Coincidence in map positions between pathogen-induced defense-responsive genes and quantitative resistance loci in rice

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Abstract Quantitative disease resistance conferred by quantitative trait loci (QTLs) is presumably of wider spectrum and durable. Forty-four cDNA clones, representing 44 defense-responsive genes, were fine mapped to 56 loci distributed on 9 of the 12 rice chromosomes. The locations of 32 loci detected by 27 cDNA clones were associated with previously identified resistance QTLs for different rice diseases, including blast, bacterial blight, sheath blight and yellow mottle virus. The loci detected by the same multiple-copy cDNA clones were frequently located on similar locations of different chromosomes. Some of the multiple loci detected by the same clones were all associated with resistance QTLs. These results suggest that some of the genes may be important components in regulation of defense responses against pathogen invasion and they may be the candidates for studying the mechanism of quantitative disease resistance in rice.

Keywords: QTL, disease resistance, mapping, rice.

Disease is one of the major restrictions for crop production. Developing resistant cultivars is considered to be the most effective way to control plant diseases. Complete or qualitative disease resistance conferred by the interaction between a disease resistance (*R*) gene and an avirulence gene is specific to a pathogen race and lifetime limited in a particular cultivar due to the rapid evolution of the pathogen. This characteristic of *R* genes has limited their value in breeding programs. Partial or quantitative resistance modulated by a group of genes is considered to be race-nonspecific[1]. These genes, when properly characterized, may provide the sources for improving the level of resistance of plants against pathogen infection.

Many quantitative trait loci (QTLs) for partial resistance to blast, bacterial blight, sheath blight and virus have been identified in different rice cultivars[2—5]1). However, the nature of these genes underlying the resistance QTLs is poorly characterized because of the complexity of the quantitative effects, which has limited the application of these genes in breeding programs.

In a previous study, we identified a group of genes that showed either enhanced expression or repressed expression after pathogen invasion in rice by cDNA array analysis[6]. Most of the de-

1) Chen, H., Population structure of *Pyricularia grisea* from central and southern China and comparative mapping of QTL for blast- and bacterial blight-resistance in rice and barley (in Chinese), Ph. D. Thesis, Wuhan, China: Huazhong Agriculture University, 2001.

fense-responsive genes appeared to be involved in resistant responses against both blast and bacterial blight, the two most devastating rice diseases worldwide. The objectives of this study were to determine the chromosomal locations for a number of defense-responsive genes and to examine their relationship with disease resistance QTLs in rice. It is believed that location correspondence between defense-responsive genes and resistance QTLs will provide the starting point for characterizing these genes.

1 Materials and methods

1.1 Experimental materials

Forty-four defense-responsive cDNA clones were studied, including 38 clones that showed enhanced expression and 6 clones that showed repressed expression after pathogen inoculation as revealed by cDNA array analysis[6] (table 1). Two segregation populations were used for mapping the cDNA clones. One population consisted of 235 individuals developed from a three-way cross, Balilla (*Oryza sativa* ssp. *japonica*) /Dular (*O. sativa* ssp. *indica*) // Nanjing 11 (*indica*). A molecular linkage map containing 158 RFLP (restriction fragment length polymorphism) markers and centromere loci was developed with this population[7,8]. Another population included 241 recombinant inbred lines developed by single seed descendent from a cross between Zhenshan 97 (*indica*) and Minghui 63 (*indica*). A molecular linkage map containing 221 RFLP and SSR (single sequence repeat) markers was constructed with this population[9].

1.2 Mapping

Several cDNA clones were assigned to the rice molecular linkage map by RFLP analysis of the mapping populations. The DNA hybridization was conducted according to the procedure described previously[10]. After hybridization, filters were washed in 1× SSC and 0.1% SDS once for 15 min at room temperature and once for 15 min at 65^{o}C. The chromosomal locations of the cDNA that detected polymorphisms between the parents of the mapping populations were determined using Mapmaker/Exp 3.0 at a LOD threshold 3.0[11].

Other cDNA clones were mapped onto the rice chromosomes by homology search of the cDNA sequences against rice genomic sequences with known chromosomal locations (http://rgp.dna.affrc.go.jp and http://www.genome.clemson.edu) using BLAST analysis[12].

2 Results

2.1 Distribution of defense-responsive genes in rice genome

The 44 genes, represented by the 44 cDNA clones, were assigned to 56 loci distributed on 9 of the 12 rice chromosomes (fig. 1). Of the 44 clones, five were mapped on the chromosomes by RFLP analysis (table 1). Another 39 clones were mapped on the molecular linkage map by homologous sequence analysis (fig. 1). Six clones, BI75E3, EI3H3, EI23E13, EI38M12, EI31M6 and EI22F22, were all single copy sequences based on Southern hybridization (data not shown). Three clones, EI39C8, EI31I7 and EI36H4, detected 2 to 3 bands after hybridization with rice total DNA digested using different restriction enzymes (data not shown). Additionally, eight clones,

Table 1 Pathogen-induced defense-responsive sequences

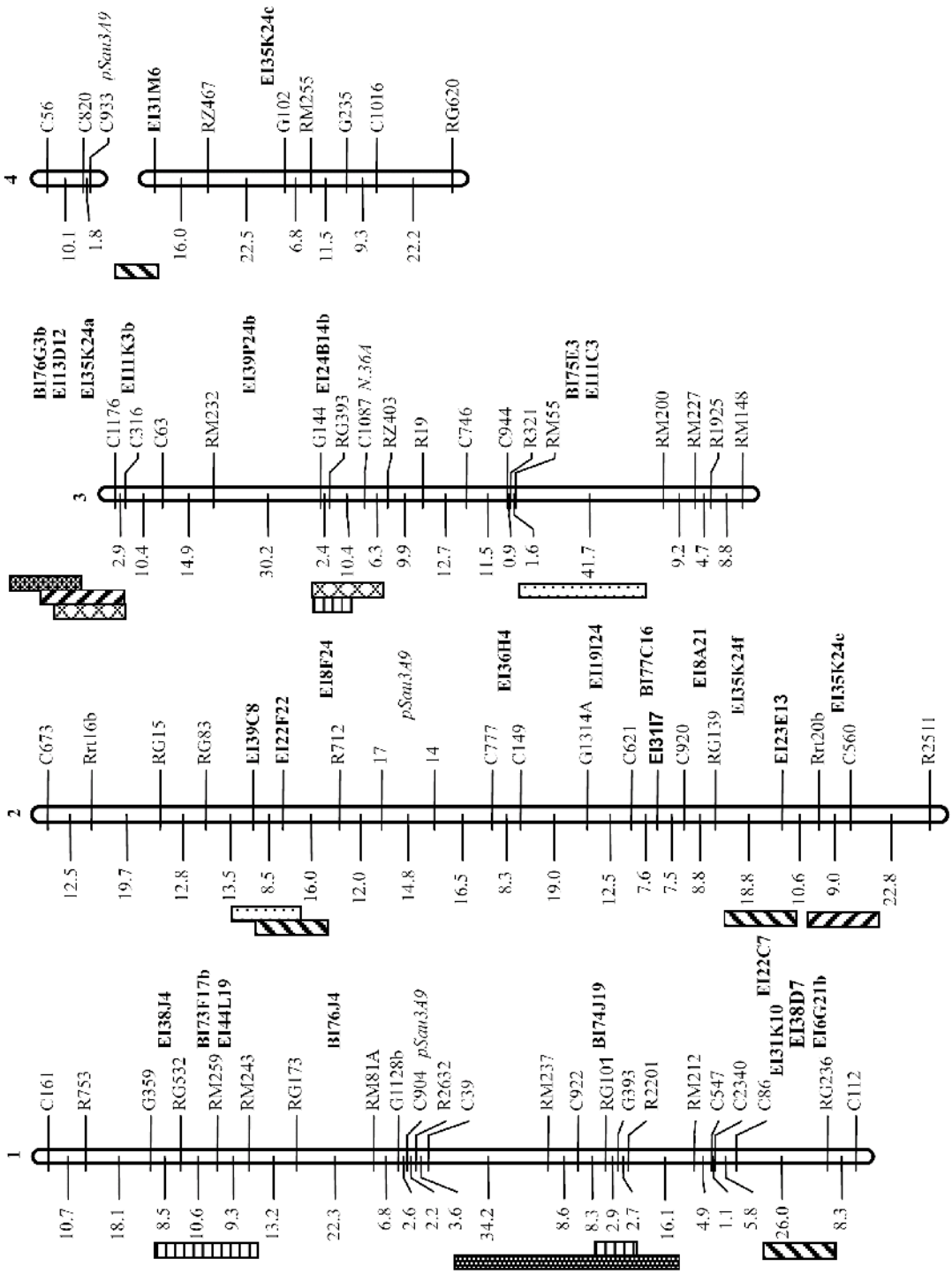
cDNA clone	Accession number	Mapping (BLAST)[a)]			Function analysis (BLAST)	
		chromosome	homologous sequence	E value	homologous sequence	E value
EI38J4	BF108354	1	AP003197	5e-17	metallothionein-like protein (AF009959)	1e-124
EI39P24	BF108327	7	AP004316	0.0	putative pectinesterase (AAF26136)	3e-22
		3	AC118668	4e-36		
EI30O11	BF108352	7	AP004384	0.0	thioredoxin h (D21836)	0.0
EI28N12	BF108322	10	AC084763	1e-128	nucleoside diphosphate kinase (AF271362)	1e-126
		7	AP004051	3e-10		
EI11K3	BF108342	7	AP003845	4e-40	alpha-tubulin (X91807)	0.0
		3	AC104474	1e-27		
EI3G1	BF108314	8	AP004228	1e-107	poly (A)-binding protein (TAU81318)	1e-23
EI24B14	BF108326	8	AP003798	2e-68	pectin methylesterase (BAA89480)	9e-24
		3	AC091787	5e-11		
BI76G3	BF108343	10	AC069300	4e-75	beta expansion (EXPB3) (AF261271)	1e-156
		3	AF485811	6e-28		
EI10N21	BF108329	6	AP004756	1e-176	ferrochelatase precursor(P42045)	2e-66
EI20C18	BF108349	10	AC074354	0.0	mucin 2 precursor (Q02817)	2e-13
BI73F17	BF108315	10	AC092172	0.0	glycine-rich RNA-binding protein (T04346)	8e-45
		1	AP003434	5e-21		
EI6G21	BF108317	10	AC060755	1e-173	putative nonsense-mediated mRNA decay protein (AAD24816)	5e-16
		1	AP004326	2e-16		
EI39F4	BF108324	10	AC051632	0.0	hydroxyproline-rich glycoprotein 1 (Q9FPQ6)	2e-07
EI38D7	BF108310	1	AP003451	1e-166	zinc finger protein (T48868)	5e-51
BI74J19	BF108334	1	AP003229	4e-29	putative S-adenosylmethionine: 2demethylmenaquinone methyltransferase (BAB84438)	3e-14
BI76J4	BF108321	1	AP003535	8e-57	zinc-induced protein (AF323612)	1e-109
EI31K10	BF108353	1	AP003436	1e-129	putative glycine-rich protein (P13728)	2e-12
EI22C7	BF108350	1	AP003316	5e-98	putative calreticulin protein(AAL07169)	1e-61
EI19I24	BF108360	2	AP004056	2e-54	unknown protein (AAK25977)	5e-75
EI36H4	BF108333	2	AP004772	9e-47	molybdopterin synthase sulphurylase (AAD18052)	3e-60
BI77C16	BF108357	2	AP004023	0.0	unknown protein (AAD26478)	2e-24
EI8A21	BF108347	2	AP005055	1e-147	LIP9 (low temperature induced protein (AB011367)	1e-135
EI8F24	BF108364	2	AP004875	0.0	acrosin precursor (P08001)	7e-05

(*To be continued on the next page*)

(*Continued*)

cDNA clone	Accession number	Mapping (BLAST)[a]			Function analysis (BLAST)	
		chromosome	homologous sequence	E value	homologous sequence	E value
EI38M12	BF108335	6	AP005413	1e-109	Disease resistance protein (AAC35544)	0.9
EI39C8	BF108323	2	Southern hybridization		protein kinase (LAMMER) (P51567)	5e-39
EI22F22	BF108319	2	Southern hybridization		putative sugar transporter (AAG46115)	1e-64
EI23E13	BF108351	2	Southern hybridization		ATP/ADP translocator (D12637)	0.0
EI13D12	BF108348	3	AC118980	0.0	heavy metal associated domain (AAF82161)	2e-10
EI11C3	BF108330	3	AC092781	1e-116	Glyoxysomal malate dehydrogenase (P46488)	2e-55
BI75E3	BF108311	3	AC090713	1e-174	ZF1 finger protein (AF332876)	1e-174
BI77K23	BF108361	3	AC092558	1e-162	aluminum-induced protein-like (BAB11312)	2e-65
EI36C19	BF108328	8	AP004460	0.0	S-adenosyl-L-methionine: caffeic acid 3-O-methyltransferase (AJ231133)	5e-71
EI43O12	BF108355	6	AP003628	6e-60	Dreg-2 like protein (AAC79147)	3e-17
EI3H3	BF108336	6	AP003614	0.0	putative Mlo protein (O80580)	1.2
EI8H23	BF108312	6	AP003517	0.0	putative homeodomain transcription factor (AAC69941)	2.9
EI31I7	BF108325	2	Southern hybridization		phospholipase like protein (CAB37511)	0.9
EI31M6	BF108363	4	Southern hybridization		no significant similarity found	
EI44L19	BF108320	1	AP000570	1e-72	Similar to epoxide hydrolases (BAA84626)	1.7
BI71N2[b]	BF145170	5	AC104709	7e-43	ubiquitin-conjugating enayme (BAA96583)	6e-25
EI28P15[b]	BF145171	6	AP003514	6e-76	ubiquitin conjugating enzyme 2 (AAL35400)	5e-36
EI21C23[b]	BF145185	8	AP003912	2e-72	26S proteasome regulatory subunit 3 (BAB78499)	2e-69
BI75M9[b]	BF145187	6	AP004685	4e-49	high mobility group protein (HMG) (AF093632)	1e-144
EI35K24[b]	BF145188	3	AC121489	0.0	putative bHLH DNA-binding protein (CAB77716)	2e-37
		10	AC079935	4e-42		
		4	AL662981	3e-38		
		8	AP004213	4e-34		
		2	AP004814	5e-24		
		2	AP004118	2e-20		
EI7O24[b]	BF145189	7	AP005197	0.0	DDX1 (putative RNA-binding protein (AAC47310)	1.8

a) The chromosomal locations of some of the cDNA sequences were determined by homologous rice genomic sequences which had been physically mapped on rice chromosomes (http://rgp.dna.affrc.go.jp/ and http://www.genome.clemson.edu). b) Sequence showing repressed expression after pathogen inoculation. The expression of other sequences was increased after pathogen inoculation.


1
C161 10.7
R753 18.1
G359 8.5
EI38J4
RG532 10.6
B173F17b
EI44L19
RM259 9.3
RM243 13.2
RG173 22.3
B176J4
RM81A 6.8
G1128b 2.6
C904 2.2
pSau349
R2632 3.6
C39 34.2
RM237 8.6
C922 8.3
RG101
B174J19
G393 2.9
R2201 2.7
RM212 16.1
C547 4.9
C2340 1.1
C86 5.8
EI22C7
EI31K10
EI38D7
EI6G21b
RG236 26.0
C112 8.3
2
C673 12.5
Rrt16b 19.7
RG15 12.8
RG83 13.5
EI39C8
EI22F22 8.5
16.0
R712 12.0
EI8F24
17 14.8
pSau349
14 16.5
C777 8.3
EI36H4
C149 19.0
G1314A 12.5
EI19I24
C621 7.6
EI31I7
B177C16
C920 7.5
EI8A21
RG139 8.8
EI35K24f
EI23E13 18.8
Rrt20b 10.6
C560 9.0
EI35K24c
22.8
R2511
3
C1176 2.9
C316 10.4
C63 14.9
RM232 30.2
EI39P24b
G144 2.4
EI24B14b
RG393 10.4
C1087 N.36A 6.3
RZ403 9.9
R19 12.7
C746 11.5
C944 0.9
R321 1.6
RM55
B175E3
EI11C3 41.7
RM200 9.2
RM227 4.7
R1925 8.8
RM148
B176G3b
EI13D12
EI35K24a
EI11K3b
4
C56 10.1
C820 1.8
C933 pSau349
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G235 9.3
C1016 22.2
RG620

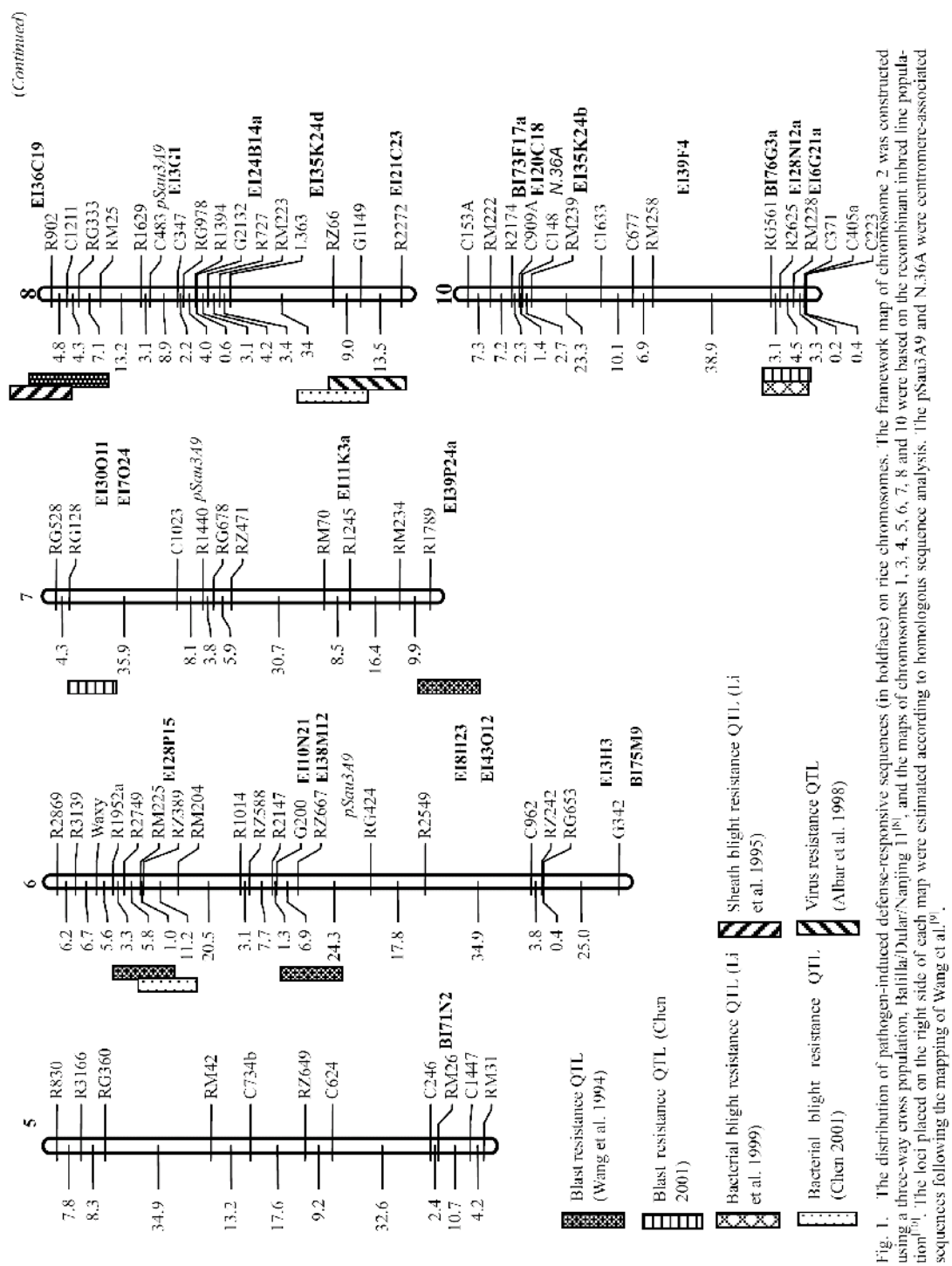

Fig. 1. The distribution of pathogen-induced defense-responsive sequences (in boldface) on rice chromosomes. The framework map of chromosome 2 was constructed using a three-way cross population, Balilla/Dular//Nanjing 11[18], and the maps of chromosomes 1, 3, 4, 5, 6, 7, 8 and 10 were based on the recombinant inbred line population[19]. The loci placed on the right side of each map were estimated according to homologous sequence analysis. The pSau3A9 and N.36A were centromere-associated sequences following the mapping of Wang et al.[9].

EI39P24, EI28N12, EI11K3, EI24B14, BI76G3, BI37F17, EI6G21 and EI35K24, detected 2 to 6 copies and the remaining 26 clones detected only one copy each in the rice genome according to the presently available data of rice genomic sequences in the public databases (table 1). The E values, which estimated the similarity between cDNA sequences under comparison and the corresponding rice genomic sequences with known chromosome locations, used in mapping ranged from 0.0 to 3e-10. The cDNA clone EI28N12 had the least sequence homology (E value = 3e-10) with one (EI28N12b) of the two copies of homologous rice genomic sequences among all the cDNA and genomic DNA sequence pairs in mapping (table 1). The cDNA sequence EI28N12 and the genomic sequence at locus EI28N12b had 2 overlapped regions (49 bp and 125 bp) with 93% and 80% sequence identify, respectively.

The loci detected by the same clone were frequently located on different chromosomes (fig. 1). An interesting observation was that sequences homologous to the same clone were frequently located in similar locations on different chromosomes, if the chromosomal arms were not considered. For example, clone EI6G21 detected two loci distributed on chromosomes 1 and 10, and both of the loci were mapped to the near terminal regions of the chromosomes. Four of the 6 loci detected by EI35K24 were located in the regions next to the peri-terminal regions of chromosomes 2, 3, 4 and 8. The loci detected by EI11K3, EI24B14, BI76G3 and BI73F17 that identified two loci each were also mapped to similar locations of different chromosomes (fig. 1).

Another striking feature of the distribution of the multiple-copy sequences was the simultaneous occurrence of loci detected by different clones across different chromosomes. For example, loci detected by EI39P24 and EI11K3 occurred simultaneously on one arm of chromosomes 3 and 7 (fig. 1). Similar concurrence was also observed between loci detected by BI73F17 and EI6G21 on chromosomes 1 and 10, and loci detected by BI76G3 and EI35K24 on chromosomes 3 and 10, as well as loci detected by EI24B14 and EI35K24 on chromosomes 3 and 8.

2.2 Comparing the locations of defense-responsive gene loci and QTLs for disease resistance

The locations of 32 loci detected by 27 cDNA clones corresponded with previously identified QTLs against rice blast, sheath blight, yellow mottle virus or bacterial blight[2—5]1). Twenty-three of the 32 loci corresponded with blast or/and bacterial blight resistance QTLs. According to the expression patterns of the defense-responsive genes after inoculation with different pathogens[6], the genes located on 19 loci, EI38J4, BI73F17b, EI44L19, BI74J19, EI39C8, EI22F22, EI13D12, EI35K24a, EI24B14b, BI77K23, BI75E3, EI11C3, EI28P15, EI30O11, EI36C19, EI35K24d, BI76G3a, EI28N12a and EI6G21a, had the same pathogen specificity as the corresponding blast or/and bacterial blight resistance QTLs[2,5]1). In several cases, two or four of the multiple loci identified by the same cDNA clone had chromosomal locations corresponding to QTLs against rice diseases. For example, the locations of two loci detected by BI76G3 and EI6G21, respectively, and 4 of the 6 loci detected by EI35K24 corresponded well with the QTLs for resistance to blast, bacterial blight, sheath blight or/and virus[2—4]1) (fig. 1).

1) See the footnote on page 518.

3 Discussion

The major accomplishment of this study is the fine mapping of 44 cDNA sequences representing 44 defense-responsive genes on rice molecular linkage map, which facilitated examination of the relationship between these genes and previously identified QTLs for rice disease resistance. The disease resistance QTLs may be valuable sources for wider spectrum and durable resistance in rice breeding programs[1]. However, the roles of these QTLs in defense responses are unknown because the genes underlying these QTLs are largely uncharacterized. The lack of the knowledge related to resistance QTLs may be the main reason that none of the previously identified QTLs has been used for improving resistance against pathogens in rice so far. Wang et al.[13] reported that the locations of several defense-responsive gene-like sequences were mapped on 3 regions containing previously identified disease resistance QTLs in rice. In this study, the locations of 32 loci detected by 27 cDNA clones were associated with about half (26) of the previously identified QTLs against rice blast, sheath blight, yellow mottle virus and bacterial blight[2—5]1). The genes with chromosomal locations similar to resistance QTLs may be good candidates for studying the mechanism of quantitative disease resistance in rice.

Quantitative disease resistance conferred by some QTLs appears race-nonspecific, although race-specific resistance QTLs for bacterial blight and blast have been identified in rice[2,5,14]1). Some previously identified rice resistance QTLs for different diseases are located in the same chromosomal regions (fig. 1). Similar situation also occurred in barley[15]. This may explain that some cDNA clones identified in defense against blast and bacterial blight[6] were located in the similar locations to previously identified resistance QTLs for sheath blight or yellow mottle virus[3,4] in this study.

Several defense-related genes, oxalate oxidase, peroxidase, superoxidase, chitinase and thaumatin, were mapped to previously identified resistance QTLs in barley[16]. Wang et al.[13] also reported that some of the sequences associated with previously identified QTLs for disease resistance were defense-like genes, such as NPR1-like protein and pathogenesis-related protein. Although the roles of the genes associated with QTLs in this study have not been experimentally examined, the concurrence of these genes with resistance QTLs suggests the likelihood that they may be important components in regulation of defense responses against pathogen invasion in rice.

Another feature of the present results is the occurrence of the loci detected by the same multiple-copy defense-responsive sequences in similar locations of different chromosomes. Some of the conserved loci, such as BI76G3a and BI76G3b, EI6G21a and EI6G21b, and EI35K24a, EI35K24d and EI35K24e, were all associated with previously identified QTLs for rice disease resistance. The distribution of similar DNA sequences in similar locations of different rice chromosomes was also observed when using retrotransposons as well as multiple-copy cDNA and genomic DNA markers as probes[17—19]. These findings lead to the proposition that chromosome du-

1) See the footnote on page 518.

plication followed by diversification may be a mechanism for the origin and evolution of the chromosomes in the rice genome[18,19]. The present results further support the chromosomal duplication-diversification hypothesis. These results also indicate that duplication followed by diversification of the chromosomes or chromosomal segments in the rice genome resulted in the diversity of the defense-responsive genes, which may be one of the causes for the complexity of quantitative disease resistance.

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