Theor Appl Genet (2002) 105:248–257
DOI 10.1007/s00122-002-0952-y

Y.Z. Xing · Y.F. Tan · J.P. Hua · X.L. Sun · C.G. Xu
Q. Zhang

Characterization of the main effects, epistatic effects and their environmental interactions of QTLs on the genetic basis of yield traits in rice

Received: 6 August 2001 / Accepted: 16 October 2001 / Published online: 19 June 2002

Abstract Main effects, epistatic effects and their environmental interactions of QTLs are all important genetic components of quantitative traits. In this study, we analyzed the main effects, epistatic effects of the QTLs, and QTL by environment interactions (QEs) underlying four yield traits, using a population of 240 recombinant inbred lines from a cross between two rice varieties tested in replicated field trials. A genetic linkage map with 220 DNA marker loci was constructed. A mixed linear model approach was used to detect QTLs with main effects, QTLs involved in digenic interactions and QEs. In total, 29 QTLs of main effects, and 35 digenic interactions involving 58 loci were detected for the four traits. Thirteen QTLs with main effects showed QEs; no QE was detected for the QTLs involved in epistatic interactions. The amount of variations explained by the QTLs of main effect were larger than the QTLs involved in epistatic interactions, which in turn were larger than QEs for all four traits. This study illustrates the ability of the analysis to assess the genetic components underlying the quantitative traits, and demonstrates the relative importance of the various components as the genetic basis of yield traits in this population.

Keywords Quantitative trait locus · Epistasis · Genotype by environment interaction · *Oryza sativa* L.

Introduction

Yield and yield-component traits are typically quantitatively inherited showing continuous variation in segregating populations. For a long time it has been assumed that quantitative traits are controlled by multiple genetic factors each having a small effect on the expression of the trait, known as the multiple factor hypothesis (East 1916). However, this hypothesis remained largely hypothetical for most of the last century as it was impossible to unravel the genetic basis of quantitative traits at the whole genome level using classical genetic methods. Recent advances in genome research involving a number of molecular-marker techniques and the availability of high-density molecular linkage maps, together with developments in analytical methods (Lander and Botstein 1989; Zeng 1994), facilitated the analysis of the genetic basis of quantitative traits at a single-locus level. A large number of studies have been conducted that identified large numbers of quantitative trait loci (QTLs) contributing to the inheritance of quantitative traits in many plant species, including yield and the agronomic performance of the most important crop species such as tomato, maize and rice (Paterson et al. 1988; Xu 1997; Zhang and Yu 1999).

Epistasis refers to the phenotypic effects of interactions among alleles at multiple loci. Our current understanding of biochemical and physiological genetics, as well as the regulation of gene expression, strongly suggests the ubiquity of interactions among gene products. There were also substantial interests in the classical quantitative genetics of epistasis, defined as the deviation from additivity of the effects between alleles of different loci (Cockerham 1954). Morphological markers were used to demonstrate the existence of digenic epistatic interactions in barley populations long before the availability of any molecular tools (Fasoulas and Allard 1962). Recent genetic analyses using molecular markers in several plant species have clearly shown that, in addition to single locus QTLs, epistatic interactions play an important role on the genetic basis of quantitative traits (Lark et al. 1995; Maughan et al. 1996; Li et al. 1997; Yu et al. 1997).

However, a common problem associated with the analyses of the data reported so far is that the analyses of single-locus QTLs and epistatic interactions were conducted separately using different analytical tools. Al-

Communicated by C. Möllers

Y.Z. Xing · Y.F. Tan · J.P. Hua · X.L. Sun · C.G. Xu · Q. Zhang (✉)
National Key Laboratory of Crop Genetic Improvement,
Huazhong Agricultural University, Wuhan 430070, China
e-mail: qifazh@public.wh.hb.cn
Tel.: +86-27-87282429, Fax: +86-27-87287092

though both of the analytical tools can provide statistical estimates for the amount of the effects and the proportions of variation explained, it is nonetheless impossible to evaluate the relative importance of the individual QTLs and epistatic interactions in determining the performance of the traits. No assessment can be made regarding the relative importance of the overall effects of single-locus QTLs as compared to epistatic interactions. Another shortcoming associated with the analyses of epistatic interactions published in previous studies is that the calculations were directly based on markers that are located at certain distances away from the QTLs involved in the epistases. The estimated effects are therefore biased depending on the distances between the marker loci and the QTLs.

Genotype by environment (GE) interaction plays an important role in determining the adaptation and fitness of genotypes to the physical environment. GE interaction has received considerable attention in crop breeding programs as they are closely related to the stability of varieties. A large number of studies were carried out in the past to estimate the amounts of GE, and to evaluate varietal stability in a number of crop species (Finlay and Wilkinson 1963; Eberhart and Russell 1966; Zhang and Geng 1986). QTL analysis in recent years has made it possible to track the performance of individual QTLs across environments in data collected from multiple environmental trials. However, because of a lack of analytical tools, all the studies to-date have involved comparing the QTLs detected in different environments (Lu et al. 1996; Tinker et al. 1996), and the ones that demonstrated significantly different behavior in different environmental conditions were considered as showing QTL by environment (QE) interaction. Additionally, because the data from different environments were analyzed separately, the results obtained could not provide estimates regarding the amounts and relative importance of QEs in the total phenotypic variation.

Recently, Wang et al. (1999a) developed a method to identify main-effect QTLs, digenic interactions and QEs by including data collected from multiple environments in the same analysis. The analysis is based on a mixed linear-model approach (Zhu and Weir 1998), and put together the QTL main effects, digenic interactions and their environmental interactions that are possible with a two-locus data set in the same model (Wang et al. 1999a). As pointed out by Wang et al. (1999a), this method can provide unbiased estimates for both positions and effects of QTLs and unbiased predicted values for QEs. It also produces high accuracy and power in mapping QTLs with epistatic effects and QEs. Wang et al. (1999b) also developed computer software (QTLMapper 1.0) for interval mapping of QTLs with additive, additive by additive epistasis, and QEs.

In the study reported in this paper, using the method of Wang et al. (1999a), we analyzed the data from replicated field tests of a recombinant inbred line (RIL) population derived from the cross between Zhenshan 97 and Minghui 63, the parents of Shanyou 63, the best hybrid in China. The objectives of the study were to resolve the genetic basis of yield-traits into their components, such as main-effect QTLs, epistatic QTLs and QEs, and to evaluate the relative magnitudes of these components in controlling the inheritance of the traits.

Materials and methods

Experimental population and phenotypic measurements

The population used in this study consisted of 240 F_9 recombinant inbred lines (RILs) derived by single-seed descendent from a cross between Zhenshan 97 and Minghui 63, the parents of Shanyou 63, an elite rice hybrid. The F_9 RILs, two parents and the F_1 were transplanted to a bird-net-equipped field in the experimental farm of Huazhong Agricultural University in the 1997 and 1998 rice growing seasons in Wuhan, China. For the 1997 test, the seeds were sown on May 15 in large plastic boxes filled with soil; and for the 1998 test, the seeds were sown on June 5 in a seedling bed. The difference in planting times between the 2 years provided very different environmental conditions for the field trials. Field experiments were carried out following the randomized complete block design with two replications within each year. Fifty seedlings of approximately 25-days-old for each entry were transplanted into a five-row plot, with a distance of 16.5 cm between plants within a row, and 26.4 cm between rows. The eight plants in the middle of the third row of each plot were harvested individually to score the following traits: the yield per plant as the total weight (g) of the grains from the entire plant, the number of tillers per plant scored as the number of reproductive tillers for each plant, the number of grains per panicle as the total number of grains from the entire plant divided by the number of tillers, and the 1,000-grain weight (g) as the yield per plant divided by the number of grains multiplied by 1,000. Trait measurements averaged over the two replications within each year were used as the raw data in the analyses.

DNA markers

Two kinds of DNA markers representing 220 polymorphic loci, including 168 RFLPs and 45 SSRs, were used to develop the genetic linkage map. The RFLP marker assay followed the method described by Liu et al. (1997), and the SSR assay was conducted essentially as described by Wu and Tanksley (1993). Apicule color controlled by the *C* gene was used as a morphological marker in the map construction. Most of the markers were the same as the ones that Yu et al. (1997) used in the analysis of $F_{2:3}$ data of the same cross, and more markers were added to regions that were under-represented with molecular markers in the analyses of Yu et al. (1997).

Data analyses

A genetic linkage map was constructed using Mapmaker 3.0 (Lincoln et al. 1992). Single-locus QTLs were analyzed by composite interval mapping (Zeng 1994) using the computer program QTL Cartographer. Two-locus analysis that tests the QTL main effects, and epistatic interactions and their environmental interactions, was conducted using the computer program QTLMapper 1.0 (Wang et al. 1999b). This included estimating and mapping QTLs for additive effects at individual loci, interactions between two different loci, and interactions between QTLs and the environments. For ease of description, we will refer to the QTLs with main effects that corresponded to QTLs detected by single-locus analysis as main-effect QTLs, and QTLs involved in digenic interactions as epistatic QTLs, although many of the main-effect QTLs were also involved in epistatic interactions. In the analyses, the likelihood ratio (LR) and the *t*-test were combined to test the

Table 1 Descriptive statistics of yield and yield-component traits of the RIL population and their parents

Traits	Mean ± SD	Range	Zhenshan 97	Minghui 63	F_1
Yield per plant (g)	21.4±4.68	7.6–60.1	17.4	24.5	27.8
Tillers per plant	9.4±2.35	3.6–20.6	8.9	9.6	8.9
Grains per panicle	96.5±24.28	40.6–200.4	83.7	95.7	126.2
1,000-grain weight (g)	24.5±2.80	15.5–33.0	23.8	27.3	25.3

hypothesis on both genetic effects (including additive effects and digenic epistatic effects) and QE effects. Estimates of QTL main-effects were obtained by the maximum-likelihood estimation method, while QE effects were predicted by the-best-linear-unbiased prediction (BLUP) method. In the mixed linear-model approach, the environmental effect was regarded as random. Thus, the significance test for the predicted QE effects by BLUP has very lower power. As a remedy, the Bayesian test was used for the estimation of QTL main effects and QE effects, and also for the significance test. In this study, the LR value corresponding to $P = 0.005$ (equivalent to LOD = 4.03 for $df = 6$) was used as the threshold for claiming the presence of putative main or epistatic QTLs. The peak points of the LR and the *t*-test statistics in the linkage map were taken as the putative positions of the QTLs. When a QTL was involved in more than one epistasis, its position and additive effect were presented as the arithmetic mean of the values obtained from the different calculations. The relative contribution of a genetic component was calculated as the proportion of phenotypic variance explained by that component.

Results

The measurements of the traits

All phenotypic values of Minghui 63 were larger than those of Zhenshan 97 (Table 1). The means of RILs for the four traits are approximately equal to the mid-parent values. All the four traits expressed transgressive segregation in both directions.

Genotyping and the linkage map

Data for a total of 213 markers including 168 RFLPs, 45 SSRs and one morphological trait, detecting a total of 221 loci, from the RIL population, were obtained to construct the linkage map. Of these markers, 14 RFLPs, detecting 17 loci, were dominant markers. Segregation ratios of the two parent genotypes in most loci fit the expected 1:1 Mendelian ratio. Segregation distortion at $P < 0.01$ was detected for 38 marker loci located in 11 contiguous regions on ten chromosomes, with the exception of chromosomes 1 and 10. The overall level of heterozygosity in the population was calculated to be 0.81% which was much higher than the expected 0.39% ($1/2^8$) on the basis of eight generations of selfing. In map construction, the heterozygotes of individual loci were treated as missing data.

Mapmaker analysis at LOD 3.0 resolved the 221 loci into 14 linkage groups, with the total length of the map spanning 1,796 cM, and an average 8.7 cM between adjacent markers (Fig. 1). The marker order on this RIL map was in good agreement with the map of the F_2 population derived from the same cross (Yu et al. 1998).

Table 2 Putative QTLs identified for yield and yield-component traits for data of 1997 and 1998 from the RIL population of the Zhenshan 97/Minghui 63 cross, using the composite interval mapping method with a LOD threshold of 2.4

Trait	QTL	Flanking markers	LOD	a[a]	Var (%)[b]
Yield (1997)	*yd1b*	C547-C2340	4.3	0.92	7.2
	yd2	RM240-RM213	3.3	0.75	4.9
	yd3	C1087-RZ403	3.3	–1.13	5.7
Yield (1998)	*yd1a*	C922-RG101	3.4	1.20	4.9
	yd1b	C547-C2340	6.8	1.72	10.0
	yd2	RM240-RM213	6.5	1.44	9.8
	yd6	RZ667-RG424	3.1	1.29	6.9
Tillers/plant (1997)	*tp1a*	RG101-G393	4.5	0.36	6.7
	tp5	RG360-C734b	2.8	0.35	6.5
	tp7	C1023-R1440	5.5	–0.49	12.7
Tillers/plant (1998)	*tp1b*	C547-C2340	2.8	0.36	3.8
	tp2	RM208-RM207	5.3	0.54	8.6
	tp7	C1023-R1440	4.5	–0.48	6.8
	tp11	C794-R2918	3.6	–0.43	5.4
Grains/panicle (1997)	*gn1*	G359-RG532	3.9	6.42	7.0
	gn3	C1087-RZ403	10.4	–9.73	15.4
	gn7	C1023-R1440	4.7	6.94	8.1
Grains/panicle (1998)	*gn1*	G359-RG532	2.9	4.26	3.7
	gn 2	RG634-R1738	2.4	–3.16	4.0
	gn3	C1087-RZ403	12.4	–7.72	17.6
	gn7	C1023-C1440	4.2	4.28	5.6
	gn 11	RM254-G4001	4.0	4.28	5.2
Grain weight (1997)	*gw1a*	G359-RG532	7.5	–0.83	10.2
	gw1b	C2340-C86	3.6	0.56	4.5
	gw1c	RG236-C112	2.6	–0.46	3.0
	gw3a	C1087-RZ403	15.6	1.19	20.8
	gw3b	C944-R321	8.3	0.80	8.3
	gw5	R3166-RG360	8.9	–1.14	19.8
	gw6	RG424-R2549	2.9	0.67	7.1
Grain weight (1998)	*gw1a*	G359-RG532	7.3	–0.86	7.9
	gw3a	C1087-RZ403	11.9	1.15	13.1
	gw3b	C944-R321	7.1	0.81	6.8
	gw5	R3166-RG360	8.4	–0.92	8.8
	gw9	RM242-RG570	2.7	–0.50	2.5
	gw11	G257-G44	2.9	0.57	2.8

[a] Additive effect: positive values of the additive effect indicate that alleles from Minghui 63 were in the direction of increasing the trait score

[b] Variance explained by the QTLs

QTLs resolved by single-locus analyses

Composite interval mapping at LOD threshold 2.4 identified a total of 25 distinct QTLs for the four traits (Table 2). Ten of the QTLs were detected in both years, and the remaining 15 QTLs were detected only in one year.

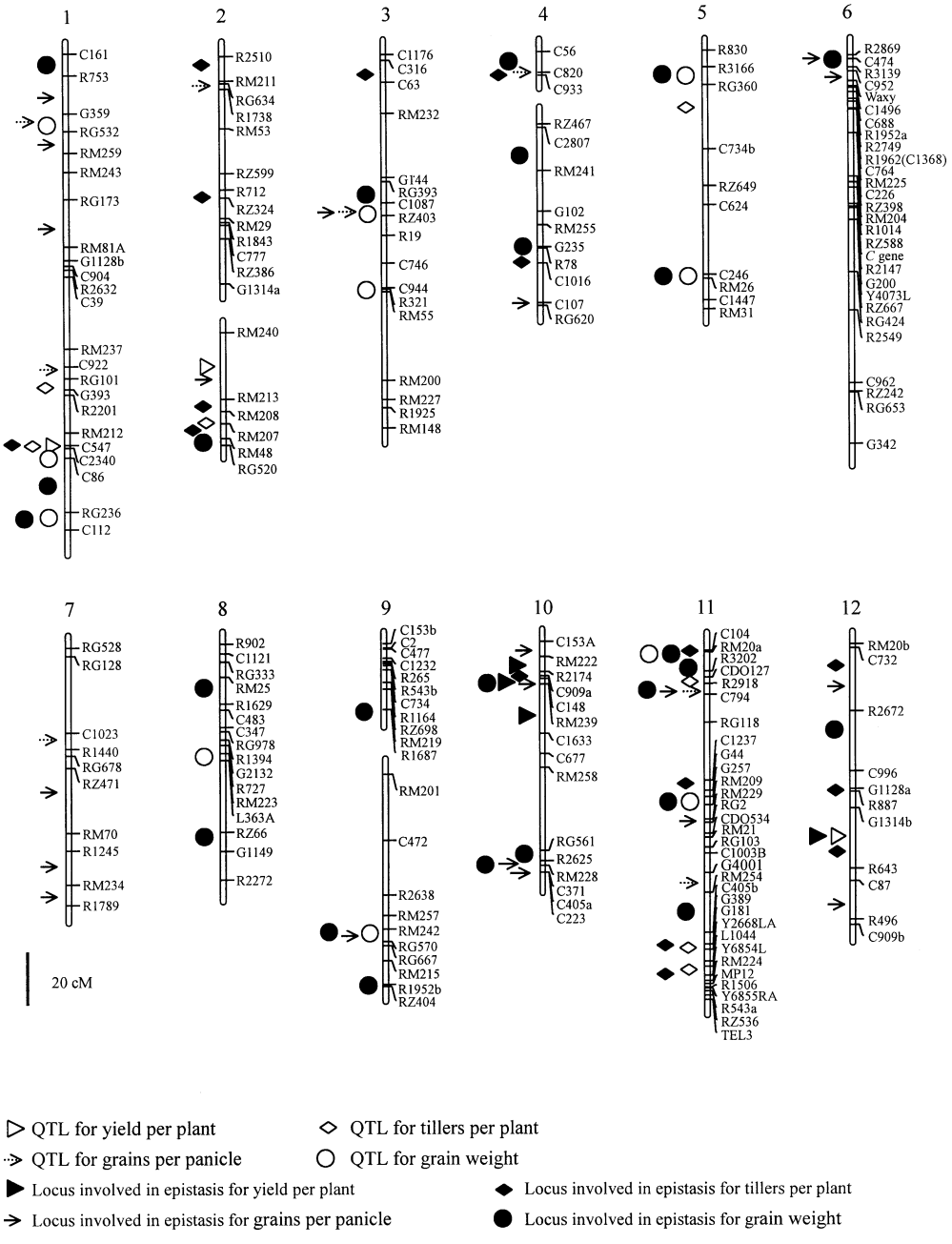

Fig. 1 Distribution of main-effect QTLs and epistatic QTLs on the molecular linkage map as detected by QTLMapper

For yield, three and four QTLs were detected in 1997 and 1998, respectively. Two QTLs, *yd1b* and *yd2*, located on chromosomes 1 and 2 respectively, were simultaneously detected in both years. The other three QTLs were detected only in one year. Alleles from Minghui 63 at four of the QTLs were in the direction of increasing yield, while alleles from Zhenshan 97 at the loci of *yd3* increased plant yield.

Three and four QTLs were resolved for tillers per plant in 1997 and 1998, respectively. Only one QTL (*tp7*) was detected in both years; the Zhenshan 97 genotype showed an increase in the number of tillers.

Of the three and five QTLs for grains per panicle detected in 1997 and 1998, three QTLs (*gn1*, *gw3* and *gn7*) were identified in both years. The QTL g*n3* showed the largest effects explaining a relatively large proportion of phenotypic variance in both years. The Zhenshan 97 genotype had an increased effect on the number of grains per panicle.

Seven and six QTLs were resolved for grain weight in 1997 and 1998, respectively. Four QTLs, *gw1a*, *gw3a*, *gw3b* and *gw5*, were detected in both years. The QTL *gw3a* had the largest effect, accounting for 20.8% and 13.1% of the phenotypic variation in the two years; the Minghui 63 genotype contributed to the increase of grain weight. The QTL g*w5* showed the second largest effect in both years; the Zhenshan 97 genotype was in the direction of increasing grain weight.

Table 3 Main effects, epistatic effects and environmental interactions of QTLs detected by two-locus analyses using QTLMapper for yield per plant at the likelihood ratio LR threshold of 18.6 (the LR value is equal to a chi-square value for $df = 6$ at $P = 0.005$) combining the data of 1997 and 1998

Ch-Ini[a]	Flanking markers	QTL	Ch-Inj[a]	Flanking marker	QTL	LR	a_i[b]	h^2a_i[e]	a_j[b]	h^2a_j[e]	aa_{ij}[c]	h^2aa_{ij}[e]	ae_i[d]	h^2ae_i[e]	ae_j[d]	h^2ae_j[e]	h^2total[f]
1-19	C547-C2340	*yp1*	10-5	C148-RM239		68.0	1.43	12.80									12.80
2-1	RM240-RM213	*yp2*	11-14	G389-C405b		43.4	0.94	5.56									5.56
10-2	RM222-R2174		10-6	RM239-C1633		24.2					0.79	3.89					3.89
10-5	C148-RM239		12-7	G1314b-R643	*yp12*	26.2			–0.51	1.61	0.61	2.36			0.16	0.16	4.13

Overall contributions: Additive: $h^2a = 19.97\%$; Epistasis: $h^2aa_{ij} = 6.25\%$; QE Interactions: $h^2ae = 0.16\%$; $h^2aae = 0$

[a] Ch-Ini and Ch-Inj represent the chromosome number-interval of the points being tested in the analysis

[b] a_i and a_j are the additive effects of testing-points i and j, respectively. A positive value indicates the Minghui 63 genotype having a positive effect on the trait

[c] aa_{ij} is the effect of additive by additive interaction between points i and j; a positive value indicates that the two-locus genotypes are the same as those in the female or the male parent with a positive effect, while the recombinants had negative effects

[d] ae_i and ae_j are effects of interactions between locus I and j and the environment; a negative value means that the effect in 1997 is larger than in 1998

[e] h^2a_i, h^2a_j, h^2aa_{ij}, h^2ae_i and h^2ae_j are the percentages of the phenotypic variations explained by a_i, a_j and aa_{ij}, ae_i, and ae_j, respectively

[f] h^2total is the total phenotypic variation explained by the genetic components included in the model

Table 4 Main effects, epistatic effects and environmental interactions of QTLs detected by the two-locus analyses using QTLMapper for the number of tillers per plant at a likelihood ratio LR threshold of 18.6 (the LR value is equal to the chi-square value for $df = 6$ at $P = 0.005$) combining the data of 1997 and 1998

Ch-Ini[a]	Flanking markers	QTL	Ch-Inj[a]	Flanking marker	QTL	LR	a_i[b]	h^2a_i[e]	a_j[b]	h^2a_j[e]	aa_{ij}[c]	h^2aa_{ij}[e]	ae_i[d]	h^2ae_i[e]	ae_j[d]	h^2ae_j[e]	h^2total[f]
1-16	RG101-G393	*tp1a*	11-6	MP12-RM224		27.3	0.28	1.07									1.07
1-19	C547-C2340	*tp1b*	4-10	R78-C1016		33.4	0.23	0.71			0.21	0.61					1.32
1-19	C547-C2340		5-4	RG360-C734b	*tp5*	27.7	0.24	0.79	0.19	0.48							1.27
2-1	R2510-RM211		11-28	G44-C1237		22.9			–0.16	0.34	0.17	0.40					0.74
2-7	R712-RZ324		3-2	C316-C63		19.4					–0.20	0.53					0.53
2-15	RM213-RM208		11-32	R2918-CDO127	*tp11c*	33.2	0.15	0.31	–0.29	1.18					0.14	0.26	1.75
2-15	RM213-RM208		12-7	G1314b-R643		24.7					–0.30	0.91					0.91
2-16	RM208-RM207	*tp2*	8-2	C1121-RG333		34.2	0.30	1.23									1.23
2-17	RM207-RM48		12-2	C732-R2672		37.4	0.22	0.7			0.23	0.74					1.44
4-2	C820-C933		11-35	RM20a-C104		21.0					0.22	0.64					0.64
10-4	C909A-C148		12-5	G1128a-R887		30.0					0.27	0.98					0.98
11-9	Y6854L-L1044	*tp11a*	11-12	G181-G389	*tp11b*	21.1	0.18	0.44	–0.16	0.37	–0.40	1.83	–0.22	1.33	0.14	0.31	4.28
11-22	RM21-CDO534		11-31	C794-R2918		22.4			–0.29	1.15					0.15	0.28	1.43

Overall contributions: Additive: $h^2a) = 8.40\%$; Epistasis: $h^2aa) = 6.64\%$; QE: $h^2ae) = 2.18\%$; $h^2aae) = 0$

[a–f] See footnotes of Table 3 for explanations

QTLs and QE interactions resolved by two-locus analyses

Yield

Three QTLs showing main effects on yield per plant were mapped on chromosomes 1, 2 and 12 (Table 3). Collectively, these QTLs explained 19.97% of the phenotypic variation. Minghui 63 alleles at two of the QTLs, *yp1* and *yp2*, were in the direction of increasing yield, while the allele from Zhenshan 97 at the third QTL, *yp12*, increased the yield per plant.

Two digenic interactions were detected for this trait, accounting for 6.25% of the phenotypic variation in the population (Table 3). One interaction occurred between two loci located on chromosome 10, neither of which showed main effects at the single-locus level. The other interaction was detected between a locus on chromosome 10 that did not detect a main effect at the single-locus level, and a locus on chromosome 12 that detected a significant main effect at the single-locus level.

Significant interaction was detected only between *yp12* and the environment, accounting for 0.16% of the variation.

Tillers per plant

Seven QTLs were identified as showing main effects on the number of tillers per plant (Table 4); three QTLs were detected on chromosome 11 (*tp11a, tp11b* and*tp11c*), two QTLs on chromosome 1 (*tp1a* and *tp1b*), and one QTL each on chromosomes 2 (*tp2*) and 5 (*tp5*). The additive effects ranged from 0.16 to 0.30 tillers per plant. Minghui 63 alleles increased the number of tillers per plant at all the QTLs except *tp11b* and *tp11c*, at

Table 5 Main effects, epistatic effects and environmental interactions of QTLs detected by the two-locus analyses using QTLMapper for the number of grains per panicle at a likelihood ratio LR threshold of 18.6 (the LR value is equal to the chi-square value for $df = 6$ at $P = 0.005$) combining the data of 1997 and 1998

Ch-Ini [a]	Flanking markers	QTL	Ch-Inj[a]	Flanking marker	QTL	LR	a_i[b]	h^2a_i[e]	a_j[b]	h^2a_j[e]	aa_{ij}[c]	h^2aa_{ij}[e]	ae_i[d]	h^2ae_i[e]	ae_j[d]	h^2ae_j[e]	h^2total[f]
1-2	R753-G359		11-31	C794-R2918	*gn11b*	41.0			3.27	1.28	1.98	0.47			2.41	1.38	3.13
1-3	G359-RG532	*gn1a*	10-7	C1633-C677		41.1	4.20	2.11									2.11
1-4	RG532-RM259		6-1	R2869-C474		58.6	3.68	1.62			3.66	1.60					3.22
1-7	RG173-RM81A		7-6	RZ471-RM70		24.7					–3.33	1.32					1.32
1-14	C922-RG101	*gn1b*	10-5	C148-RM239		30.6	–3.56	1.51									1.51
1-18	RM212-C547		11-32	R2918-CDO127		33.4			3.26	1.27					2.15	0.55	1.82
2-3	RG634-R1738		7-3	C1023-R1440	*gn7*	67.2	–2.65	0.84	4.91	2.88					0.66	0.05	3.77
2-3	RG634-R1738	*gn2*	11-14	G389-C405b		21.6	–2.72	0.88									0.88
2-14	RM240-RM213		5-12	C107-RG620		27.2					3.29	1.29					1.29
3-7	C1087-RZ403		7-8	R1245-RM234		138.9	–7.49	6.70			2.25	0.60					7.30
3-7	C1087-RZ403	*gn3*	9-16	RM242-RG570		143.2	–7.32	6.40			–3.32	1.31					7.71
4-2	C820-C933	*gn4*	11-6	MP12-RM224		23.7	–2.15	0.55					–2.88	1.98			2.53
6-4	C952-Waxy		7-9	RM234-R1789		22.9					–3.40	1.38					1.38
8-3	RG333-RM25		11-18	RM254-G4001	*gn11a*	26.3			2.84	0.96					–1.76	0.37	1.33
10-1	C153A-RM222		10-11	R2625-RM228		24.5					3.31	1.31					1.31
10-1	C153A-RM222		11-24	RG2-RM229		25.7					3.53	1.48					1.48
10-5	C148-RM239		12-9	C87-R496		18.7					–2.63	0.82					0.82
10-13	C371-C405a		12-2	C732-R2672		20.9					–2.69	0.87					0.87

Overall contributions: Additive: $h^2a = 19.96\%$; Epistasis: $h^2aa = 12.46\%$; QE: $h^2ae = 4.33\%$; $h^2aae = 0$

[a–f] See footnotes of Table 3 for explanations

which the Zhenshan 97 allele had a positive effect on the number of tillers per plant.

Eight digenic interactions were detected for the number of tillers per plant, involving 16 loci distributed on seven chromosomes (Table 4). One interaction (11-9/11-12) occurred between two linked loci on chromosome 11, both of which showed significant main effects on the trait. Three interactions (1-19/4-10, 2-1/11-28 and 2-17/12-2) each involved a locus that had a significant main effect and a locus that did not have a significant effect on the trait. The remaining four interactions occurred between loci that did not have significant main effects. For three interactions (2-7/3-2, 2-15/12-7 and 11-9/11-12) the parental two-locus combinations tended to reduce the number of tillers per plant, while for the remaining five interactions the parental genotypes appeared to increase the number of tillers.

Three QTLs on chromosome 11 (*tp11a*, *tp11b* and *tp11c*) were detected as showing significant QEs (Table 4). The QE effect of *tp11a* was larger than the QE of *tp11b* and *tp11c*. The environment condition in 1997 was more favorable than in 1998 for the Minghui 63 allele of *tp11a* to increase the numbers of tillers. Again, no interaction was detected between the epistatic QTLs and the environments.

Overall, the main effects of QTLs explained 8.40% of the phenotype variation, epistatic interactions accounted for 6.64% of the variation, and the QEs explained 2.18% of the phenotype variation.

Number of grains per panicle

Eight QTLs were detected as having main effects on the number of grains per panicle, with additive effects ranging from 2.15 to 7.32 grains per panicle (Table 5). Two QTLs were detected on each of chromosomes 1 (*gn1a* and *gn1b*) and 11 (*gn11a* and *gn11b*), and one QTL each on chromosomes 2 (*gn2*), 3 (*gn3*), 4 (*gn4*) and 7 (*gn7*), respectively. For four (*gn1b*, *gn2*, *gn3* and *gn4*) of the QTLs, the Zhenshan 97 genotypes increased the number of grains, while for the remaining four QTLs (*gn1a*, *gn7*, *gn11a* and *gn11b*), the Minghui 63 genotypes increased the number of grains.

Eleven digenic interactions were detected as showing significant effects on the number of grains per panicle, involving 20 loci dispersed on all the chromosomes except for chromosomes 4 and 8 (Table 5). Four interactions each involved a locus that had a significant main effect, and a locus that did not have a significant effect, on the trait at the single-locus level. The remaining seven interactions occurred between loci that did not have main effects on a single-locus basis. Parental genotypes for six interactions had positive effects on grain numbers while, for the remaining five interactions, recombinant genotypes had positive effects on the number of grains.

Significant environmental interactions were detected for *gn4*, *gn7*, *gn11a* and *gn11b* (Table 5). The QE effects of *gn4* and *gn11b* were larger than the QEs of the other two QTLs; the environments of the 2 years appeared to influence the effects of the two QTLs in an opposite directions in 1997 and 1998. Again, no interaction was detected between the epistatic QTLs and the environments.

Overall, the main effects of QTLs explained 19.96% of the phenotype variation, epistatic interactions accounted for 12.46% of the variation, and the QEs explained 4.33% of the variation.

Table 6 Main effects, epistatic effects and environmental interactions of QTLs detected by the two-locus analyses using QTLMapper for the grain weight at a likelihood ratio LR threshold of 18.6 (the LR value is equal to the chi-square value for df = 6 at P = 0.005) combining the data of 1997 and 1998

Ch-Ini [a]	Flanking markers	QTL	Ch-Inj [a]	Flanking marker	QTL	LR	a_i[b]	h^2a_i[e]	a_j[b]	h^2a_j[e]	aa_{ij}[c]	h^2aa_{ij}[e]	ae_i[d]	h^2ae_i[e]	ae_j[d]	h^2ae_j[e]	h^2total[f]
1-1	C161-R753		4-1	C56-C820		27.4					–0.40	1.66					1.66
1-3	G359-RG532	*gw1a*	5-2	R3166-RG360		201.2	–0.74	5.65	–0.89	8.13							13.78
1-13	RM237-C922		8-12	RM223-L363A	*gw8*	21.7			–0.41	1.72					–0.05	0.03	1.75
1–20	C2340-C86	*gw1b*	3-8	RZ403-R19		92.0	0.41	1.76	0.68	4.73					0.19	0.38	6.87
1–22	RG236-C112	*gw1c*	4-9	G235-R78		36.0	–0.21	0.47			–0.34	1.19					1.66
1–21	C86-RG236		5-2	R3166-RG360	*gw5a*	154.8			–0.94	9.02	–0.33	1.15			0.05	0.03	10.20
1–22	RG236-C112		10-5	C148-RM239		30.4	–0.32	1.05			0.32	1.07					2.12
2–4	R1738-RM53		3-11	C944-R321	*gw3b*	121.7			0.87	7.82							7.82
2-18	RM48-RG520		9-10	RM219-R1687		18.8					0.25	0.67					0.67
3–3	C63-RM232		9-16	RM242-RG570	*gw9*	20.7			–0.34	1.17					0.09	0.07	1.24
3-6	RG393-C1087		5-8	C246-RM26		104.8	0.55	3.17	0.28	0.83	–0.21	0.45					4.45
3-7	C1087-RZ403	*gw3a*	8-13	L363A-RZ66		138.1	0.93	8.90	–0.33	1.14			–0.40	3.37			13.41
4-4	RZ467-C2807		11-27	G257-G44		21.9			0.37	1.43					–0.11	0.02	1.45
4-5	C2807-RM241		8-4	RM25-R1629		24.8					0.36	1.34					1.34
5-8	C246-RM26	*gw5b*	8-14	RZ66-G1149		25.9	0.31	0.97			0.24	0.57					1.54
6-1	R2869-C474		11-34	R3203-RM20a		28.3			0.26	0.67	–0.32	1.07			–0.04	0.02	1.76
8-4	RM25-R1629		11-26	RM209-G257	*gw11*	30.0			0.36	1.35	0.22	0.51			–0.09	0.08	1.94
9-16	RM242-RG570		10-10	RG561-R2625		45.3	–0.32	1.06			–0.36	1.36					2.42
9-20	R1952b-RZ404		11-32	R2918-CDO127		18.7					0.26	0.70					0.70
10-11	R2625-RM228		12-3	R2672-C996		20.4					0.32	1.07					1.07
11-15	C405b-RM254		11-35	RM20a-C104		31.7			0.30	0.95	0.32	1.03					1.98

Overall contributions: Additive: h^2a = 57.96%; Epistasis: h^2aa_{ij} =13.82%; QE: h^2ae = 4.00%; h^2aae = 0

[a–f] See footnotes of Table 3 for explanations

Grain weight

In total 11 QTLs were detected as showing main effects on grain weight (Table 6). Three of the QTLs (*gw1a, gw1b and gw1c*) were located on chromosome 1, two on each of chromosomes 3 (*gw3a* and *gw3b*), 5 (*gw5a* and *gw5b*) and 11 (*gw11a* and *gw11b*), and one on chromosomes 8 (*gw8*) and 9 (*gw9*). The additive effects of the QTLs ranged from 0.21 to 0.94 g per 1,000 grains and explained 0.47–9.02% of the phenotypic variance. Alleles from Zhenshan 97 showed an increasing effects on grain weight at *gw1a*, *gw1c*, *gw5a*, *gw8* and *gw9*, and decreasing effects at the remaining six QTLs. The effects of two QTLs on chromosome 3 (*gw3a* and *gw3b*) and one QTL on chromosome 5 (*gw5a*) were much larger than the remaining eight QTLs.

Fourteen highly significant interactions were detected for grain weight, involving 25 loci distributed on 11 of the 12 chromosomes (Table 6). Five of the 14 interactions occurred between loci that did not have main effects at the single-locus level. One interaction occurred between two loci (3-6/5-8), both having significant main effects on the trait. The remaining eight interactions each involved one locus having a main effect at the single-locus level and another locus that did not show significant effect at the single-locus level. Interestingly, the intervals closely linked to RG236 (*gw1c*) on chromosome 1 simultaneously interacted with three loci on different chromosomes. Parental genotypic combinations for eight epistatic pairs of loci contributed to the increase of grain weight, whereas for the remaining six epistatic pairs of loci, recombinant genotypic combinations increased the grain weight.

Five QTLs (*gw3a*, *gw5a*, *gw8*, *gw9* and *gw11a*) showed significant QEs. However, only the QE effect (–0.40 g, explaining 3.37% of the variation) of *gw3a* appeared to be prominent, which indicated that the environment conditions in 1997 were more favorable than in 1998 for the Minghui 63 allele of *gw3a* to increase grain weight. No significant QE was detected between epistatic interactions with the environments.

Overall, main-effect QTLs accounted for 57.96% of the phenotype variance, epistatic QTLs explained 13.82% of the variation, and 4.00% of the variation were due to QEs.

Taken together, this analysis resolved a total of 29 QTLs as showing main effects on yield and the three traits that are components of yield, and 35 additive by additive interactions involving 58 different loci distributed on all 12 chromosomes, most of which did not have main effects at the single-locus level. QEs were detected for 13 main-effect QTLs but not for any epistatic QTLs.

Pleiotropic effects

Three loci with pleiotropic effects were observed. The locus within the genomic region C547-C2340 on chromosome 1 simultaneously influenced the yield per plant and the number of tillers per plant; in both cases, the Minghui 63 genotype had a positive effect on the traits. Two loci located in the interval G359-RG532 on chromosome 1 and the interval C1087-RZ403 on chromosome 3, respectively, had simultaneous effects on grain weight and number of grains per panicle, but in opposite directions. The Minghui 63 genotype for the locus on

chromosome 1 increased grain number but decreased grain weight, while the reverse was the case for the locus on chromosome 3, in agreement with the negative correlation between the two traits observed in this population ($r = –0.28$, $P < 0.001$).

Interestingly, the locus in the interval C148-RM239 on chromosome 10 simultaneously interacted with three loci on two different chromosomes to influence three traits respectively. The recombinant genotypes for the interaction between loci in the interval C148-RM239 and G1314b-R643 decreased the yield per plant, and between loci in the interval C148-RM239 and RG236-C112 the interaction had a negative effect on grain weight, while the recombinant genotypes for the interaction between loci in the interval C148-RM239 and C87-R496 increased the number of grains.

Comparison of single-locus QTLs detected by the two methods

For yield

Both of the QTLs, *yd1b* and *yd2*, that were detected in both years in the single-locus analysis using QTL Cartographer were also detected as main-effect QTLs in the two-locus analysis (by QTLMapper). In contrast, none of the other three QTLs that were detected only in one year in the single-locus analysis were detected in the two-locus analysis. Moreover, one QTL, *yp12*, that showed a significant main effect and was also involved in epistatic interaction as revealed in the two-locus analysis, was not detected in the single-locus analysis

For grain weight

Eight of the nine QTLs, with the exception of *gw6*, that were detected by single-locus analysis, also showed significant main effects in the two-locus analysis. Whereas, three QTLs (*gw5b*, *gw8* and *gw11b*) that showed significant main effects by the two-locus analysis were not detected in the single-locus analysis.

For the number of tillers per plant

Six of the seven QTLs detected for the number of tillers per plant in the single-locus analysis showed significant main effects in the two-locus analysis. However, the only QTL that was detected in both years in the single-locus analysis did not show a significant main effect in the two-locus analysis. By contrast, two epistatic QTLs (*tp11a* and *tp11b*), both showing significant main effects and significant QEs, were not detected in the single-locus analysis.

For the number of grains per panicle

All the QTLs detected in the single locus-analysis were also recovered as showing significant main effects in the two-locus analysis. In addition, main effects were also detected for three putative QTLs (*gn1b*, *gn4* and *gn11b*) in the two-locus analysis.

Thus, overall, these two methods appeared to be in good agreement with respect to the detection of the single-locus QTLs, despite the possible differences in the level of stringency of the statistical tests. As expected, compared with the single-locus analysis using QTL Cartographer, the two-locus analysis using QTLMapper had the obvious advantage for enabling the simultaneous detection of main effects, epistatic interactions and environmental interactions of the QTLs.

Discussion

The most-important result of this study is the statistical characterization of the genetic components that control the expression of the traits, including main effects of the QTLs, additive by additive epistatic interactions or epistatic QTLs, and QTL by environmental interactions (QEs). The analysis resolved a total of 29 QTLs as showing main effects on yield and the three traits that are components of yield, and 35 additive by additive interactions involving 58 different loci (epistatic QTLs) distributed on all 12 chromosomes. Clearly, the number of epistatic QTLs was much larger than that of the main-effect QTLs for the four traits, and most of the epistatic QTLs did not have main effects at the single-locus level. In contrast, a large proportion of the main-effect QTLs was involved in the epistatic interactions. This indicates that epistasis, in the form of additive by additive interactions, plays a very important role in controlling the expression of yield and yield-component traits. A similar conclusion has been reached in several previous studies. For example Yu et al. (1997) detected large numbers of interactions for yield and yield components in $F_{2:3}$ families derived from the same cross, and additive by additive interactions were the predominant forms of interactions. Li et al. (1997) also found epistasis as an important genetic basis of three grain yield components, and the digenic interactions were due primarily to the additive epistatic gene action.

A direct implication of epistasis, especially the involvement of QTLs in the epistatic interactions, is that the effects of the single-locus QTLs are mostly dependent on the genotypes of other loci, and, as can be seen from this analysis, the effect of a QTL can sometimes be negated by the genotypes of a second locus. Thus an attempt for utilization of the QTLs in the breeding programs has to taken into account for such epistatic effects. It should be noted that although this study revealed a large number of epistases by statistical genetic analyses, many studies are needed before we can understand the biological meaning of the epistases, as in the case of model organisms such as the *EXO1-MSH2* interaction in *Saccharomyces* (Daniel et al. 1997), and the RAS pathways involved in eye development in *Drosophila* (Rebay et al. 2000).

Another important aspect of this study is the identification of QTLs by environmental interactions. Genotype by environment interaction is a very important factor that determines the stability of crop varieties, and has received considerable attention in plant breeding programs. There has been a large number of studies for demonstrating the existence of genotype by environmental interactions and for determining varietal stability by analyzing data from varietal trials of multiple years and locations. Recent developments in QTL analyses have provided detailed information about possible loci that may perform differently under different environmental conditions, by comparing the QTLs that were detected in multiple environments. However, such a comparison could not provide direct estimates for the number of interactions of particular QTLs with the environment.

The present analysis revealed that slightly less than half (13/29) of the QTLs showed environmental interactions, indicating different effects of the same QTLs in different years; whereas the single-locus analysis showed that 15 of the 25 QTLs were detected only in one year. Thus the two analyses are consistent in that the performance of 13 or 15 of the single-locus QTLs were influenced by environmental conditions in the two years. However, the total amounts of the effects accounted for by QEs were small for all four traits, ranging from less than one percent to a few percent.

Epistasis refers to the phenotypic effects of interactions between alleles of different loci. According to our current understanding of the physiological basis of epistatic interactions, epistasis should be much-more sensitive to environmental changes, which also provided experimental evidence in recent molecular marker-based genetic analyses. For example, in the data reported by Yu et al. (1997), only very small portions (about 10%) of epistatic interactions separately detected in different years could be simultaneously detected in both years. However, the present analysis failed to detect any significant interactions between epistatic QTLs and the environment. Such a discrepancy is most-likely due to the fact that the effects of individual interactions (second-order statistics) were statistically small, and their environmental interactions (third-order statistics) would be too small to be detected individually using the current statistical analysis. Statistical detection of such interactions remains to be the subject of future studies.

Acknowledgements The authors thank Dr. Jun Zhu for kindly providing the QTLMapper software and assistance in data analysis, and the Cornell group and the Japanese Rice Genome Research Program for the RFLP probes. This study was supported in part by a grant from the National Natural Science Foundation of China and a grant from the Rockefeller Foundation.

References

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

Daniel XT, Adrienne LB, Pascale B, Nicole F, Gretchen MG, Michael FK, Richard DK (1997) Identification and characterization of *Saccharomyces cerevisiae EXO1*, a gene encoding an exonuclease that interacts with MSH2. Proc Natl Acad Sci USA 94:7487–7492

East EM (1916) Studies on size inheritance in *Nicotiana*. Genetics 1:164–176

Eberhart SA, Russell WA (1966) Stability parameters for comparing varieties. Crop Sci 6:36–40

Fasoulas AC, Allard RW (1962) Nonallelic gene interactions in the inheritance of quantitative characters in barley. Genetics 47:899–907

Finlay KS, Wilkinson GN (1963) The analysis of adaptation in a plant-breeding programme. Aust J Agric Res 14:742–754

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Lark KG, Chase K, Adelf F, Mansur LM, Orf JH (1995) Interactions between quantitative trait loci in soybean in which trait variation at one locus is conditional upon a specific allele at another. Proc Natl Acad Sci USA 92:4656–4660

Li ZK, Pinson SRM, Park WD, Paterson AH, Stansel JW (1997) Epistasis for three grain yield components in rice *Oryza sativa* L. Genetics 145:453–465

Lincoln S, Daly M, Lander E (1992) Constructing genetics maps with MAPMAKER/EXP 3.0. Whitehead Institute Technical Report, Whitehead Institute, Cambridge, Massachusctts, USA

Liu KD, Wang J, Li HB, Xu CG, Liu AM, Li XH, Zhang Q (1997) A genome-wide analysis of wide compatibility in rice and the precise location of the S5 locus on the molecular map. Theor Appl Genet 95:809–814

Lu C, Shen L, Tan Z, Xu Y, He P, Chen Y, Zhu L (1996) Comparative mapping of QTLs for agronomic traits of rice across environments using a doubled-haploid population. Theor Appl Genet 93:1211–1217

Maughan PJ, Saghai Maroof MA, Buss GR (1996) Molecular-marker analysis of seed-weight: genomic locations, gene action, and evidence for orthologous evolution among three legume species. Theor Appl Genet 93:574–579

Paterson AH, Lander ES, Had JD, Patrson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726

Rebay I, Chen FL, Hsiao F, Kolodziej PA, Kuang BH, Laverty T, Suh C, Voas M, Williams A, Rubin GM (2000) A genetic screen for novel components of the Ras/mitogen-activated proteins kinase signalling pathway that interact with the *yan* gene of *Drosophila* identities split ends, a new RNA recognition motif-containing protein. Genetics 154:695–712

Tinker NA, Mather DE, Rossnagel BG, Kasha KJ, Kleinhofs A, Hayes PM, Falk DE, Ferguson T, Shugour LP, Legge WG, Irvine RB, Choo TM, Briggs KG, Ullrich SE, Franckowiak JD, Blake TK, Graf KJ, Dofing JM, Saghai Maroof MA, Scoles GJ, Hoffman D, Dahleen LS, Kilian A, Chan F, Biyashev RM, Kudrna DA, Steffenson BJ (1996) Regions of the genome that affect agronomic performance in two-row barley. Crop Sci 36:1053–1062

Wang DL, Zhu J, Li ZK, Paterson AH (1999a) Mapping QTLs with epistatic effects and QTL ×environment interactions by mixed linear model approaches. Theor Appl Genet 99:1255–1264

Wang DL, Zhu J, Li ZK, Paterson AH (1999b) A computer software for mapping quantitative trait loci QTLs with main effects, epistatic effects and QTL ×environment interactions. Copyright by Zhejiang University, Hangzhou, China

Wu KS, Tanksley SD (1993) Abundance, polymorphism and genetic mapping of microsatellites in rice. Mol Gen Genet 241:225–235

Xu Y (1997) Quantitative trait loci: separating, pyramiding, and cloning. Plant Breed Reviews 15:85–139

Yu SB, Li JX, Xu CG, Tan YF, Gao YJ, Li XH, Zhang Q, Saghai Maroof MA (1997) Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. Proc Natl Acad Sci USA 94:9226–9231

Yu SB, Li JX, Xu CG, Tan YF, Gao YJ, Li XH, Zhang Q, Saghai Maroof MA (1998) Epistasis plays an important genetic role for heterosis in rice. Science in China Series C 41:293–302

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

Zhang Q, Geng S (1986) A method of estimating varietal stability for data of long-term trials. Theor Appl Genet 71:810–814

Zhang Q, Yu SB (1999) Molecular marker-based gene tagging and its impact on rice improvement. In: Nanda JS (ed) Rice breeding and genetics-research priorities and challenges. Science Publishers Inc, Enfield, New Hampshire, pp 241–270

Zhu J, Weir BS (1998) Mixed model approaches for the genetics of quantitative traits. In: Chen LS, Ruan SG, Zhu J (eds) Advanced topics in biomathematics: Proc Int Conf on Mathematical Biology, World Scientific Publishing Co, Singapore, pp 321–330