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Rice Blast Control Efficacy of Three Genes (*Pib*, *pi21*, and *Pb1*) Conferring Complete and Partial Resistance

Jemal Tola HORO[1], Toshio FUJII[2], Yasunori YAMASHITA[2], Sachie McGoey[3] and Shinzo KOIZUMI[4]*

[1] Pawe Agricultural Research Center, Ethiopian Institute of Agricultural Research (25 Pawe, Ethiopia)
[2] Overseas Agricultural Development Association (Akasaka, Tokyo 107-0052, Japan)
[3] Tsukuba International Center, Japan International Cooperation Agency (Tsukuba, Ibaraki 305-0074, Japan)
[4] Technical Adviser, Tsukuba International Center, Japan International Cooperation Agency (Tsukuba, Ibaraki 305-0074, Japan)

Abstract

The rice blast control efficacy of three genes—complete resistance gene *Pib* in isogenic line 'Koshihikari Toyama BL 3' and partial resistance genes *Pb1* in near-isogenic line 'Koshihikari Aichi SBL' and *pi21* in a variety 'Tomohonami'—was evaluated in pot and upland trials with spray inoculation of the blast isolate Ina 86-137 (Japanese race 007.0) or Ao 92-06-2 (Japanese race 337.1). The evaluation was conducted by assessing their leaf and panicle blast severity, and then comparing their severity with that of blasticide (probenazole or tricyclazole) applied 'Koshihikari' and three control varieties with different levels of partial resistance to blast, including 'Koshihikari'. 'Koshihikari Toyama BL3' harboring *Pib* showed very high levels of efficacy with few leaf and panicle blast lesions in both trials. The efficacy of 'Tomohonami' having *pi21* to leaf blast was also high and equal to or higher than that of the blasticide-applied 'Koshihikari' in both trials. However, 'Tomohonami' had no panicle blast control efficacy in the pot trial, although it showed high panicle blast reduction in the upland trial. The panicle blast control efficacy of 'Koshihikari Aichi SBL' with *Pb1* was moderate, while its leaf blast reduction was low to moderate in both trials. For expansion of the leaf blast lesions with punch inoculation on the uppermost leaves of rice plants in the booting stage of the pot trial, the areas (length x width) of the lesions on 'Koshihikari Toyama BL 3' with *Pib* were the smallest and significantly different from those of all other lines and varieties, including probenazole-applied 'Koshihikari'. The results showed the blast control efficacy of the three resistance genes *Pib*, *Pb1*, and *pi21*, and confirmed a quantitative reduction of blast severity with partial resistance genes *Pb1* and *pi21*.

Discipline: Plant disease
Additional key words: blasticides, inoculation, isogenic/near-isogenic lines, Koshihikari, pot and upland trials

Introduction

Rice blast disease caused by *Pyricularia oryzae*, teleomorph *Magnaporthe oryzae* (Couch & Kohn 2002) is one of the most serious fungal diseases of rice worldwide (Valent & Chumley 1991) and causes significant rice yield loss. The causal fungus can infect rice plants at any growth stage, and the blast pathosystem consists of two major interrelated phases: leaf blast and panicle blast, with leaf blast providing inoculum for panicle blast (Ou 1985). The typical lesions of leaf blast are spindle-shaped, with the leaves collapsing and sometimes being killed by the disease, while panicle blast directly prevents the grain filling of rice through blast infection on panicles.

Among the several available methods of controlling rice blast, genetic resistance is the most practical way used in developing countries as well as in developed countries, because it can effectively control the disease with less chemical application, thus reducing the environmental impact as well as production cost. Resistance to rice blast is classified as complete/true and partial/field resistance. Complete resistance (CR), controlled by a major gene, is qualitative. However, CR is race-specific, and rice varieties containing single genes conferring CR have become susceptible within several years after being released and singly cultivated due to the development of new blast races viru-

*Corresponding author: e-mail Koizumi-Shinzo@jica.go.jp
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lent to the resistance genes. In contrast, quantitative partial resistance (PR) is commonly controlled by minor genes, and PR is considered durable due to its generally non-race-specific and polygenic characteristics. Through recent advances in molecular studies, many resistance genes conferring CR and PR to blast have been identified and mapped on different rice chromosomes using molecular markers, and some resistance genes have even been cloned (Miah et al. 2013, Sharma et al. 2012). Moreover, breeding rice varieties with blast resistance has become easy through molecular marker assisted selection (MAS) using DNA markers, tightly linked to blast resistance genes (Koide et al. 2009, Miah et al. 2013). In Japan, several isogenic/near-isogenic lines, each holding CR or PR genes as well as the genetic background of the most popular rice variety 'Koshihikari' and varieties with single PR genes have been developed through MAS and conventional breeding (Kojima et al. 2004, Sugiura et al. 2004, Fujii et al. 2005, Ishizaki et al. 2005, Sunohara et al. 2007, Saka et al. 2010). However, studies on the blast control efficacy of blast resistance genes using isogenic/near-isogenic lines and varieties possessing each of the genes are limited.

Blasticides with different characteristics and several application methods have been developed, however, to control blast. Modern fungicides including blasticides have been developed through extensive evaluation for safety, and non-fungicidal disease controlling agents such as plant defense activators including probenazole have been released, as such fungicides are supposedly specific to the target organisms and less likely to lead to fungicide resistance problems (Yamaguchi 2004). However, comparison of the efficacy of blast control with blasticides with that of blast resistance genes is limited.

Under the circumstances, the blast control efficacy of two rice isogenic/near-isogenic lines with the genetic background of 'Koshihikari', each harboring the CR gene *Pib* and the PR gene *Pb1*, and rice variety 'Tomohonami' having the PR gene *pi21* was evaluated in pot and upland trials by comparing their efficacy with that of blasticide application and three rice varieties with different levels of PR.

Materials and methods

1. Rice varieties and lines

Two Koshihikari isogenic/near-isogenic lines, 'Koshihikari Toyama BL3' harboring the CR gene *Pib* (Koshi-Pib) (Kojima et al. 2004) and 'Koshihikari Aichi SBL' having the PR gene *Pb1* to panicle blast (Koshi-Pb1) (Sugiura et al. 2004), and rice variety 'Tomohonami' (Chubu 125) that was bred by being crossed twice with 'Koshihikari' and which harbors the PR gene *pi21* (Saka et al. 2010) were used to evaluate the blast control efficacy of the three resistance genes in this study. Three rice varieties ('Koshihikari', 'Shin 2', and 'Koganenishiki') showing low, medium, and high levels of PR to blast, respectively, were used as the control varieties for evaluating blast control efficacy. Rice variety 'Toride 1' with the CR gene *Piz-t* showing CR to most Japanese blast isolates was planted among the plots as a barrier in the upland trial. Seeds of Koshi-Pib were supplied by the Toyama Agricultural Research Institute, and seeds of 'Tomohonami' and Koshi-Pb1 were offered by the Aichi Agricultural Research Center.

2. Experimental design and site

In the study, both pot and upland trials were conducted in a randomized complete block design with three replications (blocks). Each block consisted of seven treatments, that is, two isogenic/near-isogenic lines each having the blast resistance genes *Pib* and *Pb1*, a variety with gene *pi21*, three control varieties with different levels of PR to blast, and blasticide-applied 'Koshihikari', which was treated with either probenazole in the pot trial or tricyclazole in the upland trial. In the pot trial, each block consisting of seven treatments (pots) was inoculated in one day during a period of consecutive three days. However, in the upland trial, one nursery bed (0.9 m in width, 14.1 m in length), consisting of seven plots (treatments) 1.2 m in length and with 50-cm spacing between the plots, composed one block. In the trial, the beds were spaced 1.3 m apart.

The trials were conducted during the rice cropping season of 2014 at the Tsukuba International Center (TBIC) of Japan International Cooperation Agency (JICA) in Ibaraki prefecture (located in the northeastern part of the Kantō region in central Japan).

3. Preparation of plants

(1) Pot trial

For the pot trial, the rice seeds were dipped and disinfected in 200 times solution of benomyl-thiuram wettable powder (20% active ingredient (a.i)) for one day. The disinfected seeds were pre-germinated in Petri dishes including water for four days at 25-30°C before sowing. Nursery boxes (5.5 cm × 15 cm × 9.5 cm in size) filled with commercial soil (Kanuma A: Kumiai granulated culture soil containing zeolite, Kanuma Sangyo Co., Ltd.) including fertilizer (N 0.4 g, P 1.1 g, and K 0.5 g per L of soil) were used for raising rice seedlings. Seeds of each variety and line were sown in the plastic boxes on April 25, 2014, and then grown up to the 3rd leaf stage in a greenhouse. Three rice seedlings were then transplanted individually about 8 cm apart into each of the 1/500,000 ha Wagner pots on May 9 and grown until inoculation in the greenhouse. The Wagner pots contain the same commercial soil as that used for raising the seedlings.

(2) Upland trial

For the upland trial, the seeds of each rice variety or

Table 1. Blast severity of rice varieties/lines with different resistance and blasticide-applied 'Koshihikari' in the pot and upland nursery trials

Variety/line	Resistance gene/applied blasticide	Type of resistance/ blasticide	Blast severity						
			Pot trial (spray/punch inoculation)					Upland trial	
			LN1 (No. of lesions/ plant)	LN2 (No. of lesions/ plant)	PLA (mm^2)	PDS1 (%)	PDS2 (%)	Leaf blast (AUDPC)	Panicle blast (AUDPC)
Koshihikari	+	Low level of PR	12.8a	76.2a	93.0a	11.0bc	15.7bc	667a	346a
			(100)	(100)	(100)	(100)	(100)	(100)	(100)
Koshihikari Toyama BL3	*Pib*	CR	0.0c	0.0e	20.3c	3.3d	4.0e	5e	5d
			(0)	(0)	(22)	(30)	(25)	(1)	(1)
Tomohonami	*pi21*	PR	0.0c	1.2de	73.1ab	16.1b	19.9b	39d	67c
			(0)	(2)	(79)	(147)	(127)	(6)	(19)
Koshihikari Aichi SBL	*Pb1*	PR	8.1ab	24.9b	83.0ab	7.5c	10.4cd	478ab	127b
			(68)	(33)	(89)	(68)	(66)	(72)	(37)
Koshihikari	Probenazole	Plant activator	0.4c	7.8cd	68.3ab	19.7a	29.4a	-	-
			(3)	(10)	(73)	(178)	(187)	(-)	(-)
Koshihikari	Tricyclazole	Melanin biosynthesis inhibitor	-	-	-	-	-	223bc	58c
			(-)	(-)	(-)	(-)	(-)	(33)	(17)
Koganenishiki	+	High level of PR	3.9bc	24.3b	81.7ab	-	-	107c	-
			(33)	(32)	(88)	(-)	(-)	(16)	(-)
Shin 2	+	Medium level of PR	2.4bc	17.9bc	59.4b	6.7cd	8.3de	139c	-
			(20)	(23)	(64)	(61)	(53)	(21)	(-)

Values in parentheses in the table show relative percentages of blast severity for respective varieties, lines, and blasticide-treated 'Koshihikari' to that of 'Koshihikari'.
Variance analyses were conducted using arcsine (PDS1 and PDS2), logarithm (PLA and AUDPC), and square root (LN1 and LN2) transformed data; the letters in each column show the result of Tukey's multiple range test with the transformed data at $p = 0.05$.
+: no effective complete resistance gene to most Japanese blast isolates, although Koshihikari is reported to possess *Pish* and *Pik-s* (Kawasaki -Tanaka & Fukuta 2014), PR: partial resistance, CR: complete resistance.
LN1 and LN2: leaf blast lesion numbers 9-11 days after spray inoculation on June 25-27 and July 10-12, PLA: lesion areas 18-20 days after punch inoculation,
PDS1 and PDS2: percentages of diseased spikelets at 16 and 21-24 days after spray inoculation, AUDPC: area under disease progress curve, -: Not tested.

line were sown by drilling in 10 rows composing each plot 90 cm in length and with 12-cm spacing between the rows on May 26. A total of 150 seeds were sown per row. Seeds of 'Toride 1' with the CR gene *Piz-t*, which shows CR to most Japanese blast isolates, were also sown the same way as those of the entry varieties and lines among the plots (50-cm width) as a barrier. In the trial, a compound fertilizer was applied at the rate of N 200 kg, P 300 kg, and K 268 kg/ha as basal dressing; ammonium sulfate was applied at N 125 kg/ha as topdressing.

4. Blasticide treatment

Two blasticides, probenazole granule (8% a.i, plant activator) and tricyclazole suspension concentrate (20% a.i., melanin biosynthesis inhibitor), were employed in the study. Probenazole was only used in the pot trial; tricyclazole was only used in the upland trial.

Probenazole granule was submerged into the pot of 'Koshihikari' at the rate of 30 kg/ha on June 9 (one month after transplanting). In contrast, 1,000 times diluted solution of tricyclazole suspension concentrate was applied to the blasticide-treated plots in the upland trial. Tricyclazole was applied three times for leaf blast at the rate of 1,200 L/ha at seven- to eight-day- intervals on July 8, 15, and 23, two times for panicle blast at the rate of 1,500 L/ha at the full heading stage (August 25), and nine days after the initial application to panicles.

5. Preparation of inoculum

Two rice blast isolates—Ina 86-137 (Japanese race 007.0, MAFF101511) and Ao 92-06-2 (Japanese race 337.1, MAFF101530) from the Gene Bank of the National Institute of Agrobiological Sciences—were used for inoculation. The blast isolates were cultured on oatmeal agar plates containing chloramphenicol (0.1 g/L) in Petri dishes (9 cm in diameter) at 25°C for about 10 days. Afterwards, aerial mycelia in the Petri dishes were gently rubbed with a paint brush after pouring sterilized water on them. The isolate

colonies were then exposed under fluorescent lights for three to four days to induce sporulation. For spray inoculation, conidial suspension was prepared by pouring sterilized distilled water containing 0.01% Tween 20 into the sporulated Petri dishes and then rubbing the plate surfaces with a paint brush. The conidial suspension was filtered through three layers of gauze mesh and adjusted to a concentration of 2-3 × 10^5 conidia per milliliter using a hemocytometer. For punch inoculation, the surfaces of the sporulated plates were rubbed with a spatula after pouring a small amount of sterilized distilled water along with a small amount of carboxymethyl cellulose (CMC) to make a paste including blast conidia, and then the conidial paste was inoculated directly to the punched leaves.

6. Inoculation and evaluation of blast severity

(1) Pot trial

Given the size of the dew chamber used for inoculation, each block containing seven treatments (pots) was inoculated separately in one day, and the inoculation for three blocks was finished within three to four days for spray inoculation and three days for punch inoculation.

The rice plants were inoculated three times for leaf blast before heading: twice by spray inoculation (June 25-27 and July 10-12) and once by punch inoculation (July 16-18). For panicle blast, rice plants at the full heading stage were spray-inoculated on July 25-28. The amount of spore suspension used for spray inoculation was 20-30 ml per pot (three plants). For punch inoculation, rice plants at the booting stage were used. The left centers of 10 leaf blades of their uppermost leaves were punched (1.9 mm in diameter) per plant, and the paste including blast conidia was placed on the wounded parts from July 16 to July 18. There was one punched point per leave.

The inoculated plants were kept in the dew chamber at 28°C for 24 hours, and then moved to a glasshouse until evaluation. Except for the first spray inoculation of leaf blast with isolate Ina 86-137, isolate Ao 92-06-2 was used for remaining inoculation.

For the spray inoculation, the numbers of susceptible type lesions per plant were counted for leaf blast 9 to 11 days after inoculation, and the panicle blast severity was assessed using the scale (0-10) of Asaga (1981) on each of the plants at 16 and 21-24 days after inoculation. The panicle blast severity was transferred through proportional distribution to the diseased spikelet percentages corresponding to blast severity on Asaga's scale, and the area under disease progress curve (AUDPC) was calculated using the diseased spikelet percentages and dates of assessment (by the American Phytopathological Society). For the punch inoculation, the lengths and widths of lesions that developed from the inoculated parts were measured with a digital-type slide caliper 18 to 20 days after inoculation, and lesion areas were calculated by multiplying their lengths by their widths.

(2) Upland trial

In the upland trial, one month after sowing (on June 26), 500 ml of the blast isolate Ina 86-137 conidial suspension (concentration 3 × 10^5 conidia/ml) was sprayed onto rice plants in each bed in the evening. The inoculated beds were then covered with plastic films for about 12 hours to induce blast fungus infection into the rice plants until the next morning. And to induce blast development, about 10 g of blast-infected rice seeds of the variety 'Moeminori', which were obtained from a severely blast-infected experiment paddy field at the Tohoku Agricultural Center of the National Agriculture and Food Research Organization (NARO), were put in a net bag, and the bag including the seeds was placed at the center of each plot on July 3.

After the first appearance of blast lesions (on July 3) in the trial beds, rice plants had been watered every afternoon to increase humidity. Blast severity was assessed on the second, fourth, sixth, and eighth rows from the left border row per plot. For leaf blast severity, total leaves were assessed six times from July 17 to August 12 using the scale (0-10) of Asaga (1981). The leaf blast severity was transferred to the diseased leaf area (DLA, Asaga 1976). Panicle blast severity was evaluated in the five treatments (Koshi-Pib, Koshi-Pb1, 'Koshihikari', 'Tomohonami', and tricyclazole-treated 'Koshihikari') by using Asaga's scale (0-10, Asaga 1981). The four rice varieties/lines in the treatments had the same heading time (of around August 22). The evaluations were conducted four times (on September 6, 12, 19, and 26) from two weeks after the heading time of the varieties/lines. The panicle blast severity was transferred to the diseased spikelet percentage as previously noted. The area under disease progress curve (AUDPC) was calculated using the diseased leaf areas, diseased spikelet percentages, and the dates of assessment (by the American Phytopathological Society).

7. Statistical analysis

Blast severity data were analyzed using the Statistical Analysis System (SAS version 9.0). Arcsine, logarithm, and square root transformations were conducted for percentage, lesion area/AUDPC, and number of lesions data, respectively, before conducting variance analyses of them. Tukey's multiple range test was used for comparisons of the mean values of treatments after variance analyses at $p = 0.05$.

Results

1. Leaf blast control efficacy

(1) Lesion number (LN) in the pot trial

In the pot trial, compared with 'Koshihikari', leaf blast reduction for the lesion number of Koshi-Pb1 was relatively

low, and smaller than that in the application of probenazole. However, the leaf blast reduction of Koshi-Pb1 was increased in the second inoculation, and similar to that of 'Koganenishiki' and 'Shin 2', whose leaf blast reduction levels for the number of lesions were moderate. Conversely, Koshi-Pib and 'Tomohonami' harboring *pi21* showed very high levels of leaf blast reduction with a few lesions, and their levels were higher than those of 'Koganenishiki' and 'Shin 2'. Furthermore, the leaf blast reduction of Koshi-Pib and 'Tomohonami' was similar to or higher than that of probenazole application (Table 1, Fig. 1).

(2) Lesion area after punch inoculation (PLA) in the pot trial

The lesions of Koshi-Pb1, 'Tomohonami', 'Koganenishiki', 'Shin 2', and probenazole-applied and non-applied 'Koshihikari' expanded from the punch-inoculated parts, and their lesion areas (lesion length × lesion width, mm^2) did not differ significantly, except that the area of 'Shin 2' was significantly smaller than that of non-applied 'Koshihikari'. In contrast, the lesion area of Koshi-Pib was smallest, and differed significantly from those of all other varieties/lines including probenazole-applied 'Koshihikari' (Table 1).

(3) Upland trial

In the upland trial, diseased leaf areas of 'Koshihikari' and Koshi-Pb1 increased relatively well from initial lesion observation (on July 3) to the booting stage of the plants (Fig. 2), although about a 30% reduction of the AUDPC against 'Koshihikari' was observed on Koshi-Pb1 (Table 1). The leaf blast development of 'Shin 2' and 'Koganenishi' was slow, and both varieties reduced the blast severity to almost the same level as that of three applications of tricyclazole on 'Koshihikari'. Very high levels of leaf blast reduction were observed on Koshi-Pib and 'Tomohonami', as shown by spray inoculation in the pot trial, although the AUDPC leaf blast severity of 'Tomohonami' was significantly greater than that of Koshi-Pib (Table 1, Fig. 2). Very few susceptible type leaf blast lesions were observed in two of the three Koshi-Pib plots.

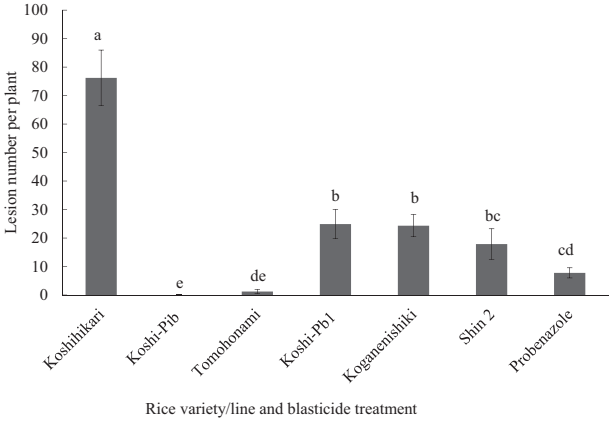

Fig. 1. Leaf blast severity of rice varieties/lines after spray inoculation in the pot trial

Inoculation: July 10-12, Evaluation: 9-11 days after inoculation, Koshi-Pib: 'Koshihikari Toyama BL3', Koshi-Pb1: 'Koshihikari Aichi SBL', Probenazole: probenazole-applied 'Koshihikari'.

Letters show the result of Tukey's multiple range test ($p < 0.05$) with square root transformed data.

Error bars: standard errors.

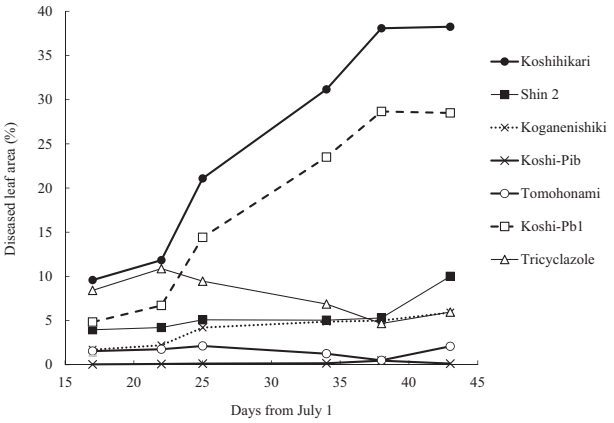

Fig. 2. Leaf blast development of rice varieties/lines in the upland trial

Tricyclazole: tricyclazole-applied 'Koshihikari'.

Koshi-Pib and Koshi-Pb1 in the figure are identical with those in Fig. 1.

Conidial suspension of the blast isolate Ina 86-137 (Japanese race 007.0) was sprayed on June 26, and blast-infected rice seeds in a net bag were placed at the center of each plot on July 3. The blast lesions were initially observed on July 3.

2. Panicle blast control efficacy

(1) Pot trial

Under spray inoculation in the pot trial, no panicle blast control efficacy was observed on the probenazole-applied 'Koshihikari' and 'Tomohonami'. The AUDPC of probenazole-applied 'Koshihikari' did not differ significantly from that of the non-applied 'Koshihikari', although the percentage of diseased spikelets (PDS) of probenazole-applied 'Koshihikari' was significantly ($p < 0.05$) higher than that of the non-applied 'Koshihikari'. Moreover, both the PDS and AUDPC of 'Tomohonami' were not statistically different from those of 'Koshihikari' (Table 1, Fig. 3). Koshi-Pb1 reduced panicle blast severity. However, the level of panicle blast reduction of Koshi-Pb1 was moderate and similar to that of 'Shin 2'. In contrast, Koshi-Pib showed a high level of panicle blast reduction (Table 1, Fig. 3).

(2) Upland nursery trial

In the upland nursery trial, the level of panicle blast reduction of Koshi-Pb1 was moderate and lower than that of tricyclazole application. High panicle blast reduction was observed on 'Tomohonami' and its reduction level was

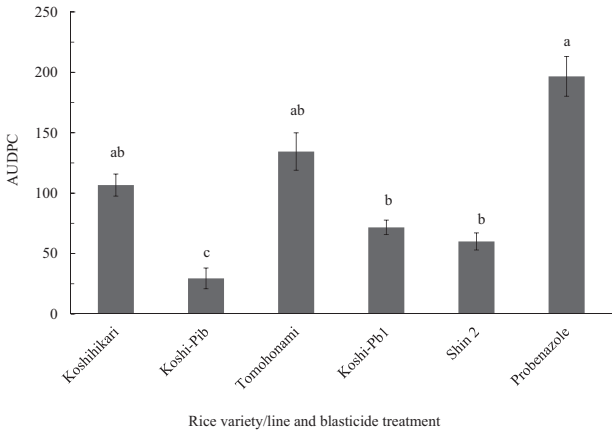

Fig. 3. Panicle blast severity of rice varieties/lines after spray inoculation in the pot trial
AUDPC: area under disease progress curve calculated by percentages of diseased spikelets.
Koshi-Pib, Koshi-Pb1, Probenazole, and error bars in the figure are identical with those in Fig. 1.
Letters show the result of Tukey's multiple range test ($p < 0.05$) with log-transformed data.

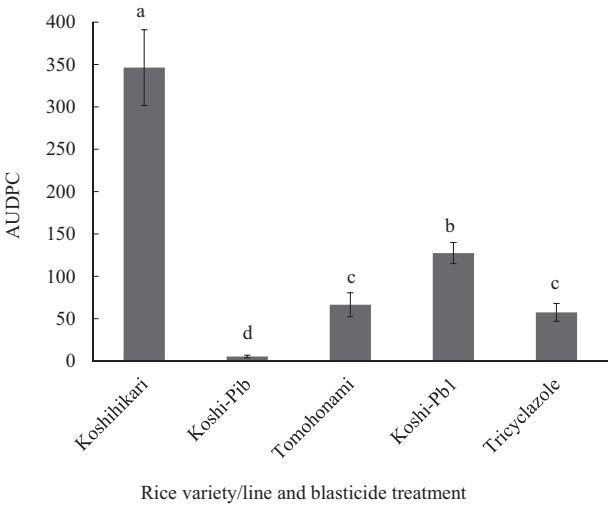

Fig. 4. Panicle blast severity of rice varieties/lines in the upland trial
Tricyclazole: tricyclazole-applied 'Koshihikari'.
AUDPC: area under the disease progress curve as calculated by percentages of diseased spikelets.
Koshi-Pib, Koshi-Pb1, and Tricyclazole in the figure are identical with those in Fig. 2.
Letters and error bars in the figure are identical with those in Fig. 3.

almost the same as that of tricyclazole application. Koshi-Pib showed a very high level of panicle blast reduction (Table 1, Fig. 4).

Discussion

In the trials conducted in this study, compared to 'Koshihikari', the highest blast control efficacy was observed on 'Koshihikari Toyama BL3' (Koshi-Pib) (Tables 1 and 2). The very high blast control efficacy of Koshi-Pib is thought to be induced by the CR gene *Pib* in the line. In the upland trial, however, a very small number of susceptible type leaf blast lesions were observed on Koshi-Pib. We obtained three blast isolates from the lesions with mono-conidial isolation and checked their virulence to the set of 26 LTH monogenic lines with different CR genes (Tsunematsu et al. 2000). From their virulence to the set, all the isolates were designated as race U33-i7-k100-z00-ta401 with virulence to *Pib* according to the international system for differentiating the blast races of Hayashi & Fukuta (2009). The number of lesions was very small on Koshi-Pib. This suggests that the density of the virulent blast race to *Pib* in the trial field was very low, and that the low density did not affect the blast control efficacy level of Koshi-Pib. However, breakdowns of the CR genes including *Pib* have been reported due to increase in amount of blast races being virulent to them (Koizumi 2007), and the results of this study imply a possible breakdown of the CR gene *Pib*.

The leaf blast lesions of rice varieties and lines used in the study expanded relatively well from the punch-inoculated parts except for Koshi-Pib (Tables 1 and 2). This suggests that CR significantly inhibits lesion development due to punch inoculation, while the inhibition with PR is limited. Yasuda et al. (2015) indicated that the length of leaf blast lesions on 'Tomohonami' with the PR gene *pi21* after spot inoculation did not differ significantly from that on 'Koshihikari', although the width of lesions on 'Tomohonami' was smaller than that on 'Koshihikari'. Our results almost agreed with their results (data not shown).

Levels of PR in 'Tomohonami' with *pi21* are reportedly very high for leaf blast and high for panicle blast, respectively (Saka et al. 2010). However, 'Tomohonami' had no panicle blast control efficacy in the pot trial, though that variety's blast reduction performance was very high for leaf blast in both the spray-inoculated pot and upland trials, and high for panicle blast in the upland trial (Table 2). We could not clarify why 'Tomohonami' had no panicle blast control efficacy in the pot trial. However, our results suggesting that the reduction level of leaf blast of 'Tomohonami' was greater than that of panicle blast agreed with the results of Saka et al. (2010). The resistance mechanisms of the PR gene *pi21* contributing to the blast reduction of 'Tomohonami' have been studied (Fukuoka et al. 2009). The difference in levels of 'Tomohonami' reduction to leaf and panicle blast might be related to the resistance mechanisms of *pi21*.

Our trial results showed that the blast control efficacy of Koshi-Pb1 was low to moderate for leaf blast and moder-

Table 2. Blast reduction levels of rice varieties/lines with different resistance and blasticide-applied 'Koshihikari' in the pot and upland nursery trials

Variety/line	Resistance gene/applied blasticide	Type of resistance/ blasticide	Level of blast reduction				
			Pot trial (spray/punch inoculation)			Upland trial	
			Leaf blast		Panicle blast	Leaf blast	Panicle blast
			Spray	Punch	Spray		
Koshihikari	+	Low level of PR	-	-	-	-	-
Koshihikari Toyama BL3	*Pib*	CR	++++	+++	+++	++++	++++
Tomohonami	*pi21*	PR	++++	+	-	++++	+++
Koshihikari Aichi SBL	*Pb1*	PR	+ ~ ++	+	++	+	++
Koshihikari	Probenazole	Plant activator	++++ ~ +++	+	-		
Koshihikari	Tricyclazole	Melanin biosynthesis inhibitor				++ ~ +++	+++
Koganenishiki	+	High level of PR	++	+		+++	
Shin 2	+	Medium level of PR	++	+ ~ ++	++	+++	

Levels of blast reduction in the respective varieties and lines were decided by comparing their blast severity with that of 'Koshihikari' in each trial.++++ : very high, +++ : high, ++ : moderate, + : low, - : no reduction.
+ in the resistance gene, and PR and CR in the type of resistance are identical with those in Table 1.

ate for panicle blast, while greater leaf blast reduction was observed with the growth of rice plants of Koshi-Pb1 in the pot trial (Tables 1 and 2). Hayashi et al. (2010) cloned the PR gene *Pb1* conferring a high level of PR to panicle blast, and clarified that the expression of *Pb1* increases from panicle initiation to the full heading stage in rice varieties with the gene, and that the increase contributes a high level of PR in the varieties to panicle blast. This agrees with our results that the level of PR of Koshi-Pb1 to leaf blast became higher with rice growth in the pot trial, and that the level of PR of Koshi-Pb1 to panicle blast was higher than that of PR to leaf blast in the upland trial (Tables 1 and 2). The levels of panicle blast reduction with *Pb1* in the study were lower than those achieved by Fujii et al. (2005), who conducted their trials in paddy fields. Differences in the levels of panicle blast reduction with *Pb1* between Fujii et al. (2005) and ours might be caused by differences in environmental conditions and the inoculation methods used by Fujii et al. (2005) and our group.

The application of probenazole could not reduce panicle blast development, although the blasticide could control leaf blast well in the pot trial (Tables 1 and 2, Fig. 3). This result is consistent with the results of Yamashita et al. (2014), and reconfirms that the blast control efficacy of probenazole is reduced before rice heading, and that the efficacy is not directly valid for panicle blast control (Yamashita et al. 2014).

This study clarified the effectiveness of three resistance genes to control rice blast. In the study, both the PR gene *pi21* and CR gene *Pib* showed very high or high levels of blast control efficacy which were greater than or equal to those of blasticide applications, except for the low efficacy of *pi21* to panicle blast in the pot trial (Table 2). Moreover, the two genes had greater leaf blast control efficacy than that of 'Shin 2' and 'Koganenishiki' holding medium, and high levels of PR to blast.

The levels of blast control efficacy of the PR gene *Pb1* were moderate to panicle blast and low to moderate to leaf blast; however, the blast control efficiency of the gene was lower than that of blasticide applications except for the efficacy of probenazole to panicle blast in the pot trial. Compared to the blast efficacy level of 'Shin 2' with the medium level of PR to the disease, the efficacy levels of *Pb1* were lower to equal for leaf blast and equal for panicle blast (Table 2).

Levels of PR of 'Koganenishiki' and 'Shin 2' to blast are generally classified as high and medium (Ezuka 1980). However, our trial results showed that the level of PR of 'Koganenishiki' to leaf blast was similar to that of 'Shin 2'. The results almost agreed with the PR level evaluations of 'Shin 2' and 'Koganenishiki' to leaf blast conducted by Koizumi & Fuji (1995). In the evaluations of Koizumi & Fuji (1995), 'Koganenishiki' showed medium levels of leaf blast PR to 16 blast isolates, although leaf blast severity of 'Koganenishiki' was significantly higher than that of 'Shin 2' after inoculation of two blast isolates (Koizumi & Fuji 1995). Ezuka (1980) summarized the results of evaluating the PR levels of 'Koganenishiki' and 'Shin 2' to leaf blast in trials conducted in three different conditions (i.e. pots, upland nurseries, and paddy fields), and reported that the levels of PR of 'Koganenishiki' and 'Shin 2' had been moderate to high and low to high, respectively. Consequently, we consider that our evaluations of PR of 'Shin 2' and 'Koganenishiki' to blast are consistent with previous evalu-

ations of PR of the two varieties to blast, although further studies might be needed to reconfirm the levels of PR of both varieties to blast.

The use of resistant varieties is recommended to manage rice blast disease for resource-poor farmers in developing countries, such as those located in Asia and Africa, because resistant varieties offer high blast control efficacy and easy utilization with cost effective and environmentally friendly characteristics (Mew 1991).

CR to blast in resistant varieties breaks down and becomes ineffective within several years after release of the resistant varieties, however, due to increase in blast races attacking CR. To prevent the breakdown of CR to blast, several methods have been proposed, such as mixing varieties or (near-) isogenic lines with different CR genes, CR gene pyramiding, using CR gene rotation, and pyramiding of CR and PR genes (Koizumi 2007). PR to blast had been considered generally stable and non-race-specific. Nevertheless, isolate-specific PR to blast, including PR controlled by recently identified or cloned PR genes, has been reported (Koizumi & Fuji 1995, Zenbayashi-Sawata et al. 2005, Mizobuchi et al. 2014, Xu et al. 2014). This suggests breakdowns of PR to blast.

Fukuoka et al. (2009) cloned *pi21*, a recessive allele conferring PR to rice blast, and reported that the response in resistant *pi21* plants after a pathogen attack is not as fast or as strong as the CR gene response. They suggested that a slower induction of defense might be another type of incompleteness that might contribute to the durability of a plant's resistance. Moreover, *Pb1*, a cloned PR gene conferring durable and broad-spectrum panicle blast resistance, encodes a coiled-coil-nucleotide-binding site-leucine-rich repeat (CC-N B-LRR) protein similar to the resistance proteins of CR to blast (Hayashi et al. 2010). Inoue et al. (2013) clarified that the resistance of *Pb1* is expressed by inhibiting the degradation of WRKY45, a transcriptional activator that plays a central role in induced resistance, by binding of WRKY 45 with the Pb1 protein, and consider that this mechanism accounts for the durability of Pb1-dependent blast resistance. Such investigation of the resistance mechanisms of PR genes to blast may contribute to selecting durable blast PR genes.

PR gene pyramiding is expected to contribute to increased blast control efficacy as well as the durability of PR genes (Koizumi 2007). Through recent advances in genetic analyses with molecular markers, blast PR gene pyramiding has become easy, and rice varieties with two PR genes such as 'Tachiharuka' and 'Chubu 134' holding *Pi39* and *Pb1* have actually been developed (Sakai et al. 2013, Aichi Agricultural Research Center 2014). The varieties have increased blast control efficacy. Moreover, two types of combined-gene interactions for leaf blast reduction were recently observed on rice lines with two pyramided PR genes including *pi21*: (i) the combination of PR genes was more effective than either of the PR genes individually, and (ii) the combination of two PR genes was similar to the level of the most effective resistance gene in the pair (Yasuda et al. 2015). Thus, understanding the blast control efficacy of each resistance gene is important for resistance gene pyramiding.

In the study, we showed the blast control efficacy of three resistance genes (*Pib*, *pi21*, and *Pb1*) conferring complete and partial resistance in pot and upland trials by comparing the efficacy of blasticide applications and two rice varieties holding medium and high levels of PR to blast. The control efficacy fluctuates due to the environment, nutrition, growth of the rice plants, blast inoculation methods, composition of blast populations, and other factors. We conducted the study in pot and upland trials, and only used two blast isolates to evaluate the blast control efficacy of the three resistance genes. Further studies are needed to confirm the blast control efficacy of respective resistance genes under different conditions, including lowland conditions with different blast isolates (populations). We expect that the accumulation of information on blast control efficacy and the characteristics of respective resistance genes will contribute to effective rice blast control with resistance.

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