QTLs for Flag Leaf Area of Rice under Multi Environments

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Abstract: QTLs with epistatic effects and environmental interaction effects for flag leaf area of rice were studied by mixed-model based QTL mapping with a doubled haploid population from IR64/Azucena in four years. The results revealed that many QTLs were involved in epistasis, and the same locus could get involved in interactions with more than one other locus. Such loci might play the role of modifying agents that tend to activate other loci or modify the action of other loci. QTL by environment (*QE*) interaction effects were detected more often than QTL main effects for flag leaf area, as might indicate that gene expression could be greatly affected by environmental factors for this quantitative trait. [Life Science Journal. 2006;3(2):79-82] (ISSN: 1097-8135).

Keywords: quantitative trait locus; epistatic effects; QTL by environment interaction effects; flag leaf area of rice

Abbreviations: DH: double haploid; QE: QTL × environment; QTL: quantative trait locus; LA: leaf area

1 Introduction

Flag leaf area is very important for grain production in rice and is genetically controlled by quantitative genes. So 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]. To dissect the quantitative inheritance of flag leaf area in rice, the QTL mapping method based on mixed linear model approaches and the software QTLMapper[5,6] were employed for detecting QTLs with additive and epistatic effects as well as their QTL by environment (*QE*) interaction effects.

2 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[7] were used in the experiments. The genetic map of this population containing 175 markers distributed among 12 chromosomes covering 2005 cm with an average distance of 11.5 cm between markers[8] 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 of China in 1995 and Hangzhou of China 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 about 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 × 20 cm. Each plot included four lines with eight plants per line. At the maturity stage, length and width of flag leaf of 6 central plants in each plot were measured. The flag leaf area (LA) was calculated according to Yoshida[9] et al: Leaf area = Leaf length × Leaf width × 0.725.

QTLs as well as their environmental interaction effects were mapped by the mixed model based QTL mapping approach and software of QTLMapper[5,6]. The likelihood ratio value of 11.5, which is equal to a LOD score of 2.5[10], was used as a threshold to declare the detection of QTL or epistasis.

3 Results and Analysis

3.1 Transgressive segregation of leaf area

The phenotypic behavior of leaf area for the DH population and its parents under four environments were described in Table 1. Leaf area (LA) 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, both skew and kurt values were less than 1.0, as suggested that the DH population were suitable for QTL analysis.

Table 1. Phenotypic behavior of flag leaf area under four environments

Environment	Parents		DH population					
	IR64	Azucena	Mean	Max	Min	Stdev	Skew	Kurt
Hainan in 95	14.9	30.1	27.97	51.42	11.66	8.05	0.34	−0.33
Hangzhou in 96	29.7	52.5	42.49	80.96	17.12	13.4	0.47	−0.43
Hangzhou in 97	32.9	57.1	45.13	70.86	20.67	10.2	−0.01	−0.82
Hangzhou in 98	31.0	37.3	32.26	55.00	21.33	5.88	0.72	0.59

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 one environment.

3.2 Quantitative trait loci for LA

Altogether 15 QTLs for leaf area with additive effects and/or additive × additive epistasis effects were found on 10 chromosomes of all the 12 chromosomes (Table 2). They were named for leaf area as "La" with the chromosomal number.

Table 2. Positions of QTLs with additive effect and/or additive × additive epistasis effect for flag leaf area

Chrom.	QTL	Marker interval	Distance(M)
1	La1 − 1	RG246 − K5	0.1
1	La1 − 2	RZ730 − RZ801	0.08
2	***La2 − 1***	***RG157 − RZ318***	***0.14***
2	La2 − 2	RZ123 − RG520	0.1
3	La3 − 1	RG348 − RZ329	0
3	*La*3 − 2	*RZ*403 − *RG*179	0.04
3	La3 − 3	CDO87 − RG910	0.02
4	La4	RZ590 − RG214	0
5	La5	RZ70 − RZ225	0.18
6	La6 − 1	RZ667 − Pgi-2	0
6	La6 − 2	Amy2A − RG433	0.02
7	La7	PGMS007 − CDO59	0.04
9	La9	RZ228 − RZ12	0
10	La10	RG134 − RZ500	0
12	La12	CDO344 − RG958	0

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, and the QTLs with only additive effects but no epistatic effects were notified with underling lines.

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

QTL	*a*	*ae1*	*ae2*	*ae3*	*ae4*
La1 − 1	−1.75**	1.94**		−1.78**	
La1 − 2		−1.17**	−0.95**	3.87**	−1.75**
La2 − 2		1.44**	0.68**	−2.76**	0.64**
La3 − 1		1.61**	−2.79**	2.77**	−1.60**
La3 − 3	−1.63**				
La4	−4.91**		−2.55**	−1.29*	2.78**
La5	−2.01**		−2.76**		1.95**
La6 − 1		0.72**	−4.58**	1.35**	2.52**
La6 − 2	1.38**		3.72*		
La7	1.90**	−3.44**	3.32**		
La9					0.51**
La10		−0.35*			
La12			−0.82**		

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 $P = 0.005$, respectively.

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. The 11 QTLs with both detectable additive effects and epistatic interaction effects were presented in regular form, while the 2 QTLs involved in epistatic interactions but without detectable additive effects were presented in bold italic letters, and the other 2 QTLs with only additive

effects but no epistatic effects were notified with underling lines. 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 4. Epistasis and epistasis by environment interaction effects of QTLs across four environments.

QTLi	QTLj	*aa*	*aae1*	*aae2*	*aae3*	*aae4*
La1-1	La1-2		0.47**			
La1-1	La6-1			0.62**		
La1-1	La10	1.15*				
La1-2	***La3-2***	1.03**				
La1-2	La5		0.37*			
La1-2	La7		-0.51*			
La2-1	La2-2	1.10*				
La2-1	La12		-2.33**	3.57**	-5.16**	3.93**
La2-2	La6-1	-1.38*				
La2-2	La7		0.54**	-2.01**	-1.34**	2.81**
La3-3	La4		1.21**	-1.50**		
La3-3	La6-1	-2.11**	1.28*	-3.48**		1.94**
La3-3	La9	-1.41**	5.12**	-3.19**	-1.92**	
La9	La10	1.39**				

Note: *aa*, *aae1*, *aae2*, *aae3*, *aae4* 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 $P=0.005$, respectively.

3.3 Analysis for QTL additive effects

13 QTLs with additive main effect (*a*) and / or additive by environment interaction effect (*ae*) were shown in Table 3. In them, 5 QTLs had both *a* and *ae* effects, while 7 QTLs with only *ae* effects in one to four environments and 1 QTL with only *a* effect. As to QTLs' *a* effects, 4 QTLs had contribution to decreasing leaf area and 2 QTLs to increasing. As to QTLs' *ae* effects, usually QTLs had opposite directions of *ae* effects in two or more environments. The additive main effect *a* was the accumulated effect expressed in the same way across different environments, while the interaction effect *ae* was 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 La4 reached maximum absolute value 4.91 cm^2, and *ae* effect from QTL La6 - 1 reached maximum absolute value 4.58 cm^2 in 1996. Maybe the environment in 1996 could influence the QTL La6 - 1 greatly. The results of *ae* effects were obviously more often detected than *a* effects, which might also suggested for quantitative traits gene expression could be modified by environmental factors easily.

3.4 Analysis for QTL epistatic effects

Altogether 14 digenic epistatic pairs with epistatic main effect (*aa*) and/or epistasis by environment interaction effect (*aae*) were detected (Table 4). Among them, only 2 pairs had both *aa* and *aae* effects, while 5 pairs had only *aa* effects and 7 pairs had only *aae* effects. The maximum absolute magnitude of *aa* and *aae* effects reached 2.11 cm^2 to 5.16 cm^2, respectively. The range of epistasis × environment interaction effects was wider than the range of epistasis main effects, and epistasis × environment interaction effects were more often detected than epistasis main effects, which might indicate that digenic interactions were more easily subjected to environmental influence.

The composition of epistatic pairs was interesting for that, the detected pairs included 2 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 to regulate 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.

4 Discussion

Both epistatic effects and QTL × environment interaction effects are important components of genetic basis for complex traits. But many of researches have been based on models assuming no epistatic effects or *QE* interaction effects[1-3]. This usually cannot give unbiased estimation for QTL parameters. Partitioning of epistasis from other genetic components of variation would help to obtain more reliable results of QTL mapping. Furthermore, the contribution of QTLs to the trait should also vary according to the growing environment. Usually, *QE* effects are treated as random effects especially in different years. They imply the extents that QTLs would be affected by unknown environment factors in different years.

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