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A genome scan for quantitative trait loci affecting grain yield and its components of maize both in single- and two-locus levels

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Abstract By adding thirty-one markers in the previous linkage map, a new genetic linkage map containing 205 markers was constructed, spanning a total of 2305.4 cM with an average interval of 11.2 cM. The genotypic errors in the whole genome were detected by the statistical method and removed manually. The precision of the linkage map was improved significantly. Main and epistatic QTL were detected by R/qtl, and main QTL were confirmed and refined by multiple interval mapping (MIM). Finally, MIM detected seven QTL for rows number, and five QTL for each grain yield, kernels per row and 100-kernel weight. The contribution to genetic variations of QTL varied from 35.3% for grain yield to 61.5% for rows number. Only kernels per row exhibited significant epistatic interactions between QTL. Twenty-four epistatic QTL were detected which distributed on almost all the ten chromosomes. About two-third epistatic QTL were observed between main QTL and another locus, which had no significant effects. These results indicate rather clearly that there are a number of QTL affecting trait expressions, not directly but indirectly through interactions with other loci. Thus, epistatic QTL effects may play a crucial role, if not more important than main QTL effects, in the genetic variation for the measured traits in present study.

Keywords: molecular markers, epistatic QTL, quantitative trait loci (QTL).

Maize is one of the most important cereal crops in the world. The hybrid yield advantage is responsible for about 10 percent of the total global maize production of 550 Mt[1]. It is exigent to study the yield traits so as to improve the hybrids *per se* in the eventful period when world populations are steadily increasing while the cultivated lands are incessantly decreasing. In the past two decades, with the development of high-density molecular marker linkage map and quantitative trait locus (QTL) mapping technologies have provided useful tools for detecting the inherence of complex traits in crops. More than 6000 and 2000 QTL for various agro-morphic, quality, and biotic and abiotic stresses etc. traits were mapped in rice and maize, respectively (www.gramene.org; www.maizegdb.org). There are two main ways to utilize these QTL: (1) use many QTL to enhance the breeding capacity through molecular marker assisted selection (MAS)[2] and, (2) clone some QTL to understand the genetic mechanism of quantitative traits. Recently, some important QTL in economically important crops like tomato (*fw2.2* for fruit weight[3]), rice (*hd1*and *hd6* for flowering time[4,5], *Gn1a* for yield[6] and *SKC1* for salt tolerance[7]) and maize (*tb1*[8] related with evolution) have been cloned.

However, most of mapping work of QTL only focused on the single-locus. According to biochemistry and physiological genetics, the interactions among gene products should be ubiquitous[9]. Recently, Brem *et al.*[10] proved that genetic interactions were widespread in the levels of genetic transcript. In addition, some evidence demonstrates that interactions between co-adapted parental species' genes may provide an important genetical foundation for the evolution and adaptation of such species[11,12]. Recently, epistatic interactions were observed in different species, such as cowpea, mung bean[13] and soybean[14]. Several reports have suggested that epistatic interaction might play an important role in the inherence of quantitative traits and the genetic basis of heterosis, especially in rice[15−18]. Some literature indicates the importance of epistatic interaction as a genetic component of inheritance for quantitative traits in maize by using conventional biometrical techniques [11,19−21] and QTL mapping using molecular markers[22−26]. In our previous study, we also found that epistasis played an important role in the inherence and heterosis of maize yield traits using 174 molecular markers and two-way ANOVA method[1)].

1) Yan J B, Tang H, Huang Y Q, et al. QTL mapping and epistatic analysis for yield and yield components using molecular markers with an elite maize hybrid. Euphytica, in press.

However, the numbers and effects of epistasis should be overestimated by two-way ANOVA method based on the markers data. The aim of this investigation was to map main QTL and epistatic QTL associated with grain yield and its components, and to analyze the inherence of yield traits using molecular markers with a new software-R/qtl in a mapping population derived from an elite maize hybrid.

1 Materials and methods

1.1 Plant materials

A total of 266 $F_{2:3}$ families derived from F_2 individuals were used as a mapping population. They came from an elite cross between Zong3 and 87-1, Yuyu22, one of the most widely grown hybrids in China. Young leaves of F_2 individuals were collected and stored at –70℃ for later use.

1.2 Field experiments

$F_{2:3}$ families, along with two parents and the F_1, were planted at the agronomy farm of Huazhong Agricultural University, Wuhan in March 21, 2000 and the Chia-Tai Agricultural Development Company experiment station in Xiangfan, March 27, 2000. A randomized complete-block design was employed with three replications. Each field plot included 20 plants grown in single 5-m long rows with a planting density of 45000 per ha. The field management followed essentially the normal agricultural practice. At maturity, only 10 plants (3rd—13th position) in each row were harvested manually for trait measurements. Harvested ears were air-dried until the grain moisture level reached 13%. Traits examined included: (1) grain yield per ear (GY), which was converted into tons/hectare (t/ha); (2) rows number (RN), denoting the number of rows in each ear; (3) kernels per row (KPR), standing for the total kernels in a row from an ear; and (4) 100-kernel weight (KW), measured as the weight of 100 kernels in gram. The average values of six replications in both locations of the four traits were used for final data analysis.

1.3 Construction of genetic linkage map

The total DNA from young plant leaves was extracted as described by Saghai-Maroof *et al.*[27]. A total of 479 markers, including 375 SSRs and 104 RFLPs, were selected from the public maize genetics map for the screening of polymorphisms. Simple sequence repeat (SSR) analysis was conducted as reported by Senior *et al.*[28], and restriction fragment length polymorphism (RFLP) analyses were performed according to Gardiner *et al.*[29]. In addition, 27 SSRs and 4 RFLPs had been included in the previous linkage map comprising 174 molecular markers[30]. All of the genotypes of each marker in all the individuals have been tested using the orders "cacl.genoprob" and "cacl.errorlod" with the software R/qtl. The genotyping should be regarded as error and replaced by missing data if the LOD≥2[31]. Ultimately, a genetic linkage map was constructed using Mapmaker 3.0[31] with all the markers excluding the error genotypes. The critical logarithm-of-odds (LOD) score for the test of independence of marker pairs was set at 3.0, and the order with the highest LOD score was then selected. The Kosambi mapping function was used for calculating the map distances.

1.4 Data analysis

(i) Phenotypic data analysis. Estimates of means and variances for the yield traits were conducted based on $F_{2:3}$ family data using SAS software[32]. Broad-sense heritabilities (h^2) for F_3 families lines were computed with an entry mean basis, and confidence intervals on h^2 were obtained according to Knapp *et al.*[33]. The heritability (h^2) was calculated as: $h^2=\sigma_g^2/(\sigma_g^2+\sigma_{gl}^2/n+\sigma_e^2/nr)$ where σ_g^2 is the genetic variance, σ_{gl}^2 is the interaction of genotype with locations, σ_e^2 is error variance, r is the number of replications, and n is the number of locations. The estimates of σ_g^2, σ_{gl}^2, and σ_e^2 were obtained from an analysis of variance (ANOVA) assuming the locations as random.

(ii) QTL analysis. The EM algorithm as well as standard interval mapping[34] in R/qtl[35] was used to map the main QTL in whole genome. One thousand permutation tests were performed to establish threshold for each trait at p=0.05 level. To confirm and refine each QTL position identified by R/qtl, multiple interval mapping (MIM) was performed with the penalty function BIC: $g(n)=\ln(n)$[36]. Haley-Knott regression methods in R/qtl[35] were used to perform epistatic QTL analysis. Marker regression is a simple linear regression of phenotypes on marker genotypes (individuals with missing genotypes were discarded). Haley-Knott regression uses the regression of phenotypes on multipoint genotype probabilities. The imputation method uses the *pseudomarker* algorithm described by Sen and

Churchill[37] (repeated 500 times). Five hundred permutation tests were performed to establish epistatic threshold for each traits at p=0.05 level. The epistatic QTL should be regarded as happening between two main QTL if the LOD value of single QTL was all ⩾2.5. The variation explained by main QTL and interaction between main QTL were counted by MIM with Windows QTL Cartographer 2.5[38].

2 Results

2.1 Population performance and relationship between marker heterozygosity and trait performance

The measurements of grain yield and three yield components for $F_{2:3}$ families lines as along with two parents and the F_1 are listed in Table 1. Grain yield and kernels per row exhibited high levels of heterosis with 245.17% and 100.60%, whereas rows number and 100-kernel weight expressed relatively low levels of heterosis with 20.63% and 21.38%, respectively. Significant variation was observed for all the traits among the $F_{2:3}$ families lines at both locations. The broad sense heritability for rows number was the highest, reaching 91%, and the 100-kernel weight was least, being 67% (Table 1). Significant ($p < 0.01$) genotype × location interactions were detected for all traits (data not shown). Highly significant positive correlation was observed between the number of kernels per row and grain yield, that indicating the highest contribution of ear length for grain yield. Correlations of heterozygosity with trait heterotic performance were calculated based on marker genotype at $p < 0.01$. For grain yield and kernels per row, there were significant relationships at $p < 0.01$, but the correlation coefficients were less than 0.3. There was no significant relationship of heterozygosity with rows number and 100-kernel weight at $p < 0.01$ (data not shown).

2.2 Construction of molecular marker linkage map

A new linkage map was constructed using 205 markers by including 31 new markers (27 SSRs and 4 RFLPs) in the previous linkage map with 174 markers[30]. With MAPMAKER/EXP3.0[31], polymorphic markers were classified into 11 groups covering all 10 chromosomes of maize, with a total length of 2395.5 cM and an average interval of 11.7 cM. Genotyping errors had been detected in 123 markers of 462 individuals based on the R/qtl (LOD⩾2.0), which distributed on all the ten chromosomes with a ratio of 0.936 % (Fig. 1). Among all the ten chromosomes, least genotyping errors was recorded in chromosome 9 involving seven markers and 13 loci, whereas chromosome 8 exhibited highest genotyping errors covering 16 markers and 100 loci (Table 2). In most of cases, up to three (0—3) genotyping error was observed in all individuals for every marker (data not shown). The linkage map was reconstructed using missing data to replace the 462 genotyping error individuals that had the same orders for all the markers with a total length of 2305.4 cM and an average interval of 11.2 cM. The QTL analysis was based on the genotypic data.

2.3 QTL analysis

(i) Main QTL analysis. Main QTL mapping in whole genome was performed by R/qtl with the EM algorithm as well as standard interval mapping. After 1000 permutations, the realistic LOD thresholds for declaring one QTL for each of the measured traits were determined, which varied between 2.9 for kernels per row and 3.1 for grain yield at a genome-wide signify-

Table 1 Means, heterosis and heritability estimates for grain yield and its components

Item	Grain yield (t/ha)	Row numbers	Kernels/row	100-kernel weight (g)
Zong3	2.37 ± 0.34	13.03 ± 0.51	21.00 ± 1.73	24.17 ± 6.81
87-1	2.81 ± 0.35	13.83 ± 0.49	15.44 ± 1.08	32.76 ± 7.15
F_1	8.94 ± 0.50	16.20 ± 0.39	36.55 ± 1.27	34.55 ± 6.19
F_3 range	2.4—6.2	11.8—8.6	18.6—31.2	24.6—35.5
$H^{a)}$ (%)	245.17	20.63	100.60	21.38
σ_g^2	0.30 ± 0.21	1.25 ± 0.32	4.83±0.95	2.96 ± 0.97
σ_{gl}^2	0.11 ± 0.29	0.05 ± 0.45	1.50 ±1.34	1.08 ± 1.37
σ_e^2	0.26	0.60	5.41	5.63
Heritability (h^2)	0.75	0.91	0.75	0.67
Confidence interval (90%)	0.71—0.79	0.89—0.92	0.70—0.79	0.60—0.72

a) H = (F_1 – MP)/MP; MP = (P1 + P2)/2. H and MP represent midparent heterosis and midparent value, respectively

Table 2 Comparison of genetic distance of different chromosomes before and after genotyping error deletion

Chromosome	1	2	3	4	5	6	7	8	9	10	Total	Average
Involved in markers	9	18	18	14	11	14	5	16	7	10	122	12.2
Involved in loci	23	97	67	43	18	51	16	100	13	34	462	46.2
Genetics distance before detection (cM)	302.1	312.2	284.1	268.4	231.6	283.5	176.6	193.5	120	223.5	2395.5	11.7
Genetics distance after detection (cM)	297.7	285.5	271.9	260.1	228.1	273.3	172.1	184.6	112.9	219.2	2305.4	11.2

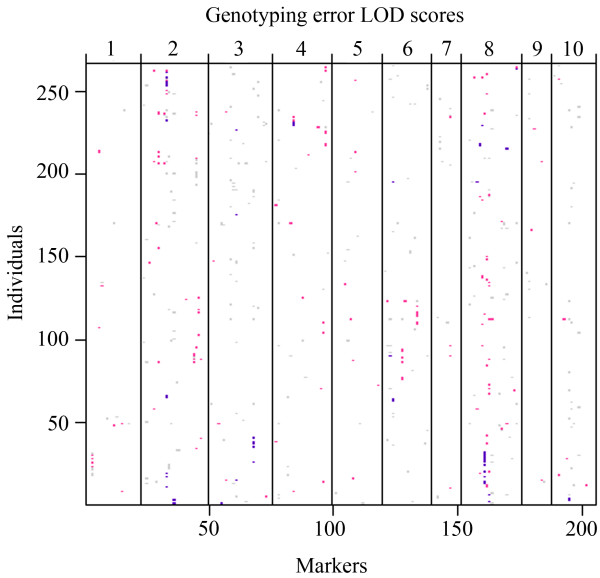

Fig. 1. The markers and individuals involved in genotyping errors in whole genome. In the plot, the Y-axis is 1−266 individuals, X-axis is the 1−205 markers; LOD<2 in white; 2≤≤LOD≤3 in gray; 3<LOD≤4.5 in pink; LOD>4.5 in purple.

cance level of 0.05. In this manner, the LOD threshold of 3.1 was used to confirm the presence of one QTL. Twenty-two QTL detected by R/qtl were found significantly associated with grain yield and its components. Eighteen QTL were confirmed and refined by MIM, while four QTL *gy6*, *kpr2a*, *rn8* and *kw4* were regarded as false positive. Moreover, using MIM approach, additional four QTL *gy10*, *kpr2a*, *rn2* and *kw7b* were detected (Table 3). Further in the study, we will refer only the QTL declared by MIM approach (Table 3). Five QTL associated with grain yield were mapped on chromosomes 1, 5, 7, 9 and 10. The 87-1 alleles for two QTL (*gy1*, *gy5*), and the Zong3 alleles for the other three QTL (*gy7*, *gy9*, *gy10*) showed positive effects on grain yield. The five QTL could explain a total of 35.5% genetic variation ranging form 3.0% to 9.2% for single QTL. Five QTL associated with kernels per row were mapped on chromosomes 1 (*kpr1*), 2 (*kpr2b*), 6 (*kpr6*), 8 (*kpr8*) and 9 (*kpr9*) and significant interaction had been found between QTL *kpr1* and *kpr8* that explained 37.4% (including the interactive QTL) of the total genetic variation. QTL *kpr2b* containing Zong3 alleles exhibited positive effects on kernels per row, while alleles from 87-1 contributed to four QTL. Seven QTL detected for rows number accounted for 61.5% of the total variances. The alleles of QTL with increasing effects on rows number were three from Zong3 (*rn2, rn9* and *qel10*), and the other four from 87-1. Five QTL detected for 100-kernel weight accounted for 39.7% of total variation. The increase in 100-kernel weight was caused by alleles from 87-1 at QTL *kw1b* and *kw3*, and the alleles from Zong3 at the remaining three QTL. Some QTL clusters were observed in different chromosomes, such as chromosome 1, where three QTL (*gy1*, *rn1*, *kpr1*) were located between 192 and 199 cM (Fig. 2 and Table 3). The peaks of LOD values for the two QTL (*gy9* and *kpr9*) were in the same region-104 cM. It is unclear whether these QTL were closely linked or if one particular QTL affected different traits. Hence, these QTL provided beneficial information for map based cloning or marker assisted selection in next step.

(ii) Epistatic QTL analysis. Epistatic QTL mapping in the whole genome was performed by R/qtl. After 500 permutations, the realistic joint LOD thresholds for each of the measured traits were determined, which varied between 6.9 and 7.1 at a genome-wide significance level of 0.05. The joint LOD threshold of 7.1 and epistasis LOD threshold of 3.0 were used to confirm the presence of one epistatic QTL. The epistatic QTL observed in this study could be partitioned into three types of combinations: QQ (epistatic interaction happened between two main QTL), QN (epistatic interaction happened between one main QTL and another locus without significant effect), and NN (epistatic interaction happened between two loci of no significant effects). Out of six epistatic QTL detected for grain yield, four belonging to QN, while the remaining two belonging to NN. The alleles of the epistatic QTL were distributed on the eight of ten chromosomes (except chromosome 3 and 8). Seven epistatic QTL affecting kernels per row were observed (including 1 QQ, 4 QN and 2 NN) on six different chromosomes located at 13

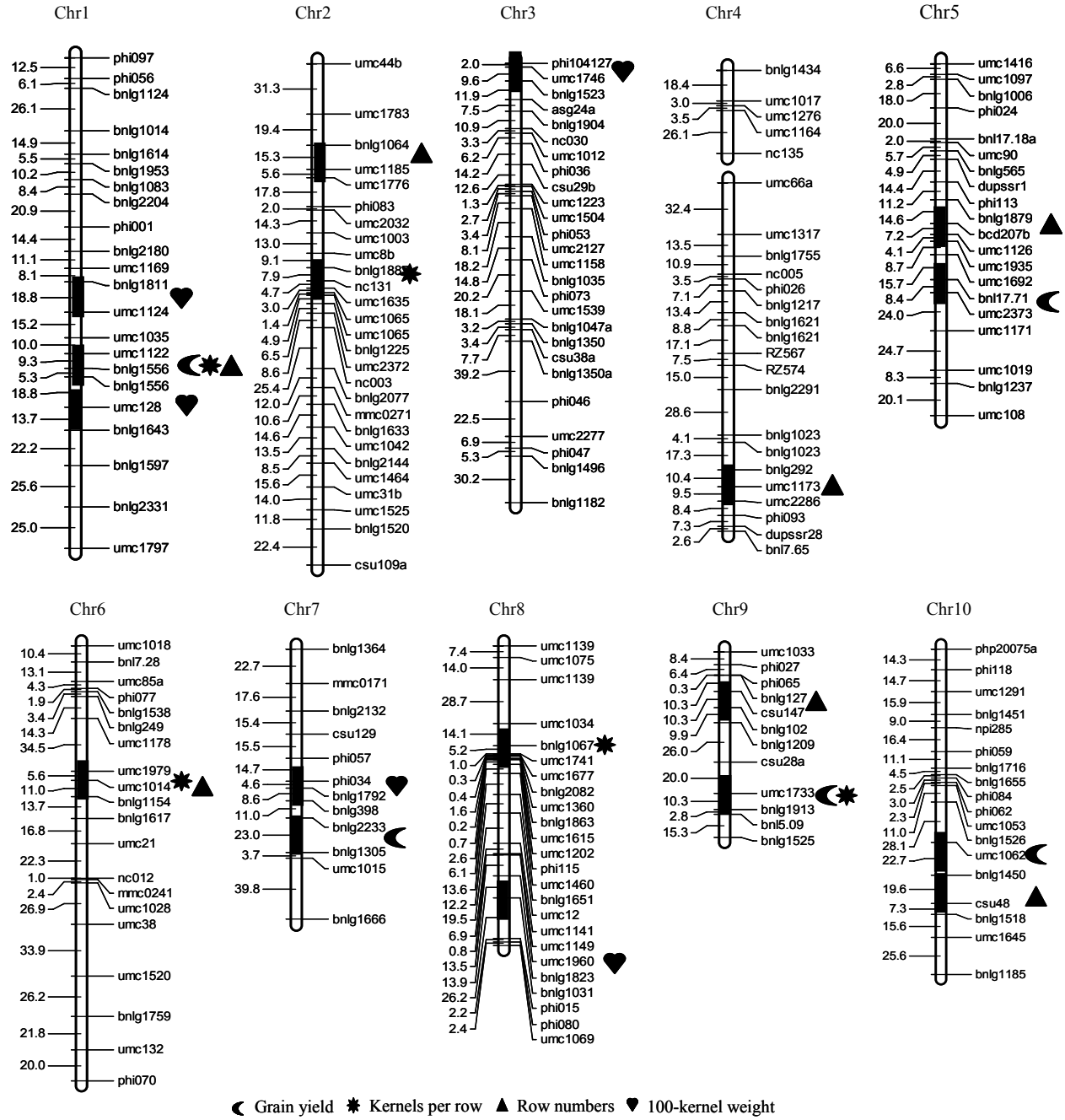

Fig. 2. Putative QTL affecting grain yield and yield components in the linkage map.

loci. Five epistatic QTL associated with rows number (4 QN and 1 NN), were identified on chromosomes 3, 4, 6, 7, 8 and 10. Five epistatic QTL for 100-kernel weight (3 QN and 2 NN) were detected at eight loci on six different chromosomes (Table 4). The epistatic interactions in the whole genome for the four traits in two-dimensional level are shown in Fig. 3, in which the different colors representing different joint LOD values. Fig. 3 illustrated two features. Firstly, the loci affected by epistatic interactions are large and almost distributed on all the chromosomes for four traits. Secondly, there are some different hot spots for four traits. For grain yield, a large number of epistatic interactions are located on nine of the ten chromosomes (except chromo-

Table 3 QTL for grain yield and yield components in $F_{2:3}$ population identified using multiple interval mapping (MIM)

Traits	QTL[a)]	Location (cM)[b)]	LOD[c)]	A	D	Variance (%)
Grain yield	*gy1*	192(190)	3.9(5.7)	–0.15	0.34	9.2
	gy5	154(152)	2.3(3.6)	–0.16	0.17	5.0
	gy6[d)]	(235)	(3.3)			
	gy7	133(132)	3.8(9.5)	0.25	0.08	8.8
	gy9	104(98)	2.1(4.6)	0.07	0.25	5.3
	gy10[e)]	122	1.9	0.04	0.32	7.0
						35.3
Kernels per row	*kpr1*	199(198)	3.5(4.8)	0.70	1.20	12.9
	kpr2a[d)]	(78)	(2.4)			
	kpr2b[e)]	149	2.0	–0.47	0.88	3.0
	kpr6	86(84)	2.0(3.9)	0.66	0.53	5.7
	kpr8	66(64)	2.9(6.0)	0.50	1.22	7.9
	kpr9	104(98)	1.6(3.6)	0.08	0.98	4.5
	kpr1:kpr8	198:64	1.8(2.5)	0.76[f)]	–1.57[g)]	3.4
						37.4
Row numbers	*rn1*	195(194)	4.7(4.5)	–0.52	0.07	9.4
	rn2[e)]	58	4	0.48	–0.20	5.8
	rn4	242(240)	3.0(3.7)	–0.47	–0.12	8.3
	rn5	120(112)	5.5(12.2)	–0.58	0.00	9.8
	rn6	103(95)	4.9(4.3)	–0.45	–0.30	9.0
	rn8[d)]	(152)	(6.2)			
	rn9	19(18)	6.3(10.1)	0.66	–0.10	13.2
	rn10	148(162)	2.8(2.7)	0.39	–0.10	6.0
						61.5
100-kernel weight	*kw1a*	151(150)	1.8(3.4)	0.04	1.30	9.6
	kw1b	218(212)	1.9(5.7)	–0.80	0.06	5.9
	kw3	2(1)	1.8(3.1)	–0.54	0.43	4.4
	Kw4[d)]	(260)	(3.5)			
	kw7a	86(86)	2.8(3.3)	0.78	–0.22	7.2
	kw7b[e)]	166	2.1	0.71	0.97	12.6
						39.7

a) The number following the two letters represents the chromosome location of the QTL and is denoted by continuous lowercase letters (a b c…) following the number if a chromosome has more than one QTL. b) The QTL location in chromosome is estimated by MIM and by R/qtl (in parentheses). c) The threshold for logarithm of odd is estimated by MIM and by R/qtl (in parentheses). d) QTL detected by R/qtl but not refined by MIM. e) Detected by MIM but not by R/qtl. f) Phenotypic value contributed by AA. g) Phenotypic value contributed by DD

some 2), and especially the loci on chromosome 1 almost interacts with the loci on the other nine chromosomes (Fig. 3(a)). There are several significant interactive hot spots for 100-kernel weight, for example, the epistatic interactions happened between chromosomes 1, 4, 5 and chromosomes 5, 8, 9 have higher frequency than others. The genomic regions of the most epistatic QTL (11∶15) with significant effects in single locus were same as that of main QTL detected by interval mapping.

3 Discussion

3.1 The accuracy of genetic linkage map

Approximately two decades have passed since the construction of the first maize molecular marker linkage map including 116 loci in 1986[39] to the publication of the highest density integrative map including 5863 loci (http://www.maizegdb.org/map.php#rep) in 2004. The rapid development of molecular marker technology made it easy to construct a high-density molecular marker linkage map for in most of major crops. However, the process of construction of molecular marker linkage map is a complex, tedious and time consuming job because most of jobs are performed manually, such as DNA isolation, DNA bands reading etc. It is hard to avoid the genotyping errors when the linkage map was constructed with a large number of markers. To get an idea of possible error rates, Falque *et al*.[40] found that 1.86% data were discordant after

Table 4 Epistatic QTL for grain yield and yield components detected by R/qtl

Traits	Chr. [a)]	QTL*i* [b)]	Chr. [c)]	QTL*j* [d)]	LOD*int* [e)]	LOD*int* [f)]	LOD*qi* [g)]	LOD*qj* [h)]
Grain yield	1	140	1	200	8.3	3.2		3.6
	1	188	2	128	9.7	3.8	5.6	
	4	176	7	100	7.7	6.3		
	5	4	6	240	7.5	3.1		2.7
	7	96	7	112	7.3	5.1		
	7	124	10	16	8.8	4	4.5	
	9	60	10	116	7.6	4		
Kernels per row	1	200	2	132	12.2	3.1	7.4	
	1	172	7	112	11.3	4.8	6.2	
	1	200	8	64	13.2	2.8	7.4	3.9
	2	16	8	72	9.6	5.4		3.0
	2	112	10	120	7.3	3.3	2.5	
	4	80	5	136	7.1	5.1		
	8	40	8	80	7.3	4.1		
Row numbers	3	208	8	152	7.3	3.3		3.8
	4	228	7	0	7.4	3.1	4.2	
	6	148	10	160	8.1	4.3		
	3	256	4	220	8.3	3.3		3.9
	4	240	3	96	11.5	3.3	4.7	
100-kernel weight	1	96	10	96	7.1	5		
	3	0	4	20	8.0	5.3		
	4	260	5	212	9.0	5.2	2.7	
	4	20	7	148	9.8	3.5		6.0
	7	148	10	36	10.6	3.4	6.0	

a) and c), The chromosomes of epistatic QTL; b) and d), the genomic regions of epistatic QTL (cM); e) joint LOD score; f) epistatis LOD score; g) and h) the conditional LOD score for a single QTL

comparing the raw segregation data of 77 markers present in IBM map and re-genotyped in their lab. Among 1000 simulations with 1.86% randomly simulated genotyping errors in the IBM_Gnp2004 framework dataset, the consecutive map size expanded about 15.3%. In the present study, we detected the genotyping errors by statistical method with R/qtl. The proportion of genotyping error data was about 1% at LOD$\geqslant$2.0. The total length of the linkage map changed from 2395.5 to 2305.4 cM after replacing the genotyping errors with missing data. There may have more genotyping errors if we use 0.05 as a significance level. Elimination of the genotyping errors by statistical method is an alternative choice in improving the precision of map construction and the resolution of QTL mapping.

3.2 The resolution of QTL mapping

Theoretically, F_2 generation derived from a cross between two diverse lines provides the most ideal and informative population for most of the genetic analysis[41]. However, it is difficult to use the F_2 population for genetic analysis of complex quantitative traits, as each individual represents a different genotype, thus replicated trials at multiple environments cannot be performed. Using the means of F_3 progeny to replace the phenotypic value of F_2 plants, a considerable amount of information can be gained to minimize the errors and improve the QTL mapping resolution. Similar design has been adopted broadly in rice[15]. Such analyses also suffer from several disadvantages that are inherent with this kind of population. Firstly, dominant effects were usually underestimated as marker data were obtained from F_2 individuals and phenotypic data were collected from F_3 families. Secondly, if the genotype of F_2 individual is heterozygous, the residual error of the mean of m F_3 plants becomes a mixture of many distributions. This mixed nature of distribution has not

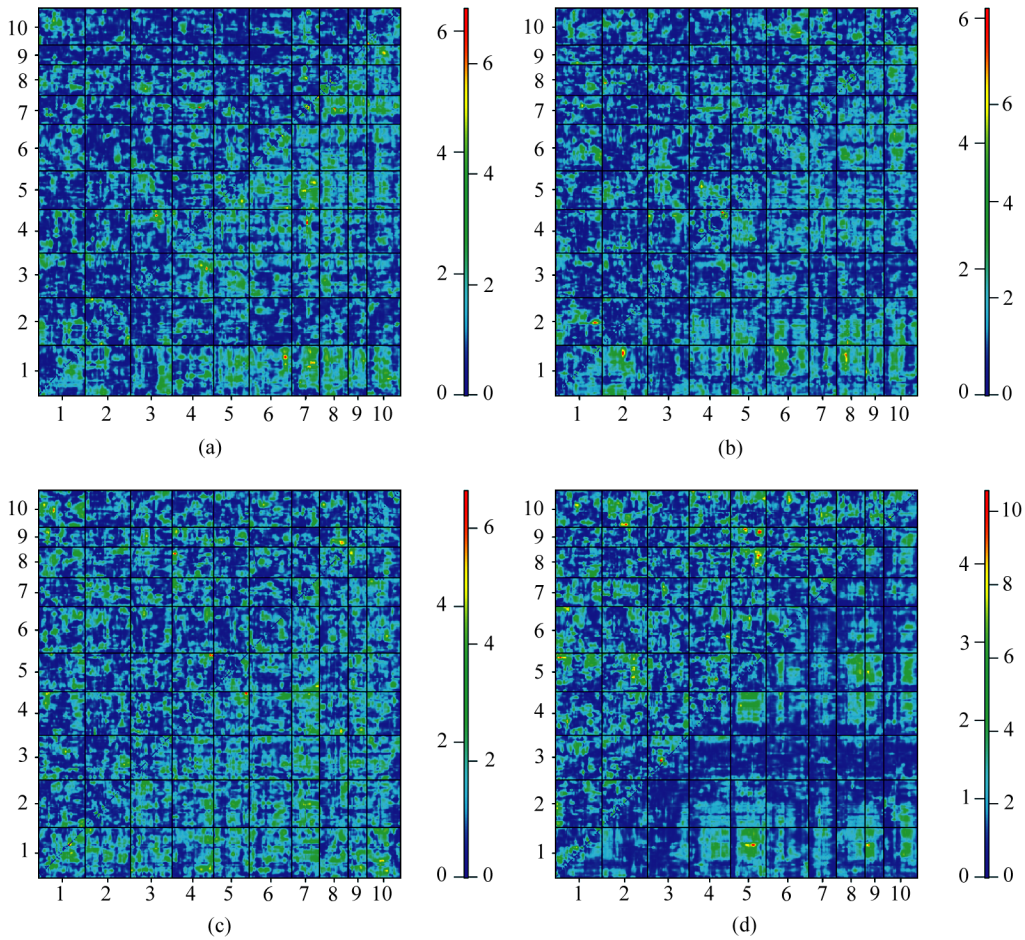

Fig. 3. Two-dimensional scanning results of epistatic QTL for grain yield and yield components. (a) Grain yield; (b) kernels per row; (c) row numbers; (d) 100-kernel weight. The epistasis LOD scores appear in the upper-left triangle; the lower-right triangle contains a LOD score in contrast to a 2-QTL versus the best 1-QTL model, though the contents of the lower-right may be changed with the lower argument. The color scale on the right indicates separate scales for the epistasis and joint LOD scores on the left and right, respectively.

been investigated in the present QTL mapping study[42]. To overcome these limitations, it was suggested to develop the "immortalized F_2" (IF_2) population because genotypes and their proportions in such population are similar to those of an F_2 population and each genotype is represented by many plants, thus permitting replicated trials in multiple environments. The idea of "immortalized F_2" (IF_2) population was put forwarded by Hua *et al*.[43] for use in rice. Recently, a similar approach called recombinant inbred intercrosses (RIX) also had been performed in mice for complex traits study[44]. Simulations demonstrated that RIX or IF_2 can provide substantially increased power for mapping QTL, and also will significantly improve our ability to genetically dissect complex epistatic interactions and gene-environment interactions[44].

3.3 Epistasis plays an important role in the inheritance of quantitative traits

In most of the previous works, epistasis was often neglected in complex trait studies because of the limitation of statistical method and detection technology[45]. In the present study, the main QTL and epistatic QTL for grain yield and its components were mapped by the software R/qtl, and the main QTL and interactions between main QTL were refined by MIM. The single QTL can explain the 35.3%, 37.4%,61.5%, and 39.7% of total genetic variation for GY, KPR, RN, and KW (Table 3). However, the broad sense heritability for all the traits is higher than the value explained by single QTL (Table 1). Although it is very difficult to evaluate accurately a total value of genetic variation caused by epistatic QTL. The number of detected epistatic QTL

was quite similar to that of single QTL. Furthermore, we revealed that about 62.5% (15∶24) of the significant epistatic interactions happened between two loci that one had significant effect and the other had no significant effect and about 33.3% (8∶24) happened between two loci of which none has significant effects on the traits (Table 3). These results indicate rather clearly that there are many QTL affecting trait expressions, not directly but indirectly through interacting with other loci. Such epistasis may reflect physiological interaction. These results are consistent with our previous study[1)] and other reports[15−18]. In general, we distinguish the presence or absence of epistatic interactions by LOD score. The ratio of Type II error should be high if the LOD score is bigger and the ratio of Type I error should be high if the LOD score is smaller. How to confirm the LOD score is still a big challenge until now. It is a good choice to display all the possible epistatic interactions in the whole genome by a two-dimensional graph. A common characteristic, as shown in Fig. 3, is that the numbers of significant epistatic interactions are large for all the traits in this study. Thus, epistatic QTL effects may play a crucial role, if not more important than the main QTL effects in the genetic variation for the measured traits. We need to further analyze these epistatic QTL in the subsequent work.

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References

1 Duvick D N. Heterosis: Feeding people and protecting natural resources. In: Coors J G, Pandey S, eds. The Genetics and Exploitation of Heterosis in Crops. Madison, WI: American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc., 1999. 19−29

2 Ribaut J M, Hoisington D. Marker-assisted selection: new tools and strategies. Trends Plant Sci, 1998, 3(6): 236−239

3 Frary A, Nesbitt T C, Grandillo S, et al. *fw2.2*: A quantitative trait locus key to the evolution of tomato fruit size. Science, 2000, 289: 85−88

4 Yano M, Katayose Y, Ashikari M, et al. *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the arabidopsis flowering time gene CONSTANS. Plant Cell, 12: 2473−2484

5 Takahashi Y, Shomura A, Sasaki T, et al. *Hd6*, a rice quantitative trait locus involved in photoperiod sensitivity, encodes the alpha subunit of protein kinase CK2. Proc Natl Acad Sci USA, 2001, 98: 7922−7927

6 Ashikari M, Sakakibara H, Lin S Y, et al. Cytokinin oxidase regulates rice grain production. Science, 2005, 309: 741−745

7 Ren Z H, Gao J P, Li L G, et al. A rice quantitative trait locus for salt tolerance encodes a sodium transporter. Nat Genet, 2005, 37: 1141−1146

8 Wang R L, Stec A, Hey J, et al. The limits of selection during maize domestication. Nature, 1999, 398: 236−239

9 Wright S. Genic and organismic selection. Evolution, 1980, 34: 825−843

10 Brem R B, Storey J D, Whittle J, et al. Genetic interactions between polymorphisms that affect gene expression in yeast. Nature, 2005, 436: 701−703

11 Allard R W. Genetic basis of the evolution of adaptedness in plants. Euphytica, 1996, 92: 1−11

12 Rieseberg L H, Sinervo B, Linder C R, et al. Role of gene interactions in hybrid speciation: Evidence from ancient and experimental hybrids. Science, 1996, 272: 741−745

13 Fatokun C A, Menancio-Hautea D I, Danesh D, et al. Young evidence for orthologous seed weight genes in cowpea and mung bean based on RFLP mapping. Genetics, 1992, 132: 841−846

14 Maughan P J, Saghai Maroof M A, Buss G R. Molecular-marker analysis of seed-weight: Genomic locations, gene action, and evidence for orthologous evolution among three legume species. Theor Appl Genet, 1996, 93: 574−579

15 Yu S B, Li J X, Xu C G, et al. Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. Proc Natl Acad Sci USA, 1997, 94: 9226−9231

16 Li Z K, Luo L J, Mei H W, et al. Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. I. Biomass and grain yield. Genetics, 2001, 158: 1737−1753

17 Li Z K, Pinson S R, Park W D, et al. Epistasis for three grain yield components in rice. Genetics, 1997, 145: 453−465

18 Luo L J, Li Z K, Mei H W, et al. Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. II. Grain yield components. Genetics, 2001, 158: 1755−1771

19 Lamkey K R, Schnicker B J, Melchinger A E. Epistasis in an elite maize hybrid and choice of generation for inbred line development. Crop Sci, 1995, 35: 1272−1281

20 Wolf D P, Hallauer AR. Triple testcross analysis to detect epistasis in maize. Crop Sci, 1997, 37: 763−770

21 Eta-Ndu J T, Openshaw S J. Epistasis for grain yield in two F_2 populations of maize. Crop Sci, 1999, 39: 346−352

22 Doebley J, Stec A, Gustus C. Teosinte branched-1 and the origin of maize: Evidence for epistasis and the evolution of dominance.

1) see footnote 1) on p.1452.

Genetics, 1995, 141: 333－346
23 Lukens L N, Doebley J. Epistatic and environmental interactions for quantitative trait loci involved in maize evolution. Genet Res, 1999, 74: 291－302
24 Barriere Y, Gibelin C, Argillier O, et al. Genetic analysis in recombinant inbred lines of early dent forage maize. I. QTL mapping for yield, earliness, starch and crude protein contents from per se value and top cross experiments. Maydica, 2001, 46: 253－266
25 Mcmullen M D, Snook M, Lee E A, et al. The biological basis of epistasis between quantitative trait loci for flavone and 3-deoxyanthocyanin synthesis in maize (*Zea mays* L.). Genome, 2001, 44: 667－676
26 Moreau L, Charcosset A, Gallais A. Experimental evaluation of several cycles of marker-assisted selection in maize. Euphytica, 2004, 137: 111－118
27 Saghai-Maroof M A, Soliman K M, Jorgensen R A, et al. Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population, and population dynamics. Proc Natl Acad Sci USA, 1984, 81: 8014－8018
28 Senior M L, Heun M, Manfred H. Mapping maize microsatellites and polymerase chain reaction confirmation of the targeted repeats using a CT primer. Genome, 1993, 36: 884－889
29 Gardiner J M, Coe E H, Melia-Hancock S, et al. Development of a core RFLP map in maize using an immortalized F_2 population. Genetics, 1993, 134(7): 917－917
30 Yan J B, Tang H, Huang YQ, et al. QTL mapping for developmental behavior for plant height in maize. Chin Sci Bull, 2003, 48(23): 2601－2607
31 Lincoln S, Daly M, Lander E. Mapping Genetic Mapping With MAPMAKER/EXP3.0. Cambridge: Whitehead institute Technical Report, 1992
32 SAS Institute, Inc. SAS Users Guide: Statistic. Cary, NC: SAS Institute, 1996
33 Knapp S J, Stroup W W, Ross W M. Exact confidence intervals for heritability on a progeny mean basis. Crop Sci, 1985, 25: 192－194
34 Lander E S, Botstein S. Mapping Mendelian factors underlying quantitative traits using TFLP linkage maps. Genetics, 1989, 121: 185－199
35 Broman K W, Wu H, Sen S, et al. R/qtl: QTL mapping in experimental crosses. Bioinformatics, 2003, 19: 889－890
36 Kao C H, Zeng Z B, Teasdale R D. Multiple interval mapping for quantitative trait loci. Genetics, 1999, 152: 1203－1216
37 Sen S, Churchill G A. A statistical framework for quantitative trait mapping. Genetics, 2001, 159: 371－387
38 Wang S C, Basten C J, Zeng Z B. Windows QTL Cartographer 2.5 user manual. North Carolina State University. 2005
39 Helentjaris T, Slocum M, Wright S, et al. Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor Appl Genet, 1986, 72: 761－769
40 Falque M, Decousset L, Dervins D, et al. Linkage mapping of 1454 new maize candidate gene loci. Genetics, 2005, 170: 1957－1966.
41 Allard R W. Formulas and tables to facilitate the calculationof recombination values in heredity. Hilgardia, 1956, 24: 235－278.
42 Zhang Y M, Xu S Z. Mapping quantitative trait loci in f2 incorporating phenotypes of F3 progeny. Genetics, 2004, 166: 1981－1993
43 Hua J P, Xing Y Z, Xu C G, et al. Genetic dissection of an elite rice hybrid revealed that heterozygotes are not always advantageous for performance. Genetics, 2002, 162: 1885－1895
44 Zou F, Gelfond A L, Airey D C, et al. Quantitative trait locus analysis using recombinant inbred intercrosses: Theoretical and empirical considerations. Genetics, 2005, 170: 1299－1311
45 Carlborg Ö, Hale C S. Epistasis: Too often neglected in complex trait studies? Nat Rev Genet, 2004, 5: 618－625