

QTL Analysis for Flag Leaf Length in a Rice DH Population under Multi Environments

CAO Gang-Qiang¹, GAO Yong-Ming², and ZHU Jun³

(¹ Department of Bioengineering, Zhengzhou University, Zhengzhou 450052, Henan; ² Institute of Crop Science, Chinese Academy of Agricultural Sciences, The National Key Laboratory for Crop Gene Resources and Genetic Improvement, Beijing 100081; ³ Department of Agronomy, Zhejiang University, Hangzhou 310029, Zhejiang, China)

Abstract: A double haploid rice population from IR64/Azucena was used to dissect the quantitative inheritance of flag leaf length. The QTL mapping method based on mixed linear model approaches was employed for detecting QTLs with additive and epistatic effects as well as their *QE* interaction effects in 4 years. The results revealed that altogether 18 QTLs for leaf length were detected on 11 chromosomes, and all of them were involved in epistasis. Three QTLs were involved in epistasis but without detectable additive effects, such loci might play the role of modifying agents that tend to activate other loci or modify the action of other loci. The same locus could get involved in interactions with more than one other locus, as might indicate the possibility of multi-QTL associations in the formation of complex traits. QTL and epistasis could have both genetic main effects that expressed stably in every environment and *QE* interaction effects. Some QTLs had *QE* effects under multi-environments but without genetic main effect, as might suggest expression of this kind of QTLs could be modified by environmental factors easily. And also some QTLs had only genetic main effect, so would not easily be affected by environment. Epistasis had wider range of epistasis × environment effects than that of main effects, might indicate that some digenic interactions were more easily subjected to environmental influence. Compared with indirect mapping method with *QE* effects but without epistatic effects, more QTLs were detected in the present study, but no major QTL was found. The *QE* effects were treated as random effects, so the sum of *QE* effects would be zero in theory, and multi environments were suitable for estimating genetic main effects better.

Keywords: Quantitative trait locus (QTL); Epistatic effects; QTL by environment interaction effects; Flag leaf length of rice

多环境下水稻 DH 群体剑叶长度的 QTL 分析

曹刚强¹ 高用明² 朱 军³

(¹ 郑州大学生物工程系, 河南郑州 450052; ² 中国农业科学院作物科学研究所, 国家农作物基因资源与基因改良重大科学工程, 北京 100081;

³ 浙江大学农学系, 浙江杭州 310029)

摘 要: 种植由籼稻品种和粳稻品种杂交衍生的 DH 群体, 连续 4 年测定剑叶长度, 运用基于混合模型的复合区间作图法, 定位其 QTL 及上位性互作, 估算遗传主效应和环境互作效应。结果表明, 全部 18 个 QTL 都参与了上位性的形成, 其中 3 个没有自身的遗传效应, 但参与了 3 对上位性互作, 这是传统方法不能发现的。另外, 一个 QTL 可与多个 QTL 发生互作, 这可能预示着存在更高阶互作。QTL 与上位性互作可以具有不受环境影响而稳定表达的效应, 以及与环境的互作效应。有些 QTL 与环境的互作效应可以在多环境下被检测到, 但却不具有主效应, 这种 QTL 可能易受环境因子的影响。QTL 与环境的互作效应为随机效应, 一个 QTL 或一对上位性与环境的互作效应总和理论上应等于零, 否则会影响对遗传效应的估算, 因此多环境下估算的遗传效应更可靠。

关键词: 数量性状位点; 上位性效应; 环境互作效应; 水稻剑叶长度

Flag leaf is very important for grain production in rice and is genetically controlled by quantitative genes.

Foundation item: Supported by the National Natural Science Foundation of China(39893354).

Biography: CAO Gang-Qiang(1972-), male, PhD, research field: crop genetics and breeding. E-mail: caogq@zsu.edu.cn

Received(收稿日期): 2006-02-16; Accepted(接受日期): 2006-07-04.

The advent of molecular genetic maps has greatly facilitated the study of complex quantitative traits and made it possible to dissect the polygenes for such traits into individual mendelian factors. To analyze the genetic background of flag leaf, genetic analysis and quantitative trait locus (QTL) mapping has been conducted, some QTLs and their effects were revealed in one environment^[1-3]. But in heritance of quantitative traits, gene expression could be modified by epistatic interaction with other genes and by environmental factors^[4-12]. Because double haploid (DH) population is stable in genotype, it can be used in different area or different environment to study gene and environment interactions. A double haploid population from IR64/Azucena was used in QTL mapping on many traits including developmental traits, panicle traits, and leaf traits^[13-17]. According to IIRRI (<http://www.iris.irri.org:8080/drought/map-detail.html>), in this population, at least hundreds of QTLs were mapped, among them 205 and 96 QTLs were for plant height and tiller number according to the QTL analysis on developmental behavior of these two traits. But most of these QTL studies have been based on models assuming no epistatic effects or *QE* interaction effects due to lacking of statistical method. With this population, an indirect method was used to map QTLs with their QTL by environment (*QE*) interaction effects but without epistatic effects on flag leaf length, using predicted total genotype \times environment interaction effects^[18]. To dissect the quantitative inheritance of flag leaf in rice in detail, the QTL mapping method based on mixed linear model approaches and the software QTL Mapper^[19-20] were employed for detecting QTLs with additive and epistatic effects as well as *QE* effects.

1 Materials and Methods

A population of 123 double 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 on 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 cm \times 20 cm. Each plot included four lines with eight plants per line. At the maturity stage, flag leaf lengths of six central plants in each plot were measured.

QTLs as well as their environmental interaction effects were mapped by the mixed model based QTL mapping approach and software of QTL Mapper^[19-20]. 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 leaf length (LI)

The phenotypic behavior of leaf length for the DH population and its parents under four environments was described in Table 1. Leaf length (LI) of parent Azucena was larger than that of IR64 in all environments. Wide variation from maximum to minimum values occurred among DH lines across all four environments. But also the population segregated continuously like normal distribution, the absolute values of skew and kurt were less than 1.0, as suggested that the DH population was suitable for QTL analysis.

Table 1 Phenotypic behavior of flag leaf length under four environments

Environment	Parents		DH population					Skew	Kurt
	IR64	Azucena	Mean	Max	Min	Stdev			
Hainan in 1995	17.1	25.9	22.81	37.8	13.4	5.06	0.42	-0.08	
Hangzhou in 1996	29.3	34.7	33.44	54.3	19.0	7.54	0.55	-0.29	
Hangzhou in 1997	32.8	46.9	38.58	61.0	24.9	6.59	0.45	0.61	
Hangzhou in 1998	30.5	35.6	34.70	52.4	23.9	6.04	0.53	-0.35	

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

2.2 Quantitative Trait Loci for LI

Altogether 18 QTLs for leaf length with additive effects and/or additive \times additive epistasis effects were found on 11 chromosomes of all the 12 chromosomes (Table 2, Fig.1). They were named for leaf length as "LI" 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. In Table 2, three QTLs presented in bold italic letters were involved in epistasis but without detectable additive effects, while the other 15 QTLs with both detectable additive effects and epistatic interaction effects were presented in regular form. The estimated additive effects and the additive \times additive epistatic effects at significance level of 0.01 or 0.005 under different environments were presented in Table 3 and Table 4, respectively.

Table 2 Positions of QTLs with additive effect and/or additive \times additive epistasis effect for flag leaf length

Chrom.	QTL	Marker interval	Distance(M)
1	LI1-1	RC246 - K5	0.12
	LI1-2	RZ730 - RZ801	0.08
2	LI2-1	RG437 - RG544	0.04
2	LI2-2	RZ123 - RC520	0.04
3	LI3-1	RZ394 - pRD10A	0
3	LI3-2	RC910 - RC418A	0
4	LI4-1	RG218 - RZ262	0
4	LI4-2	RG163 - RZ590	0.02
5	LI5-1	RZ556 - RC403	0.12
5	LI5-2	RZ70 - RZ225	0.18
6	LI6-1	RZ667 - Pgi. 2	0
6	LI6-2	Amy2A - RG433	0
7	LI7-1	RG511 - RG477	0.08
7	LI7-2	CDO418 - RZ978	0.02
8	LI8	A5J560 - A3E396	0
9	LI9	RZ422 - Amy3ABC	0.04
10	LI10	RZ625 - CDO93	0.06
11	LI11	RG1094 - RG167	0

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

QTL	a	ae1	ae2	ae3	ae4
LI1-1	-1.10**	1.22**			
LI1-2	-2.94**				
LI2-1	1.05**				
LI2-2	0.95**				
LI3-2	-1.43*	-0.97*			
LI4-1		-0.68**	1.97**		-1.27**
LI4-2	-1.16**				-0.43*
LI5-1		2.16**		-2.47**	
LI6-1				-0.31*	
LI6-2	1.21**	1.78**	-0.77**	-1.04**	
LI7-1		-0.19*	-0.96**	-1.23**	2.37**
LI7-2				1.21*	
LI9	-1.20**		-1.55**		
LI10		0.31*		-0.35**	
LI11				-0.37**	

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

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

QTLi	QTLj	aa	aae1	aae2	aae3	aae4
LI1-1	LI3-1		-0.32**			
LI1-2	LI7-2			1.36*		-1.39*
LI1-2	LI9	1.21**		-0.53*		0.61**
LI2-1	LI4-1			-0.53**		
LI2-1	LI6-1	1.34**		1.21*		
LI2-2	LI6-1	-0.83*	1.04**	-1.56**		
LI2-2	LI6-2	0.85**	0.68*		-0.79**	0.45*
LI2-2	LI7-1			0.46**		
LI3-2	LI4-2					1.10**
LI3-2	LI6-1	-0.88**				
LI3-2	LI8	-1.10*				
LI3-2	LI10	-1.43**	0.73**			
LI4-1	LI4-2				0.40**	
LI4-2	LI9	-1.18**				-1.08**
LI5-1	LI11	-1.23**	1.52**	-1.62**		
LI5-2	LI9				0.50**	-0.83**
LI7-1	LI11	0.74**				
LI9	LI10	1.12**	-1.13*			

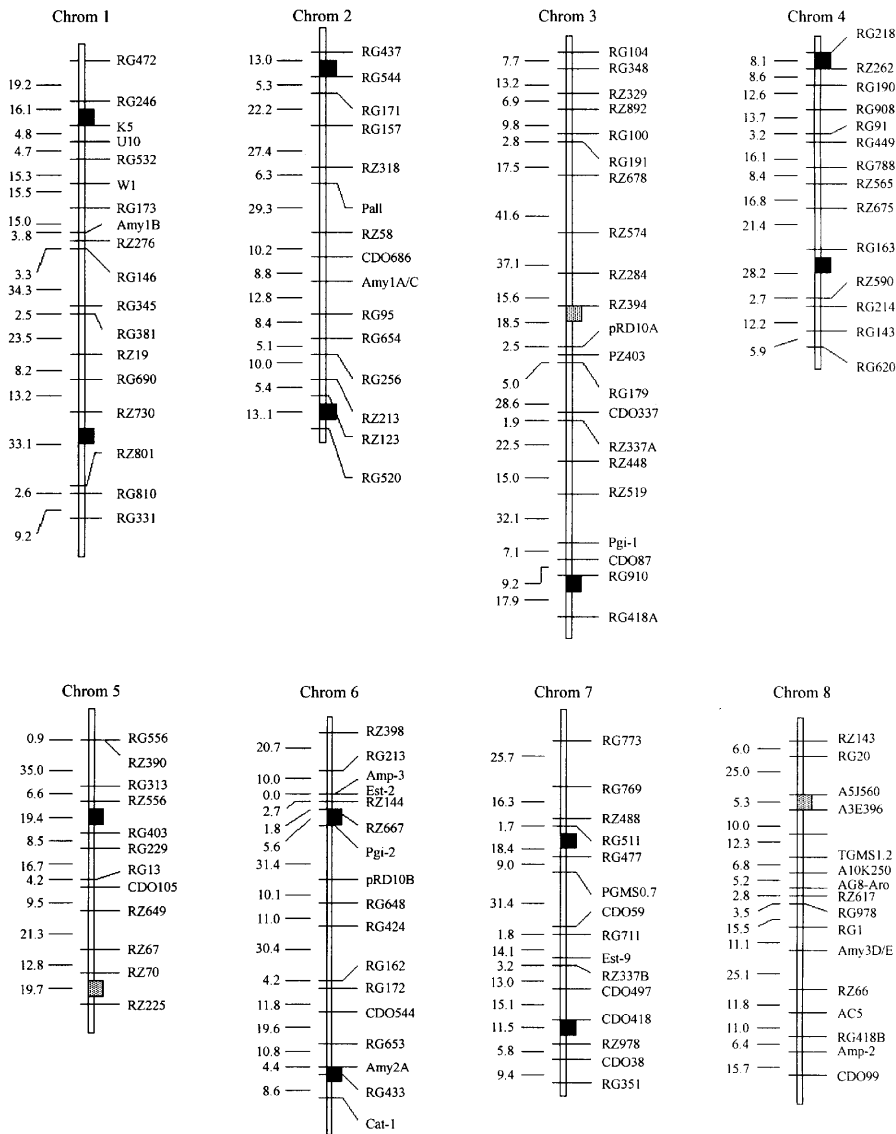
aa, aae1, aae2, aae3, and aae4 represent epistatic main effect and epistasis \times environment interaction effect at Hainan in 1995, at Hangzhou in 1996, 1997, and 1998, respectively. * and ** represent the significance at $P = 0.01$ and 0.005 , respectively.

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.

2.3 Analysis for QTL Additive Effects

Fifteen QTLs with additive main effect (a) and/or

additive by environment interaction effect (ae) were shown in Table 3. There were only five QTLs with both a and ae effects, seven QTLs with only ae effects in one to four environments and three QTLs with only a effect. As to QTLs' a effects, five QTLs had contribution to decreasing leaf length and three QTLs to increasing it. As



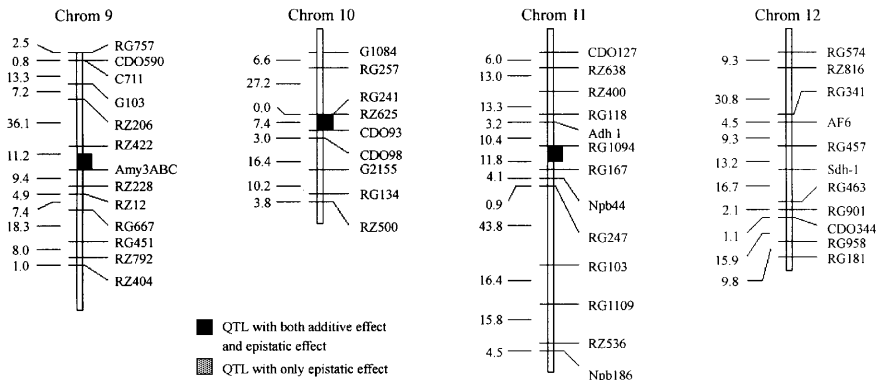


Fig. 1 Positions of QTLs with additive effect and/or additive \times additive epistasis effect for flag leaf length

to QTLs' *ae* effects, usually QTLs had opposite directions of *ae* effects in two or more environments. The additive main effect *a* is the accumulated effect expressed in the same way across different environments, while the interaction effect *ae* 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 *a* effect of QTL LI1-2 reached maximum absolute value 2.94 cM, and *ae* effect from QTL LI5-1 reached maximum absolute value 2.47 cM in 1997. Maybe the environment in 1997 could influence the QTL LI5-1 greatly. The fact that *ae* effects were obviously more often detected than *a* effects, might also suggest for quantitative traits, gene expression could be modified by environmental factors.

2.4 Analysis for QTL epistatic effects

Altogether 18 digenic epistatic pairs with epistatic main effect (*aa*) and/or epistasis by environment interaction effect (*aac*) were detected (Table 4). Among them, eight pairs had both *aa* and *aac* effects, while three pairs had only *aa* effects and seven pairs had only *aac* effects. The maximum absolute magnitude of *aa* and *aac* effects reached 1.43 cM and 1.62 cM, respectively.

The wider range of epistasis \times environment interaction effects than that of epistasis main effects, and epistasis \times environment interaction effects were more often detected than epistasis main effects, might indicate that some digenic interactions were more easily subjected to environmental influence.

It was interesting that the detected pairs included three QTLs without detectable *a* or *ae* effects (notified in bold italic form in Table 4). The role of this kind of QTL might be only regulating other QTL. Another noteworthy case was that it was fairly common for one QTL to interact with more than one QTL. This also indicated the possibility of multi-QTL associations in the formation of complex traits.

2.5 General contributions of QTL effects

In above, four kinds of QTL effects were analyzed in detail. But can they explain all the variation of the trait or can they completely control the formation of flag leaf length by detected QTLs? So general contributions of four kinds of QTL effects were calculated and presented in Table 5. The total contribution of detected QTLs reached only 0.4268, it is far from 100%.

Table 5 General contributions of four kinds of QTL effects

Kind of QTL effect	<i>a</i>	<i>ae</i>	<i>aa</i>	<i>aac</i>	Total
General contribution	0.0953	0.0852	0.1486	0.0977	0.4268

3 Discussion

IR64/Azucena is a widely used double haploid population in QTL mapping research. For the trait of flag leaf length, Yan et al. used an indirect method to map QTLs with *QE* effects using predicted total genotype \times

environment interaction effects, assuming no epistasis^[18], found seven QTLs with additive effect and/or additive \times environment effect. To compare with Yan's study, the same likelihood ratio value of 11.5 was used as a threshold to declare the detection of QTL or epistasis. Fifteen QTLs with additive effect and/or additive \times

environment effect were detected, of them six QTLs were located in the same marker interval as Yan's QTLs. The results of usual QTL mapping assuming no epistasis could be confounded by real epistatic interactions, so fewer QTLs were found with assuming no epistasis because the real epistasis would interfere with the detection of QTLs. Partitioning of epistasis from other genetic components of variation might help to obtain more reliable estimates of QTL. Therefore, the methodology based on mixed linear model approaches^[19-20], may be more reasonable for the capability to detect more QTLs by considering epistasis.

In the present study, all of the QTLs were involved in epistasis. And three loci involved in epistasis did not have any significant additive or additive \times environment effects of their own. Successful detection of this kind of QTLs without additive or additive \times environment effects indicated that some loci could affect the trait in combination with other loci, playing the role of modifying agent that tends to activate other loci or modify the action of other loci. It was common for the same locus to get involved in interactions with more than one other locus, as might indicate that more complicated multi-QTL interactions exist during the formation of quantitative traits.

QTL \times environment (*QE*) interaction is also an important component affecting quantitative traits. Significant *QE* interactions have been reported^[9,12] by comparing QTLs detected in specific environment. It was suggested that a QTL detected in one environment but not in another might indicate QTL \times environment interaction. However, in reality, even in the absence of true QTL \times environment interaction, a QTL can be detected in one environment but not in another, because the chance of simultaneous detection in both environments is naturally small. On the other hand, consistency in detection of QTLs at different environments may not conclusively indicate the absence of QTL \times environment interaction. Therefore, it is not possible to fully explore the *QE* interaction only by comparing QTLs detected in different environments separately.

Yan et al. used an indirect method to map QTLs with *QE* effects using predicted total genotype \times environment interaction effects. It was shown that some QTLs could be detected in two environments, but also had *QE* interaction effects^[18]. 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 should include the main effects plus *QE* interaction effects at that environment. The mixed-model-based composite interval

mapping (MCIM) approach allows to directly detect QTL \times environment interaction^[19,20]. In this approach, *QE* effects were treated as random effects, so the sum of *QE* effects of a QTL would be zero in theory. If there were only two environments, the *QE* effects of a QTL would have similar values but with different directions. Since the *QE* effects were treated as random effects, what is the use to estimate the *QE* effects? For practical use, the genetic main effect may be more useful. Only after partition of *QE* effect, the genetic main effect could be estimated validly.

In present results, with considering epistasis many QTLs even some without their own effects were detected, and genetic main effects and/or *GE* effects of QTLs and epistasis were also estimated using the mixed-model-based composite interval mapping (MCIM) approach, but no major QTL was found. So we might have got more reasonable results, but how to continue to use the results in practice seemed more difficult in the recent future.

4 Conclusion

In the present study, altogether 18 QTLs for leaf length with *QE* effects and/or epistasis effects were detected on 11 chromosomes of all the 12 chromosomes. Among them, 15 QTLs had additive main effect (*a*) and/or additive by environment interaction effect (*ae*), while 3 QTLs were involved in epistasis but without detectable additive effects. As to epistasis, altogether 18 digenic epistatic pairs with epistatic main effect (*aa*) and/or epistasis by environment interaction effect (*ae*) were detected. Through the result analysis, we found that *QE* effects and epistatic effects were both important in QTL mapping of the studied quantitative trait.

References

- [1] Li S-G(李仕贵), He P(何平), Wang Y-P(王玉平), Li H-Y(黎汉云), Chen Y(陈英), Zhou K-D(周开达), Zhu L-H(朱立煌). Genetic analysis and gene mapping of the leaf traits in rice (*Oryza sativa* L.). *Acta Agron Sin* (作物学报), 2000, 26(3): 261-265 (in Chinese with English abstract)
- [2] Zhong D-B(钟代彬), Luo L-J(罗利军), Mei H-W(梅捍卫), Guo L-B(郭龙彪), Wang Y-P(王一平), Yu X-Q(余新桥), Ying C-S(应存山), Li Z-K(黎志康). Mapping QTLs for total leaf number of the main stem and its related traits in rice. *Chin J Rice Sci* (中国水稻科学), 2001, 15(1): 7-12 (in Chinese with English abstract)
- [3] Wang Y-P(王一平), Zeng J-P(曾建平), Guo L-B(郭龙彪), Xing Y-Z(邢永忠), Xu C-G(徐才国), Mei H-W(梅捍卫), Ying C-S(应存山), Luo L-J(罗利军). QTL and correlation analysis on characters of top three leaves and panicle weight in rice (*Oryza sativa* L.). *Chin J Rice Sci* (中国水稻科学), 2004, 19(1): 13-20 (in Chinese with English abstract)

- [4] Atchley W R, Zhu J. Developmental quantitative genetics, conditional epigenetic variability and growth in mice. *Genetics*, 1997, 147: 765-776
- [5] Doehle J, Stec A, Gustus C. *Teosinte branched1* and the origin of maize: evidence for epistasis and the evolution of dominance. *Genetics*, 1995, 141: 333-346
- [6] Lark K G, Chase K, Adler F R, Mansur L M, Orf J H. 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*, 1995, 92: 4656-4660
- [7] Li Z K, Pinson S R M, Park W D, Paterson A H, Stansel J W. Epistasis for three grain yield components in rice (*Oryza sativa* L.). *Genetics*, 1997, 145: 453-465
- [8] Yu S B, Li J X, Xu C G, Tan Y F, Gao Y J, Li X H, Zhang Q, SaghaiMaroof M A. Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. *Proc Natl Acad Sci USA*, 1997, 94: 9226-9231
- [9] Veldboom L R, Lee M. Genetic mapping of quantitative trait loci in maize in stress and non-stress environments: I. grain yield and yield components. *Crop Sci*, 1996, 36: 1310-1319
- [10] Veldboom L R, Lee M. Genetic mapping of quantitative trait loci in maize in stress and nonstress environments: II. Plant height and flowering. *Crop Sci*, 1996, 36: 1320-1327
- [11] Lu C, Shen L, Tan Z, Xu Y, He P, Chen Y, Zhu L. Comparative mapping of QTL for agronomic traits of rice across environments using a doubled haploid population. *Theor Appl Genet*, 1996, 93: 1211-1217
- [12] Zhuang J Y, Lin H X, Lu J, Qian H R, Hittalmani S, Huang N, Zheng K L. Analysis of QTL \times environment interaction for yield components and final height of rice. *Theor Appl Genet*, 1997, 95: 799-808
- [13] Yan J Q, Zhu J, He C X, Benmoussa M, Wu P. Quantitative trait loci analysis for the developmental behavior of tiller number in rice (*Oryza sativa* L.). *Theor Appl Genet*, 1998, 97: 267-274
- [14] Yan J Q, Zhu J, He C X, Benmoussa M, Wu P. Molecular dissection of developmental behavior of plant height in rice (*Oryza sativa* L.). *Genetics*, 1998, 150: 1257-1265
- [15] Cao G Q, Zhu J, He C X, Cao Y M, Wu P. Impact of epistasis and QTL \times environment interaction on the developmental behavior of plant height in rice (*Oryza sativa* L.). *Theor Appl Genet*, 2001, 103: 153-160
- [16] He C-X(何慈信), Zhu J(朱军), Yan J-Q(严菊强), Benmoussa M, Wu P(吴平). QTL mapping for developmental behavior of leaf sheath height in rice. *Chin J Rice Sci* (中国水稻科学), 2000, 14(4): 193-198(in Chinese with English abstract)
- [17] He C-X(何慈信), Zhu J(朱军), Yan J-Q(严菊强), Benmoussa M, Wu P(吴平). QTL mapping for developmental behavior of panicle dry weight in rice. *Sci Agric Sin* (中国农业科学), 2000, 33(1): 1-9 (in Chinese with English abstract)
- [18] Yan J Q, Zhu J, He C X, Benmoussa M, Wu P. Molecular marker-assisted dissection of genotype by environment interaction for plant type traits in rice (*Oryza sativa* L.). *Crop Sci*, 1998, 39: 538-544
- [19] Zhu J. Mixed model approaches of mapping genes for complex quantitative traits. *J Zhejiang Univ* (Nat Sci) (浙江大学学报·自然科学版), 1999, 33(3): 327-335(in Chinese with English abstract)
- [20] Wang D L, Zhu J, Li Z K, Paterson A H. Mapping QTLs with epistatic effects and QTL \times environment interactions by mixed linear model approaches. *Theor Appl Genet*, 1999, 99: 1255-1264
- [21] Guiderdoni E, Galinato E, Luistro J, Vergara C. Anther culture of tropical *japonica/indica* hybrids of rice (*Oryza sativa* L.). *Euphytica*, 1992, 62: 219-224
- [22] Huang N, Pareo A, Mew T, Magpantay G, McCouch S, Guiderdoni E, Xu J, Subudhi P, Angeles, E R, Khush G S. RFLP mapping of isozymes, RAPD and QTL for grain shape, brown plant hopper resistance in a doubled haploid rice population. *Mol Breed*, 1997, 3: 105-113
- [23] Zeng Z B, Weir B S. Statistical methods for mapping quantitative trait loci. *Acta Agron Sin* (作物学报), 1996, 22: 535-549