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# Resistance QTLs controlling leaf and neck blast disease identified in a doubled haploid rice population

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**Abstract** One of the biotic constraints in rice production worldwide is blast disease which can control by planting resistant varieties. To find out effective resistance, blast resistance quantitative trait loci (QTL) were mapped against 20 and 3 virulent isolates for leaf blast and neck blast, respectively, using 111 doubled haploid lines from the cross of IR64 and Azucena. QTLs associated with leaf blast were found on chromosomes 1, 2, 4, 7, 8, 10, 11, and 12 ($\%R^2=3.6-64.3$), while neck blast linked QTLs were identified on chromosomes 1, 6, 10, and 12 ($\%R^2=6.4-22.6$). The new QTLs were identified on chromosome 1; however, most QTLs were mapped in the vicinity of resistance genes in previous references. The genetic relationship of leaf and neck blast was explained by the coincidence of detected QTLs and positive value of pathogenicity correlation ($r=4.5-4.7$). This study provides reliable QTLs locations that will benefit rice breeding programs to develop new cultivars containing durable and broad-spectrum resistance to leaf and neck blast disease.

**Keywords** Broad-spectrum resistance · Leaf blast disease · Neck blast disease · Quantitative trait loci mapping · Doubled haploid lines

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## Introduction

Blast disease caused by the fungus *Pyricularia oryzae* (teleomorph *Magnaporthe oryzae),* is one of the severe diseases in rice (*Oryza sativa*) worldwide. This fungus can survive in many host species, including barley (*Hordeum vulgare*), crabgrass (*Digitaria sanguinalis*), foxtail millet (*Setaria italica*), oat (*Avena sativa*), rice (*Oryza sativa* L.), wheat (*Triticum aestivam*), wild millet (*Eriochloa villosa*), green foxtail (*S. viridis*), and goose grass (*Eleusine indica*) (Couch and Kohn 2002; Qi et al. 2019). Therefore, the isolate purified from one host can cause the disease in others (Kongprakhon et al. 2009; Hyon et al. 2012). In addition, high genetic diversity of *P. oryzae* was observed in the region of south China, Laos, and north Thailand and identified as the center of origin (Saleh et al. 2014). Accordingly, the collection of Thai blast isolates from rice, wild rice, barley, and weed was assessed as having high genetic diversity, especially in the northern part, followed by the northeastern and central part (Mekwatanakarn et al. 1999; Sirithunya et al. 2008).

Rice blast disease is commonly observed in both vegetative (leaf blast) and reproductive (neck blast) stages and can directly cause severe yield losses (Disthaporn 1994; Khush and Jena 2009). Breeding blast-resistant cultivars are considered the most effective measure for blast management. Growing blast resistance varieties is a preferable method for protecting rice from blast disease and reducing the use of fungicides. Therefore, genetic information about resistance genes is required to advance effective breeding against the diverse blast fungi. Two types of rice blast resistance are generally classified as qualitative (complete) and quantitative (partial) resistance. Qualitative resistance is normally controlled by a single major gene and confers race-specific resistance following the gene-for-gene hypothesis. Using a single resistance gene, the improved varieties are frequently break down sooner after being released by the compatible race (Babujee and Gnanamanickam 2000) that might be the rare natural or the developed novel pathotypes. Quantitative resistance is controlled by minor genes that allow infection and lesion formation but restrict lesion expansion for non-race-specific interaction resulting in slow disease spread. Although the expression of major genes can mask the minor gene effect, partial resistance from quantitative genes was required and associated with increased resistance durability (Srivastava et al. 2017).

Several cultivars with broad spectra resistance have been reported, such as Jao Hom Nin, Moroberekan, and IR64 (Chaipanya et al. 2017; Sallaud et al. 2003; Wang et al. 1994). Nowadays, more than 100 blast resistance genes have been reported, especially on chromosome 6, 11, and 12 (Srivastava et al. 2017); however, few quantitative resistance genes for blast disease was characterized, for example, *Pi21*, *Pi35*, and *Pb1* (Fukuoka et al. 2009, 2014; Hayashi et al. 2010). Most identified/cloned resistance genes were resistance against leaf blast but less resistance for neck/panicle blast disease. The neck blast resistance genes/QTLs have been reported on rice chromosomes 5, 6, and 11 (Fang et al. 2019; Hayashi et al. 2010; Sirithunya et al. 2002). Many studies reveal the positive correlation of the resistance QTLs between leaf and neck blast (Babasaheb Aglawe et al. 2017; Kalia and Rathour 2019). According to the correlation, leaf and neck blast resistance QTLs were mapped in the same regions on chromosomes 1, 11, and 12 (Noenplab et al. 2006). However, genetic control of neck blast resistance to broad-spectrum isolates is still poorly understood. More studies related to neck blast resistance should be conducted.

The improved *indica* cultivar IR64 has been extensively grown worldwide because of its good adaptability and high-yielding potential. IR64 presented multiple genes, conferring resistance to different blast isolates in several countries (Sallaud et al. 2003; Tharreau et al. 2000). In this study, a doubled-haploid population derived from a cross of IR64 and Azucena was used to study the genetics of leaf and neck blast resistance to different races of the pathogen. The QTL mapping approach investigated the genomic locations, genetic effects, and molecular markers linked to resistance genes against diverse blast isolates. The identified QTLs and markers linked to QTLs will be informative for genetic study and durable blast resistance in the rice breeding program.

## Materials and methods

### Plant materials

Thirty-eight rice cultivars were varying in blast resistance genes, as shown in Table 1, were used in leaf

**Table 1** Broad-spectrum resistance (BSR) of 38 rice varieties, including IR64 and Azucena, tested by 120 Thai isolates (R = resistance, M = moderate, and S = susceptible)

| Varieties name | R-genes | Number of isolates | | | | BSR |
|---|---|---|---|---|---|---|
| | | R | M | S | Total | |
| C103TTP | *Pi-1* | 98 | 0 | 0 | 98 | 1.00 |
| Pi-no-4 | *Pi-ta2* | 118 | 0 | 0 | 118 | 1.00 |
| Fukunishiki | *Pi-z* | 119 | 0 | 0 | 119 | 1.00 |
| Mars | *Pi-z* | 116 | 0 | 0 | 116 | 1.00 |
| Moroberekan | *Pi-5, Pi-7* | 117 | 0 | 1 | 118 | 0.99 |
| Apura | *Pi-6* | 115 | 1 | 0 | 116 | 0.99 |
| BL-1 | *Pi-b* | 118 | 0 | 1 | 119 | 0.99 |
| Toride-1 | *Pi-zt* | 118 | 1 | 0 | 119 | 0.99 |
| Norin-22 | *Pi-sh* | 117 | 2 | 0 | 119 | 0.98 |
| Shin-2 | *Pi-ks* | 115 | 4 | 0 | 119 | 0.97 |
| **IR64** | | 107 | 2 | 2 | 111 | 0.96 |
| Nipponbare | *Pi-sh* | 113 | 5 | 0 | 118 | 0.96 |
| IAC 25 | *Pi-t* | 99 | 4 | 0 | 103 | 0.96 |
| Reiho | *Pi-a, Pi-ta* | 111 | 6 | 0 | 117 | 0.95 |
| Dawn | *Pi-d,Pi-i* | 111 | 6 | 0 | 117 | 0.95 |
| Dular | *Pi-ka* | 108 | 5 | 2 | 115 | 0.94 |
| K-3 | *Pi-kh* | 111 | 7 | 0 | 118 | 0.94 |
| Katy | *Pi-ta2* | 110 | 6 | 1 | 117 | 0.94 |
| C101A51 | *Pi-2* | 110 | 8 | 1 | 119 | 0.92 |
| Tsuyuake | *Pi-km* | 108 | 5 | 4 | 117 | 0.92 |
| Kanto-51 | *Pi-k* | 107 | 9 | 2 | 118 | 0.91 |
| Tetep | *Pi-k* | 104 | 8 | 2 | 114 | 0.91 |
| IR8 | *Pi-p* | 105 | 7 | 3 | 115 | 0.91 |
| C104LAC | *Pi-1* | 92 | 9 | 2 | 103 | 0.89 |
| K-1 | *Pi-ta* | 80 | 13 | 0 | 93 | 0.86 |
| Bluebonnet-50 | *Pi-a* | 94 | 21 | 0 | 115 | 0.82 |
| K-59 | *Pi-t* | 83 | 21 | 3 | 107 | 0.78 |
| Yashiro-Mochi | *Pi-ta* | 91 | 14 | 12 | 117 | 0.78 |
| **Azucena** | | 88 | 25 | 1 | 114 | 0.77 |
| Zenith | *Pi-a, Pi-z* | 87 | 16 | 13 | 116 | 0.75 |
| Fujisaka5 | *Pi-i* | 82 | 29 | 8 | 119 | 0.69 |
| C101TTP | *Pi-4a* | 48 | 22 | 6 | 76 | 0.63 |
| Sha-Tiao-Tsao | *Pi-ks* | 73 | 10 | 33 | 116 | 0.63 |

blast disease screening against broad-spectrum resistance. These included IR64, a well-adapted indica variety for irrigated conditions, and Azucena, an upland japonica variety from the Philippines, which was used as the parent of a mapping population. The population of one hundred and eleven doubled haploid lines (DHLs) from a cross between IR64 and Azucena (Guiderdoni et al. 1992) was used to identify QTL controlling resistance to broad spectral blast isolates. Three rice varieties, Sariceltik, KDML105, and CO39, were used as susceptible check varieties.

Blast isolates

One hundred and twenty isolates were collected from various rice, wild rice, and barley in different regions of Thailand (Table S1). Each isolates represented isolates from distinct genetic groups of blast pathogen based on AFLP (Amplified Fragment Length Polymorphism) patterns and pathogenicity evaluation (Data not shown). These isolates were used to identify virulent patterns on thirty-eight rice cultivars. For resistance QTL identification, the DHL population

was evaluated by artificial inoculation method against 20 isolates (Table S1). Of these, 20 isolates were used for leaf blast screening and 3 of 20 isolates were conducted for neck blast screening.

Evaluation of leaf blast disease in greenhouse

The disease evaluation was performed in the seedling stage at Rice gene discovery unit (RGDU), Kasetsart University, Kamphaeng Saen campus (KU-KPS), Nakhon Pathom province, in the wet season. Seven plants of each cultivar/line were randomly grown in the perforated plastic trays filled with aerobic soil using a randomized completely block design with 3 replications. 5 g/m$^2$ of ammonium sulfate $(NH_4)_2SO_4$ was applied at 10, 3, and 1 day before inoculation to increase susceptibility to blast disease. Fungal isolates were cultured in sterile Petri dishes containing rice polished agar medium (2% rice polish, 2% agar and 0.2% yeast extract) for eight days at 25 °C under fluorescent light. To induce sporulation, the mycelial mat was scraped and then transferred to an ultra-violet cabinet for 2–3 days. To prepare inoculum, distilled water was added to the petri dish, and scraped the mycelial mat with a sterilized glass spreader. The suspension was filtered through two layers of cheesecloth and adjusted concentration to $5 \times 10^4$ conidia /ml in 0.5% gelatin. 100 ml of the inoculum was sprayed on four-five leaf stage rice with an air-brush sprayer. Inoculated plants were kept inside the chamber (25 ± 3 °C and > 80% of RH) for 16–18 h and then transferred to the greenhouse. The leaf blast disease severity was visually scored on the individual plant seven days after inoculation using 7 lesions type described by Roumen et al. (1997). The disease score of 0–2 was considered resistant, with a score of 3–4 being referred to as moderate and a score of 4–6 as susceptible reactions. Broad-spectrum resistance (BSR) was used to assess the broad-spectrum resistance using the formula modified from Ahn (1994) as follows:

$$BSR = S/T$$

S = Number of isolates giving a resistance reaction

T = Total Number of isolates used for screening

T is 120 for parental and differential set screening and 20 for the mapping leaf blast experiment. The BSR ranged from 0 to 1. The BSR of 0 indicated that the rice cultivar or line was susceptible to all isolates. The BSR of 1 indicated that the rice cultivar or line was resistant to all isolates.

Evaluation of neck blast disease in the rice field

The experiment was carried out on a randomized complete block design with 2 replications (10 plants per replication for each line) in the late wet season, during which night temperature was 22–28 °C with high humidity and day temperature was 30–35 °C. The rice field was prepared in the pool at RGDU, KU KPS, Nakhon Pathom province. The susceptible controls were planted as border rows of each plot. Artificial inoculation was conducted in two steps in the evening. The first step was placing five to seven pieces of medium covered by sporulating mycelium around the neck node, then loosely covered with aluminum foil to hold it in place and retain the moisture after inoculation. The second step was dropping 2 ml of spore suspension that was adjusted to $10–30 \times 10^4$ conidia/ml on sporulating slices using a syringe. The aluminum foil and pieces of medium were removed 48 h after inoculation. The disease severity index was scored using the scale 0, 1, 3, 5, 7, and 9 as described by IRRI (1996). Neck blast severity was visually scored twice at 2 and 3 weeks after inoculation. The severity score 0 and 1 were referred to as resistance, 3 and 5 were referred to as moderate, and 7 and 9 were referred to as susceptible reactions.

Molecular map construction

The genotypic data of IR64 and Azucena DHL population was provided by Dr. S. McCouch, Cornell University, USA (Personal communication). Additional seventy-six simple sequence repeats (SSRs) markers described by Chen et al. (1997) and Temnykh et al. (2000) were genotyped on the DHL population. For SSR genotyping, genomic DNA was isolated from leaves using DNA Trap Kit (DNA Technology Laboratory, Thailand). PCR was performed in a 10 µl reaction mixture containing 20–25 ng of template DNA, 1X PCR buffer, 2.5 mM $MgCl_2$, 0.25 µM of each primer, 0.2 mM of each dNTP, and 0.5 unit of Taq polymerase. Amplification began with a denaturation

step of 94 °C for 3 min followed by 30 cycles (30 s at 94 °C, 30 s at 55 °C and 2 min at 72 °C) and completed by 7 min incubation at 72 °C. The PCR products were separated by electrophoresis in 4.5% denaturing polyacrylamide gel (80 W for 60 min), and the banding patterns were visualized using silver staining. The data of SSR markers were integrated into the framework map of Huang et al. (1997) to increase the density of the linkage map. The map contained a total of 251 markers in this study which included 141 RFLPs (restriction fragment length polymorphisms), 14 RAPDs (random amplified polymorphic DNA), 76 SSRs markers, 8 isozymes, and 12 cloned genes. Linkage analysis was carried out using the MAPMAKER/EXP3.0 program and map distance (cM) based on the Kosambi function.

### Statistical and QTL analyses

QTL analysis was performed using the MQTL software package (Tinker and Mather 1995). Simple interval mapping (SIM) and simplified composite interval mapping (sCIM) methods were used to determine the association between disease scores and marker genotypes. For MQTL, each data set was analyzed with 1000 permutations, a 5 cM walking speed, and a Type I error rate of 5%. The presence of QTL was identified when the LOD score was equal to or more than 2.4. Associations between markers and disease scores were reconfirmed using simple regression, multiple regression, and analysis of variance (ANOVA) procedures in the software STATGRA PHIC 2.1 which resulted phenotypic variance of each QTL ($R^2$). Interactions of QTL were analyzed when found associated QTL against each isolate in more than one position. ANOVA and least significant difference (LSD) determined at 95% confidence level were made based on the allelic composition of doubled haploid lines at peak marker (haplotype). $R^2$ of QTL interaction was retrieved from multiple regression analysis of the significant markers and disease scores.

### Phenotypic data analysis

Disease scores from leaf and neck blast disease screening were used in ANOVA as the calculated average of disease scores. In mapping the population, frequency distribution and pathogenicity correlation between leaf and neck blast disease were analyzed using the average disease score. These data analyses were performed on STATGRAPHIC 2.1 software. Testing a goodness of fit with the segregation ratio of 7:1, 3:1, and 1:1 by Chi-square ($\chi^2$) was done, followed $\chi^2 = \sum(O-E)^2/E$ where O = observed value (actual value) and E = expected value. Broad sense heritability is calculated from variance components following $H^2 = \sigma^2 g/[\sigma^2 g + (\sigma^2 e/r)]$ where $\sigma^2 g$ represents the genotypic variance, $\sigma^2 e$ the error variance, and r the number of replicates.

## Results

### Pathogenicity and broad-spectrum resistance (BSR)

Thirty-eight cultivars, including IR64 and Azucena, were used to screen compatible reactions against 120 local isolates. IR64 and Azucena had a broad-spectrum resistance (BSR) level of 0.96 and 0.77, respectively (Table 1). High levels of BSR were found in the rice cultivars carrying resistance genes, C103TTP (*Pi-1*), Fukunishiki (*Pi-z*, *Pi-sh*), BL-1 (*Pi-b*), Pino-4 (*Pi-ta2*), Toride-1 (*Pi-zt*), and Moroberekan (*Pi-5*, *Pi-7*), as 1.00, 1.00, 0.99, 1.00, 0.99, and 0.99, respectively and low levels of BSR as 0.03, 0.12 and 0.49 were reacted by Sariceltik, CO39, and KDML105, respectively.

Then, 20 virulent isolates were selected from 120 isolates to evaluate the DHL mapping population (Table S1). Based on the isolates that can infect IR64 and Azucena until they showed a moderate and susceptible reaction.

### Genetic map completion

Genotypic data of additional 76 polymorphic SSR markers on 111 DHLs were integrated into the polymorphic data set of 175 markers provided by Dr. S. McCouch, Cornell University, USA. Then, two hundred and fifty-one markers were used for genetic map construction. The genetic map covered a total distance of 1985 cM from twelve linkage groups, and the average distance between two markers was 8.3 cM. The markers on each chromosome were 30, 26, 28, 16, 15, 22, 19, 26, 21, 14, 20, and 14 for chromosomes 1 to 12, respectively. The marker order was

consistent with published maps constructed by Huang et al. (1997), as shown in Fig. 1.

Phenotypic distribution of blast resistance in doubled haploid lines

Evaluation of blast disease was conducted in the DHL population of IR64 and Azucena for leaf blast in the seedling stage and neck blast in the heading stage. Twenty isolates were selected from the pathogenicity test on the parental varieties of DHL. These isolates were purified from several parts of rice (leaf, neck, and collar) and barley seed (TH2 and TH3). Leaf blast screening was performed using twenty selected isolates, while only 3 of 20 isolates (nbTHL191, nbTHL557, and nbTHL949) were used in neck blast screening. Broad sense heritability was 53.7% on average. The disease score distribution is presented in Fig. 2.

**Fig. 1** The linkage map of 12 chromosomes based on 111 DHLs population from the cross of IR64 × Azucena, including 251 markers and resistance loci against 20 leaf blast isolates, 3 neck blast isolates, and BSR of leaf/neck blast

Distribution of leaf blast screening (Fig. 2 A) showed the segregation ratio of the DHL population between resistance and susceptible reaction fitted into a 1:1 ratio when performed by the isolates THL16, THL48, THL84, THL191, THL458, THL557, THL892, THL943, THL949, and THL1001 which isolated from leaf and neck tissue. The ratio of 3:1 was none significantly different from the segregation ratio of the progenies inoculated by TH2, TH3, THL112, THL364, and THL902. For THL924 and THL928, the ratio fitted a 7:1 ratio. The rest isolates were not fitted into any ratio. However, it was between the ratio of 3:1 and 7:1 for THL868 and close to a ratio of 3:1 for THL899 and THL1008.

DHL progenies were inoculated by 3 rice isolates for neck blast screening which showed the distribution in Fig. 2B. The isolates nbTHL557 and nbTHL949 were isolated from the disease that occurred on the neck. Their segregation ratio fitted into the ratio of 3:1 and 1:1, respectively. For nbTHL191, it was isolated from the leaf part and can cause the disease symptom on the neck part. But the segregation ratio of nbTHL191 screening was not fitted into any ratio but was close to a 3:1 ratio.

The phenotypic screening showed that these data were effective in discovering resistance QTL, which is controlled by a single gene according to the finding of QTL against isolating THL191, THL892, and THL943 (Table 3) and/or multi genes according to the segregation ratios.

## Correlation between neck blast and leaf blast

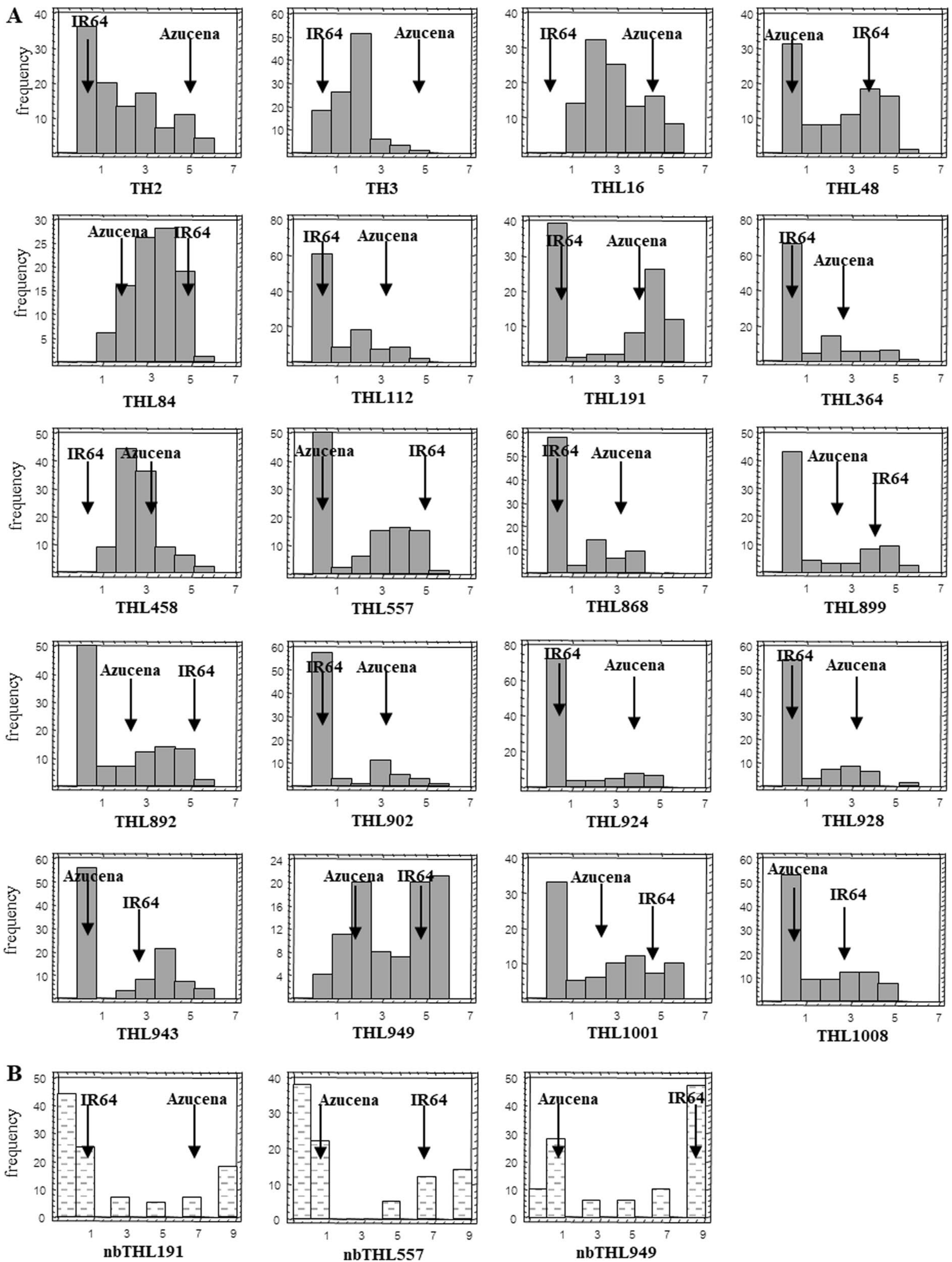
Correlation of pathogenicity was significant at $P < 0.0001$ between leaf and neck blast for THL191, THL949, and THL 557, with the value of correlation (*r*) equal to 0.45, 0.46, and 0.47, respectively. The correlation indicated that the opposite disease reactions could observe in some DHLs when evaluating leaf and neck blasts. Following inoculation by THL191, THL557, and THL949, therefore, 4, 4, and 7 DHLs were resistant to leaf blast but susceptible to neck blast, and 13, 7, and 9 DHLs were susceptible to leaf blast but resistant to neck blast, respectively. The number of DHLs showed resistance reaction to both leaf and neck blast (RR), resistance reaction to leaf blast or neck blast (RS or SR), and susceptible to both leaf and neck blast (SS) in Table 2.

## QTL detection for leaf and neck blast resistance

The analysis revealed forty-three QTLs controlling resistance against 20 isolates (leaf blast screening), 3 isolates (neck blast screening), BSR of leaf blast, and BSR of neck blast on chromosomes 1, 2, 4, 6, 7, 8, 10, 11, and 12 (Table 3 and Fig. 1). None of the QTL was detected against all traits. Fourteen and twenty-nine QTLs were inherited from Azucena and IR64, respectively. Of these, clusters of QTL were found on chromosomes 2, 11, and 12 between marker RG654-RG520 (qBLIR-C2a to qBLIR-C2f), RG1109-RZ536 (qBLIR-C11a to qBLIR-C11e), and RZ861-RG463 (qBLAZ-C12a to qBLAZ-C12j and qBLIR-C12a to qBLIR-C12h), respectively. One to three genome positions were detected to be resistant to QTL. Single QTL was identified against THL16, THL48, THL84, THL191, THL557, THL892, THL899, THL1001, THL1008, and nbTHL191. Multiple QTL was found against the rest of the isolates and affected the total phenotypic variance explained (total $R^2$) of each isolate. The total QTL effect was higher than the individual QTL effect ($R^2$) (Table S2).

For leaf blast disease evaluation, thirty-four QTLs associated with the resistance were located on eight chromosomes except chromosomes 3, 5, 6, and 9, with LOD score of 2.4–22.3 and 3.6%—64.3% of $R^2$ (Table 3). Most QTLs were detected on chromosome 12 and contributed a significant effect of resistance with an average $R^2$ of 34.4 ± 18.4%. These QTLs were located between flanking markers RZ816 to RG463 for controlling blast disease resistance against 15 isolates collected from rice. IR64 (qBLIR-C12a to qBLIR-C12h excepted qBLIR-C2f) and Azucena (qBLAZ-C12a to qBLAZ-C12h) allele were found to be involved in the reduction of disease score with isolate specificity. No QTL detection on chromosome 12 was found against the rice blast isolates TH16, THL458, and THL868, and two barley isolates, TH2 and TH3. These were mapped on various positions on chromosomes 1, 2, 7, 8, 10, and 11 with the resistant allele of IR64.

Resistance QTLs against neck blast disease were identified using screening data of the isolate THL191, THL557, and THL949 under field conditions. Six QTLs were detected on chromosomes 1, 6, 10, and 12 (Table 3). These QTLs were detected by LOD scores ranging from 2.5–6.6 with 14.6%—22.6% of $R^2$. Major QTLs of the three isolates were approximately


A
B
frequency
IR64
Azucena
TH2
TH3
THL16
THL48
THL84
THL112
THL191
THL364
THL458
THL557
THL868
THL899
THL892
THL902
THL924
THL928
THL943
THL949
THL1001
THL1008
nbTHL191
nbTHL557
nbTHL949

◂**Fig. 2** Frequency distribution graphs of 111 DHLs from the cross of IR64 and Azucena. **A** against 20 isolates from leaf blast evaluation and **B** against 3 isolates from neck blast (nb) evaluation. Y axis is frequency, and the X axis is disease score

mapped in the same region on chromosome 12 as the QTLs detected by leaf blast screening. In this region, the Azucena alleles contributed to a higher degree of neck blast resistance for THL557 and THL949, while the IR64 allele contributed resistance for THL191, as found against leaf blast screening. Most neck blast resistance QTL overlapped to leaf blast resistance QTL position. However, two QTLs, qBLIR-C10b and qBLAZ-C6, were not found in leaf blast resistance against THL557 and THL949, respectively.

QTLs detection for broad-spectrum resistance (BSR)

The frequency distribution of BSR in seedling screening supported quantitative inheritance (Fig. 3). IR64 and Azucena were in the middle of the distribution, with BSR scores of 0.8 and 0.6, respectively. Three QTLs were detected for controlling the BSR of leaf blast and BSR of neck blast, but no QTL detection for BSR against both leaf and neck blast diseases (Table 2). The QTL qBLbsr-LB with major effects on chromosome 12 was mapped between AF6-RG457 interval with LOD 2.79 and 17.4% of $R^2$. Two QTLs of BSR for neck blast were mapped on chromosome 1 (qBLbsr-NBa) between RZ276-RM34 and on chromosome 12 (qBLbsr-NBb) near Sdh-1 marker with 9.56% of $R^2$.

Effect of QTL x QTL interaction on disease severity

Among twenty-three isolates (20 isolates for leaf blast and 3 isolates for neck blast screening), only twelve of them (10 isolates for leaf blast and 2 isolates for neck blast) detected more than one QTL position controlling the resistance (Table 3). The QTL x QTL interaction was significant ($p < 0.05$) when characterized using ANOVA. The analysis was done based on the genotypes of the closest marker locus of each QTL. QTL haplotype of interacted QTL reflected the disease resistance of the DHL population (Table 4). The observed mean of disease score against isolates TH2, TH3, THL868, THL902, THL924, and THL928 showed that the composition of IR64 allele in the interaction (II, IA, AI, III, IIA, IAI, AII, IAA, AIA, and AAI) was high resistance while homozygous Azucena genotypes (AA and AAA) were more susceptible. The genotype of $A_{qBLch4}$ x $I_{qBLch12}$ (AI) showed the most resistance against THL112 and THL364. On the other hand, AI genotype conferred a susceptible reaction to THL949 ($A_{qBLch1}$ x $I_{qBLch12}$) and nbTHL557 ($A_{qBLch10}$ x $I_{qBLch12}$). The disease severity mean of THL458 was lowest when affected by the IR64 allele of the detected QTL on chromosomes 1, 7, and 8, including three combinations from 2 QTLs interaction ($I_{qBLch1}$ x $I_{qBLch7}$, $I_{qBLch1}$ x $I_{qBLch8}$, and $I_{qBLch7}$ x $I_{qBLch8}$) and one combination from 3 QTLs interaction ($I_{qBLch1}$ x $I_{qBLch7}$ x $I_{qBLch8}$). The interactions of QTLs against nbTHL949 were detected between 3 QTLs on chromosomes 1, 6, and 12. The analysis revealed that the genotype IA ($I_{qBLch1}$ x $A_{qBLch6}$ and $I_{qBLch1}$ x $A_{qBLch12}$), AA ($A_{qBLch6}$ x $A_{qBLch12}$), IIA ($I_{qBLch1}$ x $I_{qBLch6}$ x $A_{qBLch12}$), and IAA ($I_{qBLch1}$ x $A_{qBLch6}$ x $A_{qBLch12}$) were resistance.

## Discussions

Analysis of the inoculation data of 120 blast isolates on 38 rice blast varieties allowed us to identify effective resistance genes for breeding rice blast resistance in Thailand. This study revealed that the rice cultivars possess resistance genes such as *Pi1*, *Pi2*, *Pi6*, *Pib*, *Pik*, *Pika*, *Pikh*, *Pikm*, *Piks*, *Pip*, *Pish*, *Pit*, *Pita2*, *Piz*, and *Pizt* showed broad resistance (BSR ≥ 0.85) against Thai isolates. The pathogenicity test reported that *Pi1*, *Pita2*, and *Piz5* (Mekwatanakarn et al. 2000) and *Pik* and *Pik*-alleles conferred broad resistance in Thailand (Mekwatanakarn et al. 2013). Moreover, many resistance genes have been reported broad resistance, such as *Pi1*, *Pi7*, *Pi9*, *Pik*, *Pikh*, *Pikm*, *Pikp*, *Pish*, *Pita2*, and *Piz* were reported in Cambodia (Fukuta et al. 2014), *Pi-1* and *Pi-2* in southern of China (Chen et al. 2001), *Pi1*and *Pita2* in Vietnam (Nguyen 2003), and *Pi1* in Laos (Thiravong K., personal communication). These results suggest that the resistance genes *Pi1*, *Pik*, *Pik*-alleles, *Pi-sh*, *Pita2*, *Piz, and Piz*-alleles are appropriate for rice breeding in the Indo-China region.

**Table 2** Number of doubled haploid lines in each disease reaction group against THL191, THL557, and THL949 isolates when tested by leaf and neck blast disease. (RR = resistance to both leaf and neck blast; RS = resistance to leaf blast and susceptible in neck blast; SR = susceptible to leaf blast and resistance in neck blast; SS = susceptible to both leaf and neck blast)

| Isolates | Number of DHLs | | | |
|---|---|---|---|---|
| | RR | RS | SR | SS |
| THL191 | 31 | 4 | 13 | 17 |
| TH557 | 42 | 4 | 7 | 3 |
| THL949 | 18 | 7 | 9 | 32 |
| *Total* | 91 | 15 | 29 | 52 |

In this study, some DHLs from the cross of IR64 × Azucena showed higher resistance or susceptibility than parental lines. This suggested the transgressive segregation and multigenic inheritance of blast resistance that can confirm by QTL analysis (deVicente and Tanksley 1993). The improved indica cultivar IR64 and the upland japonica rice Azucena have the presence of multiple genes, conferring resistance to different blast isolates in several countries (Sallaud et al. 2003; Tharreau et al. 2000). Comparison of QTL analysis using DHLs of IR64 × Azucena from different studies showed that most of the identified QTLs were in the same regions as presented on chromosome 2, 8, 11, and 12 with a favorable allele of IR64 and chromosome 6 and 10 with IR64/Azucena allele. This indicated the stability of QTL for resistance against blast fungus across wide geographic locations; therefore, the QTLs identified from this population were appropriated to use for improving resistance in rice. Moreover, the QTLs on chromosomes 2, 11, and 12 conferred resistance to several isolates, revealing that these genes play a major role in broad-spectrum resistance in rice against Thai blast isolates. Many resistance genes/QTLs have been published (Srivastava et al. 2017). In this study, 9 of 12 rice chromosomes were found QTLs related to blast disease resistance except on chromosomes 3, 5, and 9. Three resistance loci were identified on chromosome 1. The first locus was qBLIR-C1a located near *Pit* and *Pi24*(t). The second locus assigned as qBLIR-C1b was mapped on the *Pi35*(t), *Pi37*, and *Pish* regions. The third locus is located between flanking markers RG146 and RM34, including qBLIR-C1c, qBLIR-C1d, and qBLbsr-NBa. This locus might be a new QTL that was never reported previously. The QTLs on chromosome 2 (qBLIR-C2a to qBLIR-C2f) located in analogous region of *Pi-b*, *Pitq5*, *Pig*(t), *Pid1*(t), *Pi-y1*(t), *Pi-y2*(t), and *Pi25*(t) genes. For chromosome 4, the QTLs qBLAZ-C4a and qBLAZ-C4b laid down near the region of *Pi*(t) and *Pi5*(t) while the QTL qBLAZ-C6 on chromosome 6 was close to the previously published such as *Pi2*, *Pi9*, *Piz5*, *Pizt*, *Pi8*, *Pi13*(t), and *Pi27*(t). On chromosome 7, the QTL qBLIR-C7 was located close to the QTL identified using recombinant inbred line population of KDML105 × CT9993 (Sirithunya et al. 2002). The QTL qBLIR-C8 was mapped on chromosome 8 in the region of *Pi29*(t), *Pi33*, *Pi42*(t), and *Pizh*. The QTL qBLIR-C10a and qBLIR-C10b were found near *Pi-GD-2*(t) and *Pi28*(t) on chromosome 10, respectively. On choromosome11, qBLIR-C11a to qBLIR-C11e were identified and located near the multiple blast resistance genes such as *Pi-1*, *Pi-7*, *Pi-18*, *Pi44*, *Pi47*, *Pi54*, *Pi-k*, and *Pi-k* alleles. Many QTLs were mapped on chromosome 12 in the same region of several *complete resistance genes such as Pita, Pita2, Pi-tq6, Pi12(t), Pi20(t), Pi-21(t), Pi31(t), Pi32*(t), and *Pi62*. The resistance loci on chromosome 12 (RG341 – AF6) contributed both IR64 (THL949 and THL557) and Azucena (THL191) alleles. It might be expected that IR64-QTLs and Azucena-QTLs are not the same genes or be the same gene with different allelic forms. This study supported that blast resistance genes are restricted to several regions of the rice genome, especially on chromosomes 2, 6, 11, and 12, as in previous reports. In addition, none of the QTLs was efficient against all the isolates. This showed that isolates were specific to the blast resistance genes, and the presence of various QTLs on many chromosomes also indicated the difference in pathogenic races, which can refer to high genetic diversity and pathogenicity.

The QTLs of neck blast were mapped to approximate locations as QTLs of leaf blast, which reasonably explains of the high correlation between neck and leaf blast. This might be expected that a major resistance gene conferring leaf blast would be the

**Table 3** Blast resistance QTLs against twenty blast isolates for leaf blast (lb), neck blast (nb), and broad-spectrum resistance (BSR) identified using doubled haploid lines from the cross of IR64 and Azucena

| No | Chr | QTL | Interval marker | Peak marker | LOD | $R^{2a}$ | Resistance allele | Corresponding isolate |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | qBLIR-C1a | RM84 | RM84 | 2.5 | 7.3* | I | TH3 |
| 2 | 1 | qBLIR-C1b | RG146-RM34 | RG146 | 2.5 | 12.0** | I | THL949 |
| 3 | 1 | qBLbsr-NBa | RZ276-RM34 | RM34 | 2.2 | 5.8* | A | BSR-nb |
| 4 | 1 | qBLIR-C1c | RZ276-RM34 | RM34 | 2.9 | 6.4* | I | nbTHL949 |
| 5 | 1 | qBLIR-C1d | RZ730-RM315 | RZ730 | 2.5 | 5.0* | I | THL458 |
| 6 | 2 | qBLIR-C2a | RG654-RG520 | RM213 | 6.7 | 28.4** | I | THL868 |
| 7 | 2 | qBLIR-C2b | RM208-RG520 | RZ123 | 5.7 | 24.7** | I | THL928 |
| 8 | 2 | qBLIR-C2c | RM213-RZ123 | RZ213 | 4.3 | 18.2** | I | TH3 |
| 9 | 2 | qBLIR-C2d | RM213-RZ123 | RZ213 | 2.7 | 13.7** | I | THL924 |
| 10 | 2 | qBLIR-C2e | RZ123-RG520 | RZ123 | 3.1 | 12.9** | I | TH2 |
| 11 | 2 | qBLIR-C2f | RZ123-RG520 | RZ123 | 5.6 | 13.9** | I | THL16 |
| 12 | 4 | qBLAZ-C4a | RG218-RZ262 | RG218 | 3.7 | 14.5** | A | THL112 |
| 13 | 4 | qBLAZ-C4b | RG218 | RG218 | 2.4 | 4.4* | A | THL364 |
| 14 | 6 | qBLAZ-C6 | RM50-RM253 | RM253 | 2.5 | 9.6** | A | nbTHL949 |
| 15 | 7 | qBLIR-C7 | RG477-RM214 | RM214 | 2.4 | 10.3** | I | THL458 |
| 16 | 8 | qBLIR-C8 | RM42-RM223 | RM42 | 2.5 | 3.6* | I | THL458 |
| 17 | 10 | qBLIR-C10a | RM244-RM222 | RM244 | 3.0 | 5.8** | I | THL868 |
| 18 | 10 | qBLIR-C10b | G2155-RG134 | RG134 | 2.7 | 14.6** | I | nbTHL557 |
| 19 | 11 | qBLIR-C11a | RG1109-Npb186 | Npb186 | 2.4 | 9.6** | I | TH2 |
| 20 | 11 | qBLIR-C11b | RG1109-Npb186 | RG1109 | 4.3 | 18.4** | I | THL868 |
| 21 | 11 | qBLIR-C11c | RG1109-RZ536 | RZ536 | 3.4 | 21.4** | I | THL902 |
| 22 | 11 | qBLIR-C11d | RG1109-RZ536 | RG1109 | 3.4 | 18.5** | I | THL924 |
| 23 | 11 | qBLIR-C11e | RG1109-RZ536 | Npb186 | 2.4 | 11.7** | I | THL928 |
| 24 | 12 | qBLAZ-C12a | RZ861-RG463 | AF6 | 14.6 | 47.9** | A | THL892 |
| 25 | 12 | qBLAZ-C12b | RZ861-Sdh1 | AF6 | 21.0 | 58.9** | A | THL557 |
| 26 | 12 | qBLAZ-C12c | RZ816-Sdh1 | AF6 | 17.2 | 53.9** | A | THL943 |
| 27 | 12 | qBLAZ-C12d | RZ816-Sdh1 | AF6 | 13.8 | 52.7** | A | THL949 |
| 28 | 12 | qBLAZ-C12e | RZ816-Sdh1 | AF6 | 15.4 | 59.9** | A | THL1001 |
| 29 | 12 | qBLAZ-C12f | RM247-Sdh1 | AF6 | 4.2 | 38.2** | A | THL899 |
| 30 | 12 | qBLAZ-C12g | RM247-Sdh1 | AF6 | 9.5 | 30.9** | A | THL1008 |
| 31 | 12 | qBLAZ-C12h | RM247-RG457 | AF6 | 3.7 | 11.3** | A | THL84 |
| 32 | 12 | qBLIR-C12a | RM247-Sdh1 | RG341 | 22.3 | 64.3** | I | THL191 |
| 33 | 12 | qBLIR-C12b | RM247-Sdh1 | AF6 | 5.0 | 24.1** | I | THL924 |
| 34 | 12 | qBLIR-C12c | RM247-RG457 | RG341 | 12.1 | 41.3** | I | THL48 |
| 35 | 12 | qBLIR-C12d | RM247-AF6 | RG341 | 5.0 | 16.8** | I | THL112 |
| 36 | 12 | qBLIR-C12e | RM247-AF6 | AF6 | 5.5 | 34.6** | I | THL902 |
| 37 | 12 | qBLIR-C12f | RM247-AF6 | AF6 | 6.6 | 14.6** | I | nbTHL191 |
| 38 | 12 | qBLAZ-C12i | RM247-AF6 | AF6 | 5.0 | 22.5** | A | nbTHL557 |
| 39 | 12 | qBLAZ-C12j | RM83-Sdh-1 | AF6 | 6.6 | 22.6** | A | nbTHL949 |

**Table 3** (continued)

| No | Chr | QTL | Interval marker | Peak marker | LOD | $R^{2a}$ | Resistance allele | Corresponding isolate |
|---|---|---|---|---|---|---|---|---|
| 40 | 12 | qBLIR-C12g | RG341-RZ262 | RG341 | 3.0 | 10.7** | I | THL364 |
| 41 | 12 | qBLIR-C12h | AF6 | AF6 | 2.4 | 13.1** | I | THL928 |
| 42 | 12 | qBLbsr-LB | AF6-RG457 | RG457 | 2.8 | 13.0** | I | BSR -lb |
| 43 | 12 | qBLbsr-NBb | Sdh-1 | Sdh-1 | 2.4 | 10.4 | I | BSR-nb |

[a]$R^2$, Phenotypic variation explained by QTL

same as neck blast. The segregation of the DHL population between resistant and susceptible at the seedling stage agreed with the ratio of 1:1 against most of the tested isolates suggesting that the leaf blast resistance is controlled by one major gene. While the segregation ratio between resistance and susceptibility at the reproductive stage was close to 3:1, suggesting a possibility that more than a single gene is responsible for neck blast. This was according to the detection of additional QTLs on chromosome 10 (qBLIR-C10b) and 6 (qBLAZ-C6) against nbTHL557 and nbTHL949, respectively, that did not find in the same isolate evaluated by leaf blast disease. Although many leaf and neck blast resistance QTLs were mapped in the same regions (Devi et al. 2020; Noenplab et al. 2006; Ma et al. 2015; Zhuang et al. 2002), specific QTLs of them were also reported (Hayashi et al. 2010; Ishihara et al. 2014; Zhuang et al. 2002). Therefore, some DHLs were observed the contrast disease reaction between leaf and neck

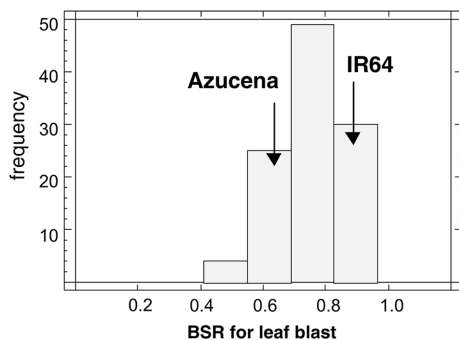



**Fig. 3** Broad-spectrum resistance (0–1) in progenies of the IR64 × Azucena cross infected with 20 *Pyricularia oryzae* isolates in the seedling stage

blast according to inconsistently occurred in some rice varieties (Noenplab et al. 2006; Puri et al. 2009). This result provided evidence that the genetic control of the blast resistance in rice may vary at different development stages.

Analysis of QTL x QTL interaction revealed that interaction of $I_{qBLch2}$ x $I_{qBLch11}$, $I_{qBLch2}$ x $I_{qBLch12}$, $I_{qBLch11}$ x $I_{qBLch12}$, and $I_{qBLch10}$ x $A_{qBLch12}$ showed highly broad-spectrum resistance. We observed that consistent correspondence of QTLs interaction showed additive gene action and no epistatic interaction. The population size and markers used in this analysis might be a limiting factor for specific interactions (Yano and Sasaki 1997). Therefore, the relatively small population (111 individuals) may not be enough to analyze of three-locus interaction and clarify the real nature of epistatic interaction of the QTLs. Estimating of these interactions would require a substantially larger population size, which would also increase the power of QTL main-effect detection (Melchinger et al. 1998).

In this study, we explored many resistance QTLs against blast isolates in Thailand and found the DHLs had high BSR than their parents. Of these, line P0489 has been selected to be a donor of blast resistance on chromosomes 2 and 12 for improving blast resistance in Thai glutinous rice variety, RD6. More SSR markers have been added to the two QTL regions and selected the polymorphic markers linked to the QTLs were before used in a breeding program. The introgression lines containing QTL on chromosomes 2 and 12 showed that it could improve the resistance in RD6 (Pinta et al. 2013; Suwannual et al. 2017). The reliable QTL data from this study will benefit molecular breeding for rice blast resistance in Thailand.

**Table 4** Disease score mean of genotype affected by the QTL x QTL interactions. II, IA, AI, AA, III, IIA, IAA, IAI, AII, AIA, AAI, and AAA refer to the allelic composition of doubled haploid lines at QTL, respectively (*A* = allele from Azucena, *I* = allele from IR64). The mean values in the same row with the same superscript do not differ statistically at 95% of LSD (Fisher's least significant difference test)

| Isolate | QTL interaction | Mean of disease score by allelic composition at QTL | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | II | IA | AI | AA | III | IIA | IAA | IAI | AII | AIA | AAI | AAA |
| TH-2 | qBLch2x qBLch 11 | 1.1$^{a}$ | 1.0$^{a}$ | 1.9$^{a}$ | 2.9$^{b}$ | | | | | | | | |
| TH-3 | qBLch1x qBLch 2 | 1.1$^{a}$ | 1.3$^{a}$ | 1.4$^{a}$ | 2.2$^{b}$ | | | | | | | | |
| THL112 | qBLch4xqBLch12 | 1.0$^{b}$ | 2.3$^{c}$ | 0.2$^{a}$ | 1.1$^{b}$ | | | | | | | | |
| THL364 | qBLch4xqBLch12 | 0.8$^{ab}$ | 1.8$^{b}$ | 0.2$^{a}$ | 1.5$^{b}$ | | | | | | | | |
| THL458 | qBLch1x qBLch 7 | 2.1$^{a}$ | 3.2$^{b}$ | 2.6$^{ab}$ | 3.0$^{b}$ | | | | | | | | |
| | qBLch1x qBLch8 | 2.2$^{a}$ | 2.8$^{ab}$ | 2.6$^{ab}$ | 3.3$^{b}$ | | | | | | | | |
| | qBLch7 x qBLch8 | 2.2$^{a}$ | 3.1$^{b}$ | 3.1$^{b}$ | 3.3$^{b}$ | | | | | | | | |
| | qBLch1 x qBLch7x qBLch8 | | | | | 1.9$^{a}$ | 2.8$^{ab}$ | 3.2$^{b}$ | 2.0$^{a}$ | 2.5$^{ab}$ | 3.4$^{b}$ | 2.9$^{b}$ | 3.3$^{b}$ |
| THL868 | qBLch2 x qBLch10 | 0.2$^{a}$ | 0.0$^{a}$ | 0.8$^{a}$ | 2.7$^{b}$ | | | | | | | | |
| | qBLch2 x qBLch11 | 0.0$^{a}$ | 0.0$^{ab}$ | 0.9$^{b}$ | 2.5$^{c}$ | | | | | | | | |
| | qBLch10 x qBLch11 | 0.4$^{a}$ | 0.5$^{a}$ | 0.0$^{a}$ | 2.5$^{b}$ | | | | | | | | |
| | qBLch2 x qBLch10x qBLch11 | | | | | 0.0$^{a}$ | 0.0$^{a}$ | 0.0$^{a}$ | 0.0$^{a}$ | 0.9$^{b}$ | 0.7$^{ab}$ | 0.0$^{a}$ | 2.9$^{c}$ |
| THL902 | qBLch11 x qBLch12 | 0.0$^{a}$ | 0.7$^{a}$ | 0.0$^{a}$ | 3.1$^{b}$ | | | | | | | | |
| Isolate | QTL interaction | Mean of disease score by allelic composition at QTL | | | | | | | | | | | |
| | | II | IA | AI | AA | III | IIA | IAA | IAI | AII | AIA | AAI | AAA |
| THL924 | qBLch2 x qBLch11 | 0.0$^{a}$ | 0.2$^{a}$ | 0.5$^{a}$ | 2.3$^{b}$ | | | | | | | | |
| | qBLch2 x qBLch12 | 0.0$^{a}$ | 0.3$^{a}$ | 0.0$^{a}$ | 2.6$^{b}$ | | | | | | | | |
| | qBLch11 x qBLch12 | 0.1$^{a}$ | 0.5$^{a}$ | 0.0$^{a}$ | 3.1$^{b}$ | | | | | | | | |
| | qBLch2 x qBLch11x qBLch12 | | | | | 0.0$^{a}$ | 0.0$^{a}$ | 0.0$^{a}$ | 0.7$^{a}$ | 0.1$^{ab}$ | 0.8$^{a}$ | 0.0$^{a}$ | 3.8$^{b}$ |
| THL928 | qBLch2 x qBLch11 | 0.0$^{a}$ | 0.2$^{a}$ | 0.9$^{b}$ | 2.3$^{c}$ | | | | | | | | |
| | qBLch2 x qBLch12 | 0.0$^{a}$ | 0.3$^{a}$ | 0.7$^{b}$ | 2.4$^{c}$ | | | | | | | | |
| | qBLch11 x qBLch12 | 0.0$^{a}$ | 1.0$^{b}$ | 0.8$^{ab}$ | 2.2$^{c}$ | | | | | | | | |
| | qBLch2 x qBLch11x qBLch12 | | | | | 0.0$^{a}$ | 0.0$^{a}$ | 0.0$^{a}$ | 0.0$^{a}$ | 0.0$^{a}$ | 1.9$^{bc}$ | 1.6$^{b}$ | 2.8$^{c}$ |
| THL949 | qBLch1 x qBLch12 | 1.0$^{ab}$ | 1.0$^{a}$ | 5.3$^{c}$ | 2.5$^{b}$ | | | | | | | | |
| nbTHL557 | qBLch10 x qBLch12 | 1.5$^{a}$ | 0.9$^{a}$ | 6.1$^{b}$ | 0.7$^{a}$ | | | | | | | | |
| nbTHL949 | qBLch1 x qBLch6 | 5.1$^{b}$ | 2.5$^{a}$ | 7.4$^{c}$ | 4.9$^{b}$ | | | | | | | | |
| | qBLch1 x qBLch12 | 6.4$^{b}$ | 2.0$^{a}$ | 6.4$^{b}$ | 4.4$^{ab}$ | | | | | | | | |
| | qBLch6 x qBLch12 | 7.6$^{b}$ | 4.8$^{ab}$ | 5.3$^{ab}$ | 3.0$^{a}$ | | | | | | | | |
| | qBLch1 x qBLch6x qBLch12 | | | | | 7.3$^{c}$ | 0.5$^{a}$ | 1.0$^{a}$ | 2.3$^{a}$ | 7.7$^{c}$ | 6.2$^{c}$ | 5.6$^{bc}$ | 3.6$^{ab}$ |

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**Conflict of Interest** The authors declare no conflict of interest.

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