RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2006.32.133

ScienceAsia 32 (2006): **133-142**

QTL Mapping for Leaf and Neck Blast Resistance in Khao Dawk Mali105 and Jao Hom Nin Recombinant Inbred Lines

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Received 4 Oct 2005
Accepted 6 Feb 2006

ABSTRACT: Blast disease caused by a fungus called *Pyricularia grisea* Sacc. (perfect stage = *Magnaporthe grisea* Sacc.) is one of the most devastating rice diseases in the world. To date, the genetic relationship of leaf and neck blast is not well understood. Some rice cultivars known to carry leaf blast resistance genes are susceptible to neck blast. Five hundred eighty-seven recombinant inbred lines (RILs) derived from a cross between Khao Dawk Mali 105 (susceptible) and Jao Hom Nin (resistant) cultivars were developed by single seed descent and used as rice materials to identify quantitative trait loci (QTL) associated with leaf and neck blast resistance. Rice materials were assayed for both leaf and neck blast resistance using three selected blast isolates, designated THL191, THL318 and THL899. Correlation coefficients between leaf and neck blast resistance were low (0.28-0.56), suggesting the presence of two different pathosystems. A chi-square (X^2) test fitted a ratio of approximately 3:1, reflecting the presence of at least 2 resistance QTL functioning against leaf and neck blast. Fourteen QTL were identified and mapped on three chromosomes: 1, 11 and 12. The QTL for resistance against leaf and neck blast on chromosome 1 were coincidentally mapped to the RM5-RM104 interval for all isolates. Jao Hom Nin contributed all resistant QTL alleles. QTL were detected on chromosome 11 with high LOD values for both leaf and neck blast resistance against THL191 and THL899. Three major genes, *Pi 7(t)*, *Pi 1* and *Pi lm*[2], have been reportedly located within the vicinity of these QTL. Jao Hom Nin contributed all resistant QTL alleles. QTL on chromosome 12 showed a race-specificity for leaf and neck blast against THL318 and THL899. The peak of resistance QTL on chromosome 12 was located between the RM179 and RM309 markers. Jao Hom Nin contributed resistant QTL alleles against THL899, while Khao Dawk Mali 105 contributed resistant QTL alleles against THL318. QTL interactions behaved additively for both leaf and neck blast resistance. Coincident, QTL on three chromosomes revealed the presence of resistance gene clustering in these genomic regions. Three QTL were detected by QTL analysis while 2 QTL were obtained from the chi-square test for THL899, which suggested that the RIL population used was large enough that even a small QTL could be detected. The coincident locations of leaf and neck blast resistance QTL on chromosomes 1 and 11 and associations of molecular markers with QTL will be useful information for marker assisted selection.

KEYWORDS: QTL mapping, blast resistance, recombinant inbred lines.

INTRODUCTION

Rice blast is one of the major rice diseases for rice growing areas all over the world. The causal agent is the fungus called *Pyricularia grisea* Sacc., with the perfect stage being called *Magnaporthe grisea* Sacc. The name *P. grisea* is more popular among researchers and will be used in this paper. Rice blast epidemics occurred in most provinces in the north and some in the northeast of Thailand in 1992[1], causing a reduction in rice production of 650,000 tonnes in rice production and a loss of approximately 3,000 million baht. Blast is

certainly a threat for farmers, particularly those who grow quality varieties, such as Khao Dawk Mali 105 (KDML105) or Kho Khor 6 (RD6), which are highly susceptible to blast. Jao Hom Nin (JHN) is a commercial non-glutinous rice variety resistant to both leaf and neck blast diseases under natural conditions. It is grown in the central and northern part of Thailand.

The use of resistant cultivars is one solution to prevent or reduce yield loss due to rice blast epidemics. However, resistant rice varieties, especially when resistance is based on single major genes, may be rapidly overcome by compatible races of the pathogen.[2] Qualitative or complete resistance shows a reaction indicating the absence of the compatible type lesion, is controlled by major genes(s)[3], has race specificity[4], and shows hypersensitivity to the pathogen. Quantitative (incomplete) resistance, also called field resistance or partial resistance, is in general, more durable than qualitative resistance.

At least 30 blast resistance loci have been identified in *Oryza sativa* L.[5] Of these, 20 are major genes and 10 are putative QTLs. Twelve of the major genes have been confirmed to be non-allelic. Eight loci have been reported on chromosome 11, i.e. *Pi-f, M-Pi-z, Pi-se-1, Pi-is-1, Pi-k, Pi-1(t), Pi-7(t) and Pi-a*. Four loci have been reported on chromosome 6, *Pi-2(t), Pi-z, Pi-3(t)* and *Pi-i*.[6] Contrasting responses between the vegetative stage and reproductive stage often occur, indicating different genes may be needed for resistance to leaf and neck blast infection. While genes resistant to leaf blast have been studied extensively, those resistant to neck blast have rather limited information. The year 2002 was a turning point when information on neck blast resistant genes was reported by 3 groups of researchers. Wu *et al*[7] found resistance to both leaf and neck blast infection under field conditions in rice cultivar Gumei 2, while Zhong156 was only resistant to that of leaf blast. Zuuang *et al*[8] followed up these results by studying a segregating population of Zhong156 and Gumei2. They found that some lines were resistant at seedling stage but susceptible at reproductive stage. This suggested that some genes responsible for leaf blast resistance were not effective at reproductive stage. In the same year, Sirithunya *et al*[9] mapped QTLs for leaf blast on chromosomes 7 and 9, while those for neck blast were mapped on chromosomes 5 and 6.

Molecular marker technology is widely used nowadays. It has been applied for the identification and mapping of genes conferring both complete and partial resistance, in particular. It has also provided insight into the genetic basis of durable resistance.[10] Many major genes for blast resistance have been identified using this method. Therefore, the QTL mapping strategy was used in this study to locate QTL for leaf blast (LB) and neck blast (NB) resistance in the rice genome and the genetic relationship between LB and NB resistance was studied.

Materials and Methods

Plant Material and Rice Blast Inoculation

Five hundred eighty-seven $F_{2:6}$ recombinant inbred lines (RIL) derived from the cross between JHN and KDML105 were developed by single seed descent at the National Center for Genetic Engineering and Biotechnology, Rice Gene Discovery Unit, Kasetsart University Kampaengsaen Campus, in 2000. This population was established for genetic mapping of blast resistance and submergence tolerance[11]. The parental lines and RIL population were assayed for both leaf and neck blast resistance using three blast isolates. Three blast isolates, designated THL191 (Thailand collection number 191), THL318 and THL899, were selected based on the basis of being aggressive to the mapping parents and being of different genetic compositions as classified by Amplified Fragment Length Polymorphism (AFLP data not shown).

Leaf blast screening was carried out by planting tested lines, ten plants each for replication, along two sides of the baskets. Three rice cultivars, designated KDML105, KTH17 and RD23, were sown as susceptible checks, while JHN was sown as a resistant check. Each of the selected blast isolates was cultured on rice polish agar and incubated at 26 ± 1 °C under light conditions for 14 days and then transferred to the near ultraviolet-light chamber for 3 days to enhance sporulation. The inoculum concentration was adjusted to $5x10^4$ conidia/ml. One percent of gelatin solution was added to the inoculum at a 1:1 ratio. Leaves of twenty-one day old seedlings were inoculated by spraying and incubated in a plastic growth chamber at 26 ± 1 °C under 12-hour alternate light and dark conditions in the growth room for 2 days, after which the cover was opened. The seedlings were maintained in the growth room for an additional 5 days under the same conditions. High humidity was provided by an automatic overhead misty-sprayed springkler. The program was set to spray for 5 seconds every hour. Leaf blast was recorded 7 days after inoculation on a 0 to 9 scale, as described by Standard Evaluation System for rice (SES).[12]

For neck blast screening, the parental lines and RIL population were sown in the field using the dibbling method. Fertilizer was applied twice during growth stages. The first application was commenced using 15-15-15 at 187.5 kg/ha (28.12 kgN/ha) approximately 30-35 days after seeding. The second one was applied 55-60 days after seeding using (46-0-0) at 125 kg/ha (57.5 kgN/ha). Neck blast inoculum was prepared as described above. The inoculum concentration was adjusted to $5x10^4$ conidia/ml. One percent gelatin

solution was added to the inoculum at a 1:100 ratio to ensure contact of the conidia to the plant surface. The inoculum was then injected into the leaf sheath at booting stage. The plants were maintained under a 60% light-allowance sarland net. High humidity was obtained from automatic misty-spray springklers. The program was setup to spray for 30 minutes 3 times a day at 11.00 am, 2.00 pm and 3.30 pm, respectively. Neck blast scoring was recorded at 21 days after inoculation on a 0 to 9 scale, as described by the Standard Evaluation System for Rice.[12]

Genetic Analysis

A linkage map was originally constructed in the F_2 population in 2003 using 111 markers, including 99 SSLPs, two SSCPs, three STSs and 7 RGAPs[11]. Sixteen SSR markers, RM5, RM246, RM237, RM319, RM212, RM104, RM21, RM206, RM254, RM224, RM139, RM144, RM179, RM309, OSR32 and AC113249, were chosen for partial linkage map construction of the 587 $F_{2:6}$ RIL population. The fourteen markers beginning with RM (Rice microsatellite markers) were developed in the Cornell University laboratory[13]. The OSR32 marker corresponded to a microsatellite marker previously reported by Akagi *et al*[14], while AC113249 was a microsatellite marker developed from the AC113249 BAC library. Selection of markers was based on the information on genomic location of blast resistance obtained from preliminary experiments (data not shown). These markers located on three genomic locations could detect blast resistance QTL effectively. Six SSRs were detected on chromosome 1, RM5, RM104, RM212, RM237, RM246 and RM319. Seven SSRs were detected on chromosome 11, RM21, RM139, RM144, RM206, RM224, RM254 and AC113249. Three SSRs were detected on chromosome 12, OSR32, RM179 and RM309. SSR assay and analysis followed that described by Panaud *et al*[15].

Linkage Map Construction and QTL Analysis

MAPMARKER/QTL software[16] was used for linkage map construction from the $F_{2:6}$ RIL population. The recombination frequency (rmax) of 0.30 and a LOD score of >2.5 were utilized to determine the final linkage map. The linkage group for corresponding chromosomes was assigned following the rice genetic

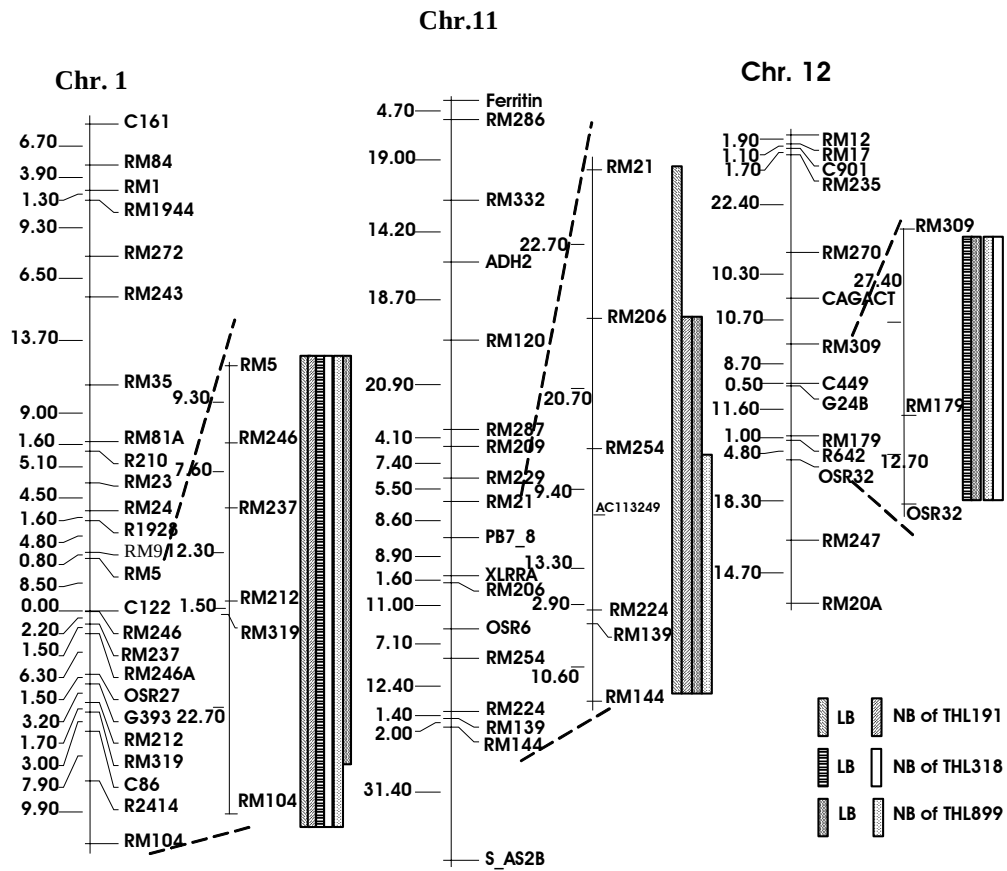

Fig 1. Linkage map from F_2 and F_6 of 587 RILs from KDML105 and JHN with sixteen SSRs markers were used to locate QTLs on chromosomes 1, 11 and 12.

map reported by Kurata *et al*[17] and Chen *et al*[18] The genetic distance (cM) was determined from recombination values using the Kosambi function.

QTL were mapped by means of the interval mapping (SIM) and simplified composite interval mapping (sCIM) procedures of NQTL, a software for interval mapping.[19,20] The phenotypic data from the inoculation with each of the three isolates were analyzed separately. For the NQTL analysis, each data set was analyzed with 1000 permutations at a 5 cM walking speed and a type I error rate of 5%. For sCIM, four background markers with approximately even spacing were specified with a maximum of three background markers per linkage group. STATGRAPHIC 2.1 software was used as a tool to reconfirm number, location and effect of the QTLs and to determine the phenotypic variance explained (PVE) by QTLs or R^2. Two loci interactions of QTLs were determined using regression analysis and ANOVA. The chi-square test was used to proof the distribution ratio of this population. Scores for resistance reactions were 0, 1, and 3, while 5, 7, and 9 were susceptible ones.

Results

Linkage Map Construction

A linkage map was constructed using MAPMAKER/QTL solfware developed by Lander *et al*[16] with distances between markers computed using the Kosambi function. The linkage on chromosome 1 consisted of 6 markers spanning 51 cM. Seven markers constituted the linkage on chromosome 11, which covered a genomic segment spanning 84.9 cM. The total distance of linkage on chromosome 12 appeared to be the shortest one, being 32.8 cM with three markers constituting this linkage group (Figure 1).

Expression of Resistance to Leaf and Neck Blast Diseases and Their Correlation with Each Other

Phenotyping reactions within the population revealed classes of resistant and susceptible reactions for leaf and neck blast inoculation (Tables 1, 2 and Figure 2). The phenotypic distribution of blast reactions did not show discreate classes. When the reaction data

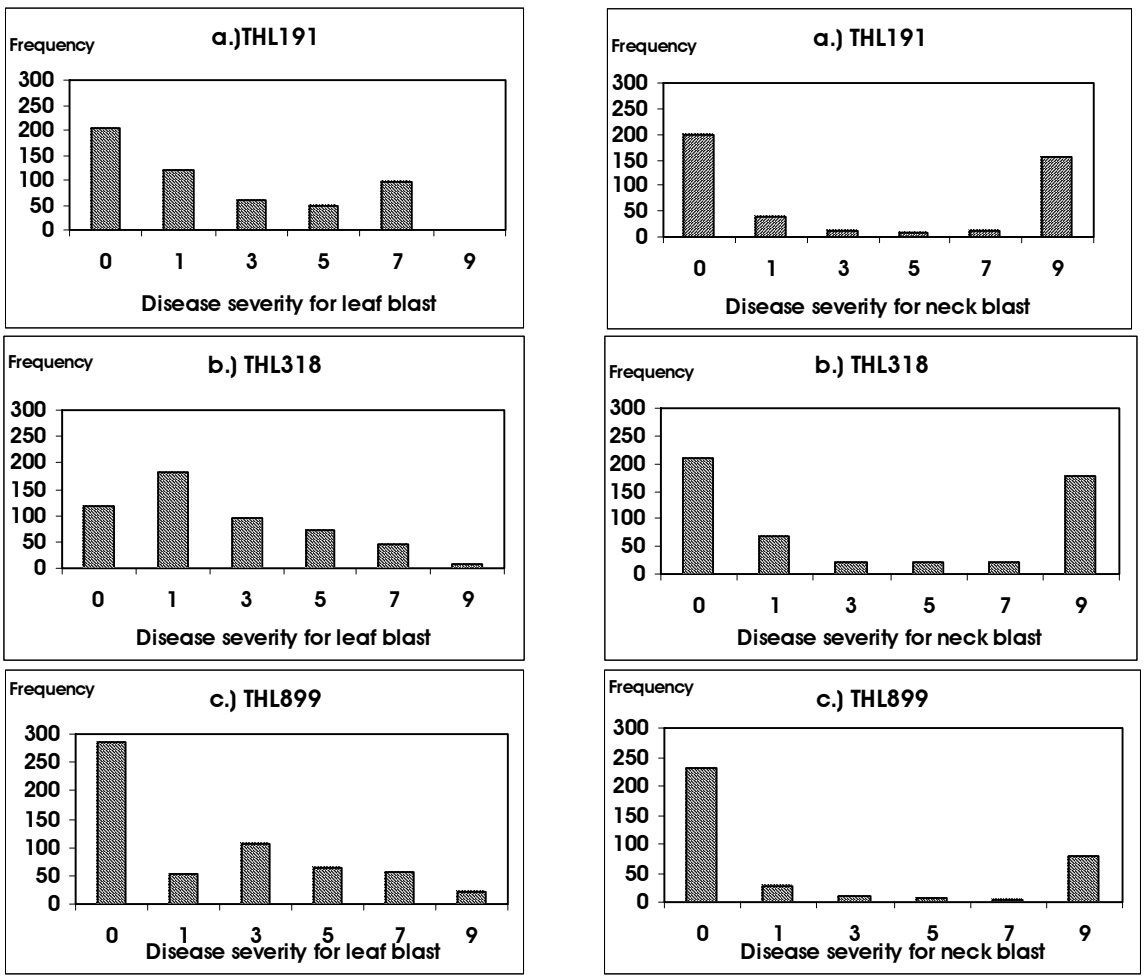

Fig 2. Frequency distribution of disease severity of leaf and neck blast resistance in the 587 RILs screened by 3 selected isolates.

Table 1. Ratios between resistant and susceptible reactions of 587 RILs screened for leaf blast resistance to 3 selected isolates with chi square values.

Isolate	R			S			Total	Ratio	X^2
	0	1	3	5	7	9		R:S	
THL191	204	121	60	47	98	2	532	3 : 1	1.96^{ns}
THL318	118	183	96	72	45	7	521	3 : 1	0.38 ^{ns}
THL899	286	52	106	64	57	21	586	3 : 1	0.18 ^{ns}

ns = non-significant.

Table 2. Ratios between resistant and susceptible reactions of 587 RILs screened for neck blast resistance to 3 selected isolates with chi square test.

Isolate	R			S			Total	Ratio	X^2
	0	1	3	5	7	9		R:S	
THL191	197	39	11	9	11	156	423	3 : 1	62.1**
THL318	210	67	22	22	20	178	519	3 : 1	85.0**
THL899	231	28	9	6	2	80	356	3 : 1	0.01 ^{ns}

** = significant at 1% level.
ns = non-significant.

were classified as resistant (scores 0-3) and susceptible (scores 5-9), the ratio of resistant:susceptible plants fitted a 3:1 ratio ($P > 0.30$ from a chi-square test) for all data sets, except for those of the neck blast data using THL191 and THL318, which fitted a 1:1 ratio. In comparison to the response given by THL191 and THL318, THL899 appeared to trigger the activation process for the same set of genes/QTL at both growing stages (leaf and neck). The effective genes triggered by THL899 during the vegetative stage remained effective at the reproductive stage, while some of effective genes triggered by THL191 and THL318 during vegetative stage were not effective at the reproductive stage. Although, THL899 could be a rather atypical and perhaps weak blast strain, it induced the expression of QTL for both leaf and neck blast, which was beneficial for this analysis.

Correlation Between Leaf and Neck Blast Severity in the RIL Populaton

Pairwise analysis of severity scores on RI lines from different isolates showed significant correlation in the phenotypic levels of resistance between leaf and neck blast (r = 0.32 for THL191, r = 0.28 for THL318, r = 0.56 for THL899). The higher correlation coefficient of 0.56 obtained from the inoculation with THL899 in comparison to those with THL191 and THL318 showed a stronger relationship between the two pathosystems, and may be explained as the possible ability of THL899 to induce the action of the same genes at both vegetative and reproductive stages. Teng *et al*[21] mentioned that leaf and neck blast are two different pathosystems due to time discontinuity, and the relationship between the two is yet to be defined. The difference in the blast reaction at seedling and reproductive stages was reported by Zuuang *et al*[8]. Interestingly, results from this section suggested that there is a genetic relationship between resistance genes against the two pathosystems.

Quantitative Trait Loci (QTL) Analysis

The numbers, genomic locations and effects of the QTL were resolved using NQTL software with a LOD threshold 2.5 or above.[19,20] Fourteen QTL were detected for leaf and neck blast resistance. The genomic location, phenotypic varience explained (PVE) and LOD score of detected QTL are shown in Table 3 and Figure 1. Through the inoculation with THL191, four QTL were identified for leaf and neck blast. Two QTL, qLB1-1 and qNB1-1, for leaf and neck blast resistance, respectively, were detected on chromosome 1. The qLB1-1 coincided with the qNB1-1 locus located at the peak between the RM212 and RM104 markers. Another

Table 3. Intervals of QTLs on chromosomes 1, 11 and 12 located by 16 SSRs markers.

Isolate	Infected Tissue	QTL	Chromosome	Contributor	Interval	LOD	R^2 (%)	Total R^2 (%)
THL191	Leaf	qLB1-1	1	JHN	RM5-RM104	13.15	2.74	41.40
		qLB11-1	11	JHN	RM21-RM144	50.82	12.8	
	Neck	qNB1-1	1	JHN	RM5-RM104	20.46	16.57	52.05
		qNB11-1	11	JHN	RM206-RM144	40.19	34.58	
THL318	Leaf	qLB1-2	1	JHN	RM5-RM104	18.17	8.28	35.77
		qLB12-2	12	KDML105	OSR32-RM309	24.64	4.25	
	Neck	qNB1-2	1	JHN	RM5-RM104	25.10	16.75	25.86
		qNB12-2	12	KDML105	OSR32-RM309	7.45	6.03	
THL899	Leaf	qLB1-3	1	JHN	RM5-RM104	12.18	8.63	42.55
		qLB11-3	11	JHN	RM206-RM144	42.63	26.94	
		qLB12-3	12	JHN	OSR32-RM309	7.72	6.79	
	Neck	qNB1-3	1	JHN	RM237-RM104	5.89	9.02	39.8
		qNB11-3	11	JHN	RM254-RM144	12.84	22.12	
		qNB12-3	12	JHN	OSR32-RM309	10.25	17.51	

two QTL, qLB11-1 and qNB11-1, for leaf and neck blast resistance, respectively, were detected on chromosome 11. These QTL were coincidently located at the peak between the AC113249 and RM224 markers and appeared to have much larger effects than the other two on chromosome 1, qLB1-1 and qNB1-1. The coefficients of determination (R^2) or PVE for THL191 in detecting QTL for leaf blast and neck blast resistance against this isolate were 41.40% and 52.05%, respectively, with JHN as the sole contributor for all QTL alleles.

Four QTL were detected on chromosomes 1 and 12 for leaf and neck blast when the second isolate, THL318, was inoculated onto the same population (Table 3 and Figure 1). Two QTL, qLB1-2 and qNB1-2 were detected on chromosome 1, with LOD scores of 18.17 and 25.10 for leaf and neck blast resistance, respectively. The genomic location of these two QTL coincided with those of qLB1-1 and qNB1-1. These QTL explained 35.77% and 25.86% of the PVE for leaf and neck blast resistance in this population. JHN contributed the resistant alleles for these QTL. The other two QTL, qLB12-2 and qNB12-2, were detected on chromosome 12 with LOD scores of 24.64 and 7.45. These QTL were coincidently located at the peak OSR32–RM309 interval. Interestingly, KDML105 contributed resistant alleles for both QTL loci. The two QTL, qLB1-2 and qLB12-2, jointly explained 35.77% of phenotypic variance for leaf blast, while qNB1-2 and qNB12-2 collectively explained 25.86% of the phenotypic variance for neck blast.

Three QTL, qLB1-3, qLB11-3 and qLB12-3, were detected with LOD scores of 12.18, 42.63 and 7.72, respectively, for leaf blast resistance against isolate THL899 (Table 3). They were located on three chromosomes, 1, 11 and 12. The qLB1-3 was mapped at the RM5–RM104 interval, and coincided with qLB1-1, qLB1-2, qNB1-1 and qNB1-2. The qLB11-3 QTL was mapped at the RM206-RM144 marker interval and coincided with the qLB11-1 and qNB11-1. The qLB12-3 was mapped at the OSR32–RM309 marker interval and coincided with the qLB12-2 and qNB12-2. These three QTL accounted for 42.55% of phenotypic varience for leaf blast resistance. For all QTL, JHN significantly reduced the severity score for leaf blast infection. The same trend of response was found for neck blast resistance. Three QTL, qNB1-3, qNB11-3 and qNB12-3, were detected with LOD scores of 5.89, 12.84 and 10.25, respectively. These QTL coincided with qLB1-1, qLB1-2, qNB1-1 and qNB1-2 on chromosome 1, qLB11-1 and qNB11-1 on chromosome 11 and qLB12-2 and qNB12-2 on chromosome 12, collectively. These QTL explained 39.8% of phenotypic varience of neck blast against THL899. Once again, for all QTL found, JHN significantly reduced the severity score for neck blast infection.

There was one interesting point involving the detection of QTL following inoculation with THL899. Although the R: S ratio from phenotypic reactions for both leaf and neck blast screenings using THL899 were equivalent to 3:1, meaning 2 QTL were present, data from the QTL analysis showed that 3 QTL were detected by this isolate. This result may be due to the fact that the population used in this study was reasonably large, so even a small QTL could be detected.

QTL x QTL Interactions

QTL x QTL interactions were characterized using ANOVA and multiple regression with the significant best-fit model ($P < 0.01$). The testing was based on genotypes with the closest marker locus of each QTL. For leaf blast resistance, a significant interaction was detected between the two main-effect QTL (qLB1-1 and qLB11-1 for THL191and qLB1-2 and qLB12-2 for THL318) and among three main-effect QTL, qLB1-3,

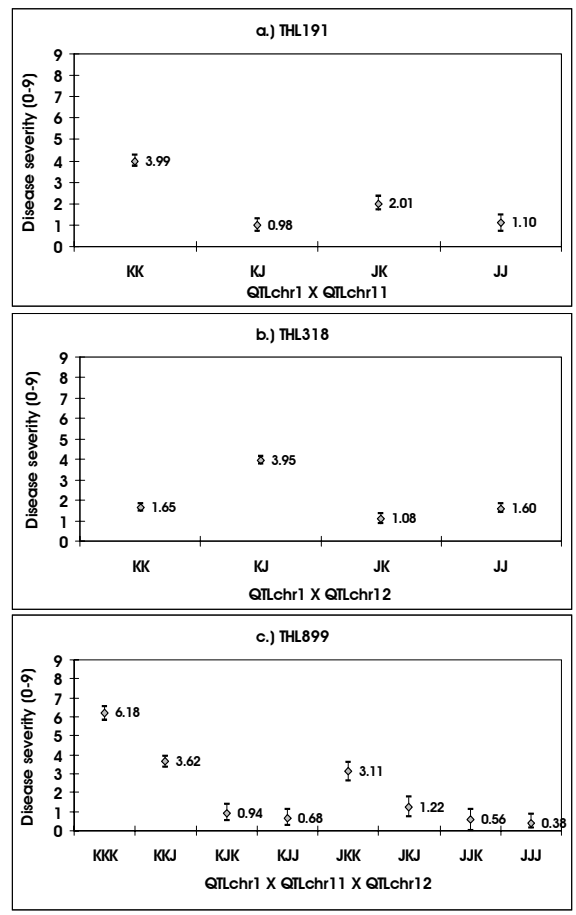

Fig 3. Interactions between QTLs of leaf blast resistance on chromosomes 1,11 and 12 obtained from inoculation with THL191, THL318 and THL899. Disease severity scores are plotted with 95.0% LSD intervals.

qLB11-3 and qLB12-3, for THL899. QTL analysis showed that qLB11-1 was the major QTL for leaf blast resistance against blast isolate THL191. This was reflected as lower mean severity scores ranging from 0.98 - 1.1 whenever the JHN allele for qLB11-1 was present on the genome, in comparison with 2.01-3.99 for the KDML105 alleles (Figure 3a). The presence of JHN alleles at the qLB1-1 locus strongly reduced the severity scores.

Both qLB12-2 and qLB1-2 are major QTL for leaf blast resistance against the blast isolate THL318. Since the qLB1-2 resistant allele was obtained from JHN while that of qLB12-2 was obtained from KDML105, transgressive segregation was observed, as seen from high disease severity score of 3.95 for recombinant lines with the KDML105 allele at qLB1-2 and JHN at qLB12-2 (Figure 3b). There were additive interactions among three QTL on chromosomes 1, 11 and 12 after inoculation with THL899. The qLB11-3 was the major QTL for leaf blast resistance against the THL899 blast isolate. Whenever the JHN allele was present at the qLB11-3 locus, the mean score of disease severity was lower than 1. The lowest mean score of 0.38 was recorded when all of the QTL loci were JHN alleles, suggesting a high degree of leaf blast resistance (Figure 3c).

Similar results were observed for the QTL x QTL interaction of the neck blast using THL191. The qNB11-1 was a major QTL and JHN allele at this locus caused low severity scores ranging from 0.5 - 1.5. The presence of the JHN allele at the qNB1-1 locus also strongly reduced the severity scores (Figure 4a). The presence of alleles from JHN at both loci helped in reducing mean score of disease severity in RIL by 73-88 %, in comparison with the presence of alleles from KDML105 at both loci.

Unlike leaf blast, qNB1-2 functioned as a major QTL for neck blast resistance against THL318. The presence of the JHN allele at this locus resulted in low severity scores, ranging from 1.17 - 1.29. qNB12-2, on the other hand, behaved as a minor QTL. RILs that carried only the KDML105 allele at this locus were moderately resistant (Disease severity score 3.69) to neck blast. Transgressive segregation towards susceptibility was also observed in the recombinant progenies (Figure 4b). The mean disease severity score of those lines was as high as 6.5.

Additive significant interactions among qNB1-3, qNB11-3 and qNB12-3 were also observed following inoculation with THL899 for neck blast screening. Since JHN alleles at all loci contributed to neck blast resistance, the presence of JHN alleles at at least one locus in the RILs showed a low range of mean disease severity score, 0.05-1.21, while RILs without any JHN QTL alleles were very susceptible, having mean disease

Table 4. Phenotypic reaction of RILs screened for leaf and neck blast with three isolates.

Isolates	LB	NB	Groups	No. of RI lines	Proportion in the RI population(%)
THL191	R	R	RR	189	36.1
	R	S	RS	85	16.3
	S	R	SR	59	11.3
	S	S	SS	190	36.3
THL318	R	R	RR	231	50.5
	R	S	RS	110	24.1
	S	R	SR	43	9.4
	S	S	SS	73	16.0
THL899	R	R	RR	269	50.9
	R	S	RS	76	14.4
	S	R	SR	70	13.3
	S	S	SS	113	21.4

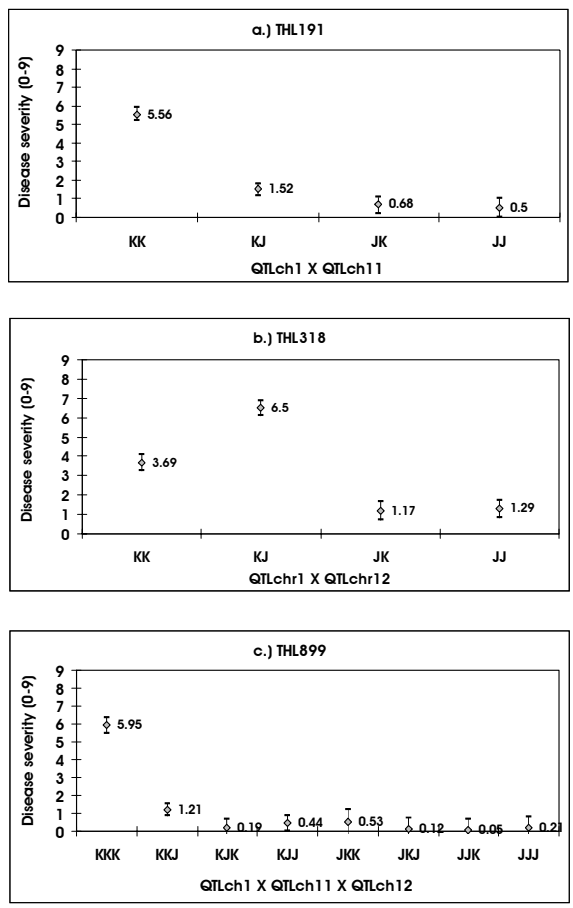

Fig 4. Interactions between QTLs for neck blast resistance on Chromosomes 1,11 and 12 obtained from inoculation with THL191, THL318 and THL899. Disease severity scores are plotted with 95.0% LSD intervals.

severity scores of 5.95 (Figure 4c).

Associations between Leaf and Neck Blast QTL

In our study, genomic locations of QTL associated with resistance to leaf and neck blast were mapped to approximately the same locations. To determine the relationship between QTL for leaf blast and QTL for neck blast, the F_6 RIL population was classified into four groups based on resistance phenotypes, as shown in Table 4.

Interestingly, approximately three quarters of the population expressed resistance to both leaf and neck blast. Another quarter expressed resistance to one but not the other. A significant number of recombinant lines responded differently to leaf and neck blast screenings using the same isolate which suggested that there might be different genes for leaf and neck blast resistance situated within the same region on the chromosome. A high amount of recombination also pointed out that these genomic gene clusters are situated in a hot spot.

Discussion

It had been said that "leaf and neck blast are two different pathosystems due to time discontinuity and the relationship between the two is yet to be defined" Teng *et al*[21] and Zuuang *et al*[8]. However, results from this study have provided more information, as they indicated that QTL associated with resistance to leaf and neck blast were located in the same genomic regions. This evidence may suggest a strong genetic relationship between these two pathosystems. Apart from this, a significant number of recombinant lines expressed different responses for leaf and neck blast, which suggested that there might be different resistance genes for leaf and neck blast situated within the same regions on the chromosomes. This part of the findings was similar to what had been reported in recent work which studied an F_8 recombinant inbred population of the cross between Zhong 156 X Gumei 2[22]. Genetic analysis in this study indicated that the resistance to leaf blast was controlled by three genes, but one of the three genes was ineffective for neck blast. Based on the RGA, AFLP and RAPD markers used in this study, 2 genes, tentatively assigned as *Pi24(t)* and *Pi25(t)*, were mapped onto chromosomes 12 and 6, respectively. *Pi24(t)* conferred resistance to only leaf blast and its resistance allele was from Zhong 156. *Pi25(t)* conferred resistance to both leaf and neck blast and its resistance allele was from Gumei 2.

In recent years, the successful cloning of more than 20 blast disease resistance genes in plants has dramatically advanced our understanding on molecular basis of disease resistance, leading to the revelation that some resistance genes are related in function and evolution. Furthermore, individual members of these multigene families are diverged to confer different specificities[23,24], and resistance genes of diverse origin with different pathogen specificity share similar structural motifs Wang *et al*[25]. Therefore, genes conferring resistance to different pathosystems and pathogens are frequently found to be colocalized in the genome. Since R genes confer resistance to a few strains of a given pathogen, each is thought to encode a receptor that recognizes directly or indirectly the corresponding avirulence-gene product from the pathogen[26]. In our study, at least two QTL for leaf and neck blast resistance were detected for a particular isolate used in the screening experiments. One has major effect and the other has minor effect on resistance phenotypes. We speculate that the major QTL might be strictly related to R gene recognition specificity and the minor QTL might be related to defense responsive genes.

The genomic segment (RM5-RM104) on chromosome 1 habouring qLB1-1, qLB1-2, qLB1-3, qNB1-1, qNB1-2 and qNB1-3 was consistently detected for leaf and neck blast resistance with a relatively small effect on resistance phenotypes. The peaks of QTL were found near the RM212 marker, which was close to the RZ19–RG331 flanking markers reported by Prashanth *et al*[27] and Wang *et al*[10], who also identified QTL associated with blast lesion number, blast disease leaf area and blast lesion size. Furthermore, in 2005, a new gene identified as *Pi37(t)* was detected by Chen *et al*[28] on chromosome 1 close to RM212. It will be of great interest to discover the molecular basis of colocalization of QTL associated with leaf and neck blast resistance. It is speculated that this may have resulted from linkage or pleiotropy. However, a cluster of genes for rice ESTs with disease-resistance gene or defense-response gene-like sequences was reported by Wang *et al*[25] and Wen *et al*[29] on chromosome 1. The genomic location coincided with the QTL for leaf and neck blast resistance detected in our study. These ESTs might be good gene candidates for these QTL. Interestingly, NPR1 (a key regulator of salicylic acid-mediated system acquired resistance) homologs were among these ESTs. This may support the idea that these QTL are presumably non-race specific and may contribute to durable leaf and neck blast resistance. The study by Wen *et al*[29] suggests that some resistance QTL are due to actions of the defense-responsive genes or genes that are allelic to them.

qLB11-1, qNB11-1, qLB11-3 and qNB11-3 associated with leaf or neck blast resistance were clustered at the RM21-RM144 interval on chromosome 11. These QTL were detected with extremely high LOD values (LOD = 50.8 for qLB11-1, 40.19 for qNB11-1,

42.63 for qLB11-3 and 12.84 for qNB11-3) and showed some degree of race specificity. This might indicate the presence of major resistance genes contributed by JHN. These genes are likely to be the key to initiation of the defense response and might be strongly related to R gene recognition specificity. As pointed out by Wu *et al*[7], some QTL are indeed a manifestation of major R genes. In this study, the QTL cluster on chromosome 11 was located in the vicinity of the genomic segment carrying the well-defined blast resistance genes *Pi-7(t)*, *Pi 1 and Pi-lm*[2] reported by Wang *et al*[10], Hittamani *et al*[30], Inukai *et al*[31] and Tabien *et al*[32] and also a major blast resistance QTL reported by Prashanth *et al*[27]. This cluster is also located in the vicinity of the major bacterial blight resistance loci *Xa21* and *Xa4*[18]. Wang *et al*[25] found some disease resistance gene clusters were located in the regions containing major QTL associated with blast, bacterial blight and sheath blight. Based on the resistance phenotype in RIL, we found that whenever RIL carried this QTL cluster with JHN alleles, the disease severity score was decreased. Since the locations of the QTL were analogous to well-defined major genes, this suggested that these QTL may be related to some previously reported major genes. Further genetic studies on these chromosomal region are needed to determine the genetic relationship between these QTL and well-defined blast resistance genes.

Unlike those on chromosomes 1 and 11, QTL on chromosome 12 had resistant alleles contributed by both KDML105 (qLB12-2 and qNB12-2) and JHN (qLB12-3 and qNB12-3). This result indicated the presence of race specificity of the QTL. A study by Li *et al*[33] showed that defeated major genes can act as QTL contributing to race specificity. The chromosomal region haboring these QTL coincided with many blast resistance genes such as *Pi ta* reported by Hittamani *et al*[30], *Pi 4a(t)* reported by Kiyosawa[34], *Pi 4b(t)* reported by Inukai *et al*[35], *Pi 4(t)* reported by Mew *et al*[36] and *Pi20(t)* reported by Imbe *et al*[37]. A major resistance QTL for leaf and neck blast reported by Sirithunya *et al*[38] is also located at the same chromosomal region as the QTL found in our study. The genetic relationship of these QTL and major blast resistant genes previously reported needs to be clarified in further genetic studies.

In conclusion, 14 QTL for both leaf and neck blast resistance were detected on chromosomes 1, 11 and 12 when the F_6 RIL population of KDML105 X JHN was inoculated with 3 selected blast isolates. Six out of fourteen QTL, 3 for leaf (qLB) and 3 for neck (qNB) blast resistance, were mapped on chromosome 1 with the peak being between RM212 and RM104. The next four QTL, 2 qLB and 2 qNB, were detected within the AC113249 – RM224 region on chromosome 11. The remaining four QTL, 2 qLB and 2 qNB, were located within the OSR32 – RM309 region on chromosome 12. In most cases, except for 1 qLB and 1 qNB on chromosome 12, resistant alleles were contributed by JHN. QTL interactions were statistically significant and behaved additively for both leaf and neck blast resistance. In addition to this, the four QTL on chromosome 11 were clustered with extremely high LOD values, had some degree of race specificity and were located in the vicinity of the genomic region carrying well-defined major genes.

Thus, in the future, clarification of the relationship between the QTL found and major blast resistance genes should be carried out. Furthermore, since resistance genes on chromosome 11 with alleles being contributed by JHN are highly likely to confer durable resistance, introduction of these genes into Thai elite lines/varieties through marker assisted selection should fulfill the objective of breeding for resistance to both rice leaf and neck blast.

Acknowledgements

We would like to express our sincere thanks to staff members and post-graduate students in the Biotechnology Unit and Rice Blast Project, National Center for Genetic Engineering and Biotechnology, Kasetsart University Kampaengsaen Campus, for their assistance in laboratory work and data analysis. We greatly appreciate the Rockfeller Foundation for the post-graduate scholarship provided for the first author.

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