

Dynamic QTL analysis for rice blast resistance under natural infection conditions

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Abstract

Dynamic analyses of rice blast resistance for the assessment of genetic and environmental effects were characterized employing a recombinant inbred lines (RILs) population. The study was conducted at three different developmental stages of rice using natural infection tests in two years. The number of main-effect quantitative trait loci (QTL), epistatic QTL and their environmental interactions greatly differed across various measuring stages. Two major QTL (*r11a* and *r11b*) both on chromosome 11 could be detected at all stages, whereas most QTL were identified only at one or two stages in the population. It was suggested that the unstable activities of most QTL identified for blast resistance may well be due to effects of major QTL, epistatic effects between different loci, the developmental status of rice, and the environments in which they were grown. Comparison of QTL analysis conducted under the conditions of natural infection and artificial inoculation was performed and drew a new conclusion that QTL analysis of plant resistance based on natural infection would have more advantages than that based on artificial inoculation.

Keywords: Rice blast resistance, Dynamic analysis, Main-effect QTL, Epistatic interactions, QTL-by-environment interactions

Introduction

Rice blast, with its genetic instability (Reddy and Bonman, 1987), is still one of the most destructive diseases of rice in both tropical and temperate countries, despite great efforts toward its control. Many studies (Wang et al., 1994; Nagato and Yoshimura, 1998; Ahn et al., 2000; Wu et al., 2005; Li et al., 2007), indicated that the genetic control of blast resistance is complex and involves both major and minor genes with complementary or additive effects (He et al., 1989), as well as their environment interactions (Bonman, 1992). Nevertheless, gene or QTL pyramiding remains to be a promising method to provide broad-spectrum and durable rice blast resistance (Tabien et al., 2002). Up to now, selection for resistance has been performed

under natural infection conditions. What is more, environmental conditions, such as temperature and moisture, greatly affect the epidemics of rice blast diseases and hamper the breeding programs for this trait. However, almost all the genetic studies including rice blast resistance was based on the artificial inoculation, and always focused on plants at a specific or a final growth stage except our former work (Li et al., 2007). Such studies could not fully capture the real gene action during the growth of plant. Moreover, breeders want to know whether the results from artificial inoculation are consistent with those obtained under natural infection conditions (Lübberstedt et al., 1999). Therefore, dynamic mapping and gene expression studies of plants at

Table 1. Descriptive statistics of the blast resistance traits in parents and the RILs population under natural infection conditions observed in 2004 (upper) and 2005 (lower).

Traits	Parent (mean ± SD)		RIL population	
	Zhenshan97	Minghui 63	Mean ± SD	Range
rs	4.0±0.5	3.0±0.3	4.0±0.9	0.3-5.0
	4.4±0.5	3.5±0	4.1±0.9	0.3-5.0
rt	4.0±0	2.8±0.4	2.6±0.7	0-4.5
	4.3±0.5	3.0±0	3.8±0.6	0.5-4.6
rh	4.0±0	3.0±0.3	2.7±0.9	0-5.0
	4.5±0.5	3.1±0.4	3.7±0.7	1.6-5.0

rs=resistance at seedling stage, rt=resistance at tillering stage, rh= resistance at heading stage

different developmental stages are needed. In the present study, we analyzed the QTL of main effects (which can show consistent effects across environments), epistatic effects (whose effects depends on a second QTL), and their environmental interactions using a RIL population at three different stages.

Materials and methods

Experimental Population and Field Planting

In the experiment, we used 241 F₁₀ RILs derived by single-seed descent method from a cross between two *indica* lines, ‘Zhenshan 97’ and ‘Minghui 63’ (Xing et al., 2002), the parents of ‘Shanyou 63’, the most widely cultivated hybrid in the last two decades in China. The RILs population and the corresponding parents were grown simultaneously during the rice-growing seasons of 2004 and 2005 in a blast hot-spot in Yuan’an County, Hubei Province (Hubei site), China. Yuan’an is a mountainous area, which has an altitude of 540m, with an average temperature of approximately 25C° and high humidity annually. The micro-climate at the site favors local rice blast disease development at epidemic proportions year after year. The field planting was the same as our former work (Li et al., 2007) except the materials. To adequately induce blast disease infection, a highly susceptible variety, CO39 (International Rice Research Institution, Los Banos, The Philippines), was planted at both sides of each row and around the population.

Field management followed essentially normal agricultural practices, with the exception of using bactericide.

Phenotypic Measurements

Eight RIL plants in the middle of each row was scored at seedling stage, tillering stage and heading stage respectively (about every 35 days), which will be referred to as three traits for ease of description, named as rs, rt, rh, respectively. In each plant we scored, the most seriously diseased leaf of the top two or three new leaves at each stage was determined by using the 0–5 scale rating system of Bonman et al., (1986), in which scores of 0–3 indicated an incompatible (resistant) reaction and scores of 4 and 5 indicated a compatible (susceptible) reaction. The phenotypic values (degree of lesion) represented the net increase of resistance level at different stages and thus reflected the dynamic responses to rice blast.

DNA Markers and Map Construction

A total of 227 polymorphic markers, covering the entire rice genome and including 168 RFLPs and 59 SSRs, were used to develop the genetic linkage map of the population. Of them, 220 were from the previous work (Xing et al., 2002) and the other 7 SSRs were added to fill the gaps in the map. The genetic linkage map was constructed using Mapmaker 3.0 (Lincoln et al., 1992) at a LOD value of 3.0.

Table 2. Correlation coefficients of the blast traits measured in the RILs population, observed in 2004 and 2005. All the correlations are significantly different from zero at the $P \leq 0.01$ level ($r_{0.01} < 0.181$)

Traits	rs(2004)	rs(2005)	rt(2004)	rt(2005)	rh(2004)
rs(2005)	0.85**				
rt(2004)	0.78**	0.39**			
rt(2005)	0.61**	0.59**	0.45**		
rh(2004)	0.64**	0.36**	0.81**	0.47**	
rh(2005)	0.50**	0.44**	0.44**	0.63**	0.50**

rs=resistance at seedling stage, rt=resistance at tillering stage, rh= resistance at heading stage.

** Significance at $P < 0.01$ level

Data Analysis and QTL mapping

The mean of two replications for each line in each year was used as the raw value for QTL analysis. To analyze genetic components of the traits, we employed QTLMAPPER VER. 1.6 (Wang et al., 1999), which is based on a mixed linear model approach (Zhu and Weir, 1998) that estimates main-effect and digenic epistatic QTL and simultaneously predicts QTL-by-environment (QE) interaction effects (Li et al., 2007).

Results and Discussion

Variation, Correlation and two-way ANOVA analyses of the Traits

Table 1 presents the phenotypic variation of the degree of lesions (a measure of blast resistance) at the three developmental stages for the RIL population and its parents in 2004 and 2005. Large differences were found in the degree of resistance between the RIL lines and their parents. The RIL population exhibited transgressive segregations in both directions for all traits, and the population showed approximately normal distributions at all stages. Correlations among the six traits measured in 2004 and 2005 are presented in Table 2. The six traits in the population were highly, significantly and positively correlated with each other at $P < 0.01$ level in both years (Table 2). In some sense, these results suggested the consistency of component and quantity of isolates across different years and also across different growth stages. Thus, phenotype data are comparable and suitable for QTL mapping.

According to two-way ANOVA (Table 3), for all of the traits in RIL lines, as well as the genotypes, showed highly significant differences between the two years (environments), and what was more, the environment factors had a highly significant effect on rice blast. This environmental effect highlights the importance of conducting the experiments under natural infection conditions.

Main-effect QTL at the Three Stages

Seven to twelve main-effect QTL were identified using the RIL population at each stage, jointly explaining the phenotypic variation of 22.1%, 14.0% and 14.7% at seedling, tillering and heading stage, respectively (Tables 4, 5 and 6). Only two QTL within the two regions RG103-CDO534 and RM229-RM209 both on chromosome 11 (Fig. 1), were simultaneously detected at all the three measuring stages in the RILs, corresponding with the three main-effect QTL, *rs11b/rt11b/rh11b* and *rs11d/rt11e/rh11c*, respectively. Thus, they were the same QTL, designated as *r11a* and *r11b* respectively. Two intervals, C161-R753 on chromosome 1 and R1952b-RZ404 on chromosome 9, were simultaneously identified at both stages in the RIL population (Fig. 1), corresponding with the two main-effect QTL, *rs1/rt1* and *rt9b/rh9a*, respectively. The other QTL were active at only one stage.

That only two QTL (*r11a* and *r11b*) were detected at all stages suggested that (1) different loci were likely to be involved in the genetic control of blast resistance at different ages, or (2) multiple genes in the same genomic region may have different expression patterns related to the trait development.

Table 3. Summary of effects resolved by two-way ANOVA of the three traits of RILs population measured in two environments

Traits	Variation ^a	SS	df	MS	F	P	F crit
rs	G	477.714	210	2.275	20.875	<0.001	1.213
	E	87.691	1	87.691	804.71	<0.001	3.864
	G×E	42.992	210	0.205	1.879	<0.001	1.213
	Error	45.986	422	0.109			
rt	G	239.753	210	1.142	7.178	<0.001	1.213
	E	287.272	1	287.272	1806.15	<0.001	3.864
	G×E	87.533	210	0.417	2.621	<0.001	1.213
	Error	67.12	422	0.159			
rh	G	411.637	210	1.96	10.613	<0.001	1.213
	E	192.332	1	192.332	1041.3	<0.001	3.864
	G×E	139.91	210	0.666	3.607	<0.001	1.213
	Error	77.945	422	0.185			

^a G, genotype; E, environment; G×E, genotype-by-environment interaction

rs=resistance at seedling stage, rt=resistance at tillering stage, rh= resistance at heading stage

Epistatic and QE Interactions at the Three Stages

The number of epistatic interactions (two to 10) and the degree of the epistatic effects (0.36% to 5.12%) observed in the RIL lines also varied considerably among different stages, although the total effects of most epistatic QTL were somewhat smaller than those of the main-effect QTL (Tables 4, 5 and 6). These results indicate that various interactions between different loci are likely to be involved in the genetic control of blast resistance at certain developmental stages.

Most QTL for rt and rh showed interactions with the environment (Tables 5 and 6), demonstrating the complex inheritance of blast resistance and the environment dependence of the gene expression at these loci. In addition, the number (0 to 8) and the effects (0.01% to 4.36%) of significant QE interactions differed at various periods. It is intriguing to note that the QTL for rs showed no obvious interaction with environment. This phenomenon could be explained by two possible reasons: (1) QTL with strong major effect (totally 22.1% of the phenotypic variation) and strong epistatic effects (collectively 18.4%) rarely interact

with environment (2) the interactions with weak effect between environment and QTL were not detected due to the limitation of the statistical method.

Comparative analyses of QTL for rice blast resistance under the conditions of natural infection and artificial inoculation

The results of QTL detected through natural infection tests could be compared with those by artificial inoculation tests conducted by Chen et al. (2003) in our lab, who used the same RILs as in our study. The number and effects of QTL detected between the two conditions were completely different. Six QTL intervals, *rs1* or *rt1*, *rh1b*, *rt2a*, *rs7*, *rs8* and *rt9a* detected by this paper, corresponding to the six QTL *rbr1a*, *rbr1d*, *rbr2*, *rbr7a*, *rbr8* and *rbr9c* detected by artificial inoculation respectively, were simultaneously identified under both conditions. However, the QTL effects detected by natural infection were much smaller than that by artificial inoculation (data published by Chen et al., 2003), possibly because of lacking single elicitors of pathotypes in the natural environment. Moreover, there were more

Table 4. Main effects, epistatic effects and environmental interactions of QTL detected by two-locus analysis using QTLMapper1.6 for rice blast resistance at the seedling stage using RIL population with the LOD threshold 4.03 (equal to a chi-square value for df=6 at P=0.005). General contributions. additive (A), $h^2a=22.06\%$; epistasis, $h^2aa=18.43\%$; QTL-by- environment interactions, $h^2ae=0\%$; $h^2aae=0$

Ch-Ini ^a	Flanking markers	QTL	Ch-Inj ^a	Flanking markers	QTL	LOD	a_i ^b	h^2a_i ^e	a_j ^b	h^2a_j ^e	aa_{ij} ^c	h^2aa_{ij} ^e	h^2total ^f
1-1	C161-R753	<i>rs1</i>	11-33	CDO127-R3203		5.85	-0.13	1.52			-0.11	1.1	2.62
1-20	C567-C2340		11-18	clone4-G4001		4.09					0.14	1.57	1.57
2-6	RZ599-R712		7-8	R1245-RM234		8.04					0.2	3.56	3.56
6-24	G200-RZ667		10-5	C148-RM239		4.8					-0.16	2.06	2.06
7-1	RG528-RG128	<i>rs7</i>	7-5	RG678-RZ471		4.44	0.11	0.99					0.99
8-1	RM25-RG333	<i>rs8</i>	10-7	C1633-C677		9.7	-0.11	1.01			0.21	3.74	4.75
11-5	R1506-MP12	<i>rs11a</i>	11-8	clone1-C405b	<i>rs11c</i>	6.24	0.3	7.83	-0.15	1.98	0.23	4.64	14.45
11-21	RG103-CDO534	<i>rs11b</i>	11-25	RM229-RM209	<i>rs11d</i>	6.11	-0.28	6.64	0.16	2.09	0.14	1.76	10.49

^a Ch-Ini and Ch-Inj represent the chromosome number-interval of the points being tested in the analysis. i and j mean two different intervals on chromosome (s).

^b a_i and a_j are the additive effects of the test points i and j, respectively. Positive values of a_i and a_j imply that the Minghui 63 genotype has a positive effect on that trait

^c aa_{ij} is the effect of additive-by-additive interaction between points i and j; a positive value indicates that the parental two-locus genotypes have a positive effect on the traits and that the recombinants had a negative effect

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

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

Table 5. Main effects, epistatic effects and environmental interactions of QTL detected by two-locus analysis using QTLMapper1.6 for rice blast resistance at tillering stage using RIL population with the LOD threshold 4.03 (equal to a chi-square value for df=6 at P=0.005). General contributions. Additive (A). $h^2a=14.03\%$; Epistasis. $h^2aa=9.86\%$; QE interactions. $h^2ae=5.08\%$; $h^2aae=0$

Ch- Ini ^a	Flanking markers	QTL	Ch- Inj ^a	Flanking markers	QTL	LOD	a_i^b	$h^2a_i^e$	a_j^b	$h^2a_j^e$	aa_{ij}^c	$h^2aa_{ij}^e$	ae_i^d	$h^2ae_i^e$	ae_j^d	$h^2ae_j^e$	h^2total^f
1-1	C161-R753	<i>rt1</i>	9-8	R1952b-RZ404	<i>rt9b</i>	8.53	-0.08	0.52	0.078	0.49	0.098	0.77			0.058	0.27	2.05
1-4	RG532-RM259		6-28	R2549-C962		4.54					0.12	1.16					1.16
1-21	C2340-C86		8-1	RM25-RG333		6.99					-0.138	1.54					1.54
2-2	RM213-RM208	<i>rt2a</i>	2-5	RM48-RG520	<i>rt2b</i>	5.09	0.146	1.72	-0.096	0.74	0.081	0.53			-0.012	0.01	3
3-11	C944-R321	<i>rt3</i>	3-15	RM227-R1925		6.01	0.069	0.38							-0.113	1.03	1.41
3-16	R1925-RM148		4-2	C820-C933	<i>rt4</i>	7.6			0.067	0.36			-0.106	1.81			2.17
3-16	R1925-RM148		10-3	R2174-C909A		6.17					0.071	0.41	-0.107	1.85			2.26
4-8	R78-C1016		11-34	R3203-RM20a	<i>rt11c</i>	4.31			0.079	0.5							0.5
5-10	C1447-RM31		6-20	RZ588-P		6.55					0.103	0.86					0.86
8-1	RM25-RG333		10-8	C677-RM258		4.96					0.091	0.67					0.67
8-2	RG333-R902		11-35	RM20a-C104	<i>rt11d</i>	4.88			0.088	0.62	0.079	0.5			-0.033	0.09	1.21
8-6	C483-C347		12-9	C87-R496		5.43					-0.086	0.6			-0.012	0.01	0.61
9-7	RM215-R1952b	<i>rt9a</i>	10-2	RM222-R2174		4.64	0.095	0.73									0.73
11-3	R543a-Y6855R	<i>rt11a</i>	11-6	MP12-RM224		10.21	0.136	1.49									1.49
11-21	RG103-CDO534	<i>rt11b</i>	11-25	RM229-RM209	<i>rt11e</i>	12.08	-0.252	5.12	0.13	1.36	0.187	2.82			-0.011	0.01	9.31

^{a-1} See footnotes of Table 4 for explanations

^d ae_i and ae_j are effects of the environmental interaction of locus i and j, respectively; a positive value implies that the effect in 2005 is larger than in 2004

Table 6. Main effects, epistatic effects and environmental interactions of QTL detected by two-locus analysis using QTLMapper1.6 for rice blast resistance at heading stage using RIL population with the LOD threshold 4.03 (equal to a chi-square value for df=6 at P=0.005). General contributions. Additive (A). $h^2a=14.71\%$; Epistasis. $h^2aa=15.54\%$; QE interactions. $h^2ae=3.02\%$; $h^2aee=0$

Ch- Ini ^a	Flanking markers	QTL	Ch-Inj ^a	Flanking markers	QTL	LOD	a_i^b	$h^2a_i^e$	a_j^b	$h^2a_j^e$	aa_{ij}^c	$h^2aa_{ij}^e$	ae_i^d	$h^2ae_i^e$	h^2total^f
1-13	RM5a-RM237	<i>rh1a</i>	6-26	C751A-RG424		4.81	-0.122	1.03			0.164	1.85			2.88
1-14	RM237-C922		9-4	RM242-RG570	<i>rh9b</i>	5.86			0.119	0.98	0.121	1.01			1.99
1-19	RM212-C567	<i>rh1b</i>	5-7	C624-C246		10.03	0.142	1.39			0.178	2.18			3.57
1-20	C567-C2340	<i>rh1c</i>	5-8	C246-RM26		5.88	0.133	1.22			0.115	0.91			2.13
1-21	C2340-C86	<i>rh1d</i>	11-33	CDO127-R3203		5.21	0.095	0.62			-0.142	1.39			2.01
2-11	C777-RZ386		2-2	RM213-RM208		5.07					0.146	1.47			1.47
2-2	RM213-RM208		4-8	R78-C1016		4.14					0.116	0.93			0.93
4-5	G102-RM255	<i>rh4</i>	4-8	R78-C1016		4.7	0.153	1.61							1.61
5-1	R830-R3166		12-10	R496-C909B		4.93					0.138	1.31			1.31
8-14	RZ66-G1149		11-18	clone4-G4001	<i>rh11b</i>	9.37			-0.098	0.66	-0.21	3.04			3.7
9-8	R1952b-RZ404	<i>rh9a</i>	10-2	RM222-R2174		8.18	0.162	1.81					0.148	3.02	4.83
11-21	RG103-CDO534	<i>rh11a</i>	11-25	RM229-RM209	<i>rh11c</i>	5.7	-0.246	4.17	0.133	1.22	0.145	1.45			6.84

^{a-c} See footnotes of Table 4 for explanations

^d ae_i and ae_j are effects of the environmental interaction of locus i and j, respectively; a positive value implies that the effect in 2005 is larger than in 2004

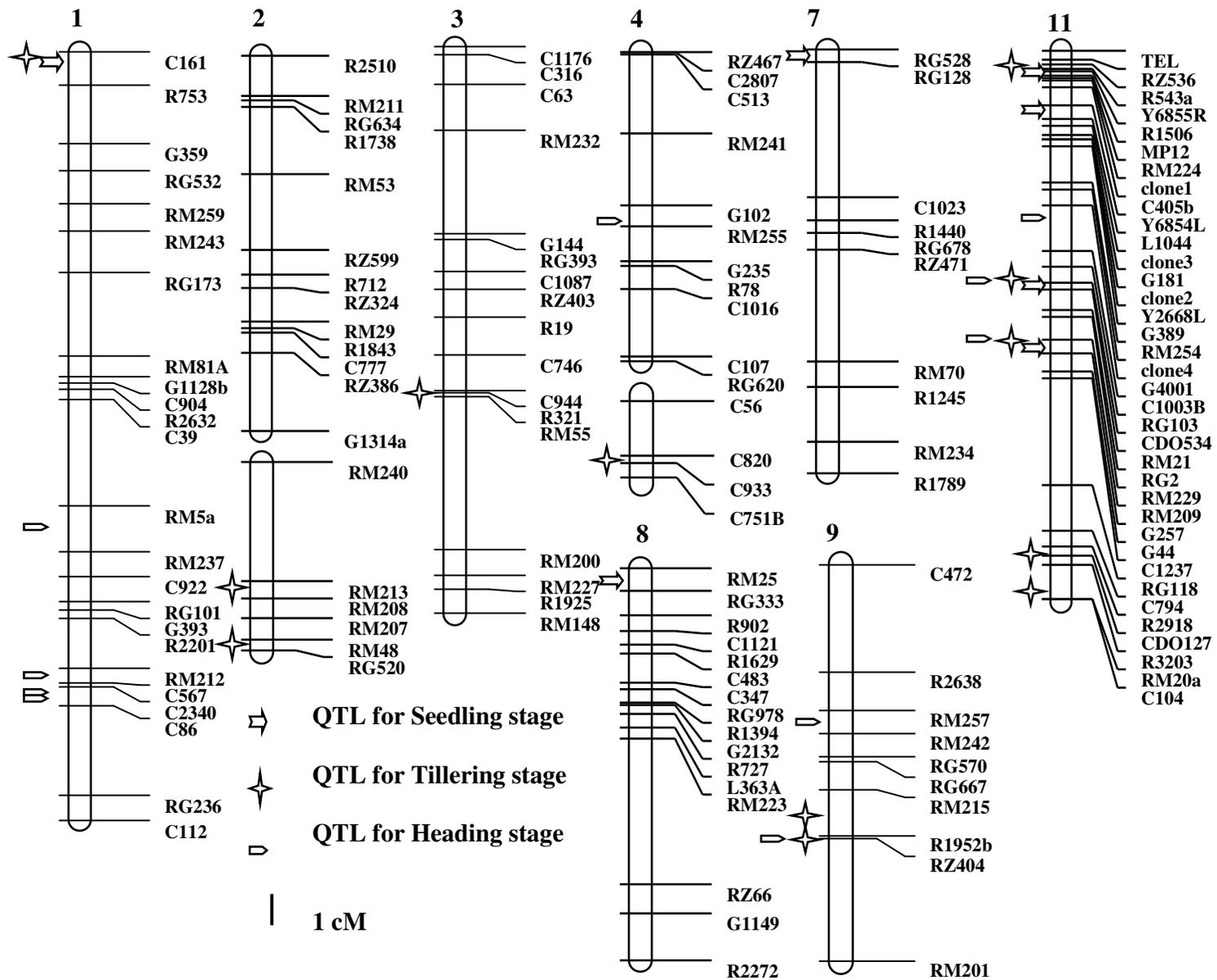


Fig1. Distribution of main-effect QTL at three developmental stage of rice on the RIL map as detected by QTL Mapper

QTL (totally 29) detected under natural infection condition in our study than that (totally 12) under artificial inoculation condition. It may be the reason that there are more unknown and complex pathotypes of *P. grisea* with their genetic instability in the natural blast nursery simultaneously involved in the inducing activities of plant resistance QTL expression than that of artificial inoculation (Chen et al., 2001; Bilgic et al., 2006). Therefore, QTL detected under natural infection condition have broad spectrum and durable resistance, which has profound implications for practical rice resistance breeding. Moreover, QTL analysis by a natural infection process could fully reflect the real gene actions in a more natural way because of lack of artificial factors. Thus, in a sense, QTL analysis of plant resistance based on natural infection has more advantages over that based on artificial inoculation.

In conclusion, the most important outcome of the present study is the dynamic characterization of the main-effect and epistatic QTL as well as their environmental interactions for rice blast resistance under natural field conditions. Our present results are clearly in agreement with our former results (Li et al., 2007) and the other study (Atchley and Zhu, 1997). Compared with those studies involved only the main effects of individual QTL at a single stage, this findings and analytical method might help to unravel more important information for molecular mechanism of plant resistance and for creating disease-resistant plants and cloning of QTL.

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