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Research

Characterization of Six Partial Resistance Genes and One Quantitative Trait Locus to Blast Disease Using Near Isogenic Lines with a Susceptible Genetic Background of Indica Group Rice (*Oryza sativa*)

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Abstract

Seven near isogenic lines (NILs) for six partial blast-resistance genes—*Pi35*, *pi21*, *Pi37*(t)(*Pi37-KRIL17*), *Pb1*, and *Pi34* (two: *Pi34-Chubu32* and *Pi34-Chugoku40*, originating from different Japonica Group rice lines, 'Chubu 32' and 'Chugoku 40', respectively)—and one quantitative trait locus (QTL), *PiPHL9*, derived from Japonica Group line 'Hokkai PL9', were developed in rice (*Oryza sativa* L.). These NILs have the genetic background of an Indica Group susceptible line 'US-2', which does not harbor complete resistance genes and can clarify the effects of partial resistance genes and the QTL. These NILs and US-2 were used to determine the effects of the partial resistance genes and the QTL based on the results of an inoculation test for leaf blast using international standard differential blast isolates in the young seedling stage and a natural infection test in the mature stage after heading at a blast field. The NILs produced mainly moderate reactions in the inoculation test and rarely exhibited "resistant" and "susceptible" reactions. Each of the partial resistance genes and the QTL had different effects in panicle blast. Two genes, *Pi35* and *Pb1*, were the most effective with respect to resistance, followed by *Pi37-KRIL17*. *Pi34-Chugoku40* was the weakest. The effects of *pi21*, *Pi34-Chubu32*, and *PiPHL9* varied among cultivation seasons and could be easily affected by environmental conditions. This is the first report clarifying the effects of partial resistance genes without the major complete genes, which will be helpful for advanced genetic and pathological studies and as a source of genes for rice breeding.

Keywords: blast (*Pyricularia oryzae* Cavara), leaf blast, near isogenic line, panicle blast, partial resistance gene, rice (*Oryza sativa* L.)

Blast, caused by the fungus *Pyricularia oryzae* Cavara, is one of the most serious diseases threatening the stable production of rice (*Oryza sativa* L.). About 10 to 30% of global rice production is lost due to damage caused by this disease (Skamnioti and Gurr 2009). The most cost-effective and sustainable way to control blast is to breed blast-resistant rice cultivars (Ashkani et al. 2015).

Recent molecular genetic research into blast resistance has identified more than 100 resistance genes or loci, 31 of which have been molecularly characterized (Kalia and Rathour 2019; Xiao et al. 2020). There are two categories: complete (qualitative) resistance and partial (quantitative) resistance, based on the heritability and characteristics of the resistance (Ezuka 1972; Parlevliet 1979). Complete resistance has been used when breeding for blast resistance because it is highly effective against blast disease. However, complete resistance is blast-race-specific and is explained by a sensitivity reaction based on the gene-for-gene theory (Flor 1971; Silué et al. 1992); that is, complete resistance is effective only against certain blast races and not others. Due to highly frequent variation in the *P. oryzae* population, if the cultivation of a rice cultivar with this type of resistance is continued, only strains of fungus that are pathogenic to the resistant variety will survive, and the resistance will lose its effectiveness because the frequency of virulent races will gradually increase, resulting in the quick breakdown of resistance (Kiyosawa 1982; Wang et al. 2021).

On the other hand, partial resistance shows the effect of quantitative control by multiple loci and is effective and durable against the broad spectrum of pathogen races (Ezuka 1972). This type of resistance is less likely to change the composition of the pathogen race in the field because the selection pressure of partial resistance is smaller than that of complete resistance (Kou and Wang 2010; Poland et al. 2009). Furthermore, complete resistance depends on the recognition of the pathogen, which can be easily lost by loss of mutations in the gene that produces the recognition protein in the pathogen. In contrast, partial resistance is governed by multiple genes; therefore, the mutations must be acquired rather than be defective if the pathogen is to overcome this resistance. Because defective mutations generally occur much more readily than acquired mutations, the loss of resistance by complete resistance is easier, and partial resistance is more persistent (Poland et al. 2009). Fujii et al (2005) reviewed the effect of the partial resistance gene *Pb1*, for which there had been no report of an outbreak of virulent blast races for more than 20 years since the release of rice cultivars harboring *Pb1* in Japan. Therefore, partial-resistance-based rice breeding should be promoted to breed rice cultivars that show durable and broad-spectrum blast resistance (Fukuoka and Okuno 2019). However, it is difficult to evaluate the effect of each gene on partial resistance because the effect of individual genes is small and can be masked by the effects of complete resistance genes present in the background of the line being studied.

Recently, advances in molecular genetic studies have allowed several partial resistance genes to be identified; these include *Pi35* (Nguyen et al. 2006) on chromosome (chr.) 1, *pi21* (Fukuoka and Okuno 2001) on chr. 4, *Pb1* (Fujii et al. 2000), *Pi34* (Maeda et al. 2010; Zenbayashi et al. 2002), *Pi37*(t) (Hirabayashi et al. 2005, 2010), and *Pi39*(t) (Terashima et al. 2008) on chr. 11. Yasuda et al. (2015) evaluated breeding lines harboring single- or two-gene combinations of partial resistance genes *pi21*, *Pi34*, and *Pi35* with the genetic background of the Japonica Group rice cultivar 'Koshihikari' to evaluate the suppression of leaf blast with a virulent blast isolate, Ao 92-06-2, and clarified that a combination of two resistance genes was more effective than either of the genes individually. Therefore, by clarifying the effects of individual partial resistance genes and examining their combined effects, it would be possible to achieve more efficient breeding for blast resistance through the introduction of partial resistance genes. However, the number of blast isolates used for the inoculation test was limited. Kawasaki-Tanaka and Fukuta (2014) reported that Koshihikari harbors the complete resistance genes *Pik-s* and *Pish* and an unknown gene in its genetic background. This means that the effects of single- and two-gene combinations were seen even in the presence of several complete resistance genes. Therefore, to clarify the effect of partial resistance genes, it is necessary to develop their corresponding near isogenic lines (NILs) harboring a single partial resistance gene without complete resistance genes in the genetic background and to exhaustively clarify the reactions with regard to resistance to various blast isolates.

Fukuta et al. (*unpublished data*) developed a susceptible line, named 'US-2', derived from the progenies of crosses between a tropical Japonica Group cultivar, 'Kencana', from Indonesia, and an improved high-yielding Indica Group cultivar, 'Takanari', bred in Japan. Because US-2 does not harbor any major resistance gene, it can minimize the influences of additional genetic factors on blast resistance. Therefore, by developing NILs of partial resistance genes with the genetic background of US-2, we can accurately evaluate the effect of the single partial resistance genes on blast resistance.

In the current study, we first describe the donor parents, the designated names, and demonstrate their genetic characterization of seven NILs for six partial resistance genes and one quantitative trait locus (QTL). Then, we clarify the effects of the partial resistance genes and the QTL in the NILs on leaf blast resistance at the young seedling stage using 25 standard differential blast isolates (SDBIs) and the effects of these on panicle blast resistance at a paddy field in the Mountainous Region Agricultural Research Institute (MARI) in Aichi prefecture, Japan. We also investigated the yield performance of these NILs through four cultivation seasons in the Tropical Agriculture Research Front (TARF) of the Japan International Research Center for Agricultural Sciences (JIRCAS) in Okinawa prefecture. The results clearly revealed the characteristics of the partial resistance genes and QTL and provided us with valuable information that contributes to advanced genetic and pathological studies for rice breeding using partial resistance genes.

MATERIALS AND METHODS

Plant materials

The broadly susceptible Indica Group line US-2 (Fukuta et al. *unpublished data*) was used as a recurrent parent for developing NILs for partial resistance genes. We developed seven NILs for six different partial resistance genes: *Pi35*, *pi21*, *Pi37*(t), *Pb1*, and *Pi34* (two donors: Chubu 32 and Chugoku 40) and one QTL, *PiPHL9* (Table 1). Partial resistance gene *pi21* was found on chr. 4 in an upland Japonica Group cultivar 'Owarihatamochi' (Fukuoka and Okuno 2001). *Pb1* was found on chr. 11 in the Indica Group cultivar 'Modan' (Fujii et al. 2000) and was introduced into the Japonica Group cultivar 'Asanohikari'. *Pi34* was found on chr. 11 in two Japonica Group lines, 'Chubu 32' (Zenbayashi et al. 2002) and 'Chugoku 40' (Maeda et al. 2010). The *Pi34* gene in Chubu 32 originated from the upland variety 'Sensho', and this gene in Chugoku 40 originated from the Japanese upland line 'Rikuto-Kanto 72'. The gene in Rikuto-Kanto 72 also originated from Sensho. A resistance gene in the Japonica Group line 'Hokkai 188' originating from the Chinese variety 'Reishiko' was found on chr. 1 and named *Pi35* (Nguyen et al. 2006). Hirabayashi et al. (2010) developed a set of chromosome segment substitution lines (CSSLs) that originated from an

Asian wild rice, *Oryza rufipogon* (IRGC-Acc. 104814), and found that a CSSL named 'KRIL17' for chr. 4 showed moderate and stable resistance to blast in an upland nursery from 2006 to 2008. A dominant resistance gene was found on chr. 4 using this introgression line and was tentatively named *Pi37*(t) (Hirabayashi et al. 2005, 2010). Lin et al. (2007) also used the same gene symbol, *Pi37*, for the blast resistance gene that was found in a rice cultivar 'St. No.1' and mapped on chr. 1. In this study, our resistance gene is renamed *Pi37-KRIL17* to avoid confusing it with that of Lin et al. (2007). 'Hokkai-PL9' was developed as a cold-tolerant line with genetic factor(s) originally from 'Norin-PL11', which was developed by backcross breeding using two cold-tolerant cultivars, the Malaysian tropical Japonica Group cultivar 'Padi Labou Alumbis' and the temperate Japonica Group cultivar 'Hayayuki' (Kuroki et al. 2007). Fukuta et al. (2019) confirmed based on inoculation tests with SDBIs that Hokkai-PL9 was a high-resistance line. A QTL for blast resistance in Hokkai-PL9 was also found on chr. 11, and it was tentatively named *PiPHL9* (I. Ando, *unpublished data*).

These cultivars and lines—Owarihatamochi, Asanohikari, Chubu 32, Chugoku 40, Hokkai 188, 'WIL23' (KRIL17), and Hokkai-PL9—were used as donor parents of resistance genes by recurrent backcross breeding with US-2 (Table 1). US-2 was crossed with each of these seven donors to generate F_1 plants. The F_1 plants were then backcrossed with US-2 to generate BC_1F_1 plants. Backcross progenies were tested by using DNA markers specific to each partial resistance gene, and then heterozygous plants were selected for crossing with US-2 again. This cycle was repeated until BC_6F_1 generation. The BC_6F_1 plants were self-pollinated to obtain BC_6F_2 plants. Plants homozygous at the targeted resistance gene locus were selected by the specific DNA marker for each partial resistance gene and were self-pollinated to achieve the BC_6F_3 generation. To evaluate morphological traits, we cultivated BC_6F_3 plants in the field and selected plants morphologically comparable to US-2. Finally, $BC_6F_{5–8}$ lines were cultivated to confirm morphological uniformity, and then fixed lines were used as NILs.

Graphical genotyping of NILs for partial resistance genes

To detect the introgression of resistance genes, we used 345 simple-sequence repeat (SSR) markers distributed throughout the 12 chromosomes of the rice genome (Supplementary Table S1) (McCouch et al. 2002). Genomic DNA of the seven NILs and US-2 was extracted using a previously described method (Obara et al. 2004), with slight modifications. Specific DNA fragments were amplified by using PCR with Quick Taq HS DyeMix (TOYOBO, Osaka, Japan) in accordance with the manufacturer's instructions, by employing the following procedure: 35 cycles (1 min at 94°C, 1 min at 55°C, 2 min at 72°C) and an extension of 5 min at 72°C. The amplified DNA fragment was separated by electrophoresis on 4.0% (w/v) agarose gel containing 0.05% (v/v) ethidium bromide. After electrophoresis, the DNA signal was scanned with a PharosFX molecular imager (Bio-Rad, Tokyo, Japan).

Inoculation and evaluation of leaf blast resistance at young seedling stage

The resistance of the seven single partial resistance gene NILs, 25 differential varieties (DVs) for 23 complete resistance genes and a susceptible cultivar 'Lijiangxintuanheigu' (LTH) (Tsunematsu et al. 2000), and US-2 was investigated with an inoculation test using 25 SDBIs (Fukuta et al. 2021) including nine isolates from Japan (Kawasaki-Tanaka and Fukuta 2014); six from the Philippines (Telebanco-Yanoria et al. 2008); one each from Nigeria (Odjo et al. 2017), China (*unpublished*), Kenya (Fukuta et al. 2019), Indonesia (Kadeawi et al. 2021), Cambodia (Fukuta et al. 2014), and Laos (Xangsayasane et al. 2020); and two each from Bangladesh (Khan et al. 2016) and Benin (Odjo et al. 2017).

The inoculation test was performed following the methods described by Hayashi et al. (2009). Three seeds of each line were sown in a plastic cell tray (φ16 × 25 mm/hole) and grown to the fourth- to fifth-leaf stage in a greenhouse at 25 to 27°C (humidity approximately 60%). Two sets of seedlings of NILs, DVs, and two susceptible controls, LTH and US-2, were grown in a glasshouse under natural light conditions. The spore concentration was standardized to 1×10^5/ml, and the suspension was sprayed onto each tray with a fine atomizer. The degree of infection of US-2, the seven NILs, and DVs by each of the blast isolates was evaluated based on the infection score of the uppermost expanded leaves at seven days after inoculation (21-day-old seedling) by assigning a score of 0 to 5, where 0 = no infection; 1 = brown specks smaller than 0.5 mm in diameter, no sporulation; 2 = brown specks about

TABLE 1

Partial resistance genes, their donor parents, chromosomes, and closely linked simple-sequence repeat markers

Partial resistance gene	Designation of NILs[a]	Donor parent	Chromosome	DNA marker for selection (position)
Pi35[b]	US2NIL*Pi35-Hokkai188*	Hokkai 188 (Reishiko)	1	RM6648 (132.8 cM: 32,337,278–32,337,484 bp)
pi21[c]	US2NIL*pi21-Owarihatamochi*	Owarihatamochi	4	RM1359 (56.1 cM: 19,860,557–19,860,797 bp)
Pi37-KRIL17	US2NIL*Pi37-KRIL17*	WIL23 (KRIL17)[d] (*Oryza rufipogon* IRGC-Acc.104814)	4	RM6909 (109.9 cM: 31,875,539–31,875,703 bp)
Pb1[e]	US2NIL*Pb1-Asanohikari*	Asanohikari (Moden)	11	RM26998 (108.6 cM: 22,164,957–22,165,094 bp)
Pi34(Chubu 32)[f]	US2NIL*Pi34-Chubu32*	Chubu 32 (Sensho)	11	RM21 (85.7 cM: 19,639,215–19,639,359 bp)
Pi34(Chugoku 40)	US2NIL*Pi34-Chugoku40*	Chugoku 40[g] (Japanese upland rice 'Rikuto-Kanto 72')	11	RM21 (85.7 cM: 19,639,215–19,639,359 bp)
PiPHL9	US2NIL*PiPHL9-HokkaiPL9*	Hokkai PL9[h] ('Padi Labou Alumbis' or 'Hayayuki')	11	RM1341 (80.2 cM: 19,677,083–19,677,265 bp)

[a] NIL = near isogenic line. NILs for partial resistance genes and one quantitative trait locus with an Indica Group rice line US-2 were developed in this study.
[b] Nguyen et al. (2006).
[c] Fukuoka and Okuno (2001).
[d] Hirabayashi et al. (2010).
[e] Fujii et al. (2000).
[f] Zenbayashi et al. (2002).
[g] Maeda et al. (2010).
[h] Kuroki et al. (2007), Fukuta et al. (2019).

0.5 to 1 mm in diameter, no sporulation; 3 = roundish to elliptical lesions about 1 to 3 mm in diameter with a gray center surrounded by brown margins, lesions capable of sporulation; 4 = typical spindle-shaped blast lesions capable of sporulation, 3 mm or longer with necrotic gray centers and water-soaked or reddish brown margins, little or no coalescence of lesions; and 5 = lesions as in 4 but about half of one or two leaf blades killed by the coalescence of lesions. The most highly infected leaf among the uppermost expanding leaves on three individual plants was investigated, and then the mean of two inoculations using same blast isolates was used as the representative datum of infection in each NIL, DV, or susceptible control.

Pathogenicity of blast isolates from MARI in Aichi

A total of 22 blast isolates were collected from rice fields located at MARI (N35°,12′ 44″, E137° 30′ 29″) in Aichi prefecture in September 2019 to understand the pathogenicity of dominant blast isolates. Single spores were isolated from infected leaves or panicles and incubated on moist filter paper in a Petri dish at room temperature for 24 h, in accordance with the protocols detailed by Hayashi et al. (2009). To clarify the pathogenicity of each isolate, we used the LTH, along with DVs in the form of 22 monogenic lines for *Pish*, *Pib*, *Pit*, *Pia*, *Pii*, *Pi3*, *Pik-s*, *Pik-m*, *Pik-h*, *Pik-p*, *Pi7*(t), *Pi9*(t), *Piz*, *Piz-5*, *Piz-t*, *Pita-2* (two lines), *Pi12*(t), *Pita* (two lines), *Pi19*(t), and *Pi20*(t) (Tsunematsu et al. 2000) and three NILs for *Pi5*(t), *Pi1*, and *Pik* (Telebanco-Yanoria et al. 2010) with the genetic background of LTH and targeting 23 resistance genes. To evaluate the pathogenicity of blast isolates, inoculation tests were conducted as described above, and scoring was performed according to the method described by Hayashi et al. (2009).

Evaluation of panicle blast resistance

To determine the degree of resistance to panicle blast, an investigation for infections was carried out at MARI in accordance with the method described by Asaga (1981) and Yoshida et al. (2021) in the three years from 2017 to 2019. In addition to the use of seven NILs for partial resistance genes, 23 NILs were employed as complete resistance genes: *Pia* (two lines), *Pish*, *Pii*, *Pi3*, *Pi5*(t), *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pik-s*, *Pi1*, *Pi7*(t), *Piz*, *Piz-t*, *Piz-5*, *Pi9*(t), *Pi12*(t), *Pita* (three lines), *Pita-2*, and *Pi20*(t) with US-2 genetic background (as US2-DVs, Fukuta et al. *unpublished data*) in 2018 and nine US2-DVs for *Pia*, *Pish*, *Pii*, *Pik*, *Piz*, *Piz-t*, *Piz-5*, *Pi9*(t), and *Pita* in 2019. A total of 37 cultivars categorized as resistant ("r", five), moderately resistant ("mr", ten), moderate ("m", nine), moderately susceptible ("ms", seven), and susceptible ("s", six) to panicle blast in MARI were used as controls.

Fifteen plants were transplanted into the paddy field in late June, with two to three replications. To enhance infection, we used infected seedlings of three cultivars (with different heading times) as spreaders: 'Wakamizu' (harboring *Pii* and early heading), 'Kusabue' (harboring *Pik* and early heading), and 'Mineasahi' (harboring *Pii* and middle heading), which were known to be susceptible in the field at MARI. In 2019, a total of 37 control cultivars, which had already exhibited panicle blast resistance at the MARI field, were also cultivated to determine appropriate timing for evaluating panicle blast resistance. The seedlings of the control cultivars, NILs, and US-2 were transplanted with a spreader at 6-cm intervals and with a 30-cm space between lines. To assess panicle blast resistance, the percentage of rice grains infected by rice blast was scored on an 11-point scale: 0 (percentage of spikelet numbers infected: 0%), 1 (5%), 3 (10%), 5 (25%), 7 (50%), 9 (90%), and 10 (100%) from heading with a 3- to 7-day interval.

We categorized these rice accessions and NILs into six categories based on each final infection score: very resistant (vr: score 0.0 to 1.9), resistant (r: score 2.0 to 2.9), moderately resistant (mr: score 3.0 to 3.9), moderate (m: score 4.0 to 4.9), moderately susceptible (ms: score 5.0 to 6.9), and susceptible (s: score 7.0 to 10.0) based on the criteria described by Yoshida et al. (2021). In addition, to compare and evaluate the dynamic progression of panicle blast severity, we calculated the area under disease progress stair (AUDPS) values (Simko and Piepho 2012).

Furthermore, to categorize the progression of infection dynamics in panicle blast, we analyzed the time series dynamics between the lines and hierarchically clustered them based on the similarity of their dynamic characters by using the "TSclust" package using statistics software "R version 4.0.1" (Montero and Vilar 2014). In this study, we used the dynamic time warping method (DTWARP), one of the model-free measures, as a method for calculating the dissimilarity measure between time series data. To complement the daily progression in panicle blast infection scores on days when the infection score was not assessed, we performed a linear regression using the scores from the days before and after those days.

Investigation of yield-related traits

We investigated eight yield-related traits: culm length (CL), panicle length (PL), number of panicles per plant (PN), panicle weight per plant (PW), culm-and-leaf weight (CW), harvest index (HI: PW/[PW + CW]), total number of spikelets in a representative panicle (SN), and percentage of fertile spikelets in a representative panicle (FS). US-2 and the seven NILs were grown four times in JIRCAS paddy fields, Ishigaki, Okinawa, Japan (24.38°N, 124.19°E) in the first season (from February to June) in 2017, 2018, and 2019 and in the second season (from July to October) in 2019. Seedlings around 28 to 30 days old from each line were transplanted, one per hill, 18 cm apart, in two rows 36 cm apart. An organic slow-release fertilizer (5.2:5.2:5.2 g/m^2 N/P/K) was used as a basal fertilizer. From 20 plants, we selected six randomly from each line for investigation. These plants were not located at the edge of a plot and not suffering from extreme poor growth or damage from disease or insects.

Statistical analyses

Statistical analyses were performed using the BellCurve Excel ver. 3.22 (Social Survey Research Information Co., Ltd., Tokyo, Japan). To categorize the differences in the frequency of infection score to 25 SDBIs in an inoculation test at the young seedling stage among NILs and DVs, hierarchical clustering analyses were conducted by employing Euclidean distance and Ward's hierarchical method (Ward 1963).

In addition, a multiple comparison test was conducted by using the Tukey-Kramer test in one-way ANOVA to determine significant differences in the yield-related traits, because the F-test and the Shapiro-Wilk test showed that these traits were equally and normally distributed.

RESULTS

Development of NILs for partial resistance genes

A total of seven NILs for six partial resistance genes, *Pi35*, *pi21*, *Pi37-KRIL17*, *Pb1*, *Pi34-Chubu32*, *Pi34-Chugoku40*, and

one QTL, *PiPHL9*, were developed by sixfold backcrossing with the susceptible line US-2 as a recurrent parent (Table 1).

These US-2 NILs were named to include the following information: genetic background, line type (NIL), resistance gene or QTL introduced, and donor cultivar. For example, the NIL with the introduced partial resistance gene *pi21* originating from the donor cultivar 'Owarihatamochi' was designated 'US2NIL*pi21-Owarihatamochi*'.

Graphical genotyping of NILs using DNA markers

To investigate the remaining donor-parent-derived chromosomal fragments, we used a total of 345 SSR markers distributed across all 12 rice chromosomes. Our survey showed that 95.9 to 99.1% (average 97.8%) of the 345 markers were non-polymorphic, and the remaining 0.9 to 4.1% were polymorphic between US-2 and NILs (Supplementary Table S1). The chromosomal segments that showed polymorphism between each NIL and US-2 were thought to be derived from the donor parents. Chromosomal segments in US2NIL*pi21-Owarihatamochi* were surely detected in the region of 56.1 cM on chr. 4 in addition to the chromosomal segments observed on chrs. 1, 5, 11, and 12 (Fig. 1). Similarly, chromosomal segments in 'US2NIL*Pi35-Hokkai188*' were detected in the region from 129.3 to 132.8 cM on chr. 1; chromosomal segments in 'US2NIL*Pi37-KRIL17*' were detected from 107.4 to 120.3 cM on chr. 4; the chromosomal segment in 'US2NIL*Pb1-Asanohikari*' was at 108.6 cM on chr.11; those in 'US2NIL*Pi34-Chubu32*' and 'US2NIL*Pi34-Chugoku40*' were at 85.7 cM on chr. 11; and that in 'US2NIL*PiPHL9-HokkaiPL9*' was detected at SSR marker RM1341, 80.2 cM on chr. 11.

Evaluation of leaf blast resistance at young seedling stage

The infection scores of US-2 to 25 SDBIs ranged from 2.0 to 5.0, with the highest value (13 isolates) at 5.0, followed by 4.5 (6 isolates) (Fig. 2, Supplementary Table S2). Those of another susceptible control, LTH, were distributed from 3.0 to 5.0, with the highest value (11 isolates) at 5.0, followed by 4.0 (7 isolates). The infection scores of US2NIL*pi21-Owarihatamochi*, US2NIL*Pb1-Asanohikari*, and US2NIL*Pi34-Chubu34* varied from 0.0 to 4.5, and the highest frequency scores were 1.0 or 1.5. US2NIL*Pi34-Chugoku40* and US2NIL*PiPHL-HokkaiPL9* had scores from 0.5 to 5.0, and the highest frequency scores were 4.0 or 4.5. The scores of US2NIL*Pi35-Hokkai188* and US2NIL*Pi37-KRIL17* varied from 0.0 or 0.5 to 5.0, and the highest frequency scores were 2.0 or 2.5 (Fig. 2, Supplementary Table S2). These reaction patterns of NILs to SBDIs were distinctly different from these of US-2 and LTH: With NILs, the numbers of isolates for scores of 0.0 (resistant) or 5.0 (susceptible) were low, and those from 1.0 to 4.5 were higher.

The distributions of infection scores in DVs for complete resistance genes were clearly different from those of NILs. The clustering analysis classified the NILs and DVs into four groups: A, B1 (were two subgroups: NILs for partial resistance genes and DVs for complete resistance genes), B2, and B3 (Supplementary Fig. S1). Cluster group A included susceptible controls US-2 and LTH and six DVs for *Pi19*(t), *Pit*, *Pish*, *Pik-s*, *Pia*, and *Pii*. All seven NILs and five DVs for *Pib*, *Piz*, *Pi12*(t), *Piz-t*, and *Pi20*(t) were classified into cluster group B1. Six DVs for *Pi19*(t), *Pita-2* (two lines), *Piz-5*, and *Pita* (two lines) fell into cluster group B2, and eight DVs for *Pi3*, *Pi5*(t), *Pik-p*, *Pi7*(t), *Pik-m*, *Pik-h*, *Pik*, and *Pi1* fell into B3.

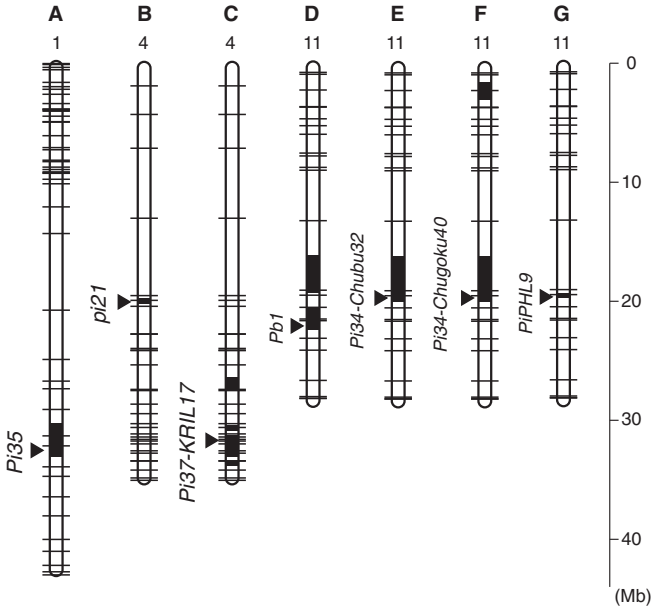

FIGURE 1
Graphical genotypes of chromosomes locating partial resistance genes and one quantitative trait locus. **A,** US2NIL*Pi35-Hokkai188*; **B,** US2NIL*pi21*-Owarihatamochi; **C,** US2NIL*Pi37-KRIL17*; **D,** US2NIL*Pb1-Asanohikari*; **E,** US2NIL*Pi34-Chubu32*; **F,** US2NIL*Pi34-Chugoku40*; **G,** US2NIL*PiPHL9-HokkaiPL9*.

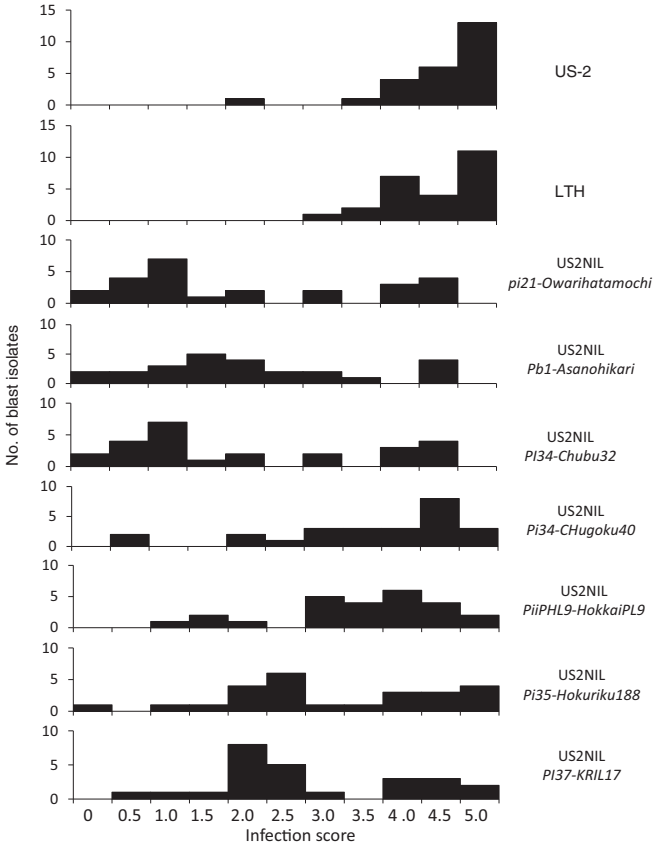

FIGURE 2
Frequency distributions of blast isolates in each infection score for US-2 and near isogenic lines. Inoculation tests were conducted using 25 standard differential blast isolates, which were collected as part of a Japan International Research Center for Agricultural Sciences research project, "Blast Research Network for Stable Rice Production" (Fukuta et al. 2021).

The infection scores in cluster group A varied from 0.5 to 5.0 (Supplementary Fig. S2). The highest frequency score (50%) was obtained at a score of 5.0, and the other scores were not high. In particular, there were a few scores from 0.0 to 2.0, which are regarded as resistance. Those of the DVs for complete resistance genes in cluster groups B1 (complete resistance genes), B2, and B3 were distributed widely from 0.0 to 5.0, and two peaks were found at 1.0 and 5.0. These seven NILs for partial resistance genes and the QTL in B1 were also distributed from 0.0 to 5.0, and those from 1.0 to 4.5 exhibited higher frequencies than those of 0.0, 0.5, or 5.0. These reactions to SDBIs of DVs for complete resistance genes clearly distinguished between resistant and susceptible, whereas NILs for a partial resistance gene were intermediate between them.

Pathogenicity of blast isolates from MARI in Aichi

A total of 22 blast isolates were collected from rice panicles in paddy fields at MARI, Aichi, in 2019 (Fig. 3). Their pathogenicity was evaluated based on their reactions to 25 DVs and two susceptible controls, LTH and US-2. The frequencies of the virulent blast isolates varied from 0 to 95.5%. Among them, 86.4 and 95.5% of the blast isolates were virulent to US-2 and LTH, respectively.

Virulent blast isolates were found at high frequency (over 60%) in the reactions of 11 DVs for *Pia*, *Pii*, *Pi3*, *Pi5*(t), *Pik-s*, *Pik-m*, *Pik-h*, *Pik*, *Pik-p*, *Pi7*(t), and *Pi19*(t); intermediate frequencies (over 20% and less than 60%) were found in five DVs for *Pish*, *Pit*, *Pi1*, and *Pita* (two lines); and low frequencies (less than 20%) were found in nine DVs for *Pib*, *Pi9*(t), *Piz*, *Piz-5*, *Piz-t*, *Pita-2* (two lines), *Pi12*(t), and *Pi20*(t).

These results indicate that at MARI, virulent blast isolates for *Pia*, *Pii*, *Pi3*, *Pi5*(t), *Pik-s*, *Pik-m*, *Pik-h*, *Pik*, *Pik-p*, *Pi7*(t), and *Pi19*(t) were dominant, and those for *Pish*, *Pit*, *Pi1*, and *Pita* were varied.

Resistance to panicle blast among NILs

In 2019, the infection scores of control cultivars reached the expected values around 30 days after heading. The average accumulated daily mean temperature in the period from heading to the day when the 37 control cultivars were assessed was 699.2°C (data not shown). Therefore, the score of the evaluation day when the accumulated daily mean temperature reached around 699.2°C was used as the infection score for US-2, each NIL, and US2-DVs. Based on this criterion, the results of the three-year field trials revealed that US-2 scored 4.5 in 2017, 5.9 in 2018, and 5.5 in 2019 (Supplementary Table S3, Supplementary Fig. S3).

The panicle blast infection scores varied among the NILs and over the three years. NILs for *Pi35*, *Pi37-KRIL17*, and *Pb1* showed scores of less than 3.0 during the three years, and these values were lower than those of the other four NILs and US-2. The NILs for *pi21* also exhibited low scores of less than 3.0, except for one score of 5.0 in 2018. The remaining three NILs for *Pi34-Chubu32*, *Pi34-Chugoku40*, and *PiPHL9-HokkaiPL9* exhibited scores over 5.0, except for *Pi34-Chube32* and *PiPHL9-HokkaiPL9* in 2019. These results indicated that the three NILs for *Pi35*, *Pi37-KRIL17*, and *Pb1* were the most efficient and stable with regard to panicle blast. In contrast, these three NILs for *Pi34-Chubu32*, *Pi34-Chugoku40*, and *PiPHL9* were weak and unstable. The NILs for *pi21* had an intermediate effect between them.

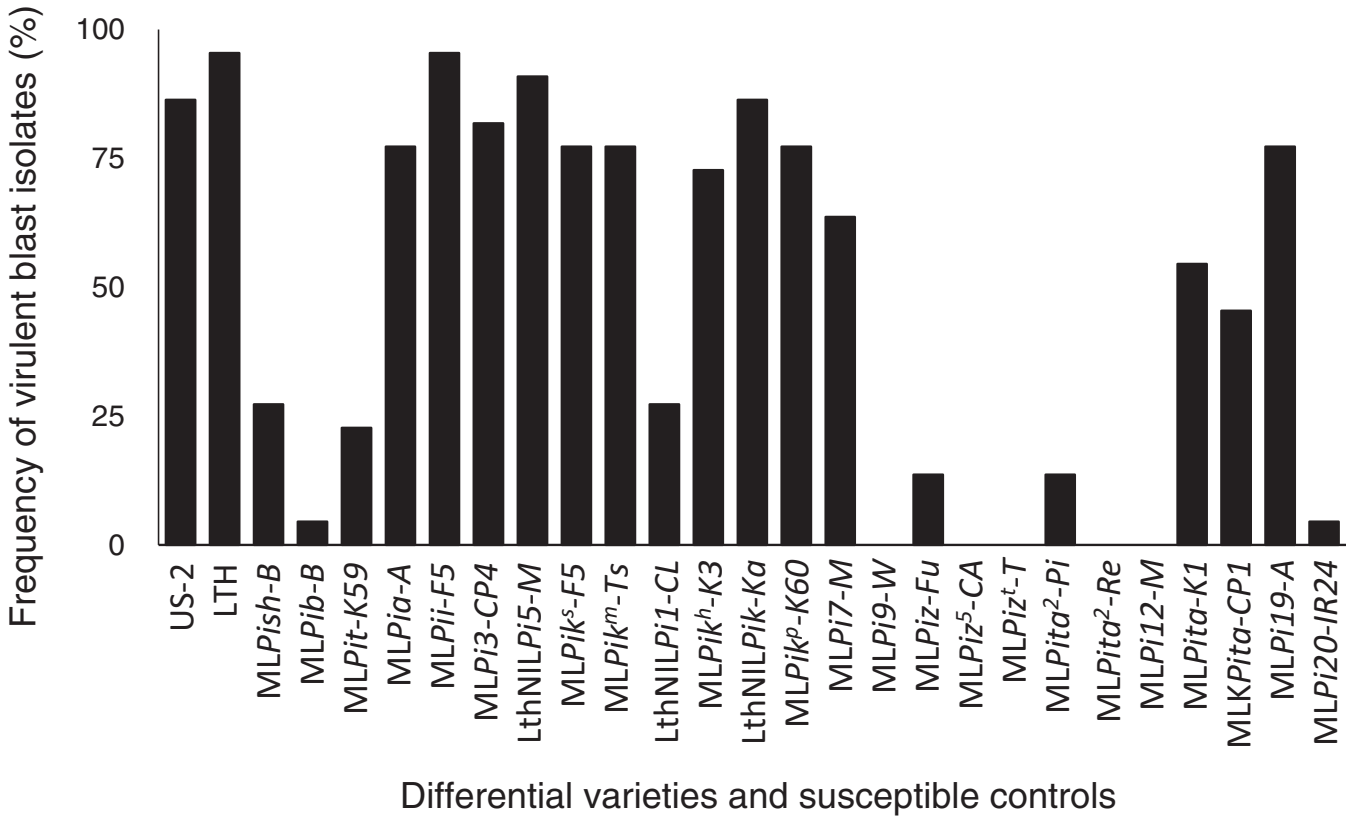

FIGURE 3
Frequency of virulent blast isolates to differential varieties. A total of 22 blast isolates were collected at the Mountainous Region Agricultural Research Institute, Aichi Agricultural Research Center, Inabu, Toyota, Japan in 2018 and 2019.

Based on the categories described by Yoshida et al. (2021), US-2 was classified as "m" in 2017 and "ms" in 2018 and 2019 (Table 2). The NIL for *Pi35* was always classified as "vr", and the NIL for *Pb1* was classified as "vr" or "r". NILs for *Pi37-KRIL17* were classified as "vr" or "mr". NILs for *pi21* were categorized as "r" or "m". The categories of the three NILs for *Pi34-Chubu32*, *Pi34-Chugoku40*, and *PiPHL9* ranged widely from "s" to "vr".

To quantitatively estimate the dynamic progression of panicle blast infection, we used AUDPS values in comparison with the control cultivars, whose own resistance level has already been clarified. The means (±95% confidence interval) of each resistant category were as shown in Supplementary Figure S4. This result shows that the range of the AUDPS values for each resistance category could be identified, although there was no difference between "ms" and "s". In accordance with these results, we categorized the resistance of each partial resistance gene (Supplementary Table S3). There was a high positive correlation between the panicle blast infection score and AUDPS in 2017 and 2018; however, the correlation was slightly lower in 2019 (Supplementary Fig. S5).

In addition, time series clustering was conducted to evaluate the similarity of the dynamic progression in addition to the classification based on the AUPDS value. In 2019, the time series clustering was classified into three groups (I, II-a, and II-b) based on the daily progressions of panicle blast infection score dynamics during the 30 days after heading (Fig. 4, Supplementary Fig. S6). A total of 18 control cultivars were classified into cluster I, which included three spreaders (Wakamizu, Kusabue, and Mineasahi) in addition to the controls for susceptible(s), 'Hitomebore' harboring *Pia*, one *Pii*, *Pi3* and *Pi5*(t), and one of *Pik* allele genes; Koshihikari (harboring *Pik-s* and *Pish*); Wakamizu (harboring *Pii* and early heading); Kusabue (harboring *Pik* and early); and Mineasahi (harboring *Pii* and middle); and 'Norin No.1', 'Norin No.29, 'Gohyakumanngoku', and 'Sasanishiki' harboring *Pia*, *Pish*, and *Pik-s*, and based on the infection scores 30 days after heading. These reactions corresponded with the pathogenicity of the dominant blast isolates in MARI. No NILs or US2-DVs were classified into this cluster.

On the other hand, cluster II-a included US-2; an NIL for *Pi34-Chugoku40*; four US2-DVs for *Pik*, *Pii*, *Pita*(CP1), and *Pia*(A); and 20 control cultivars for moderate ("m") or moderately resistant ("mr") 'Akihikari' (m), 'Kyomishiki' (m), 'Minehikari' (mr), 'Nipponbare' (m), 'Sakakimochi' (mr), and 'Todorokiwase' (mr). Cluster II-b included six NILs for *PiPHL9*, *pi21*, *Pb1*, *Pi34-Chubu32*, *Pi35*, and *Pi37-KRIL17*; five US2-DVs for *Piz*, *Piz-t*, *Pi9*(t), *Pish*, and *Piz-5*; and seven control cultivars including for resistant ("r") 'Chubu22', 'Chubu32', 'Chubu55', and 'Kokonoemochi'. The infection scores of the rice accessions in cluster I corresponded with the category, susceptible ("s"); cluster II-a harbored three categories: "s", moderately susceptible ("ms"), and "m"; and cluster II-b also included three: "mr", "r", and very resistant ("vr") (Supplementary Table S3).

In 2017, US-2 and the NILs were classified into two cluster groups (1 and 2) based on the daily progressions for infection score for 30 days from 15 days after heading because no infections were observed during the first 14 days (Supplementary Fig. S7). US-2 and four NILs for *Pi34-Chugoku40*, *pi21*, *PIPHL9*, and *Pi34-Chubu32* were classified into cluster 1. Three NILs for

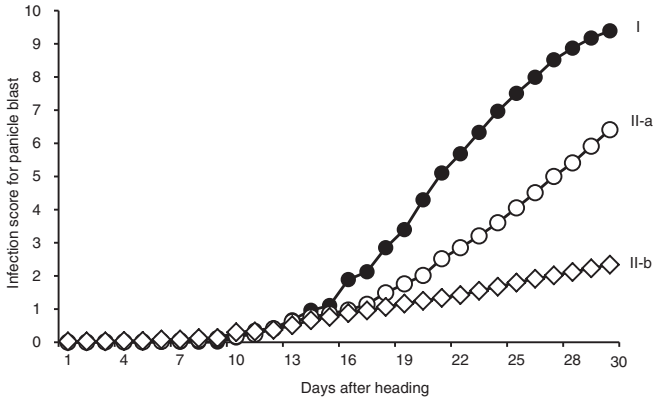

FIGURE 4

Changes of infection scores for panicle blast in near isogenic lines (NILs) for partial resistance genes and one quantitative trait locus (QTL), differential varieties (DVs), and standard control cultivars after heading in 2019. A total of 61 rice accessions including US-2, seven NILs for six partial resistance genes and one QTL, five DVs for complete resistance genes, and 44 standard control cultivars were investigated regarding the degree of infection of panicle blast and classified into three cluster groups (I, IIa, and IIb) based on the changes in the infection scores for panicle blast in the 30 days after heading at the Mountainous Region Agricultural Research Institute, Aichi Prefectural Agricultural Research Center, Inabu, Toyota, Japan. A total of 18 accessions including susceptible cultivars 'Koshihikari', 'Sasanishiki', and 'Hitomebore' were included in cluster group I. Twenty-five accessions including US-2, US-2 NILs for *Pi34-Chugoku40*, *Pik*, *Pii*, *Pia*, and *Pita* and 19 cultivars were in cluster IIa, and 18 accessions including US-2 NILs for *PiPHL9-HokkaiPL9*, *pi21-Owarihtamochi*, *Pb1-Asanohikari*, *Pi35-Hokkai188*, *Pi34-Chubu32*, *Pi37-KRIL17*, *Piz*, *Piz-t*, *Pi9*(t), *Pish*, and *Piz-5* and seven cultivars were in cluster IIb.

TABLE 2

The means of yield components of US-2 and near isogenic lines for partial resistance genes in the first season of three years

Line	Traits[a] (mean ± standard deviation)								
	DTH	CL (cm)	PL (cm)	PN	CW (g)	PW (g)	HI	SN	FS (%)
US-2	103.0 ± 4.4	70.5 ± 6.5	26.8 ± 2.5	6.9 ± 2.1	23.8 ± 8.3	23.5 ± 7.3	0.50 ± 0.05	212.1 ± 30.6	77.4 ± 8.1
US2NIL*Pi35-Hokkai188*	103.3 ± 2.1	69.0 ± 2.7	26.1 ± 2.1	7.0 ± 2.4	21.2 ± 5.7	22.8 ± 4.2	0.52 ± 0.02	198.9 ± 8.8	81.0 ± 8.6
US2NIL*pi21-Owarihatamochi*	102.7 ± 2.1	73.9 ± 3.8	26.9 ± 2.5	6.5 ± 2.6	21.2 ± 8.4	22.0 ± 4.7	0.52 ± 0.06	209.2 ± 20.0	79.5 ± 5.8
US2NIL*Pi37-KRIL17*	100.7 ± 2.3	68.0 ± 3.7	25.5 ± 1.6	7.0 ± 1.7	19.2 ± 4.8	21.0 ± 3.6	0.52 ± 0.04	179.8 ± 11.7	78.5 ± 6.9
US2NIL*Pb1-Asanohikari*	101.3 ± 1.2	72.1 ± 2.2	26.0 ± 2.3	7.1 ± 1.5	20.3 ± 5.7	23.5 ± 3.4	0.54 ± 0.04	192.9 ± 18.0	79.7 ± 7.0
US2NIL*Pi34-Chubu32*	103.3 ± 2.1	70.8 ± 3.4	25.8 ± 2.7	7.1 ± 1.4	20.6 ± 6.9	20.5 ± 2.1	0.50 ± 0.05	210.0 ± 47.9	77.1 ± 3.9
US2NIL*Pi34-Chugoku40*	101.0 ± 0.0	73.8 ± 1.5	27.2 ± 2.8	7.1 ± 1.7	20.3 ± 6.7	21.4 ± 2.1	0.52 ± 0.07	184.8 ± 12.9	70.7 ± 5.7
US2NIL*PiPHL9-HokkaiPL9*	103.3 ± 1.5	70.7 ± 1.5	26.6 ± 2.9	7.1 ± 1.7	23.0 ± 4.0	24.5 ± 4.8	0.51 ± 0.06	206.1 ± 21.1	79.3 ± 3.5
Mean	102.3 ± 1.1	71.1 ± 2.1	26.4 ± 0.6	7.0 ± 0.2	21.2 ± 1.5	22.4 ± 1.4	0.51 ± 0.01	199.0 ± 12.3	77.9 ± 3.2

[a] DTH = days to heading after sowing; CL = culm length; PL = panicle length; PN = number of panicle per plant; CW = culm and leaf weight; PW = panicle weight per plant; HI = harvest index: PW/(CW + PW); SN = total number of spikelets in a representative panicle; FS (%) = percentage of fertile spikelets per panicle. There were no significant differences between lines for any trait at the 5% level.

Pi37-KRIL17, *Pb1*, and *Pi35* were classified into cluster 2. The infection scores of clusters 1 and 2 were categorized as "ms" or "s" and "mr" or "vr", respectively (Supplementary Table S3, Supplementary Fig. S7).

In 2018, US-2 and the NILs were classified into three cluster groups (A, B-a, and B-b) based on the daily progressions of infection score for 30 days from 15 days after heading because, again, no infections were observed during the first 14 days (Supplementary Fig. S8). Cluster group A included US-2, an NIL for *Pi34-Chugoku40*, and five DVs for *Pita*(CP1), *Pita*(CT2), *Pita*(K1), *Pi5*, and *Pii*. Three NILs for *Pb1*, *Pi35*, and *Pi37-KRIL17*, and five DVs for *Piz-t*, *Pi1*, *Pita-2*, *Pi9*(t), and *Pik-m*, were classified into cluster group B-a. Three NILs for *PiPHL9*, *Pi34-Chubu32*, and *pi21*, and 13 DVs for *Pish*, *Pia*(A), *Pia*(C), *Pi3*, Piz, *Piz-5*, *Pik-s*, *Pik-p*, *Pik*, *Pik-h*, *Pi7*(t), *Pi12*(t), and *Pi20*(t) were classified into cluster group B-b. The NIL for *Pi34-Chugoku40* was categorized as "s". US-2 and three NILs for *pi21*, *Pi34-Chube32*, and *PiPHL9* were categorized as "ms"; three NILs for *Pi37-KRIL17*, *Pb1*, and *Pi35* were categorized as "vr" (Supplementary Table S3, Supplementary Fig. S8). Thus, along with the categorization by AUDPS, these results indicated that the resistance category of NILs determined by time series clustering changed depending on the cultivation years (seasons).

A two-way ANOVA for the interaction between year and resistance gene revealed that infection scores differed significantly as a result of the interaction between year and resistance gene (Supplementary Table S4). Because the year-to-year variation was attributed to temperature and precipitation, which strongly affect the infection and progression of panicle blast, we compared the yearly differences in temperature and precipitation during August and September (Supplementary Table S5). The climate condition data showed that the daily mean temperature was high in the order of 2017, 2018, and 2019, whereas the daily minimum temperature was high in 2018 and 2019 and low in 2017. The daily maximum temperature was high in 2019 and low in 2017 and 2018. It was also observed that both precipitation and the number of days of precipitation were higher in 2018 than in 2017 and 2019.

Investigation of yield-related traits in the field

The heading date and yield components in the NILs and US-2 were investigated at TARF, JIRCAS, Ishigaki, Okinawa, Japan, in the first seasons of 2017 to 2019 and the second season of 2019 (Table 3, Supplementary Table S6).

There were no significant differences between US-2 and the NILs in any trait in the three first-season cultivations (Table 3). The days to heading after sowing (DTH), culm length (CL), and percentage of fertile spikelets per panicle (FS) of US-2 and the NILs in the first seasons were larger than those of the second season (Supplementary Table S6). In contrast, the number of panicles per plant (PN), panicle weight per plant (PW), and total number of spikelets in a representative panicle (SN) in the second season exceeded those of the first season (Supplementary Table S6). These results indicate that the NILs that we developed had similar yield components and heading dates to those of US-2. Additionally, the FS of the NIL for *Pi34-Chugoku40* always had the lowest value among the NILs and US-2 in all four cultivated seasons.

DISCUSSION

Introgression of chromosome segments for partial resistance genes and QTL

We developed and characterized genetically the seven NILs for six partial resistance genes (*Pi35*, *pi21*, *Pi37-KRIL17*, *Pb1*, *Pi34-Chubu32*, and *Pi34-Chugoku40*) and one QTL (*PiPHL9*) with a genetic background of an Indica Group susceptible line US-2 (Table 1).

Our investigation of chromosomal segments derived from their donor parents was undertaken using DNA markers. More than 95% were substituted with US-2-derived chromosomes, and none of the regions of the major complete resistance loci were derived from the donor parent (Supplementary Table S1). These results indicated that the distinct effects of the six partial resistance genes and one QTL could be clarified using these NILs.

Yield components in the NILs

There were no significant differences between US-2 and the NILs with regard to heading date and traits for yield components based on the data for three first-season cultivations from 2017 to 2019 (Table 3, Supplementary Table S6). Several differences were found between US-2 and the NILs, but no specific tendency was found in these investigations, except for the NIL for *Pi34-Chugoku40*, which always showed the longest PL and the lowest FS of all four cultivations. These results indicate that additional genetic factors might have been introduced into the genetic background of *Pi34-Chugoku40*. Further studies of the NIL for *Pi34-Chugoku40* will be needed for confirmation based on clarifying the relationships with introgressions of additional chromosome segments in the genetic background.

Resistance to leaf blast at young seedling stage

The infection scores of leaf blast in the five NILs for *pi21*, *Pi35*, *Pi37-KRIL17*, *Pi34-Chugoku40*, and *PiPHL9* were lower than that for US-2 in the inoculation test using 25 SDBIs at the young seedling stage, whereas the two NILs for *Pb1* and *Pi34-Chubu32* were not (Fig. 2, Supplementary Table S2, Supplementary Fig. S1). Almost all 25 DVs for complete resistance genes including monogenic lines (Telebanco-Yanoria et al. 2010; Tsunematsu et al. 2000) appeared at high frequencies with reactions to 25 SDBIs at scores of 0.0 to 1.0 or 5.0 in the distributions (Fig. 2, Supplementary Table S2). These results indicated that the reactions of DVs to SDBIs were divided more clearly into resistant and susceptible. In contrast, those of the seven NILs were distributed mainly between 2.0 (regarded as moderately resistant) and 4.5 (regarded as susceptible), and there were few scores of 0.0 and 5.0. These results indicated and confirmed that the resistances of the NILs were different from those of DVs in the inoculation test for leaf blast at the young seedling stage.

The mean values of the infection scores in four US-2 NILs for *Pi35*, *pi21*, *Pi37-KRIL17*, and *PiPHL9*, were lower than for the other three for *Pb1*, *Pi34-Chubu32*, and *Pi34-Chugoku40* (Supplementary Table S2). Yasuda et al. (2015) clarified the effects of partial resistance genes *Pi35*, *pi21*, and *Pi34-Chugoku40* on leaf blast by using NILs with the genetic background of the Japonica Group rice cultivar 'Koshihikari' harboring some complete resistance genes, such as *Pish* and *Pik-s*, and some unknown genes (Kawasaki-Tanaka and Fukuta 2014). They found that *Pi35* reduced the number of sporulating lesions and inhibited the expansion of the lesions, *pi21* reduced the number of lesions as well, and *Pi34-Chugoku40* reduced the number of lesions but did not inhibit their expansion. The effect of *Pi34-Chugoku40* was smaller than those of *pi21* and *Pi35*. This is consistent with our results demonstrating that *pi21* and *Pi35* showed resistance to most isolates with fewer lesions, with exceptions in some isolates, and that *Pi34-Chugoku40* showed resistance to some isolates but was moderately susceptible to most isolates.

Pathogenicity of blast isolates from MARI

The presence of virulent blast races against the DVs with *Pia*, *Pii*, *Pi3*, *Pi5*(t), *Pik-s*, *Pik-m*, *Pik-h*, *Pik*, *Pik-p*, *Pi7*(t), *Pita* (two lines), and *P19*(t) was confirmed based on pathogenicity analyses of blast isolates collected from MARI in 2019 (Fig. 3).

US2-DVs for *Pii*, *Pi3*, *Pi5*(t), *Pita* (three lines), *Pi20*(t), *Pia*(C), *Pia*(A), *Pish*, *Pi1*, *Pik-m*, *Pik*, *Pik-h*, *Pik-p*, *Pi7*(t) and *Pi12*(t), and *Pita-2*(Re) were classified into categories "s", "ms", and "m" in the evaluation of panicle blast in 2018 and 2019 (Supplementary Table S3). These infections in US2-DVs for complete resistance genes corresponded with the pathogenicity test results for blast isolates at MARI, although the US2-DVs for *Pik-s* categorized as "mr" in the panicle blast did not agree with the susceptible reaction in the inoculation test. Kawasaki-Tanaka et al. (2016) and Koizumi et al. (2007) also reported that virulent blast isolates for *Pit* were not found among the 310 and 1,050 isolates from Japanese paddy fields. Our result for *Pit* showed an over

TABLE 3

Classifications of US-2 and US-2 near isogenic lines (NILs) for partial resistance genes based on the infection degrees of panicle blast in three years from 2017 to 2019[a]

	Rice accessions [cluster group] (class of blast resistance)					
Year	Susceptible (s: 7.0–10.0)	Moderately susceptible (ms: 5.0–6.9)	Moderate (m: 4.0–4.9)	Moderately resistant (mr: 3.0–3.9)	Resistant (r: 2.0–2.9)	Very resistant (vr: 0.0–1.9)
2017		**US2NIL*Pi34-Chugoku40*[1]**	**US-2[1]** **US2NIL*Pi34-Chubu32*[1]**		**US2NIL*pi21-Owarihatamochi*[1]** **US2NIL*PiPHL9-HokkaiPL9*[1]**	**US2NIL*Pb1-Asanohikari*[2]** **US2NIL*Pi35-Hokkai188*[2]** **US2NIL*Pi37-KRIL17*[2]**
2018	**US2NIL*Pi34-Chugoku40*[A]** US2NIL*Pita-CP1*[A] US2NIL*Pita-CT2*[A] US2NIL*Pi5-M*[A] US2NIL*Pita-K1*[A] US2NIL*Pi12-M*[B-b]	**US-2[A]** **US2NIL*PiPHL9-HokkaiPL9*[B-b]** US2NIL*Pii-F5*[A] US2NIL*Pish-S*[B-b] US2NIL*Pik^p-K60*[B-b] US2NIL*Pi20-IR24*[B-b]	**US2NIL*pi21-Owarihatamochi*[B-b]** **US2NIL*Pi34-Chubu32*[B-b]** US2NIL*Pita^2-Re*[B-a] US2NIL*Pi7-M*[B-b]	US2NIL*Pik^m-TS*[B-a] US2NIL*Pia-C*[B-b] USNIL*Pia-A*[B-b] US2NIL*3-CP4*[B-b] US2NIL*Pik^h-K3*[B-b] US2NIL*Pik^s-S*[B-b]	US2NIL*Pi1-CL* [B-a] US2NIL*Pik-K*[B-b]	**US2NIL*Pb1-Asanohikari*[B-a]** **US2NIL*Pi35-Hokkai188*[B-a]** **US2NIL*Pi37-KRIL17*[B-a]** US2NIL*Piz^t-T*[B-a] US2NIL*Pi9-W*[B-a] US2NIL*Piz^5-CA*[B-b] US2NIL*Piz-Fu*[B-b]
2019	US2NIL*Pii-F5*[II-a] US2NIL*Pik-K*[II-a] US2NIL*Pita-CP1*[II-a] Fujisaka 5[I] Fukei 69[I] Kusabue[I] Chubu 19[I] Nihonmasari[I] Norin No.29[I] Mangetsumochi[I] Wakamizu[I] Tsukimimochi[I] Norin No.1[I] Sasanishiki[I] Kihoh[I] Mineasahi[I] Toyomishiki[I] Himenomochi[I] Hitomebore[I] Gohyakumangoku[I] Koshihikari[I] Nipponbare[II-a] Nakateshinsenbon[II-a] Yoneshiro[II-a] Kanoto 51[II-a] Akihikari[II-a] Sakakimochi[II-a] Akitakomachi[II-a] Kiyonishiki[II-a]	**US-2[II-a]** **US2NIL*Pi34-Chugoku40*[II-a]** USNIL*Pia-A*[II-a] Hourei[II-a] Homarenishiki[II-a] Reimei[II-a] Todorokiwase[II-a] Sachiizumi[II-a]	US2NIL*Pish-S*[II-b] Ohgonbare[II-a] Tsuyuake[II-a] Chubu 7[II-a] Chubu36[II-a]	**US2NIL*Pi37-KRIL17*[II-b]** **US2NIL*Pi34-Chubu32*[II-b]** US2NIL*Piz^5-CA*[II-b] Dewanomochi[II-a] Minehikari[II-a] Chiyonishiki[II-b] Chubu 32[II-b] Chubu35[II-b] Chubu 55[II-b]	**US2NIL*Pb1-Asanohikari*[II-b]** **US2NIL*pi21-Owarihatamochi*[II-b]** Kokonoemochi[II-b] Yamabiko[II-b] Chubu 22[II-b]	**US2NIL*Pi35-Hokkai188*[II-b]** **US2NIL*PiPHL9-HokkaiPL9*[II-b]** US2NIL*Piz^t-T*[II-b] US2NIL*Pi9-W*[II-b] US2NIL*Piz-Fu*[II-b]

[a] The investigations of the infection degrees of panicle blast were carried out at the Mountainous Region Agricultural Research Institute, Inabu, Toyota, Aichi, during three years from 2017 to 2019. These infection degrees for panicle blast of rice accessions for the three years were compared when the accumulated daily mean temperatures reached around 699.2°C after heading. These were 36 days after heading in 2017, 34 days in 2018, and 30 days in 2019. Cluster analysis of US-2 NILs for partial and true resistance genes, one quantitative trait locus, and rice cultivars was performed using Ward's hierarchical method (Ward 1963). The listings for 2017 show NILs for partial resistance genes (bold); those for 2018 show NILs for partial resistance genes (bold) and NILs for complete resistance genes; and those for 2019 show NILs for partial resistance genes (bold), NILs for complete resistance genes, and the other controls.

25% higher frequency than these studies. It is known that a DV for *Pit* makes halo-type lesions after the inoculation of avirulent blast isolates (Hayashi et al. 2009), and the lesions are sometimes confused with susceptible lesions. Misevaluations might be included in the inoculation test, and a limited number of 22 blast isolates were used for the pathogenicity analysis. A pathogenicity analysis for blast isolates from MARI and continuous evaluations of the panicle blast for *Pit* and *Pik-s* must be performed.

Resistance to panicle blast at mature stage

By comparison with the results of the three years of MARI trials, the degree of panicle blast among NILs for partial resistance genes and QTL varied (Table 2, Supplementary Tables S3 and S4, Supplementary Fig. S3). The temparature in 2019 was slightly higher than in the other two years and less precipitation than 2018 (Supplementary Table S5). The tendency to show stronger resistance in 2019 could be due to the effect of high temperature. However, the same trend was not observed in all the partial resistance genes focused on in this study. These results indicated that the degree of infection of panicle blast in the field varied and was influenced by the environmental conditions and partial resistance genes.

In addition to the infection score, AUDPS values were obtained to evaluate the dynamic progress of panicle blast infection. In the analysis of the control cultivars whose partial resistance was already known, there was no significant difference between the AUDPS mean values of the two categories of "ms" and "s". Therefore, we could separate the panicle blast resistance of the NILs and US2-DVs into four categories in 2019 (Supplementary Table S3). A strong positive correlation was observed between the final infection scores and AUDPS value, although the correlation coefficient was lower in 2019 than in 2017 and 2018 (Supplementary Fig. S4). This could be attributed to a delay in the progression of infection due to lower temperatures, as many lines with US-2 background were not heading until late August or early September. Therefore, to classify resistance, based on the possibility that the progression of the infection is slowed by low temperatures, we applied a time series clustering method, which has been widely used in disease research, especially for the classification of infection trends caused by COVID-19 and the prediction of its transmission process (Rojas-Valenzuela et al. 2021). As a result, we were able to divide the results of the field trials into three major groups: a resistance group that suppresses infection, an intermediate resistance group that progresses infection slowly, and a susceptible group that progresses infection rapidly (in 2017, we were able to divide the results into the first two groups) (Fig. 4, Supplementary Figs. S6, S7, and S8). In 2019, resistance tended to be stronger, especially in NILs for *pi21*, *PiPHL9*, and *Pi34-Chubu32*, as well as in terms of infection scores and AUDPS, but the evaluations in the three-year field trials were generally consistent. In conclusion, in addition to the evaluation by infection score and AUDPS, the time series clustering method was effective for evaluating field resistance under different environmental conditions of disease development caused by the cultivation season or the different timing of the heading.

Through three years of trials, the infection scores of US-2 and the NIL for *Pi34-Chugoku40* were always the highest among NILs, and those of the two NILs for *Pb1* and *Pi35* were the lowest, followed by *Pi37-KRIL17* (Supplementary Table S3). The other three NILs for *Pi34-Chubu32*, *pi21*, and *PiPHL9* were intermediate between them, but the degree of resistance varied among NILs and seasons (Supplementary Table S3). Interestingly, *Pi35* always exhibited effective reactions on blast diseases in both the young seedling and maturing stages (Supplementary Tables S2 and S3). *Pi37-KRIL17* was also stable and exhibited degrees from moderate ("m") to moderately resistant ("mr") in both stages (Supplementary Tables S2 and S3). *Pb1* was characterized as one of the most efficient resistance genes for panicle blast, but it was the weakest among the NILs at the young seedling stage (Supplementary Tables S2 and S3). In contrast, *pi21* was one of the most efficient genes in the seedling stage, but it showed an intermediate effect and was not always resistant to panicle blast (Supplementary Tables S2 and S3). These results mean that the effects and stabilities of the partial resistance genes and QTL varied during the rice growing stages and under different environmental conditions. In other words, we could successfully characterize these partial resistance genes and the QTL based on the degree of resistance, effective rice growing stage, and stability of resistance during different seasons (environmental conditions). In addition, previous studies have carried out evaluations of partial resistance genes under the genetic backgrounds of rice cultivars harboring some additional complete resistance genes (Fukuoka et al. 2015; Yasuda et al. 2015), and their real effects could not be accurately evaluated. This was because the additional complete resistance genes in the Japonica Group's cultivars masked the effects of the partial resistance genes. Our experimental design based on the genetic background of US-2 made it possible for us to obtain valuable data regarding their effects as a single partial resistance gene in each line. Therefore, this study is the first to show the essential effect of six individual partial resistance genes and one QTL on the resistance to panicle blast under the susceptible genetic background of Indica Group rice. These NILs for partial resistance genes will be helpful in genetic and pathological studies on blast resistance.

Interaction between partial and complete resistance genes

An NIL for *Pi34-Chubu32* and a breeding line Chubu 32 harboring *Pi34*, *Pia*, and unknown genes (Kawasaki-Tanaka and Fukuta, 2014) showed moderate resistance ("mr") and resistance ("r") to panicle blast, respectively, in 2019, suggesting that complex genetic factors or interactions, such as allelic differences or combinations with other blast resistance genes (including complete resistance genes), might affect and change the panicle blast resistance. Yasuda et al. (2015) and Fukuoka et al. (2015) reported that the pyramiding of partial resistance genes leads to stronger blast resistance. By using our NILs, it will be possible to confirm the effects of such gene pyramiding.

US-2 showed susceptible reactions to most of the 25 SDBIs, and the mean infection value was 4.6, except for one isolate, which showed an infection score of 2.0 in leaf blast (Supplementary Table S2). Regarding panicle blast, US-2 was categorized as "ms" (score: 5.0 to 6.9) in 2018 and 2019 and "m" (score: 4.0 to 4.9) in 2017 (Supplementary Table S3). More susceptible rice cultivars or US2-DVs were found in these three years. Therefore, US-2 might harbor or retain minor genetic factors for blast resistance in the genetic backgrounds. The panicle blast infection degrees of the NILs for *Pi34-Chugoku40*, and US2-DVs for *Pi12*(t), *Pi5*(t), and *Pita* (three lines) were categorized as "s", whereas that of US-2 and an NIL for *PiPHL9* and DVs for *Pii*, *Pish*, *Pik-h*, *Pik-p*, and *Pi20*(t) was "ms" in 2018 (Table 3, Supplementary Table S3). These results suggested that the susceptible reactions in these US-2 NILs and US2-DVs might have occurred by the exchanges between the minor genetic factors in US-2 and these resistance genes introduced or interactions with the unknown genetic factors in the genetic background of US-2. These relationships between resistance genes and the genetic factors of the US-2 genetic background need to be confirmed by developing a more susceptible line.

Perspectives

The partial resistance gene *pi21* reportedly encodes a protein with a putative heavy-metal-binding domain and a proline-rich region (Fukuoka and Okuno 2019), and *Pi35* encodes a protein containing a nucleotide-binding site (NBS) and leucine-rich repeats (LRRs) (Fukuoka et al. 2014). Among the partial resistance genes used in this study, *Pi34-Chugoku49*, *Pi34-Chubu32*, *Pi37-KRIL17*, and *PiPHL9* have yet to be genetically cloned and their functions confirmed at the molecular level. Because our NILs were bred on a common genetic background that does not carry major complete resistance genes, further analyses using our materials will help to clarify the differences in the molecular physiological mechanisms that involve the proteins encoded by the respective resistance genes.

To evaluate leaf blast resistance, four-leaf seedlings have been used in inoculation tests according to the method described by Hayashi et al. (2009). In the evaluation of partial resistance, it is known that the degree of disease infection decreases with advancing leaf age, for example, at the seventh- or eighth-leaf stage (Yunoki et al 1970; Zenbayashi et al. 2000). Based on the above, the evaluation method in this study using four-leaf seedlings might not be able to fully evaluate the effects of partial resistance genes in an inoculation test. More detailed characterizations of partial resistance genes in the seedling stage will be possible using NILs. Although partial resistance genes have been expected to show race-nonspecific resistance, there have been a few studies reporting that some partial resistance genes showed race-specific resistance responses. In inoculation tests, all NILs for partial resistance genes and the QTL found the blast isolates for a score of 5.0 (susceptible), except for *pi21* and *Pi35* (Supplementary Table S2). The NIL for *Pi34-Chugoku40* was categorized as susceptible ("s") (Supplementary Table S3) in field trials in 2018. Zenbayashi-Sawata et al. (2005) reported that *Pi34-Chubu32* showed strong resistance to isolates of Y93-245c-2 but not to an isolate of IBOS8-1-1. Similarly, *qBFR4-Ingngoppor-tinawon* was reported to show strong resistance to many blast isolates of Japanese origin, whereas it is susceptible to isolates of M64-1-9-3-1 and V850265 from the Philippines (Mizobuchi et al. 2014). Other research reports have also demonstrated that some partial resistance genes show race-specific resistance responses (Xu et al. 2014; Yunoki et al. 1970), and some resistance responses followed gene-for-gene theory (Yasuda et al. 2008; Zenbayashi-Sawata et al. 2005). Although partial resistance has not been considered to cause breakdown, this finding of virulent blast isolates might suggest the risk of the outbreak of a virulent blast race. We clarified the partial resistance genes and QTL that had stage-specific effects in rice growing stages. Therefore, it is important to confirm that partial resistance genes really contribute to the durable resistance through further studies considering the rice growing stages using the NILs in this study.

Furthermore, these NILs are expected to be used for advanced pyramiding breeding for blast resistance and pathological studies analyzing the dynamics of blast fungus races when lines with only partial resistance genes are continuously grown.

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