Pathogenicities of Rice Blast (*Pyricularia oryzae* Cavara) Isolates From Kenya

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Abstract

A total of 99 isolates of rice blast (*Pyricularia oryzae* Cavara) were collected from 2010 to 2015 from four regions in Kenya: Kirinyaga County and Embu County, Kisumu County, Tana River County, and Mombasa County. The pathogenicities of these isolates were clarified based on the reaction patterns of Lijiangxintuanheigu and differential varieties (DVs) targeting 23 resistance genes. The frequency of virulent isolates was high for DVs for *Pib*, *Pia*, *Pii*, *Pi3*, *Pi5(t)*, *Pik-s*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, *Pi7(t)*, *Pi19(t)*, and *Pi20(t)*; low for DVs for *Pish*, *Pi9(t)*, *Piz-5*, and *Piz-t*; and intermediate for the remaining DVs for *Pit*, *Piz*,

Pita-2, *Pita*, and *Pi12(t)*. These blast isolates were classified into three cluster groups: Ia, Ib, and II. The frequencies of virulent isolates to DVs for *Pit*, *Pii*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, *Pi7(t)*, *Piz*, and *Pi12(t)* differed markedly between clusters I and II, and those of DVs for *Pib*, *Pit*, *Pia*, *Pi3*, *Pita-2*, *Pita*, and *Pi20(t)* differed between Ia and Ib. The frequencies of cluster groups in the four geographical regions were different. A total of 62 races were found, with 19 blast isolates categorized into one race (U63-i7-k177-z00-ta003), whereas the other races included only some isolates in each.

Rice blast, caused by the pathogen *Pyricularia oryzae* Cavara, is one of the most serious diseases affecting rice worldwide (Zeigler et al. 1994). The use of resistant cultivars is the most economical method to control this disease in rice (*Oryza sativa* L.). However, using such cultivars has a limited effect as a result of the breakdown of resistance genes, with more virulent blast races occurring. The interaction between host resistance and blast fungus virulence can be explained by the gene-for-gene theory: every resistance gene in the host corresponds to an avirulence gene in the pathogen (Flor 1971; Silué et al. 1992). Based on this theory, rice differential varieties (DVs) have been developed to distinguish races of the blast fungus

by their different patterns of reaction to different strains of pathogen. DVs can be used to determine the blast pathogen population structure and identify new blast races.

There are four rice cultivation regions in Kenya: Kirinyaga County and Embu County, Kisumu County, Tana River County, and Mombasa County. Widespread irrigation systems have been constructed in Kirinyaga County, Embu County, Kisumu County, and in the delta area of Tana River County, whereas small irrigated or rainfed paddy fields comprise the rice-growing areas of Mombasa County. Rice productivity in Kenya is low and this has been attributed to various factors, including biotic and abiotic stresses (Kouko 1997; Onyango 2014). With respect to biotic stresses, rice blast disease is the major constraint to rice production in Kenya because of its wide geographic distribution and destructiveness under unfavorable environmental conditions (Kouko 1997; Ministry of Agriculture 2014). Kihoro et al. (2013) surveyed rice blast in Mwea (Kirinyaga County) and found the disease to be widely distributed in that region.

Kariaga et al. (2016) collected blast fungus from Mwea (Kirinyaga County); Homabay, Yala Swamp, Ahero, and West Kano (Kisumu County); Bunyalla, Mumias, and Nambale (Busia County); and Msambweni, Kaloleni, and Tana River Delta (Tana River County) in Kenya. They tried to characterize the regional differences in fungal cultures, such as conidia and conidiophores, and they estimated the distributions of different blast populations in these regions based on the observations of infections of eight cultivars. However, the pathogenicities of these blast isolates were not clarified. Nyongesa et al. (2016) reported that DVs comprising monogenic lines with a Lijiangxintuanheigu (LTH) background (Tsunematsu et al. 2000) and near-isogenic lines (NILs) with a CO 39 genetic background (Telebanco-Yanoria et al. 2010) for resistance genes *Pib*, *Pit*, *Pish*, *Pi5(t)*, *Pik-s*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, *Piz*, *Piz-5*, *Piz-t*, *Pi12(t)*, and *Pita-2* were relatively resistant to the populations of blast fungus at Mwea (Kirinyaga County), West Kano (Kisumu County), and Gamba (Tana River County), under natural infection. The number of DVs infected by the blast fungus varied among these three regions, and different races/populations were estimated to be distributed in these regions. The study by Nyongesa et al. (2016) was the first report to detail pathogenicities of the blast fungus in Kenya. However, those surveys were limited to only observations

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Y. Fukuta and M. J. Telebanco-Yanoria contributed equally to this work.

Funding: This study was conducted under six research projects: “Development of Rice Production Technologies in Africa” from 2011 to 2015, “Rice Innovation for Environmentally Sustainable Production Systems” from 2011 to 2015, and “Development of Sustainable Technologies to Increase Agricultural Productivity and Improve Food Security in Africa” from 2016 to 2020 in the Japan International Research Center for Agricultural Sciences; the Global Rice Science Partnership Research Project “Methodologies and New Resources for Genotyping and Phenotyping of African Rice Species and Their Pathogens for Developing Strategic Disease Resistance Breeding Programs” (MENERGEP) from 2012 to 2014; and the Bilateral Joint Research Project between Japan and Kenya projects “Interdisciplinary Study on the Rice Blast Outbreak in Mwea Irrigation Scheme in Kenya” from 2011 to 2013 and “Development of Rice Blast Disease Control Technology Through Characterization of Rice Blast Fungus and Rice Cultivars in Kenya” from 2013 to 2015 supported by the Japan Society for the Promotion of Science and the National Commission For Science, Technology, and Innovation.

*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary figure and two supplementary tables are published online.

The author(s) declare no conflict of interest.

Accepted for publication 30 June 2019.

of natural infections of the DVs, and pathological evaluations of blast fungus and the differentiations of races were not clarified.

In previous pathogenicity studies, nine DVs (including Shin 2 for an unknown resistance gene, Aichiasahi for *Pia*, Ishikarishiroge for *Pii*, Kanto 51 for *Pik*, Tsuyuake for *Pik-m*, Fukunishiki for *Piz*, Yahiro-mochi for *Pita*, Pi No. 4 for *Pita-2*, and Toride 1 for *Piz-t*) were used by Yamada et al. (1976, 1979), and 12 DVs (including Shin 2, BL1 for *Pib*, Aichiasahi, K59 for *Pit*, Fujisaka 5 for *Pii*, Kusabue for *Pik*, K60 for *Pik-p*, Tsuyuake, Fukunishiki, Toride 1, K1 for *Pita*, and Pi No. 4) were used by Kiyosawa (1984) in Japan. This Japanese DV set for 12 resistance genes (*Pia*, *Pib*, *Pit*, *Pii*, *Pik-s*, *Pik*, *Pik-m*, *Pik-p*, *Piz*, *Pita*, *Pita-2*, and *Piz-t*) (Kiyosawa 1981, 1984) was used for pathogenicity tests in Vietnam (Noda et al. 1999). The CO 39 NILs for *Pi1*, *Pi1-LAC(t)*, *Pi1-TTP(t)*, *Piz-5*, *Pi3*, *Pi4a*, *Pi4b(t)*(= *Pita*), *Pi4a-PKT(t)*, and *Pi4-TTP(t)* and LTH NILs for *Pib*, *Pik-m*, *Pik-p*, *Pik*, *Pita*, and *Pita-2* were used in Thailand (Mekwatanakarn et al. 2000). Twelve DVs for *Pish*, *Pia*, *Pib*, *Pit*, *Pii*, *Pi3*, *Pi5(t)*, *Pik-s*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pi1*, *Piz*, *Piz-5*, *Piz-t*, and *Pi20(t)* were used in the Philippines (Telebanco-Yanoria et al. 2008). The LTH NILs for *Pib*, *Pik-m*, *Pik-p*, *Pik*, *Pita*, and *Pita-2* and CO 39 NILs for *Pi3*, *Pi13*, *Piz-5*, and *Pita* were used in China (Chen et al. 2001). However, several Japanese DVs were found to harbor additional resistance gene(s) to the target genes in their genetic backgrounds. Hayashi et al. (1998) identified *Pi19(t)* in Shin 2, BL1, Aichiasahi, K59, Fujisaka 5, Kusabue, K60, Tsuyuake, Fukunishiki, Toride 1, and K1. Kato et al. (2004) also reported that Shin 2 harbors *Pish* and *Pik-s*; K59 and Fujisaka 5 have *Pik-s*; and Fukunishiki, Toride 1, and BL1 have *Pish* as well. CO 39 has *Pia* in the genetic background (Tsunematsu et al. 2000). Thus, the Japanese DVs established by Yamada et al. (1976, 1979) and Kiyosawa (1981, 1984) and the CO 39 NILs have limitations in identifying the pathogenicity of blast isolates because of the reactions of additional resistance gene(s) in the genetic backgrounds.

Tsunematsu et al. (2000) developed the monogenic lines as a new set of DVs targeting 23 resistance genes: *Pish*, *Pib*, *Pit*, *Pia*, *Pii*, *Pi3*, *Pi5(t)*, *Pik-s*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, *Pi7(t)*, *Pi9(t)*, *Piz*, *Piz-5*, *Piz-t*, *Pita-2*, *Pita*, *Pi12(t)*, *Pi19(t)*, and *Pi20(t)*. These monogenic lines were used to introduce single resistance genes into the genetic background of a Chinese Japonica rice cultivar, LTH, to reduce the effect of the genetic background. As advanced DVs, Telebanco-Yanoria et al. (2010) bred NILs of LTH targeting 11 resistance genes: *Pib*, *Piz-5*, *Pi9(t)*, *Pi3*, *Pia*, *Pik-s*, *Pik*, *Pik-h*, *Pi7(t)*, *Pita*, and *Pita-2*. These monogenic lines and LTH NILs covered 23 resistance genes and are the most effective materials for use as international DVs.

Hayashi and Fukuta (2009) proposed a new international system using monogenic lines for designating races of the rice blast pathogen. Races are designated by the reaction patterns of LTH (susceptible control) and 25 monogenic lines targeting 23 resistance genes. This set of international DVs and a new designation system has been used to evaluate the pathogenicities of blast isolates and to clarify the differentiations of blast races in Western Africa (Odjo et al. 2014), northeastern China (Wang et al. 2013), Cambodia (Fukuta et al. 2014), the United States (Wang et al. 2015), Japan (Kawasaki-Tanaka et al. 2016), and Bangladesh (Khan et al. 2016). Using the set of common DVs and the designation system of blast races allows information on blast races from different countries or regions to be compared, and we can understand the global differentiation of blast races and can characterize the blast population in each area.

In the present study, the pathogenicities of blast isolates collected from different regions in Kenya were tested using the set of international DVs developed by Tsunematsu et al. (2000) and Telebanco-Yanoria et al. (2010), and races were named following the designation system developed by Hayashi and Fukuta (2009). The differences in blast races among four rice-growing regions of Kenya are discussed.

Materials and Methods

Blast sampling and single spore isolation. A total of 99 isolates of *P. oryzae* were collected from the infected leaves (95 isolates) and panicles (four isolates) of rice (*O. sativa* L.) cultivars grown in irrigated lowland and upland fields at harvest seasons, or the nursery at seeding stage, in four regions of Kenya from 2010 to 2015:

Kirinyaga County and Embu County, Kisumu County, Tana River County, and Mombasa County (Supplementary Table S1).

Single spores isolated from these samples were incubated on moist filter paper in a Petri dish at room temperature for 24 h following the protocol of Hayashi et al. (2009). Colonies from single conidia were grown on water agar for 5 to 7 days, and two to three cut pieces of each single colony were transferred onto sterile filter paper on rice straw agar medium. The fungi were grown on the filter paper and then dried and stored on the filter paper at -20°C .

Inoculation. To produce inocula, stock isolates (paper discs) were cultured on oatmeal agar medium. The inoculated plates were incubated at 25 to 28°C for 12 to 14 days. The culture was scraped with a sterilized toothbrush, and the plates were exposed to continuous light for 4 to 5 days to induce heavy sporulation. Conidia were dislodged from the incubated plates into sterilized distilled water containing 0.01% Tween 20 by gently rubbing with a paint brush. Spore suspensions were filtered through four layers of gauze mesh and the concentration was adjusted to 1×10^5 conidia/ml using a hemacytometer (Hayashi et al. 1998).

Inoculation was performed following the methods of Bonman et al. (1986) and Hayashi et al. (2009). Seedling cell trays (cells 16 mm in diameter $\times$ 25 mm deep, in a 5×7 array) containing 20- to 21-day-old seedlings (approximately four- to five-leaf stage) of three of the 25 DVs and LTH were placed on a rotating plate, and the seedlings were sprayed uniformly with 10 ml of spore suspension per tray using an electric atomizer (0.3-mm spray nozzle) at 0.1 MPa. Inoculated seedlings were incubated in a dew chamber at 25°C for 20 h and then transferred to a greenhouse maintained at a temperature of $25 \pm 1^{\circ}\text{C}$ and relative humidity of 70 to 80%. The process of inoculation and evaluation was performed twice using three plants each.

Scoring of infection and race designation. The degree of infection of each DV and LTH in each of the blast isolates was evaluated at 7 days after inoculation by assigning a score of 0 to 5, where 0 indicates no evidence of infection; 1 indicates brown specks smaller than 0.5 mm in diameter, with no sporulation; 2 indicates brown specks about 0.5 to 1 mm in diameter, with no sporulation; 3 indicates roundish to elliptical lesions about 1 to 3 mm in diameter with a gray center surrounded by brown margins, with lesions capable of sporulation; 4 indicates typical spindle-shaped blast lesions capable of sporulation, 3 mm or longer with necrotic gray centers and water-soaked or reddish brown margins, with little or no coalescence of lesions; and 5 indicates lesions as in 4 but with about half of one or two leaf blades killed by the coalescence of lesions. The most infected leaf was investigated among three plants, and then the mean of two inoculations was used as the representative data of infection in each DV or susceptible control. The plant reactions were further categorized by the method of Hayashi and Fukuta (2009) as resistant (in most cases, infection scores were 0 to 2) or as susceptible (in most cases, scores were 3 to 5). The only DV categorized as susceptible with scores of 2 to 5 was IRBL5-M for the resistance gene *Pi5(t)*, and the only DVs categorized as susceptible with scores of 4 to 5 were IRBLsh-B for *Pish* and IRBLta2-Pi for *Pita-2*.

Designation of blast isolates was determined following the international designation system using the reaction patterns of the 25 DVs and LTH. These DVs and LTH were divided into five groups as follows: U, i, k, z, and ta. Group U includes LTH and four DVs (IRBLa-A for *Pia*, IRBLsh-B for *Pish*, IRBLb-B for *Pib*, and IRBLt-K59 for *Pit*). Group i includes three DVs [IRBLi-F5 for *Pii*, IRBL3-CP4 for *Pi3*, and IRBL5-M for *Pi5(t)*] with the *Pii* locus on chromosome 9. Group k includes seven DVs [IRBLks-F5 for *Pik-s*, IRBLk-Ka [LT] for *Pik*, IRBLkm-Ts for *Pik-m*, IRBLkp-K60 for *Pik-p*, IRBLkh-K3[LT] for *Pik-h*, IRBL1-CL for *Pi1*, and IRBL7-M for *Pi7(t)*] with the *Pik* region on chromosome 11. Group z includes four DVs [IRBLz-Fu for *Piz*, IRBLz5-CA for *Piz-5*, IRBLzt-T for *Piz-t*, and IRBL9-W for *Pi9(t)*] with the *Piz* region on chromosome 6. Group ta includes seven DVs [IRBLta-Kl for *Pita*, IRBLta-CP1 for *Pita*, IRBLta2-Pi for *Pita-2*, IRBLta2-Re for *Pita-2*, IRBL12-M for *Pi12(t)*, IRBL19-A for *Pi19(t)*, and IRBL20-IR24 for *Pi20(t)*] with the *Pita* region on chromosome 12. The 21 resistance genes in groups i, k, z, and ta are multiple alleles or are located in the same

chromosomal regions. The other four genes (*Pia* on chromosome 11, *Pish* and *Pit* on chromosome 1, and *Pib* on chromosome 2) are located in different chromosomal regions and are gathered with LTH in group U. In this study, the monogenic lines IRBLk-K3 and IRBLk-Ka used by Hayashi and Fukuta (2009) were replaced with two LTH NILs, IRBLk-K3[LT] and IRBLk-Ka[LT], respectively.

Blast races were designated by using Gilmour's method (Gilmour 1973). The DVs in each of the five groups U, i, k, z, and ta were divided into one to three subunits, with one to three DVs (genes) in each subunit (see Table 1). To each of the DVs within each subunit, we applied codes 1, 2, or 4 if the respective DV showed a susceptible reaction to a blast isolate (i.e., the code 1 was assigned if the first DV in the subunit was susceptible; 2 was assigned if the second DV in the subunit was susceptible; and 4 was assigned if the third DV in the subunit was susceptible). Blast races were designated by the combined sum of the codes representing the reactions of the three DVs in each subunit. Isolates classified this way were designated as

“reaction type” within each DV group and as “races” using the set of all five reaction types.

Classification and diversity of blast isolates. Cluster analysis was performed using Ward's hierarchical method (Ward 1963) based on the data on infection scores of the 25 DVs and LTH by blast isolates, using JMP 11.2 for Windows software (2014; SAS Institute Inc., Cary, NC). Relationships of blast isolates between the cluster groups, as well as the ecosystems and geographical distributions, were evaluated.

The diversity of the pathogens was calculated using Simpson's diversity index (Simpson 1949). The index values vary from 0 to 1, where 0 represents no diversity and 1 represents maximum diversity.

Results

Collection and classification of isolates. A total of 99 isolates were collected from four geographical regions in Kenya: Kirinyaga County (18 isolates)/Embu County (14 isolates), Kisumu County

Table 1. Reaction types of *Pyricularia oryzae* isolates to 25 rice differential varieties (DV) for resistance genes and to variety Lijiangxintuanheigu (LTH) (susceptible control)

	Reaction to resistance gene in differential variety ^a										No. of blast isolates (%)			
	Cluster group										Ia (n = 24)	Ib (n = 62)	II (n = 13)	Total (n = 99)
“U” DV group reaction type	<i>Pish</i>	<i>Pib</i>	<i>Pit</i>	LTH	<i>Pia</i>									
U01	a	a	a	v	a	—	—	—	—	—	23	2		25 (25.3)
U43	a	a	v	v	v	—	—	—	—	—		9	10	19 (19.2)
U63	a	v	v	v	v	—	—	—	—	—		27	3	30 (30.3)
Sum	—	—	—	—	—	—	—	—	—	—	23	38	13	74 (74.7)
Others (U00, U03, U20, U21, U23, U40, U41, U60)	—	—	—	—	—	—	—	—	—	—	1	24	0	25 (25.3)
Simpson's index of diversity (1-D)	—	—	—	—	—	—	—	—	—	—	0.08	0.77	0.36	0.80
“i” DV group reaction type	<i>Pii</i>	<i>Pi3</i>	<i>Pi5(t)</i>	—	—	—	—	—	—	—				
i0	a	a	a	—	—	—	—	—	—	—	3	11		14 (14.1)
i3	v	v	a	—	—	—	—	—	—	—	8	3	2	13 (13.1)
i7	v	v	v	—	—	—	—	—	—	—	10	34	4	48 (48.5)
Sum	—	—	—	—	—	—	—	—	—	—	21	48	6	75 (75.8)
Others (i1, i2, i4, i5, i6)	—	—	—	—	—	—	—	—	—	—	3	14	7	24 (24.2)
Simpson's index of diversity (1-D)	—	—	—	—	—	—	—	—	—	—	0.69	0.67	0.66	0.71
“k” DV group reaction type	<i>Pik-s</i>	—	—	<i>Pik-m</i>	<i>Pil</i>	<i>Pih-h</i>	<i>Pik</i>	<i>Pik-p</i>	<i>Pik7(t)</i>					
k177	v	—	—	v	v	v	v	v	v		20	29		49 (49.5)
Others (k000, k002, k0003, k037, k055, k076, k077, k100, k102, k104, k106, k107, k120, k134, k136, k137, k152, k153, k157, k175)	—	—	—	—	—	—	—	—	—		4	33	13	50 (50.5)
Simpson's index of diversity (1-D)	—	—	—	—	—	—	—	—	—	—	0.30	0.76	0.77	0.73
“z” DV group reaction type	<i>Pi9(t)</i>	—	—	<i>Piz</i>	<i>Piz-5</i>	<i>Piz-t</i>	—	—	—					
z00	a	—	—	a	a	a	—	—	—		24	47		71 (71.7)
z01	a	—	—	v	a	a	—	—	—			7	13	20 (20.2)
Sum	—	—	—	—	—	—	—	—	—		24	54	13	91 (91.9)
Others (z02, z04, z10)	—	—	—	—	—	—	—	—	—		0	8	0	8 (8.1)
Simpson's index of diversity (1-D)	—	—	—	—	—	—	—	—	—	—	0.00	0.44	0.00	0.46
“ta” DV group reaction type	<i>Pita-2 (Pi)</i>	<i>Pita-2 (Re)</i>	<i>Pi12(t)</i>	<i>Pita (K1)</i>	<i>Pita (CP1)</i>	—	<i>Pi19(t)</i>	<i>Pi20(t)</i>	—					
ta003	a	a	a	a	a	—	v	v	—			23		23 (23.2)
ta403	a	a	v	a	a	—	v	v	—			10		10 (10.1)
ta431	a	a	v	v	v	—	v	a	—				11	11 (11.1)
Sum	—	—	—	—	—	—	—	—	—		0	33	11	44 (44.4)
Others (ta000, ta001, ta002, ta013, ra031, ta033, ta121, ta131, ta301, ta311, ta321, ta331, ta400, ta402, ta421, ta433, ta723)	—	—	—	—	—	—	—	—	—		24	29	2	55 (55.6)
Simpson's index of diversity (1-D)	—	—	—	—	—	—	—	—	—	—	0.75	0.81	0.26	0.89

^a a = avirulent and v = virulent.

(26 isolates), Tana River County (11 isolates), and Mombasa County (12 isolates), plus two isolates from an unknown region. These isolates showed different frequencies of virulence against the 25 DVs and LTH (Fig. 1). High frequencies of virulence (>50%) were seen in the 14 DVs for *Pib*, *Pia*, *Pii*, *Pi3*, *Pi5(t)*, *Pik-s*, *Pik-m*, *Pil*, *Pik-h*, *Pik*, *Pik-p*, *Pi7(t)*, *Pi19(t)*, and *Pi20(t)*; intermediate frequencies of virulence (10 to 50%) were seen in the seven DVs for *Pit*, *Piz*, *Pita-2* (two lines), *Pi12(t)*, and *Pita* (two lines); and low frequencies of virulence (<10%) were seen in the four DVs for *Pish*, *Pi9(t)*, *Piz-5*, and *Piz-t*. The control variety LTH was susceptible to 96.0% of the isolates and showed resistance to the others. The results indicate that the reactions to resistance genes in the DVs varied among the blast isolates tested.

Based on their patterns of reaction to the 25 DVs and LTH, the isolates were classified into two cluster groups (clusters I and II) and then divided again into three subgroups (Ia, Ib, and II) (Supplementary Fig. S1). These cluster groups showed different frequencies of isolates virulent for each DV (Fig. 1). Cluster groups Ia and Ib included 24 and 62 of the isolates, respectively, and showed minor differences in the frequencies of virulence for several DVs. Cluster group Ia differed from group Ib in that there were several isolates avirulent to LTH in group Ib and the frequencies of isolates virulent for DVs for *Pib*, *Pit*, *Pia*, and *Pi20(t)* were remarkably higher in group Ib than Ia, whereas the frequencies of isolates virulent for DVs for *Pi3*, *Pita-2* (two lines), and *Pita* (two lines) were higher in

group Ia than in group Ib. Cluster group II included only 13 isolates. The low virulent isolates for DVs for *Pii*, *Pik-m*, *Pil*, *Pik-h*, *Pik*, *Pik-p*, and *Pi7(t)* and highly virulent isolates for DVs for *Pib*, *Pit*, *Piz*, *Pi12(t)*, and *Pita* (two lines) were remarkably different between subgroups Ia and Ib.

Distributions of cluster groups. Distributions of the cluster groups differed among the four geographical regions in Kenya (Fig. 2). Subgroup Ib was found in all four regions (Kisumu County, Kirinyaga County/Embu County, Tana River County, and Mombasa County), with frequencies of 43.8, 90.5, 9.1, and 75.0%, respectively. Subgroup Ia was found only in two regions (Kisumu County and Kirinyaga County/Embu County), with frequencies of 56.3 and 9.5%, respectively. Group II was also distributed in only two regions (Mombasa County and Tana River County), with frequencies of 28.0 and 90.9%, respectively.

Reaction types in groups of DVs and diversity. Several predominant reaction types of isolates were found in each group of DVs, and the diversities of isolates differed among the three cluster groups and four regions for each of the five groups of DVs (Table 1; Supplementary Table S2).

A total of 11 reaction types were found in the group U DVs: U00, U01, U03, U20, U21, U23, U40, U43, U41, U60, and U63. Reaction types U01 (virulent for LTH), U43 (virulent for LTH and the DVs for *Pit* and *Pia*), and U63 (virulent for LTH, *Pib*, *Pit*, and *Pia*) had 25, 19, and 30 blast isolates, respectively. U01, U43, and U63 were

Fig. 1. Frequencies of blast isolates virulent to differential varieties and susceptible control (Lijiangxintuanhegu [LTH]) in each cluster group.

shown to be the predominant type in cluster groups Ia, Ib, and II, respectively. The diversity indexes for isolates in cluster groups Ia, Ib, and II and for all isolates as a whole were 0.08, 0.77, 0.36, and 0.80, respectively. The reaction type U01 was distributed mainly in Kirinyaga County/Embu County and U43 and U63 were distributed in Tana River County and Kisumu County, respectively. The diversity index in Tana River County was 0.17, and it was quite low compared with the other counties. A total of eight reaction types (i0, i1, i2, i3, i4, i5, i6, and i7) were found in group i DVs. The dominant reaction types in this group were i0 with 14 isolates [avirulent for all three DVs in the i group: *Pii*, *Pi3*, and *Pi5(t)*], i3 with 13 isolates [virulent for DVs for *Pii* and *Pi3*], and i7 with 48 isolates [virulent for all three of these DVs]. Reaction type i0 was found mainly in cluster group Ib, i3 was found in group Ia, and i7 was found in Ia and Ib. The diversity indexes for isolates in cluster groups Ia, Ib, and II and as a whole were 0.69, 0.67, 0.66, and 0.71, respectively. The diversity index in Kisumu County was 0.26, which had quite low values compared with the other counties. The low diversity in Kisumu County was induced according to an occurrence of the highly virulent reaction type i7.

In the group k DVs, a total of 21 reaction types were found (k000, k002, k003, k037, k055, k076, k077, k100, k102, k104, k106, k107, k120, k134, k136, k137, k152, k153, k157, k175, and k177), and the highly virulent reaction type k177 was predominant. Reaction type k177 (virulent for all DVs of group k) was found for 20 isolates in cluster group Ia and 29 in group Ib, but there were no isolates in group II. The diversity indexes for isolates in cluster groups Ia, Ib, and II and for all isolates as a whole were 0.30, 0.76, 0.77, and 0.73, respectively. The reaction type k177 was predominant in Kisumu County and Kirinyaga County/Embu County, with 26 and

20 isolates, respectively, and the diversity indexes were 0.58 in Kisumu County and 0.59 in Kirinyaga County/Embu County. These were lower than those of the other regions.

In the group z DVs, a total of six reaction types were found (z00, z01, z02, z04, z06, and z10), and two low virulent types (z00 and z01) were predominant. Reaction type z0 (avirulent for all DVs) included 24 isolates in cluster group Ia and 47 in group Ib. Reaction type z01 (virulent only for the DV for *Piz*) was found for seven isolates in cluster group Ia and 13 in group II. The diversity indexes for isolates in cluster groups Ia, Ib, and II and for all isolates as a whole were 0.00, 0.44, 0.00, and 0.46, respectively, and these were the lowest values among those of the five DV groups. The diversity indexes among the four counties were quite low (from 0.00 to 0.39), except for Mombasa (0.61) where several reaction types were found. These results suggest that there is little differentiation of blast isolates for DV group z compared with the other DV groups, but some variation was found in cluster group Ib and Mombasa County.

In the ta DV group, a total of 20 reaction types were found (ta000, ta001, ta002, ta003, ta013, ta031, ta033, ta121, ta131, ta301, ta311, ta321, ta331, ta400, ta402, ta403, ta421, ta431, ta433, and ta723), and three types among them were predominant: ta003 [virulent for DVs for *Pi19(t)* and *Pi20(t)*], ta403 [virulent for DVs for *Pi12(t)*, *Pi19(t)*, and *Pi20(t)*], and ta431 [virulent for DVs for *Pi12(t)*, *Pita* (two lines), and *Pi19(t)*]. The diversity indexes for isolates in cluster groups Ia, Ib, and II and for all isolates as a whole were 0.75, 0.81, 0.26, and 0.89, respectively. The diversity indexes varied from 0.31 to 0.86 among the four regions, and Tana River County showed the lowest value (0.31). The reaction type ta431 was dominant in Tana River County. These results indicate that the low diversities

Fig. 2. Distributions of blast isolates classified into four cluster groups in Kenya.

in cluster group II and Tana River County were induced by the occurrence of the highly virulent and predominant reaction type ta431.

Thus, three cluster groups and four regions of Kenya were characterized by the presence of predominant reaction types. The diversity indexes were also decreased according to the occurrence of predominant reaction types among the cluster groups and four regions.

Blast races. A total of 62 races were found among the 99 blast isolates: 14 races in cluster group Ia, 38 in Ib, and 10 in II (Table 2). A total of 19 blast isolates were categorized into race U63-i7-k177-z00-ta003, but the other races included only from one to six isolates each. The isolates in race U63-i7-k177-z00-ta003 were collected in Kisumu County from 2011 to 2012 and infected the nine DVs for *Pia*, *Pii*, *Pi3*, *Pik*, *Pik-m*, *Pik-h*, *Pi1*, *Pi19*(t), and *Pi20*(t), as well as Basmati 370 and a hybrid progeny derived from a cross between Habataki and Basmati 370.

Discussion

A total of 99 blast isolates from four regions in Kenya showed different frequencies of virulence against 25 DVs and LTH. The frequency of virulent isolates was high (>50%) with respect to the 14 DVs for *Pib*, *Pia*, *Pii*, *Pi3*, *Pi5*(t), *Pik-s*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, *Pi7*(t), *Pi19*(t), and *Pi20*(t); intermediate (10 to 50%) with respect to the seven DVs for *Pit*, *Piz*, *Pita-2* (two lines), *Pi12*(t), and *Pita* (two lines); and low (<10%) with respect to the four DVs for *Pish*, *Pi9*(t), *Piz-5*, and *Piz-t* (Fig. 1). Nyongesa et al. (2016) reported that DVs for *Pib*, *Pit*, *Pish*, *Pi5*(t), *Pik-s*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, *Piz*, *Piz-5*, *Piz-t*, *Pi12*(t), and *Pita-2* were relatively resistant to the population of blast fungus at three locations, based on their field observations: Mwea in Kirinyaga County and West Kano and Gamba in

Kisumu County/Busia County. Our results for the whole collection of 99 isolates from Kenya corresponded mostly with those of Nyongesa et al. (2016), but the reactions to DVs for *Pib*, *Pi5*(t), *Pik-s*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, and *Pik-p* were different. These results indicate the instability or limitations of field evaluations based on natural infection and the importance of pathological analysis based on inoculation tests.

Based on the reaction patterns to DVs and LTH, these blast isolates were classified into three cluster groups (Ia, Ib, and II), and the distributions of blast cluster groups differed among the four regions (Fig. 2). Cluster group Ia was found in two regions (Kisumu County and Kirinyaga County/Embu County), and it was the main group in Kisumu County. Cluster group Ib was found in each of the four regions, and II was distributed only in Tana River County (where it was the main group) and Mombasa County. These results indicate that Ib was distributed as a common or basic group of the blast population in Kenya, and the distributions of blast populations were completely different between the two regions of Tana River County and Mombasa County and the two regions of Kirinyaga County/Embu County and Kisumu County. Thus, the distributions of cluster groups changed among the four regions in Kenya.

The three cluster groups and four geographical regions were characterized by the predominant reaction types of isolates (Table 1). The presence or absence of reaction types i7 and k177 as well as z00 and z001 were the main differences between cluster groups I and II. The presence or absence of i0, ta003 and ta403, and U01 and U63 were the main differences between Ia and Ib. Predominant reaction types U63, i7, k177, z00, and ta003 were distributed in Kisumu County; U01, i3, k177, and z00 were in Kirinyaga County/Embu County;

Table 2. *Pyricularia oryzae* races identified in Kenya

Cluster group	Race designation	No. of isolates	Cluster group	Race designation	No. of isolates
Ia	U01-il-k177-z00-ta301	1	Ib	U23-i5-kl53-z00-ta001	1
	U01-i0-k136-z00-ta311	1		U23-i7-kl77-z00-ta402	1
	U01-i0-kl77-z00-ta321	2		U40-i4-kl00-z00-ta000	1
	U01-i2-kl77-z00-ta321	1		U41-i0-k000-z01-ta400	1
	U01-i3-kl57-z01-ta131	1		U41-i6-kl77-z04-ta003	1
	U01-i3-kl77-z00-ta301	1		U43-i0-k077-z04-ta002	1
	U01-i3-kl77-z00-ta321	3		U43-i0-kl07-z00-ta033	1
	U01-i3-kl77-z00-ta331	3		U43-i0-kl77-z00-ta002	1
	U01-i5-kl77-z01-ta121	1		U43-i0-kl77-z04-ta002	1
	U01-i7-k077-z00-ta301	1		U43-i6-k077-z04-ta002	2
	U01-i7-kl57-z00-ra031	1		U43-i7-k000-z00-ta002	1
	U01-i7-kl77-z00-ta321	1		U43-i7-kl07-z00-ta033	2
	U01-i7-kl77-z00-ta331	6		U60-i0-kl20-z01-ta000	1
	U41-i7-kl77-z00-ta723	1		U63-i7-kl06-z00-ta403	1
	Sum	24		U63-i7-kl07-z00-ta403	3
	Simpson's index of diversity (1-D)	0.88		U63-i7-kl07-z10-ta403	1
Ib	U00-i0-k077-z00-ta402	1		U63-i7-kl34-z00-ta403	2
	U00-i1-k077-z00-ta003	1	II	U63-i7-kl36-z00-ta403	1
	U00-i4-k000-z01-ta002	1		U63-i7-kl77-z00-ta003	19
	U01-i0-k037-z00-ta000	1		Sum	62
	U01-i3-kl77-z00-ta013	1		Simpson's index of diversity (1-D)	0.80
	U01-i4-k076-z00-ta402	1		U43-i3-kl00-z01-ta431	1
	U03-i7-kl77-z00-ta033	2		U43-i4-kl06-z01-ta431	1
	U03-i7-kl77-z04-ta002	1		U43-i6-kl00-z01-ta431	2
	U20-i0-k003-z01-ta000	1		U43-i6-kl02-z01-ta421	1
	U21-i0-kl77-z00-ta001	1		U43-i6-kl06-z01-ta431	2
	U21-il-kl52-z01-ta000	1		U43-i6-kl07-z01-ta431	1
	U21-i3-kl37-z00-ta000	1		U43-i7-k000-z01-ta431	1
	U21-i3-kl75-z00-ta403	1		U43-i7-kl00-z01-ta431	1
	U21-i5-k077-z00-ta000	1		U63-i7-kl04-z01-ta421	1
	U21-i5-kl57-z00-ta003	1		U63-i7-kl04-z01-ta431	2
	U21-i5-kl77-z01-ta403	1		Sum	13
	U23-il-k055-z02-ta403	1		Simpson's index of diversity (1-D)	0.89
	U23-i3-kl77-z00-ta003	1	Total		99
	U23-i5-k057-z00-ta001	1		Simpson's index of diversity (1-D) for whole	0.92

and U43, z01, and ta431 were in Tana River County. The nondominant type was in Mombasa County. These results indicated that predominant reaction types between cluster groups and geographical regions corresponded, and different reaction types were distributed among the four regions. Particularly, the reaction types of two regions (Kisumu County and Kirinyaga County/Embu County) were completely different from those of Tana River County and Mombasa County.

Low diversity indexes were found in the DV groups i, k, and z in Kisumu County; k and z in Kirinyaga County/Embu County; and U, z, and ta in Tana River County when comparing those of Mombasa County, except for those of DV group ta in Kisumu County and Kirinyaga County/Embu County. The diversity indexes were decreased according to the occurrences of predominant reaction types among the cluster groups and four regions. Particularly, the lowest diversity indexes for DV groups i (0.26) and k (0.58) were found in Kisumu County, and DV groups U (0.17), z (0.00), and ta (0.31) were found in Tana River County. Those in Kirinyaga County/Embu County and Mombasa County showed intermediate or the highest values of diversity indexes among the five DV groups. Kihoro et al. (2013) reported that serious blast infections have been observed in the Mwea, Kirinyaga/Embu region. These results suggest that there is a high risk for an occurrence of virulent blast races in Kisumu County and Tana River County, followed by Kirinyaga County/Embu County. Mombasa County maintains its high diversity for blast races, and the risk of virulent blast races might be the lowest among the four regions. Kariaga et al. (2016) and Nyongesa et al. (2016) also suggested that different blast populations were distributed in Kenya. Our study demonstrated well the distribution or differentiation of different blast populations among the four regions, and these blast races in Kisumu County, Kirinyaga/Embu region, and Tana River County, where the cultivations of limited rice cultivars have been continued, were completely different from that of Mombasa County in particular. To our knowledge, this is the first report on the pathogenicities of isolates in Kenya based on inoculation tests using international DVs. Nevertheless, the number of isolates collected and investigated in this study was limited, and more samples need to be collected and the pathogenicities evaluated for a more detailed understanding of the distributions and differentiation of races in Kenya.

The same set of DVs established by Tsunematsu et al. (2000) and Telebanco-Yanoria et al. (2010) was used for the pathogenicity analyses of blast isolates from six regions: Jilin Province in northeastern China (Wang et al. 2013), Western Africa (Odjo et al. 2014), Cambodia (Fukuta et al. 2014), the United States (Wang et al. 2015), Japan (Kawasaki-Tanaka et al. 2016), and Bangladesh (Khan et al. 2016). The frequencies of isolates virulent on DVs for *Pi9(t)* were constantly low, and those for DVs for *Pia*, *Pii*, *Pi3*, *Pi5(t)*, and *Pi20(t)* were commonly high among seven regions including Kenya in this study. However, the frequencies with respect to DVs for other resistance genes differed. These results suggest that *Pi9* is the most stable resistance gene. On the other hand, virulent isolates against the resistance genes *Pia*, *Pii*, *Pi3*, *Pi5(t)*, and *Pi20(t)* were distributed widely in these seven regions. The other virulent isolates for DVs for *Pish*, *Pib*, *Pit*, *Pik-s*, *Pik-m*, *Pil*, *Pik-h*, *Pik*, *Pik-p*, *Pi7(t)*, *Piz*, *Piz-5*, *Piz-t*, *Pita-2*, *Pita*, *Pi12(t)*, and *Pi19(t)* changed among these regions. The frequencies of virulent isolates were compared among the seven regions, and these results indicate the tendencies or differences in the variation of isolates among them, and the importance of using the common DV set.

These isolates from the four regions of Kenya, Cambodia, Japan, Bangladesh, and Western Africa were designated as reaction types for each DV group and race, based on the designation system by Hayashi and Fukuta (2009). Fukuta et al. (2014) analyzed the pathogenicities of 122 isolates from Cambodia and found that U43, U61, U63, i0, i6, i7, k000, k100, z00, z04, z06, ta002, ta033, ta403, ta421, and ta431 were the predominant reaction types. Odjo et al. (2014) clarified the pathogenicities of 96 isolates from Western Africa and found 15 reaction types (U23, U43, U63, i0, i7, k100, k106, k177, z00, z03, z04, ta003, ta031, ta403, and ta431). In Japan, Kawasaki-Tanaka et al. (2016) examined 310 isolates and found 11 predominant

reaction types (U11, U13, U33, i0, i7, k100, k177, z00, z10, ta001, and ta401). In Bangladesh, Khan et al. (2016) investigated the pathogenicities of 331 isolates and found 26 predominant reaction types (U03, U23, U43, U61, U63, U73, i0, i4, i7, z00, z02, z04, z05, z06, z07, z17, k000, k100, k102, k177, ta003, ta023, ta402, ta403, ta423, and ta431). In Kenya, 12 reaction types (U01, U43, U63, i0, i3, i7, k177, z00, z01, ta003, ta403, and ta431) were predominant. Three predominant reaction types, i0 [avirulent for *Pii*, *Pi3*, and *Pi5(t)*], i7 [virulent for *Pii*, *Pi3*, and *Pi5(t)*], and z00 [avirulent for *Pi9*, *Piz*, *Piz-5*, and *Piz-t*], were common to all five regions. These results suggest that rice cultivars harboring the three resistance genes *Pii*, *Pi3*, and *Pi5(t)* would be more vulnerable to an occurrence of virulent blast races compared with cultivars harboring the other genes, whereas an outbreak of virulent races would be more difficult in rice cultivars harboring the four resistance genes *Pi9*, *Piz*, *Piz-5*, and *Piz-t*. These results correspond with the frequencies of virulent isolates for DVs among the five regions. In addition, six predominant reaction types, U43 [virulent for *Pit*, *Pia*, and LTH], U63 [virulent for *Pit*, *Pib*, LTH, and *Pia*], k100 [virulent for *Pik-s*], k177 [virulent for *Pik-s*, *Pik-m*, *Pil*, *Pik-h*, *Pik*, *Pik-p*, and *Pi7(t)*], ta403 [virulent for *Pi12(t)*, *Pi19(t)*, and *Pi20(t)*], and ta431 [virulent for *Pi12(t)*, *Pita(K1)*, *Pita(CP1)*, and *Pi19(t)*], were also found in four regions at least. These results indicate that the virulent races for *Pit*, *Pia*, *Pib*, *Pik-s*, *Pik-m*, *Pil*, *Pik-h*, *Pik*, *Pik-p*, *Pi7(t)*, *Pi12(t)*, *Pita(K1)*, *Pita(CP1)*, *Pi19(t)*, and *Pi20(t)* differentiated widely among them. Therefore, these resistance genes are highly susceptible to the occurrence of virulent races and pose a high risk in rice breeding programs. Among nine common reaction types, eight types were found in Kenya. Thus, predominant reaction types changed among five regions with several common tendencies.

The outbreak of blast races can be explained by the gene-for-gene theory: every resistance gene in the host corresponds to an avirulence gene in the pathogen (Flor 1971; Silué et al. 1992). This means that the virulence of a blast isolate is determined by the genotypes of the resistance genes in the rice cultivar. The complex situation of blast populations might be related to the rice cultivars that have been cultivated in each region. These clarified the high risks for an outbreak of predominant reaction types in Kisumu County and Tana River County, followed by Kirinyaga County/Embu County, where monoculture has been conducted under widespread irrigation schemes using limited types of rice cultivars such as Basmati 217, Basmati 370, and ITA310. These isolates from Kenya were categorized finally into 62 races (Table 2). Race U63-i7-k177-z00-ta003 [virulent for DVs for *Pib*, *Pit*, *Pia*, *Pii*, *Pi3*, *Pi5(t)*, *Pik-s*, *Pik-m*, *Pil*, *Pik-h*, *Pik*, *Pik-p*, *Pi7(t)*, *Pi19(t)*, and *Pi20(t)*] included 19 isolates, whereas other races had few isolates with numbers ranging from 1 to 6. These isolates were collected from monogenic lines as follows: IRBLa-c for *Pia*, IRBLiF5 for *Pii*, IRBL3-CP4 for *Pi3*, IRBLk-Ka for *Pik*, IRBLkm-Ts for *Pik-m*, IRBLkh-K3 for *Pik-h*, IRBL1-CL for *Pil*, IRBL19-A for *Pi19(t)*, and IRBL20-IR24 for *Pi20(t)* and cultivar Basmati 370, and a hybrid progeny line derived from a cross between Habataki and Basmati 370, in Kisumu County. The virulence of race corresponded with the resistance genes in these DVs. This means that Basmati 370 and the hybrid progeny line might harbor some of these resistance genes in their genetic backgrounds, based on the gene-for-gene theory. Thus, the monoculture using limited types of rice cultivars might induce high risks in Kisumu County, Tana River County, and Kirinyaga County/Embu County in Kenya. On the other hand, there were no predominant reaction types with high virulence and high diversities observed in almost all DV groups in Mombasa County, where small irrigated or rainfed paddy fields comprised the rice-growing areas. Therefore, in order to develop a strategy for a stable system of protection against blast fungus, it is necessary to further understand the relationships between blast fungus and host plants in Kenya. Thus, it will also be necessary to identify the resistance genes present in rice cultivars that are growing in farmers' fields.

As the above results indicate, we were able to demonstrate the usefulness of the set of international differential DVs developed by Tsunematsu et al. (2000) and Telebanco-Yanoria et al. (2010) and the

new designation system developed by Hayashi and Fukuta (2009) as common tools for the investigation and pathological analysis of blast isolates and for understanding the differentiation of races on a global level. These findings will be further confirmed as data on the pathogenicity of isolates are accumulated from various countries around the world.

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