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Identification and Mapping of the QTL Controlling Resistance to Blast (*Magnaporthe grisea*) Disease in Rice

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The rice blast resistance of one hundred and sixty F_2-derived lines from the TNG69 x KHK cross was evaluated in greenhouse inoculation and field tests. Results from both screening methods revealed that the resistance to rice blast was mainly controlled by two major and several minor QTLs (Quantitative trait loci). Broad-sense heritability (H^2_B) was estimated according to the variance of combined analysis. The value was 85.4% in greenhouse inoculation and 73.7% in field test. The blast resistance phenotypes in six environments (2 methods x 3 years) were analyzed using Mapmaker-QTL program. All informative markers distributed within the R165-R750 interval of linkage group A (chromosome 12) were correlated with rice blast resistance. Except for slighter symptom in the field test during 1999, the phenotypic contribution of each detected QTL was ranged from 21.3% to 72.4% with LOD scores from 7.43 to 24.42 in all surveyed environments. Thus, it is suggested that a major resistance gene was located on chromosome 12. Our results not only have proved the efficiency of greenhouse test for rice blast resistance, and have located a major gene with resistance across the different environments. The DNA makers closely linked with QTL affecting blast resistance may provide a useful tool for marker-assistant -selection (MAS) in the near future.

Key words: rice (Oryza sative), rice blast, molecular marker, major resistant gene, chromosome 12

Introduction

Breeding programs usually demand to combine high quality and stable yield, and disease resistance. Rice blast, is known to be the most serious fungal disease of rice, caused by *Magnaporthe grisea*. Growing resistant cultivars has been the most economical and effective way of controlling this disease. Many major genes for resistance have been identified and successfully used for developing blast-resistant cultivar with

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complete resistance (vertical resistance) [20, 30, 31]. However, the obtained resistance is frequently frustrated by the rapid adaptation of the pathogen population to resistant cultivars. Breeding durable resistance can be accomplished by pyramiding 2-3 blast-resistance genes to generate cultivars with multiple blast resistance.

Molecular markers linked to major blast-resistance genes offer a powerful tool for marker-aided indirect selection of resistance loci in gene-pyramiding strategies. It is also noted that molecular markers can improve the efficiency and resolution of genetic analysis, particularly when multiple resistance genes are present in a single cultivar [24]. Strategies aimed at breeding for durable rice blast resistance have been focused on the possibility of using molecular markers to combine genes that confer complete and partial resistance (horizontal resistance) [30]. Several studies have been localized the genetic loci controlling blast resistance on linkage groups of rice by molecular markers.

At least 22 major genes conferring complete resistance to rice blast and 10 quantitative trait loci associated with partial resistance have been located via linkage markers [1, 19, 28]. For example, a number of blast resistance genes have been mapped relative tightly linked to RFLP markers [1, 9, 12, 15, 16, 30, 32, 33], RAPD markers [14, 22], SCAR markers [1, 21], sequenced-tagged-sites markers (STSs), and specific amplicon polymorphism (SAP) markers [12].

We have developed an F_2 population from TNG69 (blast resistant) × Koshihikari (blast susceptible) to constructe a saturated molecular map of rice, and to map the blast resistance genes on the molecular map of rice. Markers tightly linked to resistance genes can be further used for indirect selection in a disease resistant breeding program.

Materials and Methods

Plant materials

An $F_{2:4}$ population was developed from a cross between the japonica cultivars Tainung 69 (TNG 69) and Koshihikari (KHK). TNG 69, a Taiwan cultivar, was used as female parent. It is resistant to a wide range of blast races including those at IRRI Philippines, and is also resistant to bacterial leaf blight disease. Resistant to brown planthopper biotypes 1, 2 and 3, as well as to the white-blacked planthopper was also indicated [10]. KHK, the Japonica parent with good grain quality, but is susceptible to blast. The F_2 plants were grown at farm of Taiwan Agriculture Research Institute (TARI) and $F_{2:3}$ seeds were harvested. A total of 160 $F_{2:3}$ seeds representing each F_2 plant were grown during summer of 1998. $F_{2:4}$ seed was harvested from these lines. $F_{2:4}$, $F_{2:5}$ and $F_{2:6}$ populations were used as the materials for phenotypic evaluations in 1999, 2001 and 2002, respectively.

Phenotypic evaluations in the field and greenhouse

The 160 F_2-derived lines and parents were planted in the field and greenhouse at spring crop seasons from 1999 to 2001. In the field, we investigated disease incidence at mature period. In the greenhouse, the plants were inoculated with *M. grisea* at the fourth leaf stage which was about 15 days after planting. Fertilizers were applied every 3-4 days for inducing disease. Response of each plant to disease was recorded 30 days after inoculation, according to the scale described by IRRI (1988) with minor modification, i.e. scores 1-5 were classified as resistant (R), and scores 7-9 as susceptible (S).

From 1999 to 2001, the phenotypic data evaluated at the two sites (field and greenhouse planting) were used in a combined analysis of variance (ANOVA). The effect of replications, lines and years

were considered as random factors, the effect of sites was considered as fixed factors. Broad-sense heritability (H_2B) was estimated on the basis of the expected mean squares (EMS) from the combined ANOVA test [6].

Laboratory assays

DNA extraction

Rice genomic DNA was prepared from fresh-frozen leaf tissue using the Doyle et al. (1990) [7] method.

RAPD analysis

A total of 1080 arbitrary 10-nucleotide primers were surveyed for their ability to amplify the polymorphic band among parents and the F_2 individuals. The primers included 480 primers of Operon 10-mer kits, and 600 primers of University of British Columbia (UBC) from set #2 to set #7. The PCR reaction was carried out in a volume of 23 μl containing 60 ng genomic DNA, 0.2 mM dNTPs, 1.5U *Taq* polymerase, 0.3 μM primer, 1X *Taq* buffer. The mixture was performed in a PCR program of 5 min at 94 °C, then 45 cycles of 1 min at 94 °C, 2 min at 43 °C, and 3 min at 72 °C, followed by 10 min extension at 72 °C. Amplified products were electrophoretically resolved in 1.0% agarose gels, stained with ethidium bromide, and visualized under UV light.

Sequence targeted sited (STS) analysis

To identify polymorphic markers, forty primer pairs were surveyed. The positions of these amplified fragments on the rice linkage group were known since they had been mapped previously [3]. The PCR condition was the same as RAPD analysis.

Inter-simple sequence length polymorphism (ISSLP) analysis

A set of 100 ISSLP primers (University of British Columbia, set #9) were surveyed for their ability to amplify the polymorphism band among parents and the F_2 individual plants. All PCR amplifications were performed in a volume of 23 μl containing 250 μM each of dNTPs, 12 μM of each primer, 1U of *Tag* polymerase and 1X reaction buffer. All amplifications were programmed with 40 cycles of 1 min at 94 °C, 1 min at 46 °C, 2 min at 72 °C, followed by 4 min extension at 72 °C. Amplified products were examined similar to RAPD analysis except 2% agarose gels were used.

Simple sequence length polymorphism (SSLP) analysis

A total of 88 SSLP primer pairs were surveyed in this study. SSLP primer pairs (Research Genetics, Huntsville, AL), RM set as described by Chen et al. (1997) [5] and Panavd et al. (1996) [25] were used. Simple sequence repeat (SSR) of *Oryza sativa* markers -- OSR set as described by Akagi et al. (1996) [2] were also used. PCR amplification mixture was the same as ISSLP. The mixture was performed with 42 cycles of 30 sec at 94 °C, 30 sec at 50 °C, and 30 sec at 72 °C, followed by 5 min extension at 72 °C. Amplified products were analyzed on 3% MetaPhor agarose gels (FMC) and visualized by ethidium bromide staining.

Linkage between resistance gene and molecular markers

The molecular markers showing polymorphism among F_2 individuals were used to analyze their linkage to blast resistance locus by the PROC-GLM program in the Statistical Analysis Systems package [27].

Linkage values were calculated using the MAPMAKER-QTL [18]. Markers were positioned on chromosomes with a LOD > 2.0, and map distance was estimated in Kosami centiMorgans (cM) [17].

Results

Genetic and non-genetic variation of rice blast resistance

The distributions of field and greenhouse disease scales during the 3 years period are shown in Table 1. According to the normality test for frequency distributions, distributions for rice blast were skewed toward the male parent (KHK) in these three years. The F_1 plants of the cross between TNG 69 and KHK were susceptible to rice blast, indicating that resistance trait was under recessive gene control.

In general, less phenotypic variation was observed in greenhouse condition (Table 1). The mean of disease scale for 3 years were 6.00, 6.27, 6.44, respectively, while in the field they were 4.70, 6.78, 6.88. Therefore, the estimated broad sense heritability by EMS of the combined ANOVA from field was less than those of greenhouse for which the estimated heritability on an entry mean basis were 85.4%. There were highly significant differences among F_2-derived lines for rice blast and interactions of the lines × years (P=0.0001). There were no significant differences among these three years (Table 2). In the field condition, the heritability was 73.7%. There were highly significant differences among F_2-derived lines, years and interactions of the lines × years for rice blast resistance (P=0.0001) (Table 3). In these three years, the disease reaction in the first year was vividly different at the two sites tested.

The difference of disease incidence between greenhouse and field tests

It is expected that the result of artificial inoculation in greenhouse could be served as an indicator of field blast infection. Therefore, correlation analysis between the artificial infection in greenhouse and field tests was conducted. The result showed that the correlation coefficient was significant at 5% level (R^2=0.6806). Nevertheless, 33 F_2-derived lines were not consistent in disease reaction between greenhouse and field (data not shown). According to correlation coefficient analysis, artificial inoculation in greenhouse test could represent the result of field test.

We divided disease response into

Table 1. Means and ranges of disease resistance scale of F_2-derived lines investigated in the field and greenhouse for three years.

Year	F_2-derived lines			Parents	
	Site	Mean	Range	TNG69	KHK
1999	Greenhouse	6.00	1-9	1-3	5-9
	Field	4.70	1-7	1-3	5
2000	Greenhouse	6.27	1-9	3	5-9
	Field	6.78	1-9	3-5	7-9
2001	Greenhouse	6.44	1-9	1-3	5-9
	Field	6.83	3-9	1-3	7

Table 2. The combined ANOVA of resistance to rice blast for 160 F_2-derived lines of TNG69 X KHK population in the greenhouse.

Source of variation	df	MS(EMS)	F value	Pr>F
Years(Y)	2	6.84	0.40	0.7014
Replication/Y (R/Y)	3	16.97		
Lines(L)	159	18.96 (=M1)	12.51	0.0001
L×Y	318	2.67 (=M2)	1.76	0.0001
Error	477	1.52 (=M3)		

σ^2_g =(M1-M2)/RY=2.715; σ^2_{ph}=M1/RY=3.160; $h^2_B = \sigma^2_g \div \sigma^2_{ph}$=0.854

Table 3. The combined ANOVA of resistance to rice blast for 160 F_2-derived lines of TNG69 X KHK population in the field.

Source of variation	df	MS(EMS)	F value	Pr>F
Years(Y)	2	464.49	69.32	0.0031
Replication/Y	3	6.70		
Lines(L)	159	7.29 (=M1)	6.93	0.0001
L×Y	318	1.92 (=M2)	1.83	0.0001
Error	477	1.05 (=M3)		

σ^2_g =(M1-M2)/RY=0.895; σ^2_{ph}=M1/RY=1.215; h^2_B =$\sigma^2_g \div \sigma^2_{ph}$=0.737

resistant and susceptible groups, and conducted genetic analysis (χ^2-test). The genetic analysis for rice blast resistance was not consistent between greenhouse and field tests. In the greenhouse test, of 160 F_2 derived lines, 123 lines were susceptible and 37 lines were resistant. The segregation of susceptible and resistant phenotypes in the F_2 population was agreed with 3:1 ratio (χ^2=0.30). The results supported that the resistance of TNG 69 to *M. grisea* race IF2 was governed by one single recessive gene [4]. However, the F_2 segregation also showed good fit to 13:3 ratio (χ^2=2.00), indicating that the resistance might be possible controlled by two genes instead of one.

In the field test for 160 F_2-derived lines, 130 lines were susceptible and 30 lines were resistant. The segregation of susceptible and resistant in the F_2 population fitted completely for a 13:3 ratio (χ^2=0). Thus, rice blast resistance of TNG 69 might be affected by one dominance inhibitor gene and one recessive gene, with intergenic interaction.

The genetic mechanism of rice resisting to blast resistance characters have been extensively studied and two types of resistance have been described, i.e. incomplete (field) and complete (true) resistance [8, 23]. As a result, we used the evaluated results from both field and greenhouse tests to conduct genetic analysis of rice blast resistant and gene mapping.

Parental polymorphism and informative marker survey

Frequency of polymorphism within rice subspecies (i.e., japonica/japonica and indica/indica types) is generally lower than that between subspecies (i.e., japonica/indica) [29]. The parents used in this study, i.e. TNG 69 and KHK, are both japonica rice varieties. The percentage of polymorphism was relatively lower between japonica varieties (19.9%) than between indica × indica (30%) [32], japonic × japonica (24.1%) [1], and lowland japonic variety × upland japonic variety (37%) [9]. In this study, of the 1180 RAPD, 88 SSLP, 100 ISSLP, 80 STS and 162 STS + SSR primers tested, 324, 29, 13, 13, and 16, respectively were polymorphic between TNG 69 and KHK, respectively. The SSLP primer exhibited more polymorphism (29/88=33.0%) than the other type primers between parents. The lowest polymorphism was detected in SSLP + STS primers with 10.0% polymorphism (Table 4).

While testing polymorphic markers between the parents, we used the 160 F_2 derived lines to verify their segregation in the F_2 population. The total percentage of informative primers, which were segregating in the F_2 population, was 7.1% (115/1610) and the highest primer was SSLP primer (10.2%). The SSLP+STS primers were the lowest (1.9 %) (Table 4).

QTL analysis and blast resistance in F_2-derived lines

Greenhouse test

The informative markers were used to

Table 4. Summary of primers tested in RAPD, SSLP, ISSLP, STS and SSLP+STS studies for identification of DNA polymorphism in the parents and F_2 derived lines.

Type of primer	No. of primers tested	No. of polymorphic primers	Percentage. of polymorphic primers(%)	No. of informative primers (markers)	Percentage of informative primers(%)
RAPD	1180	324	27.4	98(112)	8.3
SSLP	88	29	33.0	9(14)	10.2
ISSLP	100	13	13.0	3(4)	3.0
STS	80	13	16.3	2(2)	2.5
SSLP+STS	162	16	10.0	3(3)	1.9
Total	1610	395	19.9	115(135)	7.1

construct a rice linkage map [11]. The SAS-GLM and MAPMAKER-QTL programs were employed to identify markers linked with QTL. In a total of 135 DNA markers, 23 markers were on linkage group A with 3 markers unlinked, and showed a significant correlation to the blast resistance scale at the 1 % level in the year of 1999, 2000, 2001 and the combined data from three years, while six markers showed a significant correlation at the 5 % level in each year trial following the analysis performed with SAS/GLM (data not shown).

The result of MAPMAKER/QTL analysis is shown in Table 5 and Fig 1. The analysis revealed that four intervals involved in resistance to blast were mapped on linkage groups A, C, H and L, respectively in 1999. R165~R750-1 intervals on linkage group A contributed 74.2% of the phenotypic

Table 5. Intervals significantly associated with variation in rice blast resistance in greenhouse for 160 F_2-derived lines of the TNG69 X KHK population.

Interval	Linkage group	R^2 (%)	LOD score	Means of QTL Genotypic Classes			Parental contribution
				A_1A_1	A_1A_2	A_2A_2	
1999							
R165~R750-1	A	74.2	24.42	3.21	6.72	7.31	TNG69
R760~R170	C	67.2	4.70	6.44	7.15	2.38	KHK
OPD2~R769-1	H	69.6	7.92	6.69	7.03	1.75	KHK
Rm249~R192	L	59.2	3.25	6.32	7.02	2.12	KHK
2000							
R758~R750-1	A	56.0	20.81	3.89	6.78	7.23	TNG69
R791~R760	C	64.6	3.41	6.17	7.12	3.34	TNG69
R309-2~OPD2	H	42.9	3.34	6.13	7.03	3.44	KHK
2001							
OSR32~R503	A	50.0	22.42	3.89	5.77	8.39	TNG69
Rm249~R192	L	71.8	3.11	7.51	3.99	7.98	KHK
Combined data							
R165~R750-1	A	61.0	24.20	4.40	6.92	8.07	TNG69
R309-2~R769-1	H	36.5	2.91	6.67	7.41	4.48	KHK

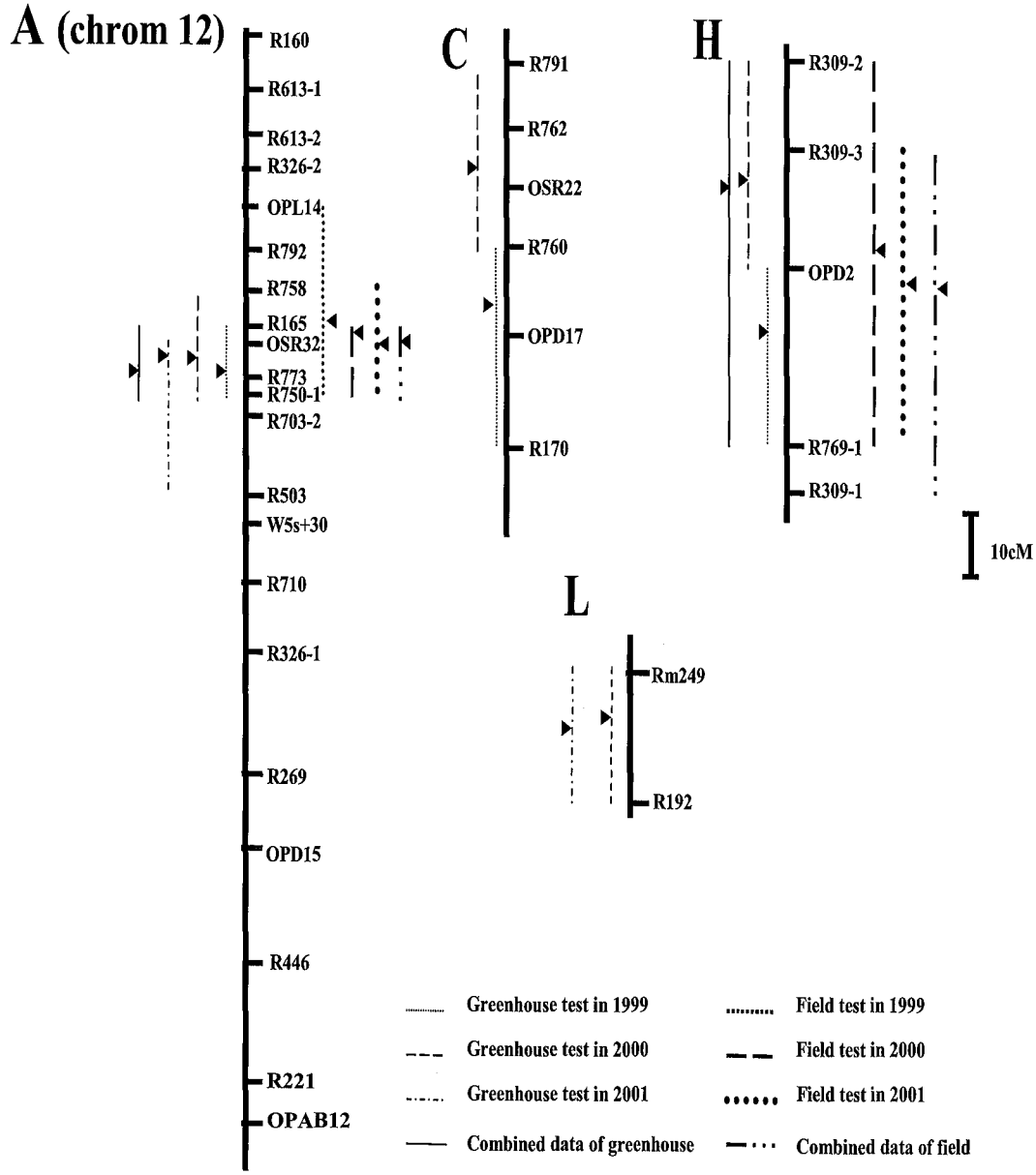

Fig 1. Locations of QTLs controlling rice blast resistance for the TNG69 x KHK population. The straight lines and arrows beside the linkage groups show the confidence intervals and locations of the detected QTL, respectively.

variation (R^2 value) and the high LOD score (24.42). The F_2 plants with A_1A_1 genotype had higher resistance scale (3.21) for rice blast than those of A_2A_2 genotype (7.31). The heterozygous (A_1A_2 genotype) was susceptible for rice blast (6.72) therefore the resistant phenotype revealed a recessive inheritance at this interval. The blast resistant allele was contributed from the female parent

TNG 69. The LOD scores of the other three intervals on linkage groups C, H and L were 4.70, 7.92 and 3.25, respectively. All of the blast resistant alleles contributed from the male parent KHK. Three intervals related to blast resistance were mapped on linkage groups A, C and H in 2000. The intervals on linkage groups A and C contributed resistance alleles from the TNG 69. R758~R750-1 intervals was located on the similar region of linkage group A to 1999. Its phenotypic contribution was 56.0%, and LOD score was 20.81.

In the trial of 2001, two intervals were mapped on linkage groups A and L. Similar interval on linkage group A was also mapped in 1999 and 2000. Rm249~R192 on the linkage group L had high R^2 value (71.8%). The combined data have shown that there were two intervals mapped on the linkage groups A and H. They both were detected at least for two years. The OSR32 ~ R750-1 interval on the linkage group A was consistent identified in each year and combined data.

The large phenotypic contribution, high LOD score, and consistent expression in each year and combined data suggested that the QTL in interval OSR32~R750-1 of linkage group A expressed as a major gene in greenhouse test. Very high LOD scores, i.e. 24.42 in 1999, 20.81 in 2000, 22.42 in 2001, and 24.20 at combined data were detected in this interval. As the major gene was located on linkage group A, all of markers on group A were significant correlation to the rice blast score at 1 % level.

Based on QTLs mapping, the results were not entirely consistent in each year for greenhouse test. In 1999 interval R760~R170 and in 2000 interval R791~R760 on the linkage C had high R^2 value (67.2 and 64.6 %), but not observed in 2001 and combined data. The interval of Rm249~R192 on linkage group L detected in 1999 and 2001 had high R^2 values (59.2 and 71.8%) but not found in 2000 and combined data. Moreover, QTLs genetic classes of the interval in 1999 and 2001 were not consistent.

Field test

For the field test, there were 8 markers on linkage group A and 2 markers unlinked, and there was significant correlation to the blast resistance at the 5 % level in each year and the combined data of all these years following the analysis performed with SAS/GLM (data not shown).

The result of MAPMAKER/QTL analysis has been shown in Table 6 and Fig 1. It is indicated that one interval was mapped on linkage group A in 1999. The R^2 value and LOD score of the interval on the linkage group A was 12.7% and 4.59, respectively. This was not same as the greenhouse result, for which the R^2 value and LOD score of intervals on the linkage group A was the higher (74.2% and 24.42). Two QTLs were mapped on linkage groups A and H in 2000. Both of them were detected in greenhouse in 2000, too. The QTL on the linkage group A had high LOD score (19.42), the R^2 value (52.3%) was similar to greenhouse in the same year, and R^2 value (47.9%) of interval on the linkage group H was similar to the R^2 value of greenhouse test in 2000 (42.9%). The LOD score was 5.64.

Two intervals were detected in 2001 for disease scale. They located at linkage groups A and H, respectively. The LOD score (7.43) of R758~R750-1 was lower than that of greenhouse condition. From combined data two QTLs were mapped on linkage groups A and H, and their R^2 value (41.9%) on the linkage group A were lower than the combined data in greenhouse test (61.0%).

The QTL on linkage group A contributed resistant allele from TNG 69. The other three QTLs on linkage group H contributed resistant alleles from KHK. They all were detected in greenhouse condition, too. However, all R^2 value and LOD sore of QTL on the linkage group A were lower than those in greenhouse condition.

Table 6. Intervals significantly associated with variation in rice blast resistance in field for 160 F_2-derived lines of the TNG69 X KHK population.

Interval	Linkage group	R^2 (%)	LOD score	Means of QTL Genotypic Classes			Parental contribution
				A_1A_1	A_1A_2	A_2A_2	
1999							
OPL14~R750-1	A	12.7	4.59	3.89	4.59	4.97	TNG69
2000							
R165~R750-1	A	52.3	19.42	4.39	7.00	7.58	TNG69
R309-2~R769-1	H	47.9	5.64	6.58	7.30	3.94	KHK
2001							
R758~R750-1	A	21.3	7.43	5.64	6.88	7.32	TNG69
R309-3~R769-1	H	51.5	3.33	6.85	7.23	4.39	KHK
Combined data							
R165~R750-1	A	41.9	14.81	5.03	7.69	6.93	TNG69
R309-2~R769-1	H	51.8	5.71	6.73	7.37	4.19	KHK

Comparison between detected QTL in both sites

Some difference for rice blast response between field and greenhouse tests was found in SAS-GLM analysis. There were seventeen markers not consistent in the two sites. Eight markers linked with QTLs were detected in field test but not in greenhouse test, while nine markers linked with QTLs were detected in greenhouse test but not in field test. Twenty-nine markers associated with QTLs were consistent in field and greenhouse tests (data not shown). The QTLs identified between field and greenhouse tests were more different. Eleven QTLs were detected in greenhouse test, but seven QTLs were detected in field test only. LOD score in greenhouse test was generally higher than that in field test (Table 5 and 6). QTLs identified in both tests were mapped in the same regions of linkage group A or H. Four QTLs were sensitive to environment on linkage groups C and L, and they were recognized in greenhouse test not in field test (Fig 1). In summary, QTLs detected in greenhouse test had stronger expression than that in field test.

Discussion

In Taiwan, the breeding programs have been planed to develop high quality varieties with resistance to disease and insect usually using japonica rice as parents. To facilitate our breeding aims, molecular linkage maps based on japonica × japonica crosses have to be developed so that marker-assisted selection (MAS) will be feasible for exploiting favorable traits in japonica rice. However, it is difficult and low efficiency for constructing a linkage map of intra-subspecific crosses because of lower level of DNA polymorphism than inter-subspecific crosses.

Recently, TNG 69 rice variety has stable resistance to blast in Taiwan. Its resistance is very complicated and is supposed to be contributed from both parents, CI5309 (American variety originated from China) and *O. rufipogon*, IRRI Acc. No. 100923 (Wild type)[10]. According to the results shown in this study, its resistance to rice blast

was controlled by two major genes with modifying genes (Tables 5, 6; Fig 1).

In our study, blast resistance genes of rice identified from field and greenhouse tests were different. However, major QTLs in both sites were mapping on the linkage group A. Because the anchored SSLP marker OSR32 has been well studied [2], the major gene for blast resistance is assigned in chromosome 12 of rice (Fig 1). Phenotypic contribution of each identified QTL was ranged from 21.3 to 72.4% with LOD score from 7.43to 24.42 in all environments except in the field test in 1999. F_2 plants with only a DNA band (A_1A_1 genotype) contributed from TNG69 had better disease resistance than those of other genotypes (A_2A_2 and A_1A_2 genotypes). It revealed that the resistance character from TNG 69 was controlled by a recessive gene (Tables 5, 6).

A total of 55 rice blast resistance genes have been reported until now (Rice Oryzabase Network, 2004). Fourteen of these genes, *IP_i (t), IP_i 3(t), P_i t, (P_i 4(t)), P_i 12(t), P_i 12(t)*, P_i 14(t), P_i 157, P_i157 (t), P_i 19(t), P_i 20, P_i 21(t), P_i tq(6), P_i 62(t), P_i 6(t)* and *P_i 24* were located on chromosome 12. Because the linked markers were different in each study a direct comparison cannot be made. However, *P_i ta (P_i – 4(t)), P_i tq(6)*, and *P_i 62(t)* were dominant gene [15, 16, 28, 33]. *Ipi(t)* and *IPi3(t)* genes contributed from *O. longistaminata* [3]. The other *P_i -12(t), P_i 12(t)*, P_i – 14(t)* and *Pi 24* were QTL and originated from *Indica* type [14]. No sufficient information to demonstrate that the remaining five genes [(*P_i 157, P_i 19(t), P_i 157(t), P_i 20* and *P_i 21(t)*] and the resistance genes detected in our study were different. Hence, it is necessary for additional allelism tests in the future.

The resistance to rice blast in this study is recessive. It is affected by a major gene on chromosome 12 and the other major gene on linkage group H (Table 6). The result is similar to the field resistance in Japanese upland rice [9], both of them have the common marker OSR32 linked with a rice blast resistant gene. But most previously reported disease resistance genes are dominant [15, 16, 19, 28, 33]. A detailed survey of this gene might be a useful approach to understanding the mechanism of defense response in TNG 69.

Field resistance usually controlled by polygenes is defined as the resistance that allows effective control of a parasite under natural field conditions and is considered to be durable when exposed to new blast races [9]. Therefore, field resistance is a very useful strategy for disease resistance breeding. The QTLs related with rice blast resistant characters were also detected in greenhouse test, and all of them were expressed at least twice in the four times tested. This indicates that disease test in greenhouse is stable and representative than field test is. The usefulness of markers linked to blast resistance genes will be discussed in the context of breeding for durable resistance. Tightly linked DNA markers may facilitate early selection for blast resistance genes in breeding programs.

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