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Analysis on additive effects and additive-by-additive epistatic effects of QTLs for yield traits in a recombinant inbred line population of rice

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Abstract A linkage map consisting of 158 DNA markers were constructed by using a recombinant inbred line (RIL) population derived from the indica-indica rice cross Zhenshan 97B × Milyang 46. Quantitative trait loci (QTLs) conditioning grain yield and five yield component traits were determined at the one-locus and two-locus levels, and genotype-by-environment (GE) interactions were analyzed. Thirty-one QTLs were detected to have significant additive effects for yield traits, of which 12 also exhibited significant epistatic effects. Sixteen significant additive-by-additive (AA) interactions were detected, of which nine occurred between QTLs with own additive effects (M_{ep}QTLs), four occurred between QTLs showing epistatic effects only (epQTLs), and three occurred between M_{ep}QTLs and epQTLs. Significant GE interactions were found for six QTLs with additive effects and one AA interaction. Generally, the contributions to the phenotypic variation were higher due to QTL main effects than to epistatic effects. The detection of additive effects and AA effects of a QTL interfered with each other, indicating that the detection of QTLs with main effects, as well as the magnitude and directions of the additive effects, might vary depending on their interactions with other loci.

Keywords Quantitative trait loci (QTL) · Additive effects · Additive-by-additive epistatic effects · Genotype-by-environment interaction effects

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Introduction

Most agronomic traits of crops are inherited in a complex manner and affected by environments. The importance of epistasis as the genetic basis for complex traits has been indicated on the basis of classical quantitative genetic studies. Experiments on changes in the allele frequencies of isozyme markers also revealed that epistasis among multiple loci might play a major role in the evolution of complex traits in plant species (Allard 1996). Recent quantitative trait locus (QTL) mapping studies revealed pronounced epistasis affecting quantitative traits (Eshed and Zamir 1996; Li et al. 1997; Yu et al. 1997; Cao et al. 2001; Zhang et al. 2001). While all three types of epistasis – the classification being based on whether the QTLs involved exhibit own main effects – were observed, the results varied with respect to the importance of epistasis between QTLs with own main effects. Although this type of epistasis has been shown to be the prevalent form of epistasis affecting the final height of rice plants (Cao et al. 2001), QTLs exhibiting own main effects were either not detected or only occasionally detected in most QTL mapping studies (Li et al. 1997; Li 1998; Liao et al. 2001).

Genotype-by-environment (GE) interaction is another important component for the genetic control of complex traits. Significant GE interactions have been reported by comparing QTLs detected in multiple environments (Stuber et al. 1992; Zhuang et al. 1997). In these studies, the appearance of QTLs being detected in one environment but not in another was considered to be an indication of GE interaction. However it has been shown that QTLs readily detected in different environments may still have significant GE effects (Yan et al. 1998).

With the recent advancements in the methodology and software for QTL mapping, direct mapping of QTLs with main effects and/or epistatic effects as well as the estimation of their GE effects have become available (Wang et al. 1999). In the investigation reported here, QTLs conditioning yield traits in rice were detected in a recombinant inbred line (RIL) population derived from

an indica-indica cross, and their genetic effects and GE effects were determined as well. The relationships of QTLs with additive effects and additive-by-additive interactions and their involvement in the detection of QTLs with additive effects were analyzed.

Materials and methods

Rice material and phenotyping

An indica/indica rice cross Zhenshan 97B (ZS97B)/Milyang 46 (MY46) was made in 1993. An RIL population derived from a single F_1 plant by single-seed descent was developed. In 1999 and 2000, 209 F_7 lines were transplanted in the paddy field of the China National Rice Research Institute, Hangzhou, China, with two replications, in a randomized complete block design. Twelve and nine plants per replication were planted in 1999 and 2000, and the middle eight and five plants were harvested, respectively.

Six traits – grain yield per plant (GYD), number of panicles per plant (NP), number of filled grains per panicle (NFGP), total number of spikelets per panicle (TNSP), spikelet fertility (SF), and 1000-grain weight (TGWT) – were measured. Mean values over two replications in each year were used for analysis.

DNA marker assay

DNAs were extracted from a leaf mixture of 20 plants for each F_7 line. The DNA marker genotypes detected thus represented the genotypes of F_6 individuals.

Restriction endonuclease digestion, electrophoresis, and Southern blot were performed as described by McCouch et al. (1988). The ECL direct nucleic acid labelling and detection systems (Amersham Pharmacia Biotech) were employed for probe labelling, DNA hybridization, and signal detection. The restriction endonucleases *Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I were used to digest the total DNA. Except that clone B10 was derived from a random amplified polymorphic DNA (RAPD) marker linked to a blast resistance gene mapped in our laboratory (Zheng et al. 2000), all of the restriction fragment length polymorphism (RFLP) probes were obtained from Dr. S.D. Tanksley's Laboratory (Cornell University, USA).

The presence of simple sequence length polymorphisms (SSLPs); was surveyed using MAPPAIR simple sequence repeat (SSR) primers (Research Genetics). The polymerase chain reaction (PCR) protocol of the manufacturer was followed, and the product was analyzed on a 3% MetaPhor agarose (BMA) gel. On the basis of the SSLP framework map (Temnykh et al. 2000), polymorphic SSLP markers which might fill the gaps in the RFLP framework map or saturate intervals containing QTLs were selected to assay the RIL.

Data analysis

Having heterozygous alleles treated as missing data, we performed linkage analysis with MAPMAKER/EXP 3.0 (Lander et al. 1987; Lincoln et al. 1992). Markers were grouped at an LOD of 3.0 and ordered at an LOD of 2.0. Distances between markers were presented in centiMorgans (cM) derived using the Kosambi function. Chromosome numbers were given to the linkage groups according to the Cornell molecular map of rice (Causse et al. 1994).

QTLMAPPER 1.01B of the mixed linear model (Wang et al. 1999) was employed to determine QTLs conditioning yield traits with the year as the environmental factor. Important markers and marker pairs were selected using stepwise regression analysis (P<0.001). Background genetic variation (BGV) due to main and epistatic effects of important markers was controlled. The threshold of LOD>3.0 was chosen for claiming a putative QTL. The significance of QTL effects, including additive effect (A), additive-by-environment interaction effect (AE), additive-by-additive epistatic effect (AA), and epistasis-by-environment interaction effect (AAE), was further tested by running the sub-menu Bayesian test (P<0.001). QTLs were designated as proposed by McCouch et al. (1997), but the prefix '*q*' was omitted for QTLs showing significant epistatic effects only. Taking into account all QTLs detected for a same trait on a same chromosome, the suffix following the chromosome number was given from top to bottom.

Results

Trait performance and linkage map

Normal distributions were observed for each of the six traits in 1999 and 2000. Highly positive significant correlations were shown between grain yield and each of its component traits, among which NFGP and TGWT were the traits showing the highest and the lowest correlations with GYD, respectively (Table 1). Highly positive significant correlations between the phenotype performances in 1999 and 2000 were also observed. The correlation coefficients obtained were 0.685 for TGWT, 0.544 for NFGP, 0.395 for NP, 0.319 for TNSP, 0.282 for SF and 0.236 for GYD.

A linkage map consisting of 158 DNA markers (122 RFLP and 36 SSLP) and spanning 1,287.8 cM was constructed (Fig. 1). Except that two segments on each of the chromosomes 4, 6, and 9 remained unlinked to each other, respectively, genetic distances between neighboring markers ranged between 0 cM and approximately 33.2 cM and averaged 9.0 cM. The average heterozygosity over the RILs was 2.1%, which was close to the expected value of 3.1% for F_6 individuals.

QTLs exhibiting significant additive effects for yield traits

A total of 31 QTLs were detected as having significant additive effects for the yield traits (Table 2), including six for GYD, one for NP, eight for NFGP, six for TNSP, two for SF and eight for TGWT. A proportion of these QTLs also exhibited epistatic effects for the same trait, which will be described in the next section. For ease of description, QTLs showing significant additive effects

Table 1 Phenotype correlations between yield and its component traits in the ZS97B/MY46 RIL population

Trait[a]	GYD		
	1999	2000	Average
NP	0.337**	0.655**	0.480**
NFGP	0.780**	0.712**	0.686**
TNSP	0.679**	0.389**	0.461**
SF	0.351**	0.501**	0.426**
TGWT	0.251**	0.291**	0.251**

[a] See Materials and methods for abbreviations
** P<0.01

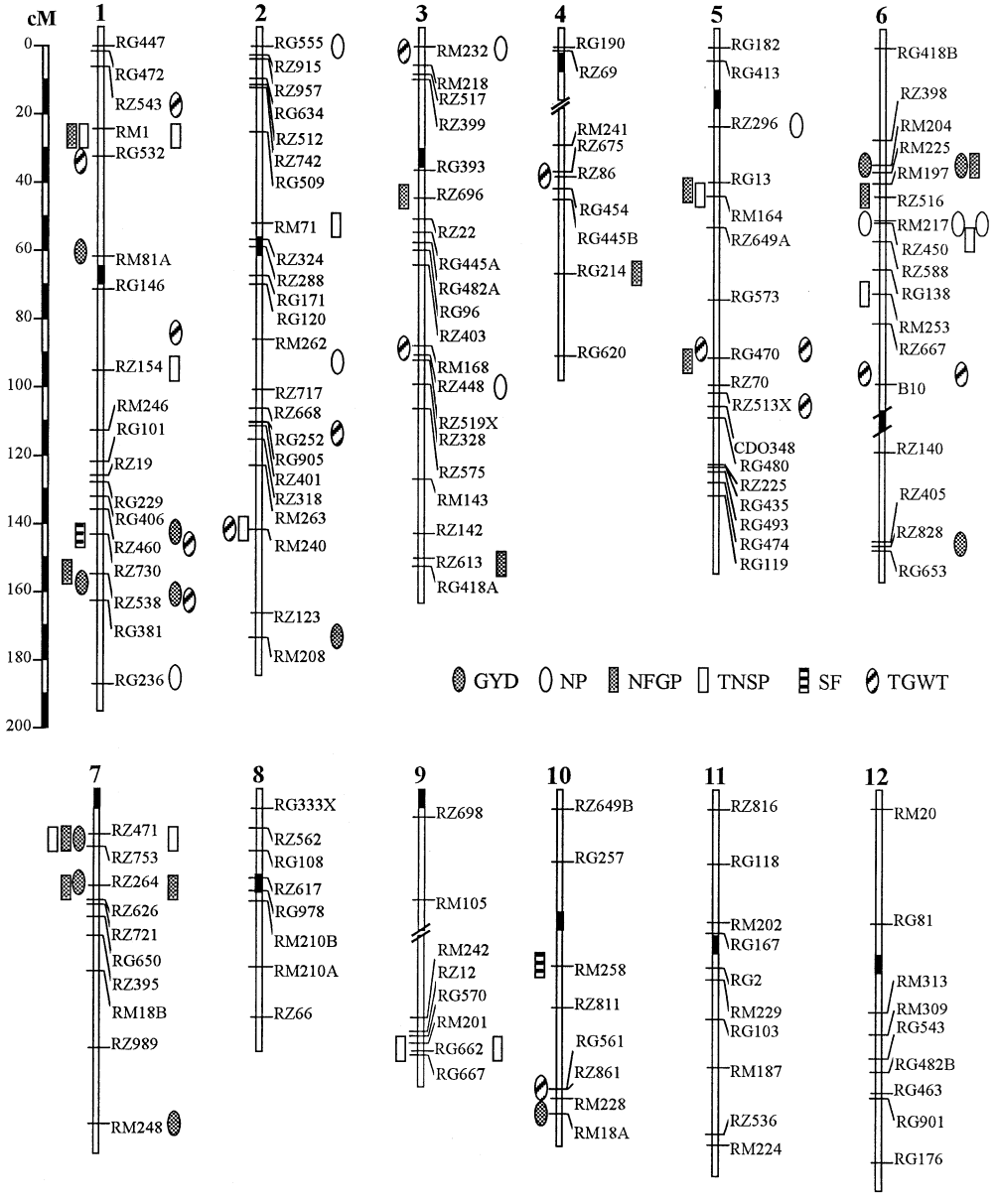

Fig. 1 Most likely positions of QTLs for yield components detected in the RIL population of Zhenshan 97B/Milyang 46. The *blank bars* indicate chromosomes, and the *solid portions* indicate approximate positions of the centromeres according to Singh et al. (1996) and Temnykh et al. (2000). For each chromosome, loci showing significant additive effects and epistatic effects are shown on the *left-hand* and *right-hand* sides, respectively. *RM#* are SSLP markers, and the others are RFLP markers

are simply referred to as QTLs in the following parts of this section.

QTLs for GYD were distributed on chromosomes 1, 6, 7, and 10, and all of them were located in chromosomal regions in which QTLs for yield component traits were detected. Similarly, clustering of QTLs for the component traits was mainly observed in these regions (Fig. 1).

Two QTLs for GYD and five QTLs for other traits were detected on chromosome 1. On the short arm, *qGYD-1-1* was loosely linked to the cluster of *qNFGP1-1*, *qTNSP-1-1*, and *qTGWT-1-1*. The alleles for increasing the trait values at *qGYD-1-1*, *qNFGP1-1*, and *qTNSP-1-1* were from MY46, while *qTGWT-1-1* had the opposite direction of the additive effect. On the long arm, *qGYD-1-2*, *qNFGP-1-2*, and *qSF-1* were located in similar positions, and the alleles for increasing the trait values were all from MY46.

One QTL for GYD and four QTLs for other traits were detected on chromosome 6. They were located in linked intervals on the short arm. The alleles for increasing the trait values were from ZS97B at *qGYD-6-1*, *qNFGP-6*, and *qTNSP-6*, while *qNP-6* and *qTGWT-6* had the opposite direction of additive effects.

Two QTLs for GYD and three QTLs for other traits were detected in two closely linked intervals on the long arm of chromosome 7. The alleles for increasing the trait values were from MY46 at *qGYD-7-1*, *qNFGP-7-1*, and *qTNSP-7* located in interval RZ471-RZ753, while *qGYD-7-2* and *qNFGP-7-2* located in another interval had the opposite direction of additive effects.

One QTL for GYD and two QTLs for other traits were detected on chromosome 10. QTLs *qGYD-10* and *qTGWT-10* were located in similar positions, and the alleles for increasing the trait values were from ZS97B

Table 2 QTLs exhibiting significant additive effects for yield traits in ZS97B/MY46 RIL population

QTL	Interval	LOD	A		AE effect [b]		Var (%) [c]	
			Effect [a]	Probability	1999	2000	A	AE
qGYD-1-1	RG532-RM81A	7.0	1.37	<0.0001			3.6	
qGYD-1-2	RZ538-RG381	5.9	1.51	<0.0001			4.4	
qGYD-6-1	RZ398-RM204	9.8	–1.11	<0.0001	–1.77**	1.77**	2.4	12.1
qGYD-7-1	RZ471-RZ753	4.7	1.19	<0.0001	1.52*	–1.52*	2.7	8.9
qGYD-7-2	RZ753-RZ264	3.4	–1.14	0.0001			1.6	
qGYD-10	RM228-RM18A	5.1	–1.36	<0.0001			3.5	
					General contribution:		16.9	21
qNP-6	RZ450-RZ588	11.1	0.53	<0.0001	–0.54**	0.54**	1.7	3.5
					General contribution:		1.7	3.5
qNFGP-1-1	RM1-RG532	7.4	5.22	<0.0001			4.1	
qNFGP-1-2	RZ538-RG381	4.1	3.63	<0.0001			2.0	
qNFGP-3-1	RZ696-RZ22	4.8	–3.14	0.0001			1.5	
qNFGP-5-1	RG13-RM164	4.4	–3.85	<0.0001			2.0	
qNFGP-5-2	RG470-RZ70	4.5	–3.74	<0.0001			2.1	
qNFGP-6	RZ516-RM217	5.5	–4.53	<0.0001			3.1	
qNFGP-7-1	RZ471-RZ753	11.5	6.39	<0.0001	5.63**	–5.63**	6.2	9.7
qNFGP-7-2	RZ264-RZ626	3.6	–3.15	0.0001			1.4	
					General contribution:		22.5	9.7
qTNSP-1-1	RM1-RG532	18.2	10.05	<0.0001			12.4	
qTNSP-2-2	RM263-RM240	3.6	–4.09	<0.0001			2.1	
qTNSP-5	RG13-RM164	5.3	–5.00	<0.0001			3.1	
qTNSP-6	RG138-RM253	17.2	–8.28	<0.0001	–5.12*	5.12*	8.4	6.5
qTNSP-7	RZ471-RZ753	6	5.41	<0.0001	5.38*	–5.38*	3.6	7.1
qTNSP-9	RM201-RG662	3.1	3.52	0.0002			1.6	
					General contribution:		31.2	13.6
qSF-1	RZ730-RZ538	5.1	2.44	<0.0001			3.9	
qSF-10	RM258-RZ811	4.2	2.05	<0.0001			2.7	
					General contribution:		6.7	0
qTGWT-1-1	RG532-RM81A	12.6	–0.68	<0.0001			9.8	
qTGWT-2-2	RM240-RZ123	8.4	–0.52	<0.0001			5.9	
qTGWT-3-1	RM232-RM218	3.9	–0.39	<0.0001			3.3	
qTGWT-3-2	RM168-RZ448	8.5	0.55	<0.0001			6.5	
qTGWT-4	RZ86-RG454	7.4	0.39	<0.0001			3.3	
qTGWT-5	RG573-RG470	10.2	0.56	<0.0001			6.8	
qTGWT-6	RZ667-B10	4.7	0.46	<0.0001			4.5	
qTGWT-10	RG561-RM228	19.6	–0.79	<0.0001			13.4	
					General contribution:		53.2	0

[a] The genetic effect of the putative QTL when a maternal allele was replaced by a paternal allele
[b]* $P<0.001$, ** $P<0.0001$; [c] Relative contributions to the phenotype variation due to A and AE effects, respectively

at both loci. Another QTL, *qSF-10*, was detected in an adjacent interval, and the allele for increasing fertility was from MY46.

Ten additional QTLs for yield component traits were detected in other chromosomes, including *qTNSP-2-2* and *qTGWT-2-2* clustered on the long arm of chromosome 2, *qNFPG-3-1*, *qTGWT-3-1*, and *qTGWT-3-2* scattered over the two arms of chromosome 3, *qTGWT-4* on the long arm of chromosome 4, *qNFGP-5-1* and *qTNSP-5* clustered in one interval and *qNFGP-5-2* and *qTGWT-5* in another interval on the long arm of chromosome 5, and *qTNSP-9* on the long arm of chromosome 9.

Significant AE interactions were observed for six QTLs. These were clustered in two regions, including *qGYD-6-1*, *qTNSP-6*, and *qNP-6* in RZ398-RZ588, and *qGYD-7-1*, *qNFGP-7-1*, and *qTNSP-7* in RZ471-RZ753. Only the QTL for NP, two of the six QTLs for GYD, and two of the six QTLs for TNSP showed significant AE effects, whereas none of the eight QTLs for TGWT and only one of the eight QTLs for NFGP exhibited such effects. As shown in the previous section, the highest correlation between phenotype performances in 1999 and 2000 was observed for TGWT, which was followed by the value for NFGP. This implies that the detection of AE interactions was related to a certain degree to the phenotype correlations between years.

QTL detection was also in accordance with the phenotype correlations between traits. NFGP was the component trait having the highest correlation with GYD, and it was also the one showing the highest association with GYD upon QTL detection. Five pairs of QTLs for NFGP and GYD were located in similar positions, and each pair had the same direction of additive effects. Similarly, TGWT was the component trait having the lowest correlation with GYD, and only one of the eight QTLs detected for TGWT coincided with a QTL for GYD in both the location and the direction of additive effects.

Table 3 Pairwise QTLs exhibiting significant additive-by-additive interaction effects for yield traits in ZS97B/MY46 RIL population

QTLi	Interval	QTLj	Interval	LOD	AAij		AAE effect[b]		Var (%)[c]	
					Effect[a]	Prob	1999	2000	AA	AAE
qGYD-1-2	RZ538-RG381	*qGYD-6-1*	RZ398-RM204	17.3	0.97	0.0008			1.1	
qGYD-1-2	RZ460-RZ730	*GYD-6-2*	RZ828-RG653	6.0	–1.11	0.0001			1.5	
GYD-2	RZ123-RM208	*GYD-7-3*	RZ989-RM248	6.3	–1.58	<0.0001			3.0	
							General contribution:		5.5	0
NP-1	RG381-RG236	*NP-3-2*	RZ328-RZ575	5.3	–0.47	<0.0001			1.1	
NP-2-1	RG555-RZ915	*NP-2-2*	RM262-RZ717	5.5	0.40	0.0003	–0.48*	0.48*	0.8	2.2
NP-3-1	RM232-RM218	*qNP-6*	RZ450-RZ588	12.9	–0.40	0.0002			0.8	
NP-5	RZ296-RG13	*qNP-6*	RZ450-RZ588	12.7	0.52	<0.0001			1.3	
							General contribution:		3.9	2.2
NFGP-3-2	RZ613-RG418A	*qNFGP-7-2*	RZ264-RZ626	6.2	2.72	0.0009			1.0	
NFGP-4	RG214-RG620	*qNFGP-6*	RM204-RM225	11.3	–6.28	<0.0001			5.4	
							General contribution:		6.4	0
qTNSP-1-1	RM1-RG532	*qTNSP-9*	RM201-RG662	21.2	–3.48	0.0006			1.6	
TNSP-1-2	RG146-RZ154	*qTNSP-7*	RZ471-RZ753	9.3	3.40	0.0007			1.5	
TNSP-2-1	RM71-RZ324	*qTNSP-6*	RZ450-RZ588	4.9	–3.75	0.0002			1.9	
							General contribution:		5.0	0
qTGWT-1-1	RZ543-RM1	*TGWT-1-3*	RZ730-RZ538	4.9	–0.47	<0.0001			3.4	
TGWT-1-2	RG146-RZ154	*TGWT-1-3*	RZ538-RG381	6.1	0.46	<0.0001			3.3	
TGWT-2-1	RZ401-RZ318	*qTGWT-5*	RZ513X-CDO348	5.5	–0.33	0.0001			1.7	
qTGWT-5	RG573-RG470	*qTGWT-6*	RZ667-B10	17.5	0.33	0.0001			1.6	
							General contribution:		10.1	0

[a] Positive value: parental type > recombinant type; negative value: parental type < recombinant type
[b]* $P<0.001$; [c] Relative contributions to the phenotype variation due to AA and AAE effects, respectively

Significant additive-by-additive interactions detected for yield traits

A total of 16 significant AA interactions were detected for the yield traits, of which seven and nine displayed effects in favor of the parental and recombinant genotype combinations, respectively (Table 3). Only one interaction for NP showed significant AAE effects.

When significant additive and AA effects for a same trait were detected in similar locations, a single QTL designation was given. For example, *qGYD-1-2* was given to the QTL showing additive effects for GYD in interval RZ538-RG381 and to QTLs showing AA effects for the same trait in RZ538-RG381 and RG460-RZ730. Similarly, a single designation, *TGWT-1-3*, was given to the two loci involved in AA interactions for TGWT and detected in intervals RZ538-RG381 and RG460-RZ730, respectively. For ease of description, QTLs exhibiting both significant additive and AA effects are referred to here as M_{ep}QTLs, and those exhibiting significant AA effects only as epQTLs.

GYD

Five QTLs were involved in three significant AA interactions for GYD. One interaction acted for increasing the values of the parental types, and the two others acted in the opposite direction. M_{ep}QTLs were involved in two of the interactions – one between M_{ep}QTLs *qGYD-1-2* and *qGYD-6-1* and the other between *qGYD-1-2* and an epQTL. The remaining interaction occurred between two other epQTLs.

NP

Seven QTLs were involved in four significant AA interactions for NP. The same number of interactions acted in favor of the parental and recombinant types, respectively. M_{ep}QTL *qNP-6* simultaneously interacted with epQTLs *NP-3-1* and *NP-5*, respectively. Four other epQTLs were involved in the remaining two interactions.

NFGP

Four QTLs were involved in two significant AA interactions for NFGP. One interaction acted for increasing the values of the parental types, and the other acted in the opposite direction. Each of the interactions occurred between an M_{ep}QTL and an epQTL.

TNSP

Six QTLs were involved in three significant AA interactions for TNSP. One interaction acted for increasing the values of the parental types, and the two others acted in the opposite direction. One interaction occurred between M_{ep}QTLs *qTNSP-1-1* and *qTNSP-9*, and the two others each occurred between an M_{ep}QTL and an epQTL.

TGWT

Six QTLs were involved in four significant interactions for TGWT. The same number of interactions acted for

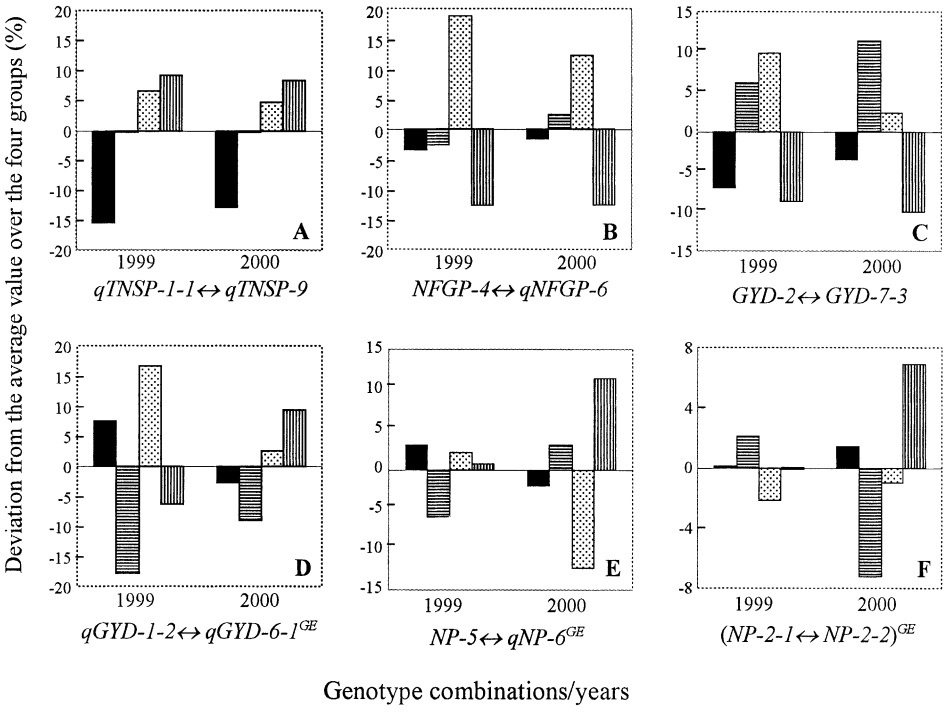

Fig. 2A–F Average phenotype values in groups classified on the basis of the genotype combinations of DNA markers linked to pairwise epistatic genes. The *superscript GE* indicates the detection of significant GE interactions for the given QTL or interaction. Genotype combinations: *solid black column* 11, *horizontally striped column* 21, *stippled column* 12, *vertically striped column* 22, where *1* is the maternal homozygote, *2* is the paternal homozygote, the *first number* is the left-side QTL, the *second number* is the right-side QTL

increasing the values of the parental and recombinant types, respectively. M_{ep}QTLs were involved in three of the interactions, including one between M_{ep}QTLs *qTGWT-5* and *qTGWT-6*, and two others between epQTLs and M_{ep}QTLs *qTGWT-1-1* and *qTGWT-5*, respectively.

Altogether, 28 QTLs were detected that exhibited significant AA effects, including 12 M_{ep}QTLs and 16 epQTLs. Of the 16 AA interactions detected, three occurred between M_{ep}QTLs, nine occurred between M_{ep}QTLs and epQTLs, and four occurred between epQTLs.

While the genetic effects and GE interaction effects that were detected contributed a considerable proportion to the phenotypic variation for traits other than NP and SF, the contribution due to AA and AAE effects was generally much smaller than the contribution due to A and AE effects. This was not only because of the lower amount of epistasis detected; it also resulted from the smaller contributions of each epistasis. The contributions of the A and AE components of a single QTL ranged from 1.4% to approximately 13.4% and from 3.5% to 12.1%, respectively, but AA and AAE components of a single epistasis only contributed 0.8% to approximately 5.4% to the phenotypic variation.

Relationships between the additive effects and AA effects of QTLs

Based on the genotype combinations of those markers mostly tightly linked to each pairwise QTLs involved in a same interactions, the RIL population was classified into four groups. By comparing the mean values of the individuals in a same group to the average value over the four groups in a same interaction, we were able to analyze the impact of AA interactions on the detection of QTLs with additive effects. Typical examples are shown in Fig. 2.

For QTLs involved in epistasis between M_{ep}QTLs exhibiting no significant GE effects, the epistatic effect was consistent in direction with the additive effects in both years. As shown in Fig. 2A, additive effects in favor of the paternal alleles were observed at *qTNSP-1-1* and *qTNSP-9* in both years, no matter which genotypes were present at the counterpart QTL. However, the additive effect of *qTNSP-9* was much smaller in the presence of the paternal genotype at *qTNSP-1-1* than in the presence of the maternal genotype at *qTNSP-1-1*. This suggested that the additive effect of *qTNSP-9* might not be detected in populations having a single genotype of the paternal homozygote at *qTNSP-1-1*, while larger additive effects would be expected in populations having a single genotype of maternal homozygote at *qTNSP-1-1*.

When an M_{ep}QTL exhibiting no significant GE effects interacted with an epQTL, it had a performance similar to *qTNSP-9*. As shown in Fig. 2B, the additive effect of *qNFGP-6* was only displayed in the presence of the paternal genotypes at *NFGP-4*. Whether the additive effect of *qNGFP-6* can be detected would thus depend on the genotype at *NFGP-4*. For epQTL *NFGP-4*, the additive effects were shown when the genotypes at *qNFGP-6* were fixed. However, the additive effects at *NFGP-4* were in favor of the paternal allele in the presence of the maternal genotype at *qNFGP-6* but in favor of the maternal allele in the presence of the paternal genotype at *qNFGP-6*. Therefore, the additive effects of *NFGP-4* could not be detected with the segre-

gation at *qNFGP-6*. The effects might be detected in populations by fixing genotype at *qNFGP-6*, but the direction would vary depending on the genotypes at *qNFGP-6*.

When an epQTL was involved in epistasis between epQTLs showing no significant GE effects, it had a performance similar to *NFGP-4*. As shown in Fig. 2C, the additive effects at *GYD-2* and *GYD-7-3* could not be detected in the undivided population because they were in the reverse directions with respect to the epistatic effects. If the genotype at one locus was fixed, then the additive effect at another locus would be detected. Obviously, whether the two QTLs exhibited significant additive effects only or significant epistatic effects only would depend on the genotype compositions of the counterpart locus.

More complicated relationships were observed when significant GE effects were detected. M_{ep}QTL *qGYD-6-1* exhibited significant AE effects, and it interacted with *qGYD-1-2* having no significant AE effects. With the shift of the maternal genotypes to paternal genotypes at *qGYD-1-2*, the additive effect of *qGYD-6-1* remained unchanged in both magnitude and direction in 1999, but it reversed in direction in 2000 while keeping similar magnitudes (Fig. 2D). Whether the additive effects of *qGYD-6-1* could be detected was related to both the genotypes at *qGYD-1-2* and the environmental conditions.

M_{ep}QTL *qNP-6* exhibited significant AE effects, and it interacted with epQTL *NP-5*. The additive effects of *qGYD-6-1* were in favor of the maternal allele in 1999 but in favor of the paternal allele in 2000 despite the genotypes at *NP-5*, but higher magnitudes were shown in the presence of the maternal genotype and paternal genotype at *NP-5* in 1999 and 2000, respectively (Fig. 2E). The additive effect of *qGYD-6-1* was much more evident in the presence of the paternal genotype at *NP-5* in 2000 than in the other three groups. The detection of the additive effect of *qGYD-6-1* varied greatly depending on the joint effects of the AA interaction and GE interaction in which *qGYD-6-1* was involved.

Epistasis between epQTLs *NP-2-1*, and *NP-2-2* was the only AA interaction showing significant AAE effects. No evidence for the interaction between *NP-2-1* and *NP-2-2* was shown in 1999 (Fig. 2F). Small additive effects were observed for *NP-2-1* and *NP-2-2* in the same year; these were similar in magnitude but reversed in direction. In 2000, the directions of the additive effects of *NP-2-1* and *NP-2-2* were reversed with the alternation of the genotypes at its counterpart locus, respectively. In addition, *NP-2-2* exhibited a strong additive effect in the presence of the paternal genotype at *NP-2-1*, although the effect was barely displayed in the presence of the maternal allele at *NP-2-1*.

Overall, the detection of QTLs with additive effects, as well as the magnitude and the direction of the additive effects, may vary greatly depending on their interactions with other loci, and the influence of the epistasis on the additive effects of QTLs may be affected by GE interactions.

Discussion

In the present study, QTLs having significant genetic effects and GE effects for six yield traits in rice were determined using an RIL population derived from the indica-indica cross ZS97B/MY46. A total of 31 QTLs with significant additive effects and 16 significant AA interactions were detected. The AA interactions detected included all three types of epistasis classified on the basis of whether the QTLs involved exhibited own main effects or not. These types were termed as epistasis between M_{ep}QTLs, epistasis between M_{ep}QTLs and epQTLs, and epistasis between epQTLs, which are equivalent to the terms of interactions between QTLs, interactions between QTLs and background loci, and interactions between complementary loci used by Li (1998).

All three types of epistasis were also reported by Yu et al. (1997), but epistasis between M_{ep}QTLs was not detected in other mapping studies for yield traits in rice (Li et al. 1997; Liao et al. 2001). In addition, M_{ep}QTLs only accounted for 13% of the QTLs involved in digenic interactions detected by Li et al. (1997), which was much smaller than the proportion of 43% found in the present study. It should be noted that inter-subspecies crosses were used in the studies of Li et al. (1997) and Liao et al. (2001), while intra-subspecies crosses were employed in our study and in the study of Yu et al. (1997). As described in the Results an epQTL can be detected as a QTL with main effects when the genotype at the other QTL it interacts with becomes fixed. A proportion of QTLs showing epistatic effects only in a more diverse background e.g, inter-subspecies crosses) might exhibit main effects in a less diverse genetic background (e.g, intra-subspecies crosses). This may be a major reason why epistasis between M_{ep}QTLs was detected in some studies but not in others, and why M_{ep}QTLs were involved in a higher proportion of digenic interactions in some studies than in others.

In a previous QTL mapping study employing an F_2 population derived from another F_1 plant of ZS97B/MY46 (Zhuang et al. 2001), it was found that the overdominance action of a number of QTLs were only displayed when the genotypes at one or more other loci were fixed to heterozygotes. Similarly, in the present study the additive effects of a number of QTLs were only displayed when the genotype at the other QTL it interacted with was fixed to a specific parental type. This could be one of the major reasons why many QTLs have been shown to be cross-specific (Mackill 1999).

Since the main effect of a QTL is measured by a deviation in trait values from the average value of the population, and epistasis between pairwise genes from that expected value is based on the main effects of the two loci (Li 1998), the main effects may be overestimated and the epistatic effects underestimated. For example, *qNFGP-6* was detected as an M_{ep}QTL, and its additive effect was enlarged and reduced by interactions with the paternal genotype and maternal genotype at *NFGP-4*,

respectively (Fig. 2B). However, the result may be interpreted in another way. QTL *qNFGP-6* alone did not have additive effects and it did not interact with the maternal genotype at *NFGP-4*; however, it did have effects when it interacted with the paternal genotype at *NFGP-4*. If this premise is true, then the AA effect would have been underestimated, while the additive effect was overestimated. Such bias might be amplified in populations having segregation at *qNFGP-6* and a fixing genotype of MY46 homozygote at *NFGP-4*. In such populations, the epistasis between *qNFGP-6* and *NFGP-4* can not be detected, whereas larger additive effects at *qNFGP-6* is expected. It is conceivable that a proportion of the QTLs would have performances similar to *qNFGP-6*. This will result in a general underestimation of the relative contributions due to epistatic effects and an overestimation of the relative contributions due to main effects.

It has been reported that whether a QTL exhibits either or both main effects and epistatic effects is influenced by GE interactions. Cao et al. (2001) found that some QTLs for plant height had both main and epistatic effects at specific developmental stages, but they had main effects or epistatic effects only at other stages. A similar alternation due to the GE interaction was also evident in the present study. For example, although *qGYD-6* was detected to have both significant additive effects and AA effects, its interaction with *qGYD-1-2* was observed in 2000 but not in 1999, and its additive effect was displayed in 1999 but not in 2000 (Fig. 2D).

In the present study, significant GE effects were observed for only 1 out of 16 significant AA interactions, while they were detected for 6 of the 31 QTLs with significant additive effects. However, the detection of significant AE effects might partially result from the presence of the AAE effects. For example, although *qGYD-6* was detected to have significant AE effects due to the presence and absence of its additive effect in 1999 and 2000, respectively, the absence of the additive effect of *qGYD-6* in 2000 was partially due to the direction alternation with the shift of the maternal genotypes to paternal genotypes at *qGYD-1-2*. Similarly, the detection of significant AE effects for *qNP-6* was partially due to the presence of a strong additive effect when the genotype at *NP-5* was fixed to the paternal type in 2000 (Fig. 2E). Similar to the detection of the genetic effects of QTLs, overestimation of AE effects and underestimation of AAE effects may have been encountered.

It may be concluded that while a substantial proportion of QTLs conditioning complex traits have both main and epistatic effects, they may display both types of effects or a single type of effect depending on the genetic background and environmental conditions. Even when a QTL is detected to have significant main effects, the magnitude and directions of the additive effects may vary due to alternations of the genotypes at other QTLs it interacts with. Similarly, the detection of the QTL-by-environment interaction for a QTL may also be related to the performance of epistasis-by-environment interaction of the epistasis in which the QTL was involved.

One of the final goals for QTL mapping studies is to employ marker-assisted selection (MAS) for varietal development. While understanding GE interaction is clearly of importance to the MAS scheme, genetic drag among traits would also have great impact on the MAS practice. In tomato, near-isogenic lines carrying the target chromosomal segment did not always have superior performance over the control line (Bernacchi et al. 1998), and genetic drag was revealed to be the major reason (Monforte and Tanksley 2000). The two clusters of QTLs on chromosome 7 detected in the present study also provide an example of genetic drag. The effect of the paternal alleles was to increase the trait values at the three QTLs in interval RZ471-RZ753 but to decrease the trait values at two other QTLs located in the adjacent interval. In other chromosomal regions in which multiple QTLs were detected, alternations in the direction of the additive effects were also commonly observed. The two possible factors for close linkage and gene pleiotropism will only be determined by using populations with larger sample sizes and with a more uniform genetic background.

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