Diversity and Distribution of Rice Blast (*Pyricularia oryzae* Cavara) Races in Japan

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Abstract

Kawasaki-Tanaka, A., Hayashi, N., Yanagihara, S., and Fukuta, Y. 2016. Diversity and distribution of rice blast (*Pyricularia oryzae* Cavara) races in Japan. *Plant Dis.* 100:816-823.

In total, 310 rice blast (*Pyricularia oryzae* Cavara) isolates from Japan showed wide variation in virulence. Virulence on rice (*Oryza sativa* L.) differential varieties (DV) harboring resistance genes *Pish*, *Pia*, *Pii*, *Pi3*, *Pi5(t)*, *Pik-s*, and *Pi19(t)* ranged from 82.9 to 100.0%. In contrast, virulence on DV possessing *Pib*, *Pit*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, *Pi7(t)*, *Pi9(t)*, *Piz*, *Piz-5*, *Piz-t*, *Pita-2*, *Pita*, *Pi12(t)*, and *Pi20(t)* ranged from 0 to 21.6%. Cluster analysis using the reaction patterns of the DV classified isolates into three groups: I, virulent to *Pik*, *Pik-h*, *Pik-p*, *Pi1*,

and *Pi7(t)*; IIa, avirulent to the preceding 6 genes and virulent to *Pia*, *Pii*, *Pi3*, and *Pi5(t)*; and IIb, avirulent to all 10 genes. Group I was limited to northern Japan and group IIb to central Japan, while group IIa was distributed throughout Japan. We estimate that group IIa represents the original population and that groups I and IIb arose from it through minor changes in pathogenicity. We classified these isolates into 123 races by a new designation system and conclude that the rice blast races in Japan are less diverse than previously thought.

Rice blast is a serious disease caused by the fungal pathogen *Pyricularia oryzae* Cavara (syn. *Magnaporthe oryzae* B. C. Couch) (Couch and Kohn 2002) in rice (*Oryza sativa* L.)-growing regions worldwide. The use of resistant cultivars is one of the most efficient ways of protecting crops from the disease. However, disease control is not always durable, because the resistance of new rice cultivars often breaks down a few years after they are released. The relationship between the avirulence genes of the pathogen and the resistance genes of rice cultivars is explained by the gene-for-gene theory (Flor 1971; Silué et al. 1992). On the basis of this theory, differential systems consisting of resistant rice cultivars and blast isolates have been developed. These systems can be used in advanced studies to identify the virulence of the rice blast pathogen, infer resistance genes in rice cultivars, and clarify pathogenic variation.

Pathogenicity of blast isolates in many places has been identified using differential varieties (DV) with different numbers and kinds of resistance genes. In Vietnam, Noda et al. (1999) clarified those from the Mekong River Delta using Japanese DV carrying 12 resistance genes: *Pia*, *Pik-s*, *Pii*, *Pik*, *Pik-m*, *Piz*, *Pita*, *Pita-2*, *Piz-t*, *Pik-p*, *Pib*, and *Pit* (Kiyosawa 1981, 1984; Yamada et al. 1976). In Thailand, Mekwatanakarn et al. (2000) used near-isogenic lines (NIL) carrying each of 10 genes—*Pi1*, *Pi1-LAC(t)*, *Pi1-TTP(t)*, *Piz-5*, *Pi3*, *Pi4a* and *Pi4b(t)* (*Pita*), *Pi4a-PKT(t)*, *Pi4a-TTP(t)*, and *Pia*—with the genetic background of Indica-type susceptible ‘CO 39’, and NIL carrying each of 6 genes—*Pik-m*, *Pita*, *Pita-2*, *Pib*, *Pik-p*, and *Pik*—with the genetic background of Japonica-type susceptible ‘Lijiangxintuanheigu’ (LTH), as DV. In Bhutan, Thinlay et al. (2000) used CO 39 NIL carrying each of five genes—*Pi4b* (*Pita*), *Pi2* (*Piz-5*), *Pi3*, *Pi4a* (*Pita*), and *Pi1*—and LTH NIL carrying *Pita*, *Pib*, *Pita-2*, *Pik-m*, and *Pik-p*. In China, Chen et al. (2001) used LTH NIL carrying each of six genes—*Pita-2*, *Pib*, *Pik*, *Pik-m*, *Pita*, and *Pik-p*—and CO 39 NIL carrying each of four genes—*Pita*, *Piz-5*,

Pi3, and *Pi13*. In the Philippines, Telebanco-Yanoria et al. (2008a) used 19 DV carrying each of 18 resistance genes: *Pia*, *Pib*, *Pii*, *Pit*, *Pita*, *Pish*, *Piz-t*, *Pi3*, *Piz-5*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pik-s*, *Pita-2*, *Piz*, *Pi1*, and *Pi20(t)*. In Japan, Yamada et al. (1976, 1979) and Yamada (1985) used nine DV (Shin 2 with no resistance genes, Aichi-Asahi for *Pia*, Ishikari-Shiroke for *Pii*, Kanto 51 for *Pik*, Tsuyake for *Pik-m*, Fukunishiki for *Piz*, Yashiro-Mochi for *Pita*, Pi No. 4 for *Pita-2*, and Toride 1 for *Piz-t*), and Kiyosawa (1984) used six DV (Fujisaka 5 for *Pii*, Kusabue for *Pik*, K1 for *Pita*, K60 for *Pik-p*, BL1 for *Pib*, and K59 for *Pit*) to characterize blast fungus. Using these DV, researchers found that the dominant blast race found at high frequency in Japan was virulent to cultivars carrying *Pii* and *Pia* (Koizumi et al. 2007; Naito et al. 1999; Yamada 1985; Yamada et al. 1979).

These DV covered only 11 resistance genes. However, several Japanese DV harbor additional genes. Imbe and Matsumoto (1985) identified *Pish* in some Japanese DV. Hayashi et al. (1998) detected *Pi19(t)* in all except two Japanese DV (Yashiro-Mochi for *Pita* and Pi No. 4 for *Pita-2*). Kato et al. (2004) reported that Shin 2 (with no resistance genes) harbors *Pik-s* and *Pish*; K59 (*Pit*) and Fujisaka 5 (*Pii*) also harbor *Pik-s*; Fukunishiki (*Piz*), Toride 1 (*Piz-t*), and BL1 (*Pib*) also harbor *Pish*; and Reiho (*Pita-2*) also harbors *Pia*. The presence of these additional resistance genes thus complicates pathogenicity studies of blast isolates.

The system by which these blast isolates were designated was also complicated because the relationships and locations of the resistance genes in these DV were not considered. It was known that the multiple-allele genes *Piz* and *Piz-t* are located on chromosome 6; *Pik-s*, *Pik*, *Pik-m*, and *Pik-p* on chromosome 11; and *Pita* and *Pita-2* on chromosome 12; however, Yamada et al. (1976) and Kiyosawa (1984) categorized some multiple-allele genes into different groups. For example, *Pik-s* was provided the code number “1” and these of *Pik* and *Pik-m* were “10” and “20”, respectively. *Pi-z* and *Piz-t* were “40” and “400”, respectively. It is preferable to manage multiple-allele genes in the same chromosome region in a set of DV to analyze the pathogenicity of blast isolates and to understand the relationships among virulence genes.

Recently, a new set of international DV was established from 25 monogenic lines harboring each of 23 resistance genes—*Pish*, *Pib*, *Pit*, *Pia*, *Pii*, *Pi3*, *Pi5(t)*, *Pik-s*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, *Pi7(t)*, *Pi9*, *Piz*, *Piz-5*, *Piz-t*, *Pita-2*, *Pita*, *Pi12(t)*, *Pi19(t)*, and *Pi20(t)* (Kobayashi et al. 2007; Tsunematsu et al. 2000)—by backcrossing with Chinese LTH rice as the recurrent parent. LTH is not known to possess any major blast resistance genes. Using six times of backcrossing, Telebanco-Yanoria et al. (2010) developed NIL carrying 11 genes—*Pib*, *Piz-5*,

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*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary table is published online.

Accepted for publication 24 November 2015.

<http://dx.doi.org/10.1094/PDIS-04-15-0442-RE>
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Pi9, *Pi3*, *Pia*, *Pik-s*, *Pik*, *Pik-h*, *Pi7(t)*, *Pita*, and *Pita-2*—as advanced DV with the same LTH genetic background. These DV make more precise evaluation of the pathogenicities of blast isolates possible. In addition, Hayashi and Fukuta (2009) proposed a new international designation system for blast races using the new set. Thus, the pathogenicity of blast isolates can be characterized by the reaction patterns of the DV and LTH, and classified by the new designation system, which takes into consideration the grouping of the 23 resistance genes and their chromosome locations. This technique will gain us a better understanding of the relationships between races and resistance genes, with minimum influence of the genetic backgrounds of the DV.

Blast isolates from Asia and Africa were collected under a Japan International Research Center for Agricultural Sciences (JIRCAS) research project with the aims of differentiating blast races by using the new set of DV at a global level and developing and distributing the differential system, which consists of the DV and standard differential blast isolates. The system now makes it possible to evaluate blast virulence and rice resistance. This will allow durable resistance to blast disease to be built up. In this study, we characterized rice blast isolates and analyzed the geographic distribution and differentiation of blast races in Japan by using the new set of international DV and the new designation system. We demonstrate the unique nature of blast race differentiation in Japan and discuss the reasons behind it.

Materials and Methods

Collection and preparation of isolates. We used a total of 310 blast (*P. oryzae* Cavara) isolates from eight regions in Japan: A, subarctic Hokkaido ($n = 23$ isolates); B, Tohoku, northern Japan ($n = 29$; Miyagi); C, Hokuriku, northern Japan ($n = 61$; Niigata); D, Kanto, central Japan ($n = 65$; Ibaraki, Tochigi, and Gunma); E, Tokai, central Japan ($n = 43$; Aichi); F, Chugoku/Shikoku, southern Japan ($n = 23$; Tottori, Shimane, Okayama, Hiroshima, Yamaguchi, Ehime, and Kochi); G, Kyushu, southern Japan ($n = 25$; Fukuoka, Saga, Nagasaki, Kumamoto, Ohtita, and Kagoshima); and H, subtropical Okinawa ($n = 41$) (Supplementary Table S1).

All isolates were collected from irrigated lowland fields, with the exception of one from an upland field in the Kanto region. Of the 310 isolates, 54 were collected during 2001 to 2003 and conserved by the National Agricultural Research Center for the Kyushu Okinawa Region, and 174 were collected from Hokkaido during 2000 to 2009 and conserved by the National Institute of Agrobiological Sciences, the Furukawa Agricultural Experiment Station in Miyagi Prefecture, and the Niigata Crop Research Center in Niigata Prefecture. The other 82 isolates were newly collected from the Kanto and Okinawa regions during 2009 and 2010 and conserved at JIRCAS. The numbers of isolates sampled were limited, and some areas in each region were not covered, but all regions from north to south were represented.

Single spores were obtained from infected leaves or panicles incubated on moist filter paper in a petri dish at room temperature for 24 h according to Hayashi et al. (2009). Colonies were grown on rice flour agar and stored on filter paper for repeat access to the same isolates.

DV. The set of DV comprised 23 monogenic lines (Kobayashi et al. 2007; Tsunematsu et al. 2000) carrying 21 resistance genes, two NIL with the LTH genetic background (Telebanco-Yanoria et al. 2010), and LTH as the control. The virulence of the isolates was evaluated from the reaction patterns of the 25 DV and LTH.

Inoculation and evaluation. Seed of the 25 DV and LTH were surface sterilized, soaked for 2 to 3 days, and then sown three to a cell in plastic cell trays (approximately 16 by 25 mm, 5 by 7 holes), which were then placed in a greenhouse. Each cell tray also contained LTH for susceptibility checking. Seedlings were inoculated by the method of Hayashi et al. (2009). The spore concentration was standardized to 1×10^5 spores/ml of sterile water. Plants were inoculated 3 weeks after sowing, at the fourth- to fifth-leaf stage, by spraying 15 to 20 ml of spore suspension onto each tray from a fine atomizer. Inoculated plants were kept in a dew chamber at 25°C for 20 to 24 h and then transferred to a greenhouse at 25 to 30°C. The degree of infection of each seedling was evaluated 7 or 8 days after inoculation, as described below. The process of inoculation and evaluation was performed twice.

Blast lesions were evaluated on a scale of 0 to 5 according to Hayashi et al. (2009). Lines were considered resistant when rated 0 to 2 and susceptible when rated 3 to 5, with the exception of IRBLta2-Pi for *Pita-2* (resistant = 0 to 3) and IRBL5-M for *Pi5(t)* (resistant = 0 to 1). Lesion sizes in the resistance reaction varied according to the resistance genes, ranging from type 0 to type 3, where 0 = no lesion, 1 = brown specks, 2 = small lesion (<1 mm), and 3 = eyespot lesion (<1.5 mm). Lesions on lines harboring *Pita-2* sometimes expanded to type 3. On the other hand, lesions on IRBL5-M, harboring *Pi5(t)*, manifested as type 1, perhaps because of minor genes also present.

Virulent blast isolates were designated by the new system (Table 1). The DV were categorized into five groups: “U” = LTH and four DV possessing *Pia*, *Pish*, *Pib*, and *Pit*; “i” = three DV possessing *Pii*, *Pi3* and *Pi5(t)*, with the *Pii* locus on chromosome 9; “k” = seven DV possessing *Pik-s*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p* and *Pi7(t)*, with the *Pik* region on chromosome 11; “z” = four DV possessing *Pi9*, *Piz*, *Piz-5* and *Piz-t*, with the *Piz* region on chromosome 6; and “ta” = seven DV possessing *Pita-2(Re)*, *Pita-2(Pi)*, *Pi12(t)*, *Pita(K1)*, *Pita(CP1)*, *Pi19(t)* and *Pi20(t)*, with the *Pita* region on chromosome 12. In groups i, k, z, and ta, multiple-allele genes or resistance genes located in the same chromosome regions were included. The other four genes (*Pia* on chromosome 11, *Pish* and *Pit* on chromosome 1, and *Pib* on chromosome 2) are located in different regions and were gathered in group U with LTH. We replaced lines IRBLkh-K3 and IRBLk-Ka with two LTH NIL: IRBLkh-K3[LT] and IRBLk-K[LT], respectively. These groups consisted of one to three variety units, and we allocated three DV (genes) in each unit. Codes 1, 2, and 4, were allocated to susceptible reactions of each of the three DV to the blast isolates. Blast races were designated by the combination of the codes representing the reactions of the three DV in each unit, using the method of Gilmour (1973). Isolates classified this way were designated as “reaction type” within each DV group, and as “races” using the set of all five reaction types which corresponded with the five DV groups.

Classification of blast isolates. We used the reaction scores (Hayashi et al. 2009) of the 25 DV and LTH to the 310 blast isolates in a cluster analysis for their classification. The classification was performed by using Ward’s hierarchical clustering method in JMP software (v. 7.0.2; SAS Institute, Inc., Cary, NC). The relationships between the virulence of the cluster groups and the regions where the samples were collected were evaluated to determine the geographic distributions of each blast race. The diversity of blast isolates in each cluster group was calculated by using the index method of Simpson (1949).

Results

Variation in virulent blast isolates. The frequency of virulence in isolates to the 25 DV and the susceptible control varied widely (Fig. 1). The frequency of isolates virulent to seven DV for *Pish*, *Pia*, *Pii*, *Pi3*, *Pi5(t)*, *Pik-s*, and *Pi19(t)* and to LTH ranged from 82.9 to 100.0%. The frequency of isolates virulent to the other 18 DV was low (0 to 21.6%). Notably, the frequency of virulence to DV for *Pit*, *Piz-5*, *Piz-t*, *Pita-2(Pi)*, *Pita-2(Re)*, *Pita(K1)*, and *Pi20(t)* was <10%. All blast isolates were virulent to LTH and IRBA19-A for *Pi19(t)* and were avirulent to IRBLt-K59 for *Pit*.

The frequencies of isolates virulent to DV and LTH were similar among the eight regions, although there were a number of differences. Frequencies of isolates virulent to six DV for *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, and *Pi7(t)* varied from 10.3 to 95.7% in Hokkaido (A), Tohoku (B), Hokuriku (C), and Kanto (D), but were <10% or not found in Tokai (E), Chugoku/Shikoku (F), Kyushu (G), and Okinawa (H). The highest percentage of virulent isolates was found in Hokkaido. The frequencies of isolates virulent to four DV for *Pia*, *Pii*, *Pi3*, and *Pi5(t)* varied from 63.1 to 76.9% in Kanto and Chugoku/Shikoku, and were lower than those in the other regions. The frequencies of isolates virulent to DV for *Pita(K1)* and *Pita(CP1)* were 21.7 and 30.4%, respectively, in Chugoku/Shikoku, and were higher than those in the other regions. The frequency of isolates virulent to DV for *Pi12(t)* was 4.7% in Tokai, and was the lowest among the eight regions. The frequencies of isolates virulent to DV for *Piz* (30.4%), *Piz-5* (26.1%), and *Pi20(t)* (30.4%) in Hokkaido were the highest among the eight regions. All

isolates were virulent to DV for *Pish*, with the exception of four (1.3% of the total) from Okinawa. These results indicate that isolates virulent against *Pish*, *Pia*, *Pii*, *Pi3*, *Pi5(t)*, *Pik-s*, and *Pi19(t)* and avirulent against *Pit* were widely distributed, and the others varied in their distribution.

Classification of blast isolates. The 310 blast isolates were classified into two major groups (I and II), including two subgroups (IIa and IIb), by cluster analysis using the reactions to the 25 DV and LTH (Fig. 2). The analysis clustered 57 isolates (18.4%) into group I and 253 isolates (81.6%) into group II (210 in IIa and 43 in IIb). Several differences in the frequencies of virulent isolates were evident among these groups. Isolates with high frequencies of virulence to the DV of the k group—*Pik*, *Pik-h*, *Pik-p*, *Pik-m*, *Pi1*, and *Pi7(t)*—were categorized mainly into group I. The frequencies of isolates virulent against the DV of the k group were similar between subgroups IIa and IIb, and those of isolates virulent against *Pia*, *Pii*, *Pi3*, and *Pi5(t)* in IIa were higher than those in IIb.

These results indicate that groups I and II were divided mainly by the reactions of *Pik*, *Pik-h*, *Pik-p*, *Pik-m*, *Pi1*, and *Pi7(t)*, and IIa and IIb were divided by those of *Pia*, *Pii*, *Pi3*, and *Pi5(t)*. Thus, virulence associated with *Pia* and *Pik* on chromosome 11 and *Pii* on chromosome 9 contributed mainly to the differentiation of blast races in Japan.

Geographic distribution of cluster groups. The frequencies of blast isolates in these cluster groups differed among the eight regions (Fig. 3). Isolates of group IIa were found in all regions, and were the only isolates found in Okinawa. The distribution of group I was limited to Hokkaido, Tohoku, Hokuriku, and Kanto (northern Japan). In particular, 95.7% of isolates in Hokkaido were in group I. Isolates of group IIb were distributed only Hokuriku, Kanto, Tokai, Chugoku/Shikoku, and Kyushu (central Japan), at different frequencies.

Characterization of blast isolates on the basis of reaction type of DV groups. In DV group U, the 310 blast isolates were classified into six reaction types—U01, U03, U11, U13, U31, and U33—based on the reactions to DV for *Pish* on chromosome 1, *Pib* on chromosome 2, *Pit* on chromosome 1, LTH, and *Pia* on chromosome 11 (Table 1). In total, 245 blast isolates were classified into reaction type U13, which was virulent to two DV for *Pish* and *Pia* and to LTH. This type had the highest frequency of occurrence (79.0% in total). U11 (virulent to DV for *Pish* and LTH; 9.7%) and U33 (virulent to DV for *Pish*, *Pib*, and *Pia* and to LTH; 7.1%) had higher values than the other three types. The occurrences of these three reaction types differed little among the cluster groups. U11 and U13 were found in all three cluster groups but U33 was not found in group IIb. Simpson's diversity index (Simpson 1949) had values of 0.22 for cluster group I, 0.29 for IIa, 0.62 for IIb, and 0.36 for the whole set. These results indicate that the virulence genes of blast fungus against DV for *Pish* and *Pia* are distributed widely and together in Japan.

In DV group i, the blast isolates were classified into seven reaction types: i0, i1, i2, i3, i4, i5, i6, and i7 (Table 1). In total, 253 (81.6%) isolates were classified into i7, virulent to DV for *Pii*, *Pi3*, and *Pi5(t)*, and 41 (13.2%) isolates into i0 (avirulent). The other five reaction types had low frequencies of occurrence (<3%). Reaction type i7 occurred most frequently in group I (91.2%) and subgroup IIa (95.7%). In contrast, i7 was not present in subgroup IIb but i0 was high (88.4%). These results indicate that cluster group I and subgroup IIa did not differ greatly, whereas subgroup IIb differed from both. The diversity indexes had values of 0.16 for group I, 0.08 for IIa, 0.22 for IIb, and 0.32 for the whole set. These results indicate that these isolates with genes virulent or avirulent against DV for *Pii*, *Pi3*, and *Pi5(t)* were distributed together in groups I and IIa (virulent) and group IIb (avirulent).

Table 1. Number of *Pyricularia oryzae* isolates in each type categorized on the basis of the reactions of differential variety (DV) groups^a

DV group, types ^b	Reactions to resistance genes shown					
Group U	<i>Pish</i>	<i>Pib</i>	<i>Pit</i>	LTH	<i>Pia</i>	...
U11	v	a	a	v	a	...
U13	v	a	a	v	v	...
U33	v	v	a	v	v	...
Others (U01, U03, U31)	...	...	...	...	...	...
Diversity index	...	...	...	...	...	...
Group i	<i>Pii</i>	<i>Pi3</i>	<i>Pi5(t)</i>	...	...	...
i0	a	a	a	...	...	...
i7	v	v	v	...	...	...
Others (i1, i2, i3, i4, and i5)	...	...	...	...	...	...
Diversity index	...	...	...	...	...	...
Group k	<i>Pik-s</i>	...	...	<i>Pik-m</i>	<i>Pi1</i>	<i>Pik-h</i>
k100	v	...	...	a	a	a
k177	v	...	...	v	v	v
Others (k000, k101, k102, k104, k106, k107, k110, k117, k120, k137, and k157)	...	...	...	...	...	...
Diversity index	...	...	...	...	...	...
Group z	<i>Pi9</i>	...	...	<i>Piz</i>	<i>Piz-5</i>	<i>Piz-t</i>
z00	a	...	...	a	a	a
z10	v	...	...	a	a	a
Others (z01, z02, z03, z04, z07, z11, z12, zz13, and z14)	...	...	...	...	...	...
Diversity index	...	...	...	...	...	...
Group ta	<i>Pita-2 (Re)</i>	<i>Pita-2 (Pi)</i>	<i>Pi12(t)</i>	<i>Pita (K1)</i>	<i>Pita (CP1)</i>	...
ta001	a	a	a	a	a	...
ta401	a	a	v	a	a	...
Others (ta003, ta011, ta013, ta021, ta031, ta101, ta131, ta203, ta301, ta331, ta403, ta421, ta431, ta433, ta501, and ta731)	...	...	...	...	...	...
Diversity index	...	...	...	...	...	...

(continued on next page)

^a Blast isolates were evaluated by the reaction types to differential variety groups classified by Hayashi and Fukuta (2009); v = virulent to DV or Lijiangxintuanheigu (LTH) and a = avirulent.

^b Reaction types in each DV group. Diversity index was calculated by the method of Simpson (1949).

In DV group k, the blast isolates were classified into 13 reaction types (k000, k100, k101, k102, k104, k106, k107, k110, k117, k120, k137, k157, and k177), of which 233 (75.2%) were included in k100 (Table 1). This reaction type was virulent to only *Pik-s*, and most isolates in subgroups IIa and IIb were classified into k100. In group I, 30 isolates (9.7%) were classified into reaction type k177, with most of them virulent to *Pik-s*, *Pik*, *Pik-p*, and *Pi7(t)*. Thus, virulence against *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, and *Pi7(t)* differentiates between groups I and II but there was little difference between subgroups IIa and IIb. The diversity indexes had values of 0.60 for group I, 0.16 for IIa, 0.09 for IIb, and 0.42 for the whole set.

In DV group z, blast isolates were classified into 11 reaction types (z00, z01, z02, z03, z04, z07, z10, z11, z12, z13, and z14; Table 1). In total, 252 blast isolates (81.3%) were classified into z00 (avirulent to DV for *Pi9*, *Piz*, *Piz-5*, and *Piz-t*). Reaction type z00 had the highest frequencies among the cluster groups. The frequencies of the other 10 reaction types were low (<10%). Reaction type z10 (virulent to *Pi9*) was found in subgroups IIa and IIb but not in group I. These results indicate that z00 is dominant in Japan but there is little differentiation or variation in the other reaction types, except for z10. The diversity indexes had values of 0.50 for group I, 0.26 for IIa, 0.38 for IIb, and 0.33 for the whole set.

In DV group ta, the blast isolates were classified into 18 reaction types (ta001, ta003, ta011, ta013, ta021, ta031, ta101, ta131, ta203, ta301, ta331, ta401, ta403, ta421, ta431, ta433, ta501, and ta731; Table 1). In total, 188 (60.6%) isolates were categorized into ta001, virulent to *Pi19(t)*, and 61 (19.7%) isolates into ta401, virulent to *Pi12(t)* and *Pi19(t)*. Reaction type ta001 had the highest

occurrence in all cluster groups. Reaction type ta401 was also commonly found in all cluster groups (14.0 to 22.8%). The frequencies of the other 16 reaction types were low (<4.0% each). There were no significant differences in frequencies of blast isolates among the three groups. These results indicate that the blast isolates virulent against DV for *Pi19(t)* and *Pi12(t)* are widely distributed in Japan. The diversity indexes had values of 0.67 for group I, 0.55 for IIa, 0.63 for IIb, and 0.59 for the whole set; thus, the degree of diversity is similar among groups.

We identified 11 major reaction types—U11, U13, U33, i0, i7, k100, k177, z00, z10, ta001, and ta401—which occurred at higher frequencies than the other reaction types in each DV group, and which were virulent to 16 resistance genes—*Pish*, *Pib*, *Pia*, *Pii*, *Pi3*, *Pi5(t)*, *Pik-s*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, *Pi7(t)*, *Pi9*, *Pi12(t)*, and *Pi19(t)*—in Japan (Table 1). The cluster groups were characterized by the occurrences of these reaction types: group I had the major reaction types U13, i7, k177, z00, and ta001; subgroup IIa had U13, i7, k100, z00, ta001, and ta401; and subgroup IIb had i0, k100, z00, and ta001. The reaction type z00, avirulent to genes in DV group z, was common among the three groups. Reaction types U11, U13, and U33 were commonly virulent to *Pish*; k100 and k177 to *Pik-s*; and ta4001 and ta001 to *Pi19(t)*. These results indicate that isolates virulent to *Pish*, *Pik-s*, and *Pi19(t)* and avirulent to *Pi9*, *Piz*, *Piz-5*, and *Piz-t*, are widely distributed together in Japan. U11, U13, and U33 were different reactions to *Pib* and *Pia*; i0 and i7 were to *Pii*, *Pi3*, and *Pi5(t)*; k100 and k177 were to *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, and *Pi7(t)*; z00 and z10 were to *Pi9*; and ta001 and ta401 were to *Pi12(t)*. The isolates virulent to these resistance genes were mainly differentiated.

Table 1. (continued from preceding page)

Reactions to resistance genes shown			Number of virulent isolates (%)				Total
			Cluster group			Sum	
			I	IIa	II b		
...	...	...	2 (0.6)	11 (3.5)	17 (5.5)	28 (9.0)	30 (9.7)
...	...	...	50 (16.1)	176 (56.8)	19 (6.1)	195 (62.9)	245 (79.0)
...	...	...	5 (1.6)	17 (5.5)	0 (0.0)	17 (5.5)	22 (7.1)
...	...	...	0 (0.0)	6 (1.9)	7 (2.3)	13 (4.2)	13 (4.2)
...	...	...	0.22	0.29	0.62	0.39	0.36
...	...	...					
...	...	...	3 (1.0)	0 (0.0)	38 (12.3)	38 (12.3)	41 (13.2)
...	...	...	52 (16.8)	201 (64.8)	0 (0.0)	201 (64.8)	253 (81.6)
...	...	...	2 (0.6)	9 (2.9)	5 (1.6)	14 (4.5)	16 (5.2)
...	...	...	0.16	0.08	0.22	0.35	0.32
Pik	Pik-p	Pi7(t)					
a	a	a	0 (0.0)	192 (61.9)	41 (13.2)	233 (75.2)	233 (75.2)
v	v	v	30 (9.7)	0 (0.0)	0 (0.0)	0 (0.0)	30 (9.7)
...	...	...	27 (8.7)	18 (5.8)	2 (0.6)	20 (5.5)	47 (15.2)
...	...	...	0.60	0.16	0.09	0.15	0.42
...	...	...					
...	...	...	39 (12.6)	180 (58.1)	33 (10.6)	213 (68.7)	252 (81.3)
...	...	...	0 (0.0)	15 (4.8)	7	(2.3)	22 (7.1)
...	...	...	18 (2.8)	15 (4.8)	3 (1.0)	18 (5.8)	36 (11.6)
...	...	...	0.50	0.26	0.38	0.28	0.33
Pi19(t)	Pi20(t)	...					
v	a	...	29 (9.4)	134 (43.2)	25 (8.1)	159 (51.3)	188 (60.6)
v	a	...	13 (4.2)	42 (13.5)	6 (1.9)	48 (15.5)	61 (19.7)
...	...	...	15 (4.8)	34 (11.0)	12 (3.9)	46 (14.8)	61 (19.7)
...	...	...	0.67	0.55	0.63	0.57	0.59

In total, 99 races included only one blast isolate, and the other 19 races had fewer than six isolates.

Discussion

On the basis of the reactions of 25 DV harboring 23 resistance genes—*Pish*, *Pit*, *Pib*, *Pia*, *Pii*, *Pi3*, *Pi5(t)*, *Pik*, *Pik-s*, *Pik-h*, *Pik-m*, *Pik-p*, *Pi1*, *Pi7(t)*, *Pi9*, *Piz*, *Piz-5*, *Piz-t*, *Pita-2*, *Pi12(t)*, *Pita*, *Pi19(t)*, and *Pi20(t)*—and LTH, we found high frequencies of isolates virulent to seven resistance genes: *Pish*, *Pia*, *Pii*, *Pi3*, *Pi5(t)*, *Pik-s*, and *Pi19(t)*. The distribution of blast races was surveyed in Japan in 1976 (Yamada et al. 1979) and 1980 (Yamada 1985), with 9 Japanese DV carrying 9 resistance genes (*Pik-s*, *Pia*, *Pii*, *Pik*, *Pik-m*, *Piz*, *Pita*, *Pita-2*, and *Piz-t*), and in 1994 (Naito et al. 1999) and 2001 (Koizumi et al. 2007), with 12 DV carrying 11 resistance genes (*Pia*, *Pii*, *Pik*, *Pik-m*, *Piz*, *Pita*, *Pita-2*, *Piz-t*, *Pik-p*, *Pib*, and *Pit*). Isolates virulent to *Pia* were dominant in the 1976 and 1980 surveys. Isolates virulent to *Pii* were dominant in the 1994 and 2001 surveys. Naito et al. (1999) showed that the dominant race virulent to *Pia* and *Pii* accounted for more than 50% of all surveyed isolates. Koizumi et al. (2007) found four dominant blast races (007.0, 001.0, 003.0, 005.0) which were virulent to *Pia*, *Pii*, or both, totaling 92% of isolates sampled. The high frequencies of occurrence of isolates virulent to *Pia* and *Pii* in our study agree with the results of Naito et al. (1999) and Koizumi et al. (2007). We also found high frequencies of virulence against *Pik-s*, *Pish*, *Pi3*, *Pi5(t)*, and *Pi19(t)*. These results suggest that the resistance genes in rice cultivars grown in Japan are limited. A limited number of rice cultivars (such as ‘Koshihikari’, ‘Hitomebore’, ‘Hinochikari’, ‘Akitakomachi’, ‘Kirara 397’, and ‘Kinuhikari’) are grown over a wide area of Japan, and few resistance genes—such as *Pish*, *Pia*, and various combinations of *Pii*, *Pi3*, *Pi5(t)*, or some *Pik* alleles—have been used in breeding programs (Kawasaki-Tanaka and Fukuta 2014). Therefore,

the distributions of long-term, dominant blast races may correspond to the distribution of these cultivars.

Very few blast isolates were virulent to *Piz*, *Piz-5*, *Piz-t*, *Pita*, *Pita-2*, and *Pi20(t)*, and no isolate was virulent to *Pit*. The frequencies and distributions of isolates virulent to *Pik-h*, *Pi1*, *Pi7(t)*, *Piz-5*, *Pi9*, *Pi12(t)*, and *Pi20(t)* had not previously been evaluated. In addition, we found some new isolates avirulent to *Pish* in Okinawa. Our results cannot be compared directly with previous studies in Japan (Koizumi et al. 2007; Naito et al. 1999; Yamada 1985; Yamada et al. 1979) because we used different DV. However, we can confirm that the distribution of Japanese blast races has not changed greatly since those studies.

We classified 310 blast isolates from Japan into 123 races. Noda et al. (1999) identified 12 kinds of blast races among 129 isolates collected from all over the Mekong River Delta in Vietnam, using Japanese DV carrying 12 resistance genes. Mekwatanakarn et al. (2000) investigated the virulence of 527 blast isolates from Thailand, using 10 CO 39 NIL and 6 LTH NIL, and classified them into 175 races. In Bhutan, Thinlay et al. (2000) differentiated 110 isolates into 53 races on the basis of the reactions of five CO 39 NIL and five LTH NIL. In China, Chen et al. (2001) classified 792 isolates into 344 races by using five LTH NIL and four CO 39 NIL. In the Philippines, Telebanco-Yanoria et al. (2008a) characterized 119 blast isolates by using 19 DV and categorized them into 70 races. Recently, using the same set of 25 DV selected by Tsunematsu et al. (2000) and Telebanco-Yanoria et al. (2010), plus LTH, Fukuta et al. (2014) categorized 122 isolates from Cambodia into 92 races, and Odjo et al. (2014) categorized 96 isolates from West Africa into 76 races. Thus, many blast races have been found, each with few isolates, and dominant races have not been found. Our finding of many races agrees with the results from the tropics and China but our results differ in that several

Fig. 1. Frequency of virulent isolates of *Pyricularia oryzae* in each region of Japan. In total, 23 monogenic lines (Tsunematsu et al. 2000) and two near-isogenic lines with the genetic background of Lijiangxintuanheigu (LTH) (Telebanco-Yanoria et al. 2010) were used as differential varieties (DV). LTH was used as a susceptible control. In total, 310 blast isolates from Japan were investigated for pathogenicity against these DV and LTH. A: Hokkaido ($n = 23$); B: Tohoku ($n = 29$); C: Hokuriku ($n = 61$); D: Kanto ($n = 65$); E: Tokai ($n = 43$); F: Chugoku/Shikoku ($n = 23$); G: Kyushu ($n = 25$); and H: Okinawa ($n = 41$).

dominant races were found in Japan. These previous studies found that the number of blast races increased with the number of DV (i.e., the number of different resistance genes) used in the pathogenicity analyses. Our finding of many blast races in Japan might be related to the use of 25 DV with 23 resistance genes. Yet in spite of the large number of DV, the presence of dominant races and the lower numbers of races than in previous studies suggest that the diversity of blast races in Japan is lower than that in other countries.

These blast races had similarities of reaction types: namely, U13, i7, k177, z00, and ta001 in group I; U13, i7, k100, z00, z10, ta001, and ta401 in group IIa; and U11, U13, i0, k100, z00, and ta001 in group IIb. We identified five major blast races, which accounted for 46.5% of all 310 isolates: U13-i7-k100-z00-ta001 (27.4% of the whole set), U13-i7-k100-z00-ta401 (10.0%), U13-i0-k100-z00-ta001 (3.5%), U13-i7-k177-z00-ta001 (2.9%), and U11-i7-k100-z00-ta001 (2.6%). These results also suggest that blast races in Japan are less differentiated than elsewhere, and indicate that races harboring virulence against genes *Pish*, *Pia*, *Pii*, *Pi3*, *Pi5(t)*, *Pik-s*, and *Pi19(t)* are distributed widely. It is possible to differentiate races on the basis of virulence against genes *Pi12(t)*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, or *Pi7(t)* and avirulence against genes *Pia*, *Pii*, *Pi3*, or *Pi5(t)* in Japan.

Almost all of the blast isolates in cluster group I were found in northern Japan, especially in Hokkaido, where the distribution differed greatly from that in other regions. Isolates in subgroup IIa were found in all regions. Isolates in subgroup IIb were distributed mainly in central Japan (Fig. 3). The distributions of group I and subgroup IIb were restricted. Subgroup IIa had the most isolates and many reaction types but the lowest diversity. On the other hand, group I had significantly higher diversity in DV groups k and z, and subgroup IIb in U and i. These results suggest that subgroup IIa represents the original population with low diversity, and that group I and subgroup IIb differentiated from it and became distributed additionally in northern and central Japan, respectively, with minor changes in virulence.

Thus, the new designation system proposed by Hayashi and Fukuta (2009) demonstrates well that several dominant blast races are distributed throughout Japan, and that several similar races with minor changes in virulence are distributed within regions. This situation might be unique, in that several similar dominant blast races are distributed over a wide area. The genotypes of recommended Japanese cultivars with blast resistance genes are quite simple: most cultivars harbor only *Pia* and *Pii* (Yaegashi et al. 1983). Koshihikari rice, with *Pish*, is a high-quality, good-tasting cultivar that is grown on around

38% of the cropping area in Japan. Moreover, the top 10 recommended cultivars, which are grown widely, were bred from Koshihikari (Kyoya et al. 1998, 2002, Saito et al. 1989; Yagi et al. 1990). The distributions of the dominant reaction types U13 (virulent to *Pish* and *Pia*) in all cluster groups and i7 (*Pii*, *Pi3*, and *Pi5(t)*) in group I and subgroup IIa correspond to the genotypes of the resistance genes in these top cultivars (Kawasaki-Tanaka and Fukuta 2004). Therefore, the limited source of cultivars carrying *Pia*, *Pii*, and *Pish* used in Japanese rice breeding might restrict the genetic diversity of our cultivars. Japanese rice cultivars tend to be more susceptible, with lower diversity of blast resistance, than those of other countries, quite unlike the Indica-type cultivars grown in the tropics (Telebanco-Yanoria et al. 2008b). In Hokkaido, almost all recommended rice cultivars harbor *Pik* (Sasaki et al. 1990; Shinbashi et al. 2003; Yaegashi et al. 1983; Yokoo et al. 2005). This uniformity suggests that the high frequency of blast isolates virulent to DV containing k group resistance genes (with the exception of *Pik-s*) in Hokkaido corresponds to this resistance genotype in the rice cultivars. In other words, our findings confirm the codifferentiation of rice cultivars and blast races in Japan. However, we still have insufficient detailed information on the resistance genotypes of Japanese cultivars.

Rice *indica*-type cultivars have been introduced as breeding parents for the development of high-yielding rice and feed rice in Japan (Imbe et al. 2004). Multiline cultivars with 'Sasanishiki' and Koshihikari genetic backgrounds have been put to practical use in Miyagi prefecture (Matsunaga 1996) and in Niigata and Toyama prefectures (Ishizaki et al. 2005, Kojima et al. 2003a,b) but the cultivation of these genotypes is still limited. Use of these different blast resistance genotypes and rice populations with combined genotypes may change the distribution of blast races in the future. The basic information on blast pathogens provided here may be useful for building up a durable system of protection against rice blast by breeding, as well as in creating an approach to disease pathology that will adapt to the distributions of new rice and cultivation systems.

We are collecting blast isolates from tropical and temperate countries to create a set of international standard differential isolates. The pathogenicity data and isolates collected here will support this work. This will allow us to identify genetic variation and genotypes of resistance genes in rice cultivars, and clarify the relationship between rice cultivars and blast races in Japan and worldwide. Our DV are different from those used in previous studies; therefore, we cannot compare our results directly with previous results. Imbe and Matsumoto (1985) reported that five Japanese DV,

Fig. 2. Frequency of virulent isolates of *Pyricularia oryzae* in each cluster group. Cluster analysis using Ward's hierarchical clustering method was used to classify 310 blast isolates by the reaction patterns of 25 differential varieties and susceptible Lijiangxintuanheigu (LTH).

Fig. 3. Distribution of pathogenicity groups of *Pyricularia oryzae* isolates in eight regions of Japan. Charts show percent frequency of blast isolates in each group (and number of isolates).

carrying *Pik-s*, *Piz*, *Pita-2*, *Pib*, and *Pita-2*, also harbored *Pish*. Hayashi et al. (2009) detected *Pi19(t)* in all Japanese DV except for two DV for *Pita* and *Pita-2*. Most isolates from Japan were virulent to DV carrying *Pish* and *Pi19(t)*. Therefore, previous research by Yamada et al. (1979), Yamada (1985), Naito et al. (1999), and Koizumi et al. (2007) might not be big problem to isolates by the additional resistance gene(s) in the genetic background of CVs in Japan. However, the existence of *Pish* and *Pi19(t)* in the DV might complicate the evaluation of blast pathogenicity in the tropics. For example, Noda et al. (1999) found it difficult to analyze the pathogenicity of blast isolates from Vietnam by using Japanese DV. Instead, we used monogenic lines (Kobayashi et al. 2007; Tsunematsu et al. 2000) and LTH NIL (Telebanco-Yanoria et al. 2010) to analyze pathogenicity without the influence of these additional genes. These common materials will need to be used in future studies using international DV to characterize blast isolates. Use of the designation system for blast races proposed by Hayashi and Fukuta (2009) will make it possible to compare blast races among countries and regions and, thus, to enhance our understanding of the differentiation and diversity of blast races and rice at a global level.

Acknowledgments

We thank the Miyagi Prefectural Plant Protection Office, the Furukawa Agricultural Experiment Station, the Niigata Crop Research Center, and the National Agricultural Research Center for the Kyushu Okinawa Region for providing blast isolates; and S. Arase (Shimane University) and H. Tsujimoto (Arid Land Research Center, Tottori University) for their comments and suggestions. This study was performed as part of the JIRCAS research projects "Blast Research Network for Stable Rice Production" (2006 to 2010) and "Rice Innovation for Environmentally Sustainable Production Systems" (2011 onward).

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