QTL Analysis for Epistatic Effects and QTL × Environment Interaction Effects on Final Height of Rice (*Oryza sativa* L.)

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Abstract: QTLs with epistatic effects and environmental interaction effects for final height of rice were studied by mixed-model based QTL mapping with a doubled haploid population from IR64 / Azucena in four environments. The results demonstrated the importance of epistasis as a genetic basis of the quantitative traits and also revealed several important features of this phenomenon. In the results, 100 per cent of QTLs were involved in epistasis, of which 64 per cent were found with significant additive effects. This might mean that the usual estimates of the QTL additive effects could be confounded by epistatic interactions and result in biased estimation unless epistatic effect is separated. The other 36 per cent did not have any significant additive effects of their own but were involved in 48 per cent of the identified epistatic interactions. Such loci might play the role of modifying agents that tend to activate other loci or modify the action of other loci. The other features of epistasis include as follows: it was fairly common for the same locus to get involved in interactions with more than one locus; the QTLs with relatively high magnitude of effects might also be involved in epistasis; and epistasis was sensitive to environmental interactions for their expression. QTL × environment (*QE*) interaction effects were detected more often than QTL main effects for plant height behavior, as might indicate that gene expression could be greatly affected by environments.

Key words: quantitative trait locus (QTL); epistatic effects; QTL by environment interaction effects; final height of rice

Plant height of rice is generally considered to be controlled by both qualitative and quantitative genes[1]. At least 60 dwarfing genes, designated d-1 to d-60, have been identified in rice by classical genetic analysis[2]. The polygenes for quantitative behavior of plant height and its components in rice were recently mapped on molecular linkage groups[1,3~6]. In heritance of quantitative traits, gene expression could be modified by epistatic interaction with other genes and by environmental factors[7]. Studies in classical quantitative genetics have strongly suggested the importance of epistasis[8], recently QTL mapping experiments have also provided some results

regarding the importance of epistasis affecting the phenotypic behavior of quantitative traits in crop population[9~12]. Nevertheless, the epistasis for each trait aforementioned was revealed by two-way analysis of variance using all possible two-marker interactions, not the interactions between QTLs.

QTL × environment (QE) interaction is another important component affecting quantitative traits. Significant QE interactions have been reported[6,13~17] by comparing QTLs detected in specific environment. But QTL detected separately in each environment is not the real QE interaction and also could be biased. Zhu[18] proposed an indirect method to map QTLs with QE effects using predicted total genotype × environment interaction effects. It was shown that some QTLs had both genetic main effects and QE interaction effects, although they could be detected in two environments[6]. The QTL main effect is the accumulated effect expressed in the same way across different environments, while the QE interaction effect is the deviation due to specific environment. At a specific environment, the total effect of a QTL could include the main effects plus QE interaction effects at that environment.

Recently, a new methodology was proposed for directly mapping QTLs with additive and epistatic effects as well as their QE interaction based on mixed linear model approaches[19,20] and the software QTL Mapper version 1.0 was developed[20] for analyzing the experiment data. To dissect the quantitative inheritance of final height of rice, the above method was employed for detecting QTLs with additive and epistatic effects as well as their QE interaction effects in the present research. The gene expressions including additive effects, additive × additive epistatic effects and their QE interaction effects for plant height were also discussed.

1 Materials and methods

A population of 123 doubled haploid (DH) lines derived from a cross between an irrigated *indica* variety IR64 and an upland *japonica* variety Azucena[21] was used in the experiments. The genetic map of this population containing 175 markers distributed among 12 chromosomes covering 2 005 cM with an average distance of 11.5 cM between markers[22] was used for QTL mapping.

The 123 DH lines and their parents, IR64 and Azucena, were grown in a randomized complete design with two replications at both Hainan in 1995 and Hangzhou in 1996, 1997 and 1998. Hainan Island is located in the Southern China Sea at 18° North latitude while Hangzhou is located in Eastern China at ~30° North latitude. These two places show great difference in climate, soil conditions, day length, and even rice growing seasons. At Hangzhou, there were remarkable divergences of temperature, soil conditions among the three years. The experiment was conducted from early December 1995 to late April 1996 at Hainan where rice can grow well all year round. At Hangzhou, experiments were carried out from late May to early

November in 1996, 1997 and middle May to middle October in 1998. In all environments, the germinated seeds were sown in a seedling bed and the seedlings were transplanted to a paddy field 30 days later, with a single plant per hill spaced at 15 × 20cm. Each plot included three to four lines with eight plants per line. At the maturity stage, plant height (from soil surface to the tip of the highest grain excluding awn), were measured in 5 central plants of each plot.

QTLs with additive and additive × additive epistatic effects as well as their environmental interaction effects for final height of plant were mapped by the QTL mapping approach[19,20] and software of QTL Mapper version 1.0[20]. The likelihood ratio value of 11.5, which is equal to a LOD score of 2.5[23], was used as a threshold to declare the detection of QTL or epistasis.

2 Results and analysis

2.1 Transgressive segregation of phenotypic behavior

The phenotypic behavior of final height of plant for the DH population and its parents under four environments were described in Table 1. The parent Azucena was higher than IR64 in all environments. Although the mean of DH population under four environments were almost the same except that in Hangzhou 1996 showed slightly higher, wide variation occurred among DH lines and transgressive segregants were observed across all four environments with some lines higher than the tall parent Azucena, or lower than the short parent IR64. The final height of the DH population segregated continuously and both absolute values of skew and kurt were less than 1.0 (Table 1), as suggested that final height of the DH population were suitable for QTL analysis.

Table 1 Phenotypic behavior of plant height under four environments

Environment	Parents		DH population					
	IR64	Azucena	Mean	Max	Min	Stdev	Skew	Kurt
Hainan in 1995	73.7	140.1	104.3	159.9	58.6	23.70	0.26	−0.69
Hangzhou in 1996	88.1	146.6	113.5	159.0	72.0	22.50	0.04	−0.62
Hangzhou in 1997	80.9	154.4	103.8	168.4	56.9	23.77	0.25	−0.49
Hangzhou in 1998	94.2	135.4	102.9	148.3	67.4	18.52	0.28	−0.28

Note: Mean, Max, Min, Stdev, Skew and Kurt are the average, maximum, minimum, standard deviation, skew and kurt of all observations for DH lines in an environment

2.2 Quantitative trait loci for final height of plant

Altogether 22 QTLs with additive effects and/or additive × additive epistasis effects were found to be associated with final height of plant on all the 12 chromosomes (Table 2). They were named for final height as "Fh" with the chromosomal number. If there were more than one QTL in a chromosome, the serial number was added after chromosomal number separated by a hyphen. The positions of these QTLs were indicated by the marker interval bracketing the concerned QTL with the estimated distance in morgon (M) from the left marker.

Table 2 Positions of QTLs with additive effect and/or additive×additive epistasis effect for plant height

Chrom.	QTL	Marker interval	Distance(M)	Chrom.	QTL	Marker interval	Distance(M)
1	Fh1-1	RG532-W1	0.14	7	***Fh27-1***	***RG769-RZ488***	**0.16**
1	Fh1-2	RZ730-RZ801	0.22	7	Fh7-2	RG711-Est-9	0.14
2	Fh2	RG157-RZ318	0.14	7	***Fh7-3***	***CDO418-RZ978***	**0.08**
3	Fh3-1	RZ574-RZ284	0.36	8	***Fh8-1***	***A18A1120-TGMS102***	**0.12**
3	Fh3-2	Pgi-1-CDO87	0.06	8	Fh8-2	Amy3DE-RZ66	0.10
4	Fh4-1	RZ262-RG190	0.08	9	Fh9-1	RZ422-Amy3ABC	0.24
4	Fh4-2	RG163-RZ590	0.2	9	Fh9-2	RG667-RG451	0.16
5	Fh-5	RG403-RG229	0.04	10	***Fh10-1***	***G1084-RG257***	**0.06**
6	***Fh6-1***	***RZ144-RZ667***	**0.00**	10	Fh10-2	RG134-RZ500	0.02
6	***Fh6-2***	***RG424-RG162***	**0.22**	11	***Fh11***	***RG118-Adh1***	**0.00**
6	Fh6-3	RG653-Amy2A	0.10	12	***Fh12***	***Sdh1-RG463***	**0.16**

Note: QTLs with both detectable additive effects and epistatic effects were presented in regular form while the QTLs involved in epistasis but without detectable additive effects were presented in bold italic form

The 14 loci (64% of all the 22 putative loci) with both detectable additive effects and epistatic interaction effects were presented in regular form while the other eight loci (36% of all the 22 putative loci) involved in epistatic interactions but without detectable additive effects were presented in bold italic letters. The estimated additive effects and the additive × additive epistatic effects at significance level of 0.01 or 0.005 under different environments were presented in the Table 3 and Table 4, respectively.

Table 3 Additive and/or additive×environment interaction effects of QTLs across four environments

Ch-Ini	a	$ae1$	$ae2$	$ae3$	$ae4$	Ch-Ini	a	$ae1$	$ae2$	$ae3$	$ae4$
Fh1-1			1.05*	-1.65**		Fh-5		2.23**	3.11**	-4.27**	-1.07**
Fh1-2	-22.67**	-11.58**	4.70**		5.63**	Fh6-3				-2.78**	1.46**
Fh2				2.15**	-1.30**	Fh7-2		-5.18**	-2.41**	5.25**	2.34**
Fh3-1		-1.75**	-3.73**	6.74**	-1.25**	Fh8-2			2.68*		-2.82**
Fh3-2	-9.06**	2.73*	-2.38**	-11.26**	10.91**	Fh9-1	-3.29*				
Fh4-1	3.80**		3.99**			Fh9-2			-0.95**		0.94**
Fh4-2	-4.27**	3.37**			-3.45*	Fh10-2		2.48**		-4.18**	2.22**

Note: a, $ae1$, $ae2$, $ae3$, $ae4$ represent additive main effect and additive × environment interaction effect at Hainan in 1995, at Hangzhou in 1996, 1997 and 1998, respectively. * and ** represent the significance level of $P = 0.01$ and 0.005, respectively

2.3 Analysis for QTL additive effects   Fourteen QTLs with additive main effect (a) and/or additive by environment interaction effect (ae) were shown in Table 3. All the 14 QTLs were found with significant ae effects in one to four environments while only four QTLs were identified with a main effects. The additive main effects of the four QTLs ranged from 3.8 cm to − 22.67cm. The alleles decreasing plant height were from IR64 at three QTLs (Fh1-2, Fh3-2 and Fh4-2) and from Azucena at Fh4-1. This might suggest that alleles for plant height might be dispersed within the two parents. So parading of all alleles decreasing plant height from the two parents

will produce the segregants lower than the short parent. A major QTL Fh1-2 flanked by markers RZ730 and RZ801 on chromosome 1 with 22 cM away from the left marker RZ730, had additive main effect of 22.67 cm. As to QTLs with *ae* effects, Fh4-1 and Fh9-1 were detected in only one environment, while the other twelve QTLs had opposite directions of *ae* effects in two or more environments. Notably, the four QTLs (Fh1-2, Fh3-2, Fh4-1 and Fh4-2) with additive main effects had also additive by environment interaction effects. The additive main effect is the accumulated effect expressed in the same way across different environments, while the interaction effect is the deviation due to specific environment. At a specific environment, the total effect of a QTL should include the main effects plus QE interaction effects at that environment. The results of additive × environment interaction effects were obviously detected more often than additive main effects and the major QTL Fh1-2 also had environmental interaction effects, might suggest that additive by environment interaction, which caused by gene expression in spatial pattern, be an important component of genetic basis of quantitative traits.

2.4　Analysis for QTL epistatic effects　　Altogether 21 digenic epistatic interactions with epistatic main effect (*aa*) and / or epistasis by environment interaction effect (*aae*) were detected to be associated with plant height (Table 4).

Among them, 13 pairs had *aa* effects and 18 pairs had *aae* effects in one to four environments while 10 pairs had both *aa* and *aae* effects. The absolute magnitude of the effects for the detected epistatic interactions varied from 2.16cm to 5.89cm for epistatic main effects and from 0.7cm to 10.49cm for epistasis by environment interaction effects. The wider range of epistasis × environment interaction effects than that of epistasis main effects along with that likewise in additive effects, epistasis × environment interaction effects were more often detected than epistasis main effects, might indicate that although digenic interactions could have both main effects and environmental interaction effects, they were more easily subjected to environmental influence. The composition of epistatic interactions was interesting for that, although all QTLs with additive effects were engaged in digenic interactions, they were not enough to bring into being all the detected epistatic interactions. The detected interactions largely included the loci without detectable QTL additive effects. There were nine interactions (43 per cent) involved one locus with QTL additive effect and one locus without, and even one interaction was resulted completely by QTLs (*Fh7-3* and *Fh12*) without additive effects. The results might suggest that the epistatic interactions be largely due to induction of the loci without detectable QTL additive effect, as signify the importance of keeping the concept in mind that the loci without detectable QTL additive effect can also be putative QTLs when doing QTL analysis. Another noteworthy case was that it was fairly common for one locus to interact with

Table 4　Epistasis and epistasis by environment interaction effects of QTLs across four environments

QTLi	QTLj	aa	aae1	aae2	aae3	aae4
Fh1-1	Fh1-2	-5.89**	-10.49**	7.73**	-0.91*	3.67**
Fh1-1	**Fh8-1**			-3.21*		
Fh1-1	Fh9-2	-2.16*				
Fh1-2	Fh3-1	-2.69*	2.22**	-4.42**		2.60**
Fh1-2	Fh-5	-3.77**	4.18**		-4.51**	2.65*
Fh1-2	**Fh11**			1.76**		
Fh2	Fh3-2				2.15**	-2.68**
Fh2	Fh9-1			4.58**		-2.88*
Fh3-1	Fh8-2	-3.28**	3.11**			
Fh3-1	Fh10-2	-2.53*	3.32**		-7.63**	3.48**
Fh3-2	**Fh7-1**		0.70**			-1.25**
QTLi	QTLj	aa	aae1	aae2	aae3	aae4
Fh3-2	Fh8-2	3.12*	1.73**	-2.95*		
Fh3-2	Fh9-1				-1.24**	1.88**
Fh3-2	**Fh11**				3.76**	
Fh4-1	**Fh10-1**			1.23**		
Fh4-2	**Fh6-1**	2.50**	1.18**			
Fh6-2	Fh6-3	2.37**				
Fh7-2	**Fh8-1**	2.26*				
Fh7-2	**Fh9-1**	4.52**	-3.14*			
Fh7-3	**Fh12**	-2.63*	-1.83**	1.45*		
Fh8-2	Fh9-1	-3.46*			-2.33**	2.29**

Note: *aa*, *aae*1, *aae*2, *aae*3, *aae*4 represent epistatic main effect and epistasis × environment interaction effect at Hainan in 1995, at Hangzhou in 1996, 1997 and 1998, respectively. * and * * represent the significance level of $P = 0.01$ and 0.005, respectively

more than one non-allelic locus. In the present study, the 21 interactions were composed of 22 interacting loci with 10 loci (45 per cent) being involved in more than one distinct interaction. This might indicate the possibility of multi-locus associations for the trait development.

3　Discussion

Both epistatic interaction effects and QTL × environment interaction effects are important components of genetic basis. But many of researches have been based on models assuming neither epistatic effects nor *QE* interaction effects due to lacking of valid statistical method. To infer epistasis between QTLs, interaction effects between molecular markers were widely assayed by two-way analysis of variance[10~12]. But this method usually cannot give unbiased estimation for QTL parameters. For indicating the possibility of *QE* interaction, QTL mapping results in different environments were simply compared[13~17]. Here we adopt mixed model approach based QTL mapping to detect QTLs with epistasis and *QE* interaction and estimate their effects. The study demonstrated the importance of epistasis as a genetic basis of the quantitative traits and also revealed several important features of this phenomenon.

Partitioning of epistasis from other genetic components of variation would, no doubted, help to obtain more reliable estimates of the QTL effects. Moreover, consideration of epistasis in the QTL analysis would enhance our understanding about the inheritance of quantitative traits.

In the present study, 100 per cent of the QTLs were involved in epistasis, of which, 64 per cent were found with significant additive effects. This means that the usual estimates of the QTL effects could be confounded by epistatic interactions and resulted biased estimation unless epistatic effect is separated. In fact, the actual genetic effect of many QTLs are reasonably dependent on other loci. The other 36 per cent of the loci involved in epistasis did not have any significant additive effects of their own and 48 per cent of the identified epistatic interactions were restricted to such loci. Successful detection of significant epistatic effects resulting from QTLs without additive effects indicated that many loci even without significantly affecting the traits on their own could still affect the trait in combination with other loci. Such loci might play the role of modifying agents which tend to activate other loci or modify the action of other loci.

Because, it was fairly common for the same locus to get involved in interactions with more than one locus, the real genetic effect of any locus would be necessarily different from genotype to genotype due to involvement of different interacting loci. This was alike that the interaction between QTL and background or modifying loci might be prevalent epistasis affecting the behavior of quantitative traits[9,12]. Futhermore, as interactions were observed to be greatly affected by environments, the contribution of any locus to the trait, should also vary according to the growing environment.

In quantitative genetics, the trait behavior is resulted from the combined effects of many genes under different environments[7]. Usually, *QE* effects are treated as random effects especially in different years. They imply the extents that QTLs would be affected by unknown environments. At a specific environment, the total effects of a QTL should include all the genetic main effects and *QE* interaction effects at that environment. It was implied, by the fact of some QTLs having only *QE* effects, that gene expression of these QTLs could be mainly induced by environments.

In the present study, a major QTL Fh1-2 with a distance of 22 cent Morgan to marker RZ730 for plant height were located very near the reported location of the qualitative semi-dwarfing gene *sd*-1, thus indicating some relationship of this gene with the detected QTL. The *indica* parent IR64 is known to have inherited the semi-dwarfing gene, *sd*-1[24]. The QTL Fh1-2 were identified in the position reported to have been occupied by *sd*-1 and moreover, the IR64 allele in this QTL has been observed to significantly reduce plant height. Thus, it might be apparent that Fh1-2 represent the *sd*-1 gene. However, significant epistasis and *QE* interaction effects were

also found at this locus. It was suggested that major gene or QTL could also interact with other genes under different environments.

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水稻株高上位性效应和 QE 互作效应的 QTL 遗传研究

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摘要：利用基于混合模型的QTL定位方法研究了由籼稻品种 IR64 和粳稻品种 Azucena 杂交衍生的 DH 群体在 4 个环境中的 QTL 上位性效应和环境互作效应。结果表明，上位性是数量性状的重要遗传基础，并揭示了上位性的几个重要特点。所有的 QTL 都参与了上位性效应的形成，64% 的 QTL 还具有本身的加性效应。因此传统方法对 QTL 加性效应的估算会由于上位性的影响而有偏。其他 36% 的 QTL 没有本身的加性效应，却参与了 48% 的上位性互作，这些位点可能通过诱发和修饰其他位点而起作用。上位性的特点还包括，经常发现一个 QTL 与多个 QTL 发生互作；大效应的 QTL 也参与上位性互作；上位性互作受环境影响。QTL 与环境的互作效应比 QTL 的主效应更多地被检测到，表明数量性状基因的表达易受环境影响。

关键词：数量性状位点；上位性效应；环境互作效应；水稻株高

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