotic loci (HL) were detected. These included three HL for grain yield, seven for ear length, one for ear row number and two for 100-kernel weight. A total of 143 digenic interactions contributing to mid-parent heterosis were detected at the two-locus level involving all three types of interactions (additive × additive = *AA*, additive × dominance = *AD* or *DA*, dominance × dominance = *DD*). There were 25 digenic interactions for grain yield, 36 for ear length, 31 for ear row number and 51 for 100-kernel weight. Altogether, dominance effects of HL at the single-locus level as well as *AA* interactions played an important role in the genetic basis of heterosis for grain yield and its components in Yuyu22.

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Introduction

Heterosis is a phenomenon, which has greatly contributed to the production of high-yielding varieties in some crops during the past century. Several main hypotheses including dominance (Bruce 1910; Jones 1917), over-dominance (Shull 1908; East 1936) and epistasis (Powers 1944; Williams 1959) were proposed to explain heterosis. However, the genetic and molecular causes underlying heterosis are still not fully understood.

The development of molecular markers and saturated linkage maps has provided new useful tools to analyze the genetic basis of heterosis. Since many important agricultural traits exhibiting heterosis display a continuous distribution and are governed by multiple genes with small effects, some researchers dissected heterosis by analyses of quantitative trait loci (QTL). By employing this approach with a Design III produced from F_3 families, Stuber et al. (1992) studied heterosis in the elite maize hybrid B73 × Mo17. They concluded that overdominance (or pseudo-overdominance) was the major cause of heterosis for grain yield. Using near-isogenic lines for a region on chromosome 5 reported by these authors to carry a major QTL for grain yield, Graham et al. (1997) dissected this region into two QTL tightly linked in repulsion phase, thus,

supporting the hypothesis of pseudo-overdominance. By re-analyzing the experimental data of Stuber et al. (1992), Cockerham and Zeng (1996) observed that most of the chromosomes harbored more than one QTL with significant effects on grain yield and others traits. They concluded that dominance of favorable genes is the main cause of heterosis for grain yield in maize. In another single cross of maize, Lu et al. (2003) reported that 24 of the 28 QTL detected for grain yield showed over-dominance or pseudo-overdominance. Schön et al. (2010) compared QTL mapping results from three previous studies (Stuber et al. 1992; Lu et al. 2003; Frascaroli et al. 2007) with maize hybrids from the Iowa Stiff Stalk Synthetic × Lancaster Sure Crop heterotic pattern. In two of these Design III studies, data were re-analyzed by advanced statistical methods (Melchinger et al. 2007b). For grain yield, heterotic QTL accounted for a large proportion of the genetic variance (~70%) and many QTL showed congruent positions across the three populations. Further, almost all congruent QTL were located in the same or an adjacent bin harboring the centromere. The authors concluded that different alleles have been fixed in each heterotic pool, which in combination with the allele(s) from the opposite heterotic pool lead to high heterosis for grain yield in maize.

Xiao et al. (1995) investigated the genetic basis of heterosis in rice with two BC_1F_7 populations between recombinant inbred lines (RILs) and their parents (Design III). They observed that the heterozygotes were superior to the respective homozygotes at most QTL, and proposed that dominance was the genetic basis of heterosis in hybrid rice. Garcia et al. (2008) re-analyzed data sets from maize (Stuber et al. 1992) and rice (Xiao et al. 1995) using multiple-interval mapping (MIM). Their results demonstrated that in maize, heterosis is mainly due to dominant gene action, while in rice additive × additive (*AA*) epistatic effects could be the main cause.

Epistasis, the interaction among genes at different loci, plays a substantial role in the genetics of complex traits such as human diseases, crop yields or growth rates (Carlborg and Haley 2004). Cockerham and Zeng (1996) reported that epistatic effects among linked loci could have played an important role in the expression of heterosis in maize hybrid B73 × Mo17. Yu et al. (1997) detected a large number of digenic interactions for yield and its component traits in hybrid rice based on an $F_{2:3}$ populations derived from a widely grown hybrid in China. Li et al. (2001) and Luo et al. (2001) also suggested that epistasis and overdominance should be the primary genetic basis of inbreeding depression and heterosis for grain yield and biomass in hybrid rice. More recently, studies by Kusterer et al. (2007a, b) and Melchinger et al. (2007a) revealed a significant role of epistasis for heterosis of growth-related traits in Arabidopsis.

To dissect the heterotic effects directly, Hua et al. (2003) introduced a novel segregating population developed from pair crosses of RILs, which they called "immortalized F_2" (IF_2) population. The main advantage of the IF_2 population design is its ability to analyze the genetic basis of heterosis directly because both the genotype and phenotype of two parents for each cross among the IF_2 population are known. Using this design, they concluded that single-locus heterotic effects and dominance × dominance (*DD*) interactions could explain the genetic basis of heterosis in an elite rice hybrid, Shanyou63, to a large extent. Hitherto, there is no report on dissecting the genetic basis of heterosis using IF_2 populations in maize.

Our objective was to dissect the genetic basis of heterosis for grain yield and its components in maize with molecular markers at both the single- and two-locus levels using an IF_2 population derived from elite maize hybrid, Yuyu22, which is widely grown in China.

Materials and methods

Development of the immortalized F_2 population

A population of RILs derived from the single cross hybrid Yuyu22 by single-seed descent until the F_8 generation was used to develop an IF_2 population. Yuyu22 is an elite hybrid in China and exhibits a high level of heterosis for grain yield. Its parents are a dent-inbred line Zong3 (P_1), selected from a synthetic population with Chinese domestic germplasm, and a flint-inbred line 871 (P_2), selected from exotic germplasm. Similar to the procedure described by Hua et al. (2002), 294 RILs were randomly divided into two groups, each group including 147 RILs. Then, pair crosses were made randomly between the lines of two groups without replacement so that 147 different crosses were generated. Every RIL was used only once in each group of mating to generate crosses. This procedure was repeated three times. Finally, 441 (147 × 3) pair crosses between two RILs formed the IF_2 population.

Trait evaluation and statistical analysis

The IF_2 population and hybrid Yuyu22 were planted in 2003 and 2004 on the Agronomy Farm of China Agricultural University (Beijing, China) located in North China (average daily temperature of 11.8°C, average annual rainfall of 585 mm) and Xunxian Agricultural Institute (Henan, China) located in the center of the North China Plain (average daily temperature 14.2°C, average annual rainfall 784 mm). Owing to seed shortage in a few crosses, 426 and 433 IF_2 crosses were evaluated in 2003 and 2004,

respectively The crosses were planted in a randomized complete block design with three replications at each location. Each plot included one row, 4 m long, with 0.67 m distance between rows. The population density was 45,000 plants per ha. At both locations, neighboring the IF_2 experiment, the 294 RILs and the two parental inbreds of Yuyu22 were also planted using the same experimental design. After maturity, ten ears from consecutive plants in each plot were harvested by hand and air-dried until the grain moisture reached 13%. The traits measured were (1) shelled grain yield (t/ha), (2) ear row number, (3) ear length (cm) and (4) 100-kernel weight (g). All traits except grain yield were measured on individual ears and plot means were used for all further computations.

A combined ANOVA over four environments (locations × years) was calculated to estimate variance components. Broad-sense heritability (H_B^2) and its confidence interval were computed according to Knapp et al. (1985) as:

$$H_B^2 = \sigma_g^2 / \left(\sigma_g^2 + \sigma_{ge}^2 / n + \sigma^2 / nb \right),$$

where σ_g^2 is the genetic variance, σ_{ge}^2 is the genotype × environment interaction variance, σ^2 is error variance, n is the number of environments, and b is the number of replications in each experiment. The means averaged across the four environments were used to detect heterotic loci (HL) and digenic interactions.

Molecular markers and genetic linkage maps

In total, 846 pairs of SSR markers were selected from the maize genome database (http://www.maizegdb.org) to screen for polymorphism between the two parents, Zong3 (P_1) and 871 (P_2). Of these, 283 polymorphic markers were used to analyze the individual RILs. The genotypes of each cross of the IF_2 population were deduced from the marker genotypes of their RIL parents. Molecular linkage maps for the RILs and IF_2 population were constructed by using Mapmaker 3.0 (Lander et al. 1987) with a logarithm-of-odds (LOD) threshold of 3.0. The Kosambi mapping function was used for calculating map distances.

Mapping heterotic loci

Heterotic effects were determined from mid-parent heterosis, which was computed as $\text{mph} = F_1 - (P_1 + P_2)/2$ (Hua et al. 2003), where F_1 is the performance of each cross in the IF_2 population, and P_1 and P_2 are the measurements of the corresponding two RIL parents. A modified composite interval mapping (CIM) model $\text{mph} = d^*z^* + \sum_i d_i z_i + \varepsilon$ (Hua et al. 2003) was employed to detect HL, where mph refers to the mid-parent heterosis of each cross in the IF_2 population, d is the difference between the heterozygote (a_1a_2) and the mean of the two homozygotes (a_1a_1 and a_2a_2), z is an indicator variable taking values 1 and 0 for heterozygote and homozygote IF_2 genotypes, respectively, and the asterisk indicates the putative HL to be tested, whereas the subscript i expresses the ith cofactor marker to account for HL in the genetic background, and ε is a residual. Cofactors were selected through stepwise regression with F tests at a significance level $P \leq 0.05$. A window size of 2 cM was used with composite interval mapping (Zeng 1994). The empirical threshold levels for declaring significant HL were determined by performing 1,000 permutations of the data with a significance level of $P \leq 0.05$. Since HL were detected using mid-parent heterosis for each measured trait in this study, it is assumed that each HL should only have a dominant effect (d) in the IF_2 population, which reflects its contribution to mid-parent heterosis.

Analysis of digenic interactions

Following the method of Hua et al. (2003), digenic interactions were analyzed with the average mid-parent heterosis across the four environments using co-dominant markers and a two-way ANOVA performed by a procedure in SAS (SAS Institute, version 8.0, 1999). First, the whole genome was searched for the effects of digenic interactions for each trait, using all possible two-locus combinations of marker genotypes. To decrease the Type I (false positive) errors, a significance level $P \leq 0.0005$ was applied. The calculations were based on unweighted cell means (Snedecor and Cochran 1980) and the sums of squares were multiplied by the harmonic means of the cell sizes to form the test criteria. Only two-locus data sets with all of the marker genotypic classes containing five or more crosses in the IF_2 population were included in the calculation. More than 33,000 tests were conducted for each trait. A number of significant interactions may not be truly significant due to Type I error. Thus, each significant digenic interaction was further assessed through a randomization test. The entry order of the heterosis data in the analysis was randomly permutated and the F-statistics for the digenic interaction were recalculated using the same data. This procedure was repeated 10,000 times and the resulting distribution of F values was compared with the F statistics from the real data. If no more than five F values from the random permutations were larger than the F value from the real data (corresponding to $P \leq 0.0005$), the digenic interaction was regarded as true.

Each significant digenic interactions was further partitioned into four types based on SSR markers: additive effect at both loci (AA); additive effect at the first locus and dominance effect at the second locus (AD); dominance effect at the first locus and additive effect at the second locus (DA); and dominance effect at both loci (DD) following Cockerham (1954). Statistical significance for each

of the terms was assessed at $P \leq 0.01$ by using an orthogonal contrast test provided by the statistical package STATISTICA (Statsoft 1997).

Results

Heterosis for grain yield and its components

Within the IF_2 population, the mid-parent heterosis for grain yield averaged 112.5% and had a range of 7.2–246.8% (Table 1). Average mid-parent heterosis was 26.4% for ear length, 14.7% for ear row number and 6.6% for 100-kernel weight. The original hybrid exhibited 223.1% mid-parent heterosis for grain yield, and 67.5–6.8% for yield components. The variance components (σ^2_g, σ^2_{ge}, σ^2) were highly significant ($P \leq 0.01$) for all traits. Estimates of H^2_B were high for all traits and ranged between 80.7% for grain yield and 92.4% for ear row number.

Heterotic loci (HL) of grain yield and its components

A genetic linkage map of the IF_2 population was constructed by using 253 polymorphic SSR markers based on the genotypes of molecular markers in the RIL population (Fig. 1). This map was the basis for all subsequent QTL analyses.

According to the permutation test, the LOD threshold for declaring significant HL for each trait ranged from 2.90 to 2.97. A total of 13 HL were identified for the four measured traits using the modified composite interval mapping model at the given values of LOD for each trait (Fig. 1). The contribution of individual HL to the variation of mph values was small and varied between 3.30 and 0.61% (Table 2).

For grain yield, three HL (*gy1a*, *gy1b* and *gy8*), two on chromosome 1 and one on chromosome 8, were detected (Table 2). Seven HL, each on a different chromosome, were identified for ear length. One HL for ear row number located on chromosome 3, and two HL for 100-kernel weight located on chromosomes 1 and 7 were detected. All HL for grain yield had positive heterotic effects, varying from 0.20 to 0.45 t/ha. QTL *el5* contributed most to mid-parent heterosis for ear length (0.50 cm), whereas the effect of *el4* was negative (−0.33 cm). In total, all HL for mid-parent heterosis of ear length added up to 1.85 cm. For 100-kernel weight, the contribution of HL *kw1* and *kw7* to mid-parent heterosis for this trait was about 1 g.

According to the experimental design of an IF_2 population, each HL theoretically has only a dominance effect (d), and the additive effect (a) of the same locus can be estimated using the RIL data. The ratio d/a for a given HL reflects the degree of dominance. When the ratio is larger than 1, it indicates over-dominance or pseudo-overdominance of the HL. Interestingly, all 13 HL detected for grain yield and its components had a ratio $d/a \geq 1.2$. The highest ratio was observed for *gy8* (267.3), followed by *gy1b* (43.7) and *kw7* (11.6).

Digenic interactions across the entire genome and their effects

Based on the permutation tests, a total of 143 significant interactions were identified for the four traits (Table 3). Among epistatic components, *AA* interactions had the highest frequency (65.0%) for all traits, followed by *AD*/*DA* (26.2%) and *DD* (8.7%). Further, the number of marker pairs showing interactions was lowest for grain yield among the four traits.

Table 1 Heterosis for grain yield and yield components in the F_1 maize hybrid Yuyu22 and an immortalized F_2 (IF_2) population derived from it

Mean performance/heterosis	Grain yield (t/ha)	Ear length (cm)	Ear row number	100-kernel weight (g)
F_1				
Mean F_1 performance	10.63 ± 0.53	21.08 ± 0.44	16.51 ± 0.25	35.46 ± 0.62
Mid-parent heterosis	7.34 ± 0.54	8.50 ± 0.42	3.04 ± 0.6	2.25 ± 1.0
IF_2				
Mean mid-parent heterosis	3.46 ± 0.07	3.41 ± 0.09	1.88 ± 0.06	2.04 ± 0.20
Range of mid-parent heterosis	1.09–6.32	−0.37–8.65	−0.46–6.60	−10.32–12.26
σ^{2a}_g	0.71**	1.83**	1.72**	10.68**
$\sigma^{2\ a}_{ge}$	0.44**	0.77**	0.42**	5.35**
σ^{2a}	0.71	0.67	0.43	2.61
Heritability (H^2_B)(%)	80.7	88.0	92.4	87.3
95% Confidence interval of H^2_B	78.0–83.0	86.3–89.4	91.4–93.3	85.5–88.8

** $P \leq 0.01$

[a] For abbreviations, see "Materials and methods"

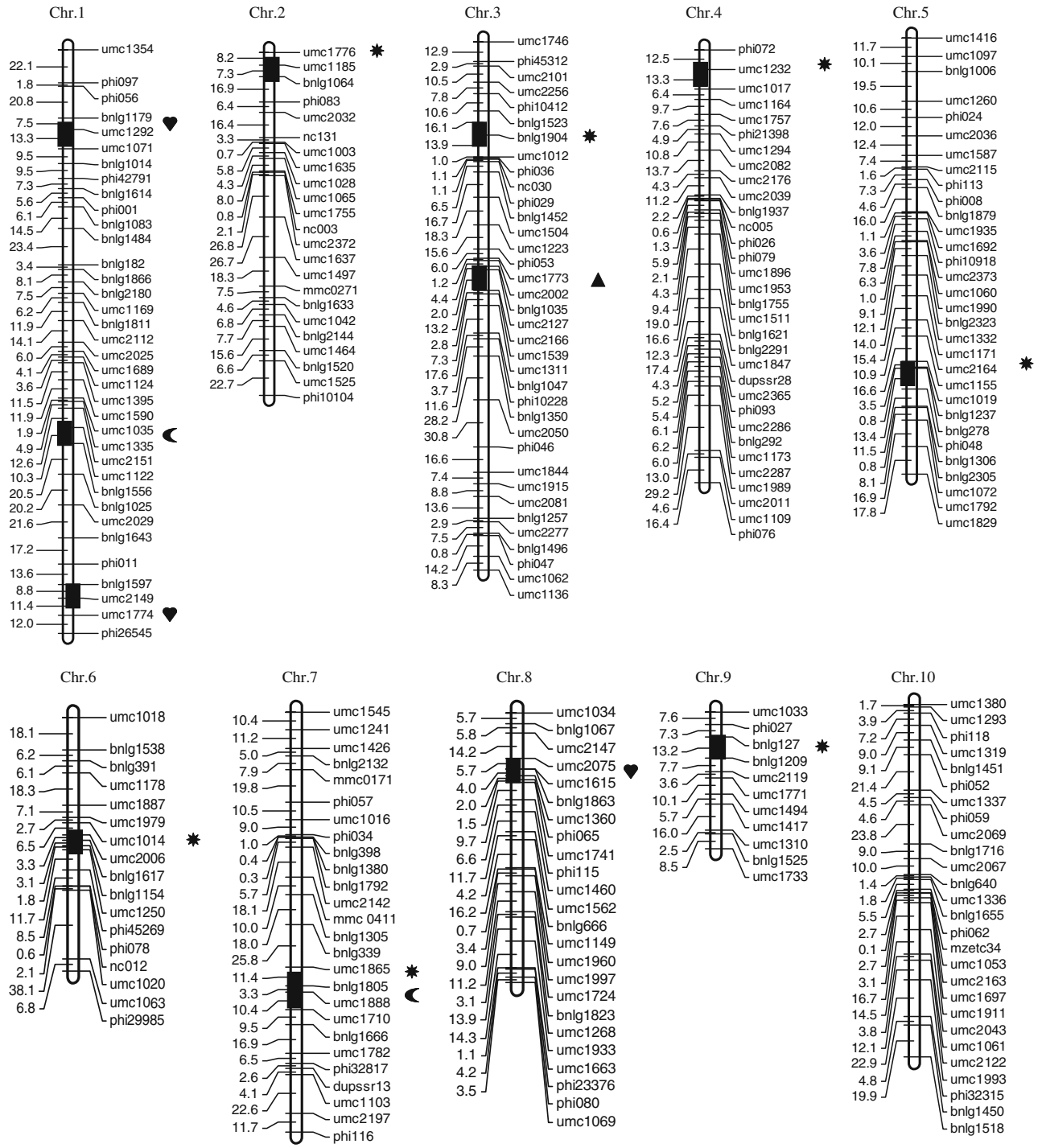

Fig. 1 Chromosomal location of heterotic loci (HL) for grain yield and its components maize hybrid Yuyu22. The genetic distance in cM is listed on the *left side* of each chromosome. The HL location is indicated by the *black boxes* on the chromosomes: ♥ HL for grain yield, ✸ HL for ear length, ▲ HL for ear row number, and ☾ HL for 100-kernel weight

Among the three yield components, ear length had a high heritability (88.0%) and highest mid-parent heterosis (67.5%). Additionally, it had significant correlation with grain yield ($r = 0.66$, $P < 0.01$). Therefore, ear length was taken as an example to illustrate the effects of digenic interactions contributing to mid-parent heterosis. In the *AA* interaction type (data not shown), most complementary homozygotes (11/22 or 22/11) had distinct advantages over the means of two parental genotypes (11/11 and 22/22), and were always the best genotypes. Although many *AD*/*DA* interactions of two loci had distinct advantages over the means of the two parental genotypes (11/11 and 22/22), only

Table 2 Heterotic loci (HL) identified for grain yield and its components in the immortalized F_2 population derived from the elite maize hybrid Yuyu22

HL	Flanking markers	Distance (cM)[a]	Peak position (cM)	LOD	d[b]	R^{2c}
Grain yield						
gy1a	umc1071-bnlg1014	9.5	74	3.20	0.26	1.69
gy1b	umc2149-umc1774	11.4	367	3.87	0.45	3.30
gy8	umc1360-umc1741	3.5	38	3.00	0.2	1.00
Ear length						
el2	umc1776-umc1185	8.2	0	3.20	0.27	0.87
el3	bnlg1523-bnlg1904	16.1	52	3.52	0.31	0.61
el4	phi072-umc1232	12.5	12	3.67	−0.33	1.28
el5	umc1072-umc1792	16.9	252	4.34	0.5	1.98
el6	umc2006-bnlg1617	3.3	65	3.24	0.38	1.83
el7	bnlg1805-umc1888	3.3	154	5.27	0.29	0.82
el9	bnlg127-umc2119	20.9	31	4.53	0.43	1.63
Ear row number						
rn3	umc1773-bnlg1035	5.6	142	3.72	0.25	1.78
100-kernel weight						
kw1	umc1122-bnlg1556	10.3	253	5.16	1.01	1.83
kw7	umc1888-umc1710	10.4	175	3.78	0.94	1.72

[a] Genetic distance of heterotic loci from the nearest flanking markers

[b] Dominance effect d of the heterotic locus (the units are t/ha for grain yield, cm for ear length, number for ear rows, g for 100-kernel weight), see "Materials and methods" for details

[c] Variance of mid-parent heterosis explained by each QTL

Table 3 Numbers of significant interactions among marker pairs for mid-parent heterosis of grain yield and its components in the immortalized F_2 population derived from the elite maize hybrid Yuyu22

	Grain yield	Ear length	Ear row number	100-kernel weight
Interaction pairs[a]	25 (44)	36 (54)	31 (45)	51 (72)
AA[b]	23	30	26	40
AD/*DA*[b]	4	13	7	24
DD[b]	1	1	4	10

[a] Numbers of significant interactions among maker pairs detected through 10,000 permutations at $P \leq 0.0005$, and numbers in parentheses refer to the significant interactions before application of the permutation test

[b] For abbreviations, see "Materials and methods"

a part of these interactions corresponds to the best genotype among the nine-two-locus combinations (data not shown). Only one *DD* interaction (12/12) between two loci was significantly associated with ear length heterosis (Table 3).

Discussion

In this study, a total of 13 HL associated with mid-parent heterosis for grain yield and its components were detected and mapped on nine of the ten maize chromosomes by using mid-parent heterosis per se, which was measured for each cross in the IF_2 population and its two parents in the RIL population. Seven HL identified for ear length contributed 1.85 cm to mid-parent heterosis at the single-locus level (Table 2). Among digenic interactions, *DD* epistasis contributed 0.82 cm to heterosis for ear length (data not shown). These results suggested that both dominance effects and epistatic interactions contributed to mid-parent heterosis in the maize hybrid Yuyu22.

If the effects from each polymorphic locus and the digenic interactions among polymorphic loci would act independently on heterosis and in the absence of higher-order interactions, single-locus heterotic effects (d) and digenic interactions should account for the total heterosis in the hybrid. However, taking ear length as an example, the cumulative effect of single-locus HL and two-locus *DD* interactions was 2.67 cm (1.85 + 0.82 cm) and thus accounted only for 31.4% of mid-parent heterosis for this trait. This raised the question of how to explain the rest of the heterosis in the F_1 hybrid. In this study, the contributions to heterosis from all three types of digenic interactions were tested in the IF_2 population on the basis of polymorphic loci in F_1. Among the three types of digenic interactions, *AA* effects were of greatest importance. As demonstrated by Melchinger et al. (2007b), they contribute

to mid-parent heterosis via the augmented dominance effects. Furthermore, we cannot rule out that higher-order interactions among more than two loci may have also appreciable effects on heterosis.

A comparison of the results in this study with those in rice reported by Hua et al. (2003), both conducted by using IF_2 populations, revealed some striking common features for the genetic basis of heterosis between maize and rice. At the two-locus level, a large number of digenic interactions, which contributed to heterosis for grain yield and its components, were detected in both crops. All three kinds of digenic interactions, *AA*, *AD*/*DA* and *DD*, were detected with *AA* interactions having the highest and *DD* interactions the lowest frequency in rice as well as in maize. More interestingly, based on the comparative genetic map of cereal crops, nine HL for grain yield and its components detected by us in maize are located in similar regions of corresponding chromosomes mapped by Hua et al. (2003) in rice (data not shown). For instance for grain yield, the chromosome region of HL *gy1* detected on chromosome 1 of maize corresponds to that of HL *yd3* detected on the chromosome 3 of rice. This implies that maize and rice may share some collinearity of heterotic loci at the single-locus level. However, all HL at the single-locus and the *DD* interactions at the two-loci levels accounted for 86% heterosis for grains per panicle in rice hybrid Shanyou 63 (Hua et al. 2003). In contrast, these two types of gene action could only explain 34.1% heterosis for ear length in maize hybrid Yuyu22.

Another interesting feature revealed by this study was that for yield components, the largest number of HL was detected for ear length that had the highest mid-parent heterosis, and a smaller number of HL was found for ear row number and 100-kernel weight, which had lower heterosis. Together with the QTL mapping results from RILs, these results suggest that the genetic basis underlying the three major yield components are distinct. While the inheritance of ear length in hybrid was controlled by additive and non-additive effects, additive effects mainly contributed to the inheritance of ear row number and 100-kernel weight. Though grain yield had the highest heterosis among the four measured traits, only three HL were identified, probably because these had the highest genetic complexity.

Our results may have been affected by the use of a randomized block design, because spatial variability within large blocks may have contributed to the experimental error variance. Another complication was that the experiments were not balanced, because a small number of crosses were missing ($\approx 3.5\%$). Considering the high heritabilities in our study, we concluded that these drawbacks had only a minor effect on our results. More importantly, our method of estimating the ratio *d*/*a* directly from the estimates of the numerator and denominator may have resulted in biased estimates. Unbiased estimates for the ratio *d*/*a* could be obtained by using a non-linear model, in which the dominance effect is expressed as a product of the dominance ratio and the additive effect *a* rather than the linear model used by us. However, this would require the development of new software for QTL analysis. Moreover, in cases where the additive effect *a* is not significantly greater than zero, this type of non-linear model cannot be applied. In spite of these limitations, our study provided useful information on the nature of gene action contributing to heterosis as well as HL for grain yield and its components. There were a large number of digenic interactions contributing to heterosis. These involved all three kinds (*AA*, *AD*/*DA*, *DD*) of interactions, but *AA* interactions were more frequent. Further, our study suggested collinearity of HL at the single-locus level between maize and rice.

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References

Bruce AB (1910) The Mendelian theory of heredity and the augmentation of vigor. Science 32:627–628

Carlborg Ö, Haley CS (2004) Epistasis: too often neglected in complex trait studies? Nat Rev Genet 5:618–625

Cockerham CC (1954) An extension of the concept of partitioning hereditary variance for analysis of variances among relatives when epistasis is present. Genetics 39:859–882

Cockerham CC, Zeng ZB (1996) Design III with marker loci. Genetics 143:1437–1456

East EM (1936) Heterosis. Genetics 21:375–397

Frascaroli E, Canè MA, Landi P, Pea G, Gianfranceschi L, Villa M, Morgante M, Pè ME (2007) Classical genetic and quantitative trait loci analyses of heterosis in a maize hybrid between two elite inbred lines. Genetics 176:625–644

Garcia AAF, Wang SC, Melchinger AE, Zeng ZB (2008) Quantitative trait loci mapping and the genetic basis of heterosis in maize and rice. Genetics 180:1707–1724

Graham GI, Wolff DW, Stuber CW (1997) Characterization of a yield quantitative trait locus on chromosome five of maize by fine mapping. Crop Sci 37:1601–1610

Hua JP, Xing YZ, Xu CG, Sun XL, Yu SB, Zhang QF (2002) Genetic dissection of an elite rice hybrid revealed that heterozygotes are not always advantageous for performance. Genetics 162:1885–1895

Hua JP, Xing YZ, Wei WR, Xu CG, Sun XL, Yu SB, Zhang QF (2003) Single-locus heterotic effects and dominance by dominance interactions can adequately explain the genetic basis of heterosis in an elite rice hybrid. PNAS 100:2574–2579

Jones DF (1917) Dominance of linked factors as a means of accounting for heterosis. Genetics 2:466–479

Knapp SJ, Stroup WW, Ross WM (1985) Exact confidence intervals for heritability on a progeny mean basis. Crop Sci 25:192–194

Kusterer B, Muminovic J, Utz HF, Piepho H-P, Barth S, Heckenberger M, Meyer RC, Altmann T, Melchinger AE (2007a) Analysis of a triple testcross design with recombinant inbred lines reveals a significant role of epistasis in heterosis for biomass-related traits in Arabidopsis. Genetics 175:2009–2017

Kusterer B, Piepho H-P, Utz HF, Muminovic J, Meyer RC, Altmann T, Schön CC, Melchinger AE (2007b) Heterosis for biomass-related traits in Arabidopsis investigated by a novel QTL analysis of the triple testcross design with recombinant inbred lines. Genetics 177:1839–1850

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincon SE, Etoh T (1987) Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Li ZK, Luo LJ, Mei HW, Wang DL, Shu QY, Tabien R, Zhong DB, Ying CS, Stansel JW, Khush GS, Paterson AH (2001) Overdominance epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. I. Biomass and grain yield. Genetics 158:1737–1753

Lu H, Romero-Severson J, Bernardo R (2003) Genetic basis of heterosis explored by simple sequence repeat markers in a random-mated maize population. Theor Appl Genet 107:494–502

Luo LJ, Li ZK, Mei HW, Shu QY, Tabien R, Zhong DB, Ying CS, Stansel JW, Khush GS, Paterson AH (2001) Overdominance epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. II. Grain yield components. Genetics 158:1755–1771

Melchinger AE, Piepho H-P, Utz HF, Muninovic J, Wegenast T, Törjek O, Altmann T, Kusterer B (2007a) Genetic basis of heterosis for growth-related traits in Arabidopsis investigated by testcross progenies of near-isogenic lines reveals a significant role of epistasis. Genetics 177:1827–1837

Melchinger AE, Utz HF, Piepho H-P, Zeng ZB, Schon CC (2007b) The role of epistasis in the manifestation of heterosis: a systems-oriented approach. Genetics 177:1815–1825

Powers L (1944) An expansion of Jones's theory for the explanation of heterosis. Am Nat 78:275–280

Schön CC, Dhillon BS, Utz HF, Melchinger AE (2010) High congruency of QTL positions for heterosis of grain yield in three crosses of maize. Theor Appl Genet. doi:10.1007/s00122-009-1209-9

Shull GH (1908) The composition of a field of maize. Am Breed Assoc Rep 4:196–301

Snedecor GW, Cochran WG (1980) Statistical methods. Iowa State University Press, Ames

StatSoft (1997) Statistica. StatSoft, Tulsa

Stuber CW, Lincoln SW, Wolff DW, Helentjaris T, Lander ES (1992) Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132:823–839

Williams W (1959) Heterosis and the genetics of complex characters. Nature 184:527–530

Xiao JH, Li JM, Yuan LP, Tanksley SD (1995) Dominance is the major genetic basis of heterosis in rice as revealed by QTL analysis using molecular markers. Genetics 140:745–754

Yu SB, Li JX, Xu CG, Yan YF, Gao YJ, Li XH, Zhang QF, Saghai Maroof MA (1997) Importance of epistasis as the genetic basis of the heterosis in an elite rice hybrid. PNAS 94:9226–9231

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468