RESEARCH ARTICLE

Genomewide mapping reveals a combination of different genetic effects causing the genetic basis of heterosis in two elite rice hybrids

LANZHI LI[1,2], XIAOHONG HE[3], HONGYAN ZHANG[1], ZHIMING WANG[1], CONGWEI SUN[1], TONGMIN MOU[4], XINQI LI[5], YUANMING ZHANG[3] and ZHONGLI HU[2*]

[1]*Hunan Provincial Key Laboratory for Biology and Control of Plant Disease and Insect Pests, College of Plant Protection, Hunan Agricultural University, Changsha 410128, People's Republic of China*
[2]*State Key Laboratory of Hybrid Rice, College of Life Science, Wuhan University, Wuhan 430072, People's Republic of China*
[3]*Section on Statistical Genomics, State Key Laboratory of Crop Genetics and Germplasm Enhancement/Chinese National Center for Soybean Improvement, College of Agriculture, Nanjing Agricultural University, Nanjing 210095, People's Republic of China*
[4]*National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, People's Republic of China*
[5]*Department of Breeding, China National Hybrid Rice Research and Development Center, Changsha 410125, People's Republic of China*

Abstract

North Carolina design III (NCIII) is one of the most powerful and widely used mating designs for understanding the genetic basis of heterosis. However, the quantitative trait mapping (QTL) conducted in previous studies with this design was mainly based on analysis of variance (ANOVA), composite interval or multiple interval mapping methods. These methodologies could not investigate all kinds of genetic effects, especially epistatic effects, simultaneously on the whole genome. In this study, with a statistical method for mapping epistatic QTL associated with heterosis using the recombinant inbred line (RIL)-based NCIII design, we conducted QTL mapping for nine agronomic traits of two elite hybrids to characterize the mode of gene action contributing to heterosis on a whole genomewide scale. In total, 23 main-effect QTL (M-QTL) and 23 digenic interactions in *IJ* (*indica* × *japonica*) hybrids, 11 M-QTL and 82 digenic interactions in *II* (*indica* × *indica*) hybrid QTLs were identified in the present study. The variation explained by individual M-QTL or interactions ranged from 2.3 to 11.0%. The number of digenic interactions and the total variation explained by interactions of each trait were larger than those of M-QTL. The augmented genetic effect ratio of most M-QTL and digenic interactions in (L_1–L_2) data of two backcross populations (L_1 and L_2) showed complete dominance or overdominance, and in (L_1+L_2) data showed an additive effect. Our results indicated that the dominance, overdominance and epistatic effect were important in conditioning the genetic basis of heterosis of the two elite hybrids. The relative contributions of the genetic components varied with traits and the genetic basis of the two hybrids was different.

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Introduction

Despite the extensive research on quantitative genetics, physiology and molecular approaches in the last 100 years, the genetic basis of heterosis or hybrid vigour, remains to be fully characterized.

Of the several hypotheses proposed to explain the genetic basis of heterosis, the 'dominance' model at the single-locus level, assumes complementation of slightly deleterious recessive alleles as its cause (Bruce 1910; Jones 1917; Xiao *et al.* 1995; Cockerham and Zeng 1996), whereas the 'overdominance' model proposes it to be due to that an overexpression of certain genes in the heterozygous offspring compared to the homozygous parents (Shull 1908; Crow 1948; Stuber

*For correspondence. E-mail: huzhongli@whu.edu.cn.
Lanzhi Li and Xiaohong He contributed equally to this work.
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et al. 1992). With more than one locus, the 'epistasis' model has been invoked to explain interlocus interactions (Stuber *et al.* 1992; Yu *et al.* 1997; Li *et al.* 2001; Luo *et al.* 2001; Syed and Chen 2005). In recent years, several experiments suggested that heterosis is the orchestrated outcome of different gene actions (Li *et al.* 2008; Zhou *et al.* 2012). This is consistent with different loci affecting heterosis for different traits and in different hybrids (Schnable and Springer 2013).

Comstock and Robinson (1952) devised one of the most powerful and widely used experimental designs that can be used for partitioning the genetic variance: the North Carolina design III (NCIII). The NCIII design is to cross the *i*th individual ($I = 1,2,...,n$) of a base population (F_2, RIL, NIL, etc.) to both parental lines (P_1 and P_2) to produce two backcross populations (L_{1i} and L_{2i}). It provides an estimation of the average level of dominance of genes affecting the evaluated traits. However, the conventional approach only deals with the collective effects of all the polygenes. The development of molecular markers facilitated the detection of individual QTL and epistatic interactions for heterosis.

Recently, with the suitable genetic mating design NCIII, many quantitative trait loci (QTL) mapping studies of rice have provided insight into the genetic basis of heterosis.

Xiao *et al.* (1995) separately analysed QTL of two BC_1F_7 populations, which were derived from crosses between RILs and the parents, for 12 agronomic traits using both single point analysis and the interval mapping method. Their results suggest that dominance is the major genetic basis of heterosis in rice. Using a set of 254 F_{10} RILs from a cross between Lemont (*japonica*) and Teqing (*indica*), two backcross (BC) and two testcross populations derived from crosses between the RILs and the parents plus two testers as material, Li *et al.* (2001) and Luo *et al.* (2001) identified main-effect and epistatic QTL of data from the RIL population, the mean midparental heterosis, and the mean values of individual backcross and testcross F_1 hybrids for grain yield and its components by the mixed linear model using the software QTLMapper 1.0 (Wang *et al.* 1999). They found that overdominance and epistatic effects are the primary genetic basis of heterosis in rice. In our previous study, we detected the main-effect QTL by the composite interval method and epistatic QTL by the mixed linear approach of (L_1+L_2) and (L_1-L_2) data of two backcross populations (L_1 and L_2) in two elite hybrids (Li *et al.* 2008). The research demonstrated that heterosis was the result of a combination of different types of gene actions. The approaches used in the above studies were based on interval mapping or composite interval mapping. Garcia *et al.* (2008) developed a multiple-interval mapping in NCIII design, which can detect augmented additive and dominance effects, and epistatic interactions (*aa*+*dd* and *ad*+*da*) of QTL simultaneously. Later on, He *et al.* (2012) developed a statistical method for mapping epistatic QTL associated with heterosis using the RIL-based NCIII design. This method provides a platform to estimate the number, genomic positions, augmented additive and dominance effects and digenic interactions of QTL, simultaneously. First, the genetic expectations of two backcross populations were calculated. Then, an epistatic genetic model included all markers on the whole genome simultaneously, and all the parameters were estimated in the model by empirical Bayes of Xu (2007).

In general, despite numerous marker-aided studies, the genetic basis of heterosis has not been sufficiently documented to reach a clear conclusion, and further investigations are required to detect QTL contributing to heterosis. In the present study, with the approach developed by He *et al.* (2012), we reanalysed the datasets of $Z_1(L_1+L_2)$ and $Z_2(L_1-L_2)$ in our previous study (Li *et al.* 2008) to characterize the mode of gene action contributing to heterosis on a whole genome-wide scale and assess the congruency of the results across studies.

Materials and methods

Populations

The materials used in this study were documented in detail in our previous research (Li *et al.* 2008). Two elite hybrids, one intersubspecific between 9024 (*indica*) and LH422 (*japonica*) and one intrasubspecific between Zhenshan97 (*indica*) and Minghui63 (*indica*), were studied. The first one is designated as *II* hybrid and the latter as *IJ* hybrid hereafter. An F_2 population was derived from two inbred lines (P_1 and P_2) that differed significantly in the quantitative traits of interest and possessed abundant polymorphic molecular markers. A random sample of n ($n = 194$ in *II* and *IJ* hybrids, $n = 194$ and $n = 222$, respectively) F_2 individuals were selected to develop n recombinant inbred lines (194 F_7 lines and 222 F_{12} lines for II and *IJ* hybrid, respectively) by consecutive self-crosses. The recombinant inbred (RI) populations were backcrossed to the two parental lines to produce $2n$ families (L_{1i} and L_{2i}).

Phenotype variation

All materials described above were laid out in a field in a randomized complete block design with two replications (plots) for phenotypic evaluation. Material planting was documented in detail in Li *et al.* (2008). The nine quantitative traits investigated were: heading date (HD) (in days), plant height (PH) (in centimetres), tillers per plant (TP), panicle length (PL) (in centimetres), filled grains per panicle (FGPP), percentage of seed set (SS), grain density (GD) (in grain numbers per centimetre of panicle length), 1000-grain weight (KGW) (in grams) and grain yield (YD) (in tons/hectare). The means of replications, for each trait, for each of two backcross populations, were used for QTL and other analyses.

Genetic linkage maps

For the *IJ* hybrid, Xiao *et al.* (1995) constructed a linkage map of the recombinant population, in which a subset of 141 polymorphic RFLP markers was used. For the *II* hybrid, a

linkage map was constructed by Xing *et al.* (2002), which consisted of 221 marker loci and covered a total of 1796 cM.

QTL mapping

QTL mapping was conducted in the $Z_1 = L_1 + L_2$ and $Z_2 = L_1 - L_2$ by a two-step approach to detect main-effect and epistatic-effect QTLs in the RIL-based NCIII design using the F_∞ metric model (He *et al.* 2012). A LOD score of 2.0 was selected as the threshold for the presence of a main-effect QTL based on the total map distance and the average distance between markers. A threshold of LOD $\geq$ 3.0 ($P \leq 0.001$) was used for declaring the presence of a putative pair of epistatic QTL. The genetic effect symbols adopted in this study are according to Kao and Zeng (2002).

The degree of dominance of a M-QTL was estimated as $|d^*/a^*|$, where d^* and a^* denote the augmented dominance and additive effect resolved in Z_2 and Z_1, respectively. Under the F_∞ metric, $a_k^* = a_k + \frac{1}{2}\sum_{l\neq k}^{q}(i_{a_k d_l} - i_{d_k a_l})$ and $d_k^* = d_k - \frac{1}{2}\sum_{l\neq k}^{q}(i_{a_k a_l} - i_{d_k d_l})$, respectively, where a_k and d_k are additive and dominance effects of the kth QTL (k = 1,2,. . ., q); $i_{a_k a_l}$, $i_{a_k d_l}$, $i_{d_k a_l}$, and $i_{d_k d_l}$ are respectively additive × additive, additive × dominance, dominance × additive and dominance × dominance epistatic effects between QTL k and l. q is the number of QTL-controlled quantitative traits. We calculated $|d^*/a^*|$ and classify the QTL as additive (A) ($|d^*/a^*| < 0.2$), partial dominance (PD) ($0.2 \leq |d^*/a^*| < 0.8$), dominance (D) ($0.8 \leq |d^*/a^*| < 1.2$), and overdominance (OD) ($|d^*/a^*| \geq 1.2$) according to Stuber *et al.* (1987).

The degree of dominance of a digenic interaction was estimated as $|\tilde{i}_{kl}/\overleftrightarrow{i}_{kl}|$, where $\tilde{i}_{kl} = i_{a_k d_l} + i_{d_k a_l}$ and $\overleftrightarrow{i}_{kl} = i_{a_k a_l} + i_{d_k d_l}$ are augmented epistatic effects between QTL k and l resolved in Z_2 and Z_1, respectively. $i_{a_k a_l}, i_{d_k d_l}, i_{a_k d_l}$ and $i_{d_k a_l}$ are the same as above. We calculated $|\tilde{i}_{kl}/\overleftrightarrow{i}_{kl}|$ and classify the epistatic QTL as additive (A) ($|\tilde{i}_{kl}/\overleftrightarrow{i}_{kl}| < 0.2$), partial dominance (PD) ($0.2 \leq |\tilde{i}_{kl}/\overleftrightarrow{i}_{kl}| < 0.8$), dominance (D) ($0.8 \leq |\tilde{i}_{kl}/\overleftrightarrow{i}_{kl}| < 1.2$) and overdominance (OD) ($|\tilde{i}_{kl}/\overleftrightarrow{i}_{kl}| \geq 1.2$).

Table 1. Main-effect QTL resolved in *IJ* hybrid.

			Z_1			Z_2			
Trait	Chr.[a]	Interval-L[b]	Effect	LOD	R^2 (%)	Effect	LOD	R^2 (%)	$\|d^*/a^*\|$[c]
HD	1	XNPB302	0.61	2.35	2.14				PD
HD	3	XNPB249	−0.64	2.23	2.37				PD
HD	3	CDO1081	−0.76	3.99	3.27				PD
HD	7	RG711	−0.92	5.68	4.83				A
HD	8	RG333	2.52	27.45	36.12	0.95	5.62	12.29	PD
HD	8	RG136	0.61	2.55	2.13				PD
PH	1	XNPB302	2.03	2.61	3.39				A
PH	5	RG480	−2.81	4.93	6.46				PD
PH	6	RZ682	−3.06	5.18	7.66				PD
PH	7	CDO533				1.67	2.94	5.73	OD
PH	8	RG333	3.48	6.15	9.94				A
TP	3	CDO1081	52.01	2.19	2.87				A
PL	4	CDO456				0.35	3.12	4.84	OD
FGPP	3	CDO1081	4.61	3.1	5.38				A
FGPP	4	RG864	4.41	3.17	4.92				A
FGPP	5	RG360	−4.59	3.31	5.33				A
SS	7	RG528	−1.59	2.63	3.06				PD
SS	11	XNPB179	−1.43	2.18	2.46				PD
GD	6	RG162				−0.2	2.38	5.07	OD
KGW	2	CDO1091	−0.61	2.84	4.08				A
KGW	3	RZ993				0.45	4.62	9.43	D
KGW	5	RZ296	1.02	7.5	11.27				A
YD	8	RG333	230.01	2.26	4.95				PD

[a]Chromosome number interval of the QTL detected in the study.
[b]The left marker of the interval where QTL located in.
[c]The degree of dominance for all M-QTL declared as significant in any dataset was determined after estimating their additive and dominance effects, in SUM and DIFF datasets. QTL were classified according to their $|d^*/a^*|$ ratio as additive (A) ($|d^*/a^*| < 0.2$), partial dominance (PD) ($0.2 \leq |d^*/a^*| < 0.8$), dominance (D) ($0.8 \leq |d^*/a^*| < 1.2$), and overdominance (OD) ($|d^*/a^*| \geq 1.2$) according to Stuber *et al.* (1987).

Results

M-QTL

QTL detected in Z_1 and Z_2 in *IJ* and *II* hybrids are shown in tables 1 and 2, respectively. In total, 23 and 11 QTLs were revealed in *IJ* and *II* hybrids, respectively. Except one QTL for heading date detected in *IJ* hybrids (36.12% in Z_1 and 12.29% in Z_2), QTL accounted for phenotypic variation less than <10% individually.

HD: In the *IJ* hybrid, six QTLs were detected. Five showed a partial-to-complete dominant effect and one showed an additive effect. Half of the six QTLs showing a partial-to-dominant effect were positive, with alleles from 9024 increasing the trait value. In the *II* hybrid, three QTL were found. One of them exhibited an additive effect and two overdominant effect. Two of the three were positive, with alleles from Minghui63 increasing the trait value.

PH: In the *IJ* hybrid, five QTLs were identified with two showing an additive effect, two a partial-to-dominant effect and one an overdominant effect. In the *II* hybrid, two QTLs were found; one was classified as partial-to-dominant and the other one as overdominant.

TP: Only one QTL was detected in Z_1 in *IJ* hybrids, which was classified as additive. In the *II* hybrid, each QTL was respectively identified in Z_1 and Z_2. One was classified as additive and the other one as dominant.

PL: Only one QTL was identified in Z_2 in *IJ* hybrids displaying an overdominant effect and with alleles from 9024 increasing the trait value. No QTL was found in *II* hybrids.

FGPP: Three QTLs were detected in Z_1 in *IJ* hybrids displaying an additive effect. No QTL was identified in *II* hybrids.

SS: Two QTLs were found in *IJ* hybrids exhibiting a partial-to-dominant effect. Only one QTL was detected in *II* hybrids showing an additive effect.

GD: Each hybrid identified one QTL. The QTL detected in *IJ* hybrids showed an overdominant effect and the other one in *II* hybrids showed an additive effect.

KGW: In *IJ* hybrids, three QTLs were found. Two of them exhibited additive effects and one was dominant. In *II* hybrids, one QTL was identified showing a partial-to-dominant effect.

YD: Each QTL was detected respectively in *IJ* and *II* hybrids.

Digenic interactions

Table 3 shows the digenic interactions resolved in *IJ* hybrids. A total of 23 interactions were found in Z_1 and Z_2 in *IJ* hybrids (table 3). The variation explained by individual interactions ranges from 2.3 to 11.0%. Except for KGW, the proportion of total variation explained by all digenic interactions was less than 20% in other traits. The number of digenic interactions detected for each trait varies from 2 to 4. No interaction was simultaneously detected in Z_1 and Z_2 datasets.

Digenic interactions resolved in *II* hybrids are listed in table 4. In total, 82 interactions were found (table 4). As with *IJ* hybrids, each interaction generally showed modest variation (<10% for all significant interactions except two interactions with 19.0 and 17.9%). However, in the *II* hybrids, the total variation explained by all digenic interactions for traits ranged from 21.9 to 70.4%. Most of them were more than 40%. Among these interactions, only one interaction was identified simultaneously in Z_1 and Z_2 for seeding set.

Table 5 summarizes the main QTL effect and digenic interactions resolved in *IJ* and *II* hybrids. In *IJ* hybrids, 19 and 5 M-QTL were detected in Z_1 and Z_2, respectively. Only one M-QTL was found simultaneously in Z_1 and Z_2. With the same material, we identified 17 and 14 M-QTL in Z_1 and

Table 2. Main-effect QTL resolved in *II* hybrid.

			Z_1			Z_2			
Trait	Chr.[a]	Interval-L[b]	Effect	LOD	R^2 (%)	Effect	LOD	R^2 (%)[b]	$\|d^*/a^*\|$[c]
HD	2	RM48	1.82	2.18	2.31				A
HD	5	RM31				−1.20	2.14	2.59	OD
HD	7	R1789				1.22	2.13	2.67	OD
PH	9	RG570				1.24	2.15	2.51	OD
PH	10	RG561	2.94	3.97	4.89				PD
TP	1	RG532				0.34	2.14	3.05	D
TP	7	R1789	−3.08	2.24	2.46				A
SS	6	RM204	2.91	5.03	5.75				A
GD	12	R887	0.17	2.37	2.74				A
KGW	3	RZ403	0.60	2.19	2.67				PD
YD	7	R1789				1.28	2.98	2.93	OD

For explanation on superscripts a–c, see footnote of table 1.

Table 3. Digenic interactions in Z_1 and Z_2 dataset in *IJ* hybrid.

Trait	Chr_i^a	Marker	Chr_j^a	Marker	Z_1 Effect	Z_1 LOD	Z_1 R^2 (%)	Z_2 Effect	Z_2 LOD	Z_2 R^2 (%)[b]	$\lvert\tilde{i}_{kl}/\overleftrightarrow{i}_{kl}\rvert$[c]
HD	4	RG864	8	RZ562	1.29	3.01	2.37				PD
HD	5	RZ556	8	RZ562	1.47	3.94	3.10				PD
PH	1	RG462	9	XNPB385	4.76	3.54	4.64				PD
PH	6	XNPB317	9	XNPB103				3.68	3.84	6.94	OD
TP	2	RG544	9	RG358				0.48	3.16	7.14	OD
TP	4	RZ590	8	RG136	−0.53	3.29	5.71				A
PL	2	RZ123	5	RG573				0.78	3.72	5.83	OD
PL	2	RZ825	9	XNPB385	−1.17	4.12	7.24				PD
PL	6	CDO109	8	RZ562				−0.69	3.04	4.65	OD
FGPP	1	RG541	4	XNPB271				−12.71	4.69	8.78	OD
FGPP	2	TW500	10	RZ400	−14.97	3.12	5.15				PD
FGPP	8	XNPB56	9	XNPB385				−10.41	3.22	5.89	OD
SS	3	CDO1081	8	RZ562	−3.98	4.30	4.80				A
SS	6	RZ450	11	RZ597	−3.21	2.78	3.12	4.19	4.22	9.57	OD
GD	1	RG375	10	RZ811	0.64	3.18	6.21				PD
GD	1	RZ776	12	RZ76	9.59	3.85	5.81				PD
GD	5	RG480	6	RG1028				7.45	4.52	7.43	OD
KGW	1	RZ776	12	RZ76	−1.44	4.08	5.67				A
KGW	1	RG811	2	RG152	1.28	3.29	4.45				A
KGW	3	XNPB232	3	RZ16	−2.01	7.69	11.00				A
KGW	3	RZ16	5	RG573				0.88	5.67	8.88	OD
YD	1	RG541	2	RZ825	602.58	4.59	8.49				A
YD	1	RG541	6	CDO78	491.46	3.08	5.65				PD

[a] Chr_i and Chr_j represent chromosomes that are located on loci *i* and *j*, respectively.

[b] Percentage of the total variation explained by AA*ij*.

[c] The epistasis dominance degree (EDD) of digenic interaction. The degree of dominance of a digenic interaction was estimated as $|\tilde{i}_{kl}/\overleftrightarrow{i}_{kl}|$, where $\overleftrightarrow{i}_{kl} = i_{a_k a_l} + i_{d_k d_l}$ and $\tilde{i}_{kl} = i_{a_k d_l} + i_{d_k a_l}$ are augmented epistatic effect between QTL *k* and *l* resolved in Z_2 and Z_1, respectively. $i_{a_k a_l}$, $i_{d_k d_l}$, $i_{a_k d_l}$ and $i_{d_k a_l}$ are additive × additive, dominance × dominance, additive × dominance and dominance × additive epistatic effects between QTL *k* and *l*. We calculated $|\tilde{i}_{kl}/\overleftrightarrow{i}_{kl}|$ and classify the epistatic QTL as additive (A) ($|\tilde{i}_{kl}/\overleftrightarrow{i}_{kl}| < 0.2$), partial dominance (PD) ($0.2 \leq |\tilde{i}_{kl}/\overleftrightarrow{i}_{kl}| < 0.8$), dominance (D) ($0.8 \leq |\tilde{i}_{kl}/\overleftrightarrow{i}_{kl}| < 1.2$), and overdominance (OD) ($|\tilde{i}_{kl}/\overleftrightarrow{i}_{kl}| \geq 1.2$).

Z_2 in *IJ* hybrids, respectively, in a previous study (Li *et al.* 2008). There were 13 M-QTL simultaneously found in both of the studies. Meanwhile, eight of the 13 M-QTL exhibited the same dominance degree. In *II* hybrids, six and five M-QTL were found in Z_1 and Z_2, respectively. While in a previous study, eight and nine M-QTL were resolved in Z_1 and Z_2, respectively. Among them, six M-QTL were detected in both the studies.

For digenic interactions, 15 and 9 interactions were identified in Z_1 and Z_2 in *IJ* hybrids, respectively. However, 43 and 46 interactions were found in Z_1 and Z_2 in our previous study, respectively. Four of the interactions were detected in Z_2 in both the studies simultaneously. The number of interactions was significantly more than those found in *IJ* hybrids.

In this study, 42 and 40 interactions were respectively detected in Z_1 and Z_2 in *II* hybrids. In a previous study, 67 and 81 interactions were respectively resolved in Z_1 and Z_2. Eleven interactions were simultaneously identified in Z_2 in both the studies.

Discussion

Methods comparison

Compared to the previous studies on the methodologies for NCIII, the method described by He *et al.* (2012) and the method used in this study offers some advantage over some previous approaches. With Z_1 or Z_2, all augmented main and epistatic effects were included simultaneously in one genetic

Table 4. Digenic interactions in Z_1 and Z_2 datasets in *II* hybrid.

Trait	Chr_i^a	Marker	Chr_j^a	Marker	Z_1 Effect	Z_1 LOD	Z_1 R^2 (%)	Z_2 Effect	Z_2 LOD	Z_2 R^2 (%)^b	$\lvert\tilde{i}_{kl}/\overleftrightarrow{i}_{kl}\rvert^c$
HD	1	R2632	10	RM258				−3.30	3.71	4.87	
HD	1	C39	5	C246	5.39	5.04	5.06				A
HD	2	RM240	6	RZ667	4.51	3.46	3.54				A
HD	3	C1087	11	C1003B	5.92	5.57	6.11				A
HD	3	C746	10	C153A	−6.27	6.41	6.85				A
HD	7	RG678	7	R1789	−4.62	3.69	3.72				A
HD	8	C483	10	RM228				3.57	4.75	5.72	OD
HD	11	R3203	14	R1687	4.62	3.54	3.72				A
HD	11	RM229	12	C87				3.50	4.37	5.48	OD
PH	1	RG173	12	R2672				4.52	7.50	8.31	OD
PH	1	RM237	9	RG570				2.99	3.68	3.65	OD
PH	1	RM212	10	C148				3.23	4.09	4.26	OD
PH	2	R1738	5	C734b	5.88	3.89	4.90				A
PH	3	R1925	11	R3203				2.85	3.00	3.30	D
PH	5	C246	13	C56				3.19	3.96	4.15	OD
PH	6	C962	12	R643	5.71	3.63	4.61				A
PH	7	R1245	11	CDO127	5.53	3.38	4.33				PD
PH	11	C1003B	12	G1128a	−5.17	3.06	3.78				PD
TP	1	R753	8	C347	1.28	6.33	6.94				A
TP	1	RM212	12	C909B	0.85	3.06	3.11				A
TP	1	C112	3	C1176				0.86	3.62	5.03	OD
TP	1	C112	5	RG360	0.84	3.00	3.01				A
TP	2	RZ599	9	R2638				0.81	3.07	4.44	OD
TP	4	C1016	10	C148	1.06	4.40	4.74				A
TP	5	C1447	9	RZ404	0.89	3.49	3.40				A
TP	8	R2272	10	C1633				−0.81	3.09	4.45	OD
TP	9	RM257	9	RZ404				1.12	4.48	8.42	OD
PL	1	C161	3	R1925	−0.92	3.06	3.88				PD
PL	1	G1128b	1	C904				−1.42	3.15	18.96	OD
PL	3	R1925	10	C1633				−0.70	3.79	4.56	OD
PL	4	RZ467	10	R2174				0.72	4.30	4.82	OD
PL	4	G235	7	RM70	1.17	4.78	6.22				A
PL	5	RZ649	7	RG128	−0.99	3.74	4.48				A
PL	7	RG678	11	RG118				0.68	3.80	4.28	OD
PL	7	RZ471	11	RM254	−0.98	3.37	4.33				A
FGPP	2	RG520	9	RM257	−12.33	4.27	5.81				A
FGPP	3	RM200	14	RM219	−10.61	3.26	4.31				A
FGPP	4	RG620	12	G1128a				−8.60	3.75	5.98	OD
FGPP	9	RZ404	12	G1128a	−12.35	4.39	5.83				A
SS	1	RG532	9	C472				−3.92	3.36	3.90	OD
SS	1	RG101	9	RG667				4.26	3.75	4.60	OD
SS	1	G393	1	R2201				−8.41	3.02	17.91	OD
SS	2	RZ599	5	RM26	4.77	3.59	3.86				PD
SS	6	C1496	9	C472	−6.41	6.00	6.96				PD
SS	6	RG424	13	C933				−4.06	3.65	4.17	OD
SS	7	RM70	9	RZ404				3.87	3.29	3.78	OD
SS	8	G1149	9	RG667				4.27	3.93	4.61	OD
SS	8	G1149	12	C87				4.68	4.88	5.54	OD
GD	1	RM259	1	RM237	−0.40	3.44	3.75				PD
GD	1	G1128b	6	C962	−0.44	3.86	4.55				A
GD	2	RM208	6	RG653	0.40	3.52	3.89				A
GD	3	C63	11	CDO127				0.26	3.46	5.18	D
GD	5	R3166	6	RZ667	−0.40	3.23	3.89				PD
GD	8	R2272	10	RM239	−0.43	3.65	4.42				A
GD	9	RZ404	12	R887	−0.41	3.35	3.93				A

Table 4 (*contd*)

Trait	Chr_i^a	Marker	Chr_j^a	Marker	Z_1 Effect	Z_1 LOD	Z_1 R^2 (%)	Z_2 Effect	Z_2 LOD	Z_2 R^2 (%)^b	$\lvert\tilde{i}_{kl}/\overleftrightarrow{i}_{kl}\rvert^c$
KGW	2	RZ324	5	R830				0.73	3.84	4.78	OD
KGW	3	RM232	4	RM255				0.74	3.91	4.93	OD
KGW	3	RZ403	8	G1149				−0.66	3.35	3.90	OD
KGW	5	RZ649	7	RG528				−0.65	3.08	3.78	OD
KGW	6	RM204	8	RG978	−1.61	4.19	4.88				A
KGW	6	RG424	12	C909B	−1.96	5.95	7.24				A
KGW	6	G342	12	G1128a				0.94	6.07	7.88	OD
KGW	7	R1245	11	G4001				−0.78	4.58	5.44	OD
KGW	7	R1245	12	C909B	−1.46	3.35	4.00				A
KGW	8	R727	8	RM223				1.14	3.04	11.61	D
KGW	11	R3203	12	R496	1.83	5.12	6.31				A
KGW	12	RM20b	12	G1128a				0.71	3.68	4.53	OD
YD	1	R753	11	G257	−2.70	3.22	3.43				A
YD	1	R2201	9	RG570				2.61	3.57	3.06	OD
YD	1	RG236	3	RM148	3.11	3.96	4.58				A
YD	2	RM211	10	RM222				3.16	4.83	4.48	OD
YD	2	RM53	9	R2638				2.58	3.35	3.00	OD
YD	2	RM207	6	P				−4.05	7.72	7.39	OD
YD	3	RM232	4	RM255				3.81	6.87	6.55	OD
YD	4	G102	6	R2147	−2.69	3.26	3.42				A
YD	5	R3166	6	RG424	−2.90	3.78	3.97				PD
YD	5	RM31	12	RM20b	−2.65	3.08	3.31				A
YD	6	RM204	8	R1629	2.91	3.63	3.99				A
YD	6	RM204	10	R2174	−2.79	3.40	3.69				A
YD	7	RG528	11	CDO127				−2.71	3.41	3.31	OD
YD	10	RM222	10	RM239				3.96	3.69	7.05	OD
YD	10	C677	11	RM229	4.40	7.93	9.16				A

For explanation on superscripts a–c, see footnote of table 3.

model and estimated together by the E-Bayes approach. Although, only augmented and not pure additive or dominant effects could be detected in this model, the power and precision of genetic effect estimation is higher than that in previous studies (Stuber *et al.* 1992; Melchinger *et al.* 2007; Garcia *et al.* 2008; Li *et al.* 2008; He *et al.* 2012). The augmented additive effect just precisely measures the net contribution of the *k*th QTL to parental difference, and the augmented dominant effect measures the net contribution of the QTL to midparent heterosis (Melchinger *et al.* 2007; He *et al.* 2012).

Dominance and overdominance in M-QTL

Similar to the results of previous studies (Li *et al.* 2008), we found that the proportion of M-QTL with an augmented additive or an augmented dominant effect is different between the two hybrids (table 5). Among the 23 M-QTL detected in the *IJ* hybrid, nine (39.1%) exhibited an additive gene action, 10 (43.5%) showed a partial-to-dominant effect, one (4.4%) showed a complete dominant effect, and three (13.0%) showed an overdominance; while among the 11 M-QTL detected in *II* hybrid, four (36.4%) showed an additive effect, two (18.2%) showed a partial-to-dominant effect, one (9.1%) exhibited a complete dominant effect, and four (36.4%) exhibited an overdominance. The results are consistent with the three other approaches (ANOVA, interval mapping (IM) and composite interval mapping (CIM)), the proportions of QTL detected with partial-to-complete dominance and with overdominance were more than 25% (Li *et al.* 2008). These results indicated that dominance and overdominance were important in conditioning the genetic basis of heterosis of the two elite hybrids.

In addition, the relative importance of dominance and overdominance conditions in heterosis is consistent with previous studies (Xiao *et al.* 1995; Li *et al.* 2008). In the *IJ* hybrids, there was more M-QTL with partial-to-complete dominance than with overdominance. While in the *II* hybrids, the proportion of M-QTL exhibiting overdominance was more than that with partial-to-complete dominance. This suggested that the genetic basis of agronomic traits in the two hybrids is different.

Some QTLs colocalized with that previously reported for heterosis. In the two studies, 13 of 23 (56.5%) and 6 of 11 (54.5%) M-QTLs were simultaneously identified in *IJ* and *II* hybrids, respectively. However, only eight of the 13 M-QTL and one of the six M-QTL showed the same dominance degree in both studies. Other M-QTL exhibiting a different dominance degree perhaps due to a pure dominant effect resolved in a previous study, but an augmented dominant

Table 5. Summary of main-effect and epistatic-effect QTL in *IJ* and *II* hybrids.

	IJ main-effect QTL		*IJ* epistatic-effect QTL		*II* main-effect QTL		*II* epistatic-effect QTL	
	No.	Rate (%)	No.	Rate (%)	No.	Rate (%)	No.	Rate (%)
A	9	39.13	6	26.09	4	36.36	34	41.46
PD	10	43.48	8	34.78	2	18.18	8	9.76
D	1	4.35	0	0	1	9.09	3	3.66
OD	3	13.04	9	39.13	4	36.36	37	45.12
SUM	23		23		11		82	

effect in this study. Interestingly, the dominance degree of most of these QTL in the present study was higher than that previously reported. This suggested that on the whole-genome level, both dominant and epistatic effects influenced the genetic basis of heterosis for the trait. Further, the epistatic effect perhaps improved hybrid vigour for these complex traits.

Among the identified M-QTL, a few of them were pleiotropic. In *IJ* hybrids, the region XNPB302 on chromosome 1 detected for heading date appeared to be involved in plant height. The region CDO1081 on chromosome 3 simultaneously identified for heading date, tillers per plant and filled grains per plant, while the region RG333 on chromosome 8 for heading date, plant height and yield. In *II* hybrids, the region R1789 on chromosome 7 investigated for heading date also involved in tillers per plant and yield. These apparent pleiotropic effects were consistent with results found in the literature (Xiao *et al.* 1995; Li *et al.* 2008). These regions deserve further attention especially in marker-assisted breeding.

We noted that, for both hybrids studied here, augmented dominant effects of the majority of detected QTL were positive. In *IJ* hybrids, we found dominance effects for plant height, plant length, kilo grains weight and heading date were positive but negative for grain density. Interestingly, the former four traits displayed significant positive midparent heterosis whereas the last one displayed negative midparent heterosis. In *II* hybrids, except one QTL for heading date, the dominant effects of M-QTL for other traits were positive. All the traits exhibited positive heterosis. These results are also consistent with those from Frascaroli *et al.* (2007) and Larièpe *et al.* (2012). They conducted QTL mapping in Maize with a NCIII design. Among the studied traits, the proportion of 'dominant' QTL was globally coherent with the heterosis level and the augmented degree of dominance of the trait, indicating a good consistency of phenotypic and QTL analyses. Surprisingly, there was a poor relationship observed between marker heterozygosity and trait expression, only a high correlation between Z_2 and hybrid heterozygosity (Li *et al.* 2008). However, Frascaroli *et al.* (2007), Schön *et al.* (2010) and Larièpe *et al.* (2012) found that the relationship observed between hybrid performance for these traits and hybrid heterozygosity was high. On one hand, this might indicate that the genetic basis of heterosis differs between autogamous (rice) and allogamous (maize) plants. On the other hand, this result also confirmed that, in rice, heterosis not only results from dominance or overdominance, but also from epistasis. This is because an augmented dominance effect of M-QTL in the Z_2 dataset involved dominance, and *aa* and *dd* epistatic effects were shown here.

Epistatic effects of digenic interactions

Several digenic interactions were found in the two hybrids. The number of digenic interactions was significantly more than those of M-QTL. And the total variation explained by interactions of each trait was larger than that of M-QTL. Compared to the epistatic effect, QTL detected in the two hybrids, we found that the number of interactions in *II* hybrids was significantly more than that in *IJ* hybrids. In addition, the total variation explained by all digenic interactions in *II* hybrids was much greater than that in *IJ* hybrids affecting the same trait. This result further confirmed that epistatic interactions between two loci played an equal, if not more important role than single locus interaction effects as the genetic basis of heterosis, and the two hybrids have a different genetic basis of agronomic traits.

It should be noted that the number of digenic interactions resolved in this study is less than that by the mixed linear approach with computer software QTLMapper ver. 1.0 (Li *et al.* 2008). This perhaps resulted from the different methods conducted in the two studies, for only a pure epistatic effect (*aa* in Z_1 and *dd* in Z_2) of a single locus was detected by ANOVA (Li *et al.* 2008) and an augmented epistatic effect (*ad* and *da* epistatic effect in Z_1, and *aa* and *dd* epistatic effect in Z_2) was investigated by E-Bayes in RIL-based NCIII design (He *et al.* 2012). In our study, the opposite sign of epistatic effects possibly led to the cancellation of an augmented effect of an interaction for a certain trait, which resulted in some interactions that could not be identified in this research. A series of Monte Carlo simulation experiments carried out by He *et al.* (2012) also confirmed that the statistical powers in the detection of augmented epistatic effects were substantively affected by the signs of pure epistatic effects.

With QTLMapper ver. 1.0 (Wang *et al.* 1999) and He *et al.* (2012) method, digenic interactions between two complementary loci, no matter whether the detectable significant effect was at a single locus, were identified for quantitative traits. It also provides a means to test for epistatic effects between individual QTL and the genetic background. In this research, only one interaction in *IJ* hybrids and three

interactions in *II* hybrids occurred between M-QTL and other complementary loci. However, using the same population of *IJ* hybrids reported here, Xiao *et al.* (1995) were unable to detect epistasis due to the unavailability of appropriate mapping methodology (Li *et al.* 2001). Therefore, they studied only the interaction that occurred between M-QTLs.

When the epistasis is absent, the analysis of the Z_1 dataset mainly identifies M-QTL with an additive effect (*a*), whereas the analysis of the Z_2 dataset detects M-QTL with a dominance effect (*d*) (Frascaroli *et al.* 2007; Li *et al.* 2008). In the present study, with the method developed by He *et al.* (2012), we calculated the dominant effect of M-QTL and digenic interactions of agronomic traits in the Z_2 dataset in two hybrids to assess the relative contributions of different types of genetic effects to heterosis. On the whole, the augmented genetic effect ratio of M-QTL ($|d^*/a^*|$) and digenic interactions ($|\tilde{i}_{kl}/\overleftrightarrow{i}_{kl}|$) in the Z_2 dataset showed complete dominance or overdominance, and in Z_1 dataset they showed additive effects. The contribution of digenic interactions showing overdominance was higher than M-QTL in *II* hybrids. In *IJ* hybrids, M-QTL and digenic interactions were important contributors to the genetic basis of heterosis, although the number of digenic interaction was less than that in *II* hybrids.

It should be noted that our results showed that the relative contributions of the genetic components varied with traits. In *II* hybrids, digenic interactions showing overdominance was the most important contributor for yield, kilo grains weight, seeding set, panicle length and plant height. Interactions with overdominance also contributed to heading date and filled grains per panicle, and with dominance contributed to plant height, grain density and kilo grains weight. M-QTL exhibiting overdominance plays a role in heading date, plant height and yield.

In *IJ* hybrids, digenic interactions showing overdominance is an important factor for plant length, filled grains per panicle, plant height, tillers per plant, seeding set, grain density and grain weight. Another important contributor for heading date, plant height, plant length, kilo grains weight is M-QTL with overdominance.

In present study, the genetic effect of digenic interactions investigated in the Z_2 dataset included *ad* and *da* epistatic effects, and in the Z_1 dataset included *aa* and *dd* epistatic effects between two QTLs. To explicitly elucidate the influence of pure single-locus (dominance, overdominance) and two-loci (*aa*, *ad*, *da*, *dd* epistatic effect) genetic effects on the heterosis of agronomic traits, models or genetic mating design (e.g., RIL-based TTC design) (Melchinger *et al.* 2008; He and Zhang 2011), which can be used to study how interactions among multiple genes can lead to the phenotypic manifestations of heterosis are probably the most relevant. Recent findings resulting from genomic, proteomic, metabolic, epigenetic and network studies, in hybrids and polyploids also highlight some testable models for heterosis (Chen 2013). The recent study by Riedelsheimer *et al.* (2012) provides an example of how genomic approaches to understand heterosis may also provide value for the prediction of heterotic traits.

Conclusion

Our results indicated that the dominance, overdominance and epistatic effects were important in conditioning the genetic basis of heterosis of two elite hybrids. The relative contributions of the genetic components varied with traits and the genetic basis of the two hybrids was different.

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