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Research Paper

Genetic variation of blast (*Pyricularia oryzae* Cavara) resistance in rice (*Oryza sativa* L.) accessions widely used in Kenya

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A total of 47 rice accessions collected from Kenya were investigated the genetic variations and classified into two cluster groups, A and B, by polymorphism data of 65 simple sequence repeat (SSR) markers. Clusters A and B corresponded to Japonica and Indica Groups, respectively. The number of Japonica Group accessions was limited in comparison with those of the Indica Group. Based on their patterns of reaction to standard differential blast isolates (SDBIs), these accessions and 57 control cultivars including differential varieties and several accessions harboring partial resistance genes were classified again into three cluster groups: Ia (high resistance), Ib (intermediate resistance) and II (susceptible). The rice accessions from Kenya were classified only into groups Ia and Ib. The accessions from Kenya were finally classified into three categories, A-Ia, B-Ia and B-Ib, based on the two classifications of polymorphism of SSR markers and resistance. The Indica Group accessions had wider genetic variation for blast resistance than did the Japonica Group accessions. The three leading cultivars (Basmati 217, Basmati 370 and ITA 310) categorized into Cluster group Ia were susceptible to some SDBIs from Kenya. The genetic variation for blast resistance in Kenya was demonstrated as the first report using SDBIs.

Key Words: genetic variation, resistance, rice (*Oryza sativa* L.), blast (*Pyricularia oryzae* Cavara), Kenya.

Introduction

Rice blast, caused by the pathogen *Pyricularia oryzae* Cavara is one of the most serious diseases affecting rice (*Oryza sativa* L.) worldwide (Zeigler *et al.* 1994). The use of resistant cultivars is the most economical method to control this disease in rice. However, using such cultivars has limited effect owing to the eventual 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 the gene-for-gene theory, Tsunematsu *et al.* (2000) and Telebanco-Yanoria *et al.* (2010) developed several monogenic lines and near isogenic lines (NILs) with the genetic background of a susceptible Japonica Group cultivar, Lijiangxintuanheigu (LTH), as a new set of international differential varieties (DVs). These DVs targeted 23 kinds of resistance genes and made it possible to efficiently characterize the pathogenicity of blast isolates. In Japan, these monogenic lines have been used to clarify the pathogenicities of standard differential blast isolates (SDBIs) selected by Hayashi (2005). Pathological studies of the blast fungus have been performed by using the monogenic lines and have

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been used to select SDBIs in the Philippines (Telebanco-Yanoria *et al.* 2008). SDBIs are a basic tool for genetic analysis for blast resistance in rice, and SDBIs have been used in studies into genetic variation in rice cultivars and in genetic studies of resistance gene(s) across the globe: the Philippines (Telebanco-Yanoria *et al.* 2008), Japan (Kawasaki-Tanaka and Fukuta 2014), West Africa (Odjo *et al.* 2014) and Bangladesh (Khan *et al.* 2016).

There are four main rice cultivation areas in Kenya: Kirinyaga County, Kisumu and Busia Counties, Tana River County, and Kwale, Kilifi, Taita-Taveta and Mombasa Counties. Extensive irrigation schemes have been constructed in Kirinyaga County, Kisumu and Busia Counties, and Tana River County, and small irrigated or rainfed paddy fields are prevalent in Kilifi, Kwale, Taita Taveta and Mombasa Counties.

Rice production in Kenya is low, and this has been attributed to various factors including biotic and abiotic stresses (Onyango 2014). As a biotic stress, blast is the major constraint to rice production in Kenya because of its wide geographic distribution and destructiveness (MOA 2014). Kihoro *et al.* (2013) surveyed blast disease in Mwea (Kirinyaga County) and found it to be widely distributed in this region. In Mwea and in Gamba (Tana River County), several rice cultivars, such as IR 50, Mutant-Z, Tana 1 and UPR-103-80-1-2, were used by local farmers from 1997 to 2009, but since then, ITA 310 and Basmati 370 have been planted mainly at Tana River County and Kiringaya County, respectively. Nyongesa *et al.* (2016) suggested that the continuous cultivation of few kinds of rice cultivars induced the outbreaks of blast disease in wide areas of Kirinyaga County. Kariaga *et al.* (2016) evaluated nine cultivars by inoculation tests using nine blast strains in the fields of Kisumu and Ahero Irrigation Research Station, Kisumu County, and they found that several cultivars including Basmati 370, NERICA 13 and NERICA 14, showed susceptible reactions to some strains, and three cultivars (Dourado Precoce, NERICA 4 and NERICA 9) showed resistance to all. These studies were carried out in field evaluations, and the resistance of rice cultivars was not evaluated in detail using SDBIs to clarify the pathogenicity based on the reaction patterns of differential varieties.

DNA polymorphism data such as simple sequence repeat (SSR) markers have been used for classification of the rice germplasm. Okoshi *et al.* (2004) classified 73 Japanese landraces into Indica and Japonica Groups and into upland and paddy rice subgroups. Yamasaki and Ideta (2013) clarified the differences in genetic diversities in landraces and improved cultivars using 104 accessions. Kawasaki-Tanaka and Fukuta (2014) classified 324 Japanese accessions using 65 SSR markers into Japonica and Indica Groups, and into irrigated lowland and upland groups. Thus, SSR markers are a useful tool for understanding genetic variation and for differentiating between Indica and Japonica Groups or lowland and upland cultivars in rice.

In Kenya, several research for blast fungus were conducted and outbreaks of virulent blast races were reported in wide areas of irrigated lowland, but genetic study into blast resistance in rice germplasm was limited to only a few cultivars, and the details of resistance gene(s) in the genetic backgrounds has not been clarified. As a first step in building up a stable system of protection against blast disease in Kenya, in this study we classified Kenyan rice accessions using SSR markers, and clarified the genetic variation of blast resistance by inoculation tests with SDBIs.

Materials and Methods

Plant materials

A total of 47 local accessions were collected from 2010 to 2015 from farmers' fields in four regions in Kenya: Kirinyaga County, Kilifi County, Kwale County, Taita Taveta County and Tana River County (**Fig. 1**). These accessions included Basmati 217 and Basmati 370, two improved cultivars (IR 2793-80-1 and ITA 310) and landrace cultivars. Masmathi 217 was been grown in Mwea, Kirinyaga County from late 1980s to early 2000s, but it has been replaced by Basmati 370 (**Table 1**). ITA 310 is a major variety at the irrigated lowland rice fields of the Tana and Athi River Development Authority (TARDA), located at the delta of Tana River County. IR 2793-80-1 has been introduced in Mwea in early 1990s, but less popular in the area. This variety is widely cultivated in the irrigation schemes around Lake Victoria in Kenya, such as Kisumu and Busia Counties (**Fig. 1**).

These local accessions were investigated together with 30 differential varieties (DVs) including 26 monogenic lines (Tsunematsu *et al.* 2000) for targeting 23 resistance genes; two near isogenic lines, IRBLkh-K3[LT] for *Pik* and IRBLk-Ka[LT] for *Pik-h*, each with the genetic background of a Chinese Japonica Group cultivar, Lijiangxintuanheigu (LTH) (Telebanco-Yanoria *et al.* 2010); and two NILs; US2NIL*Pi5*(t)-M for *Pi5*(t) and US2NIL*Pi12*(t)-M for *Pi12*(t) with the genetic background of a blast-susceptible Indica Group line, US-2 (unpublished). LTH and US-2 were also used as blast-susceptible control cultivars, and one accession, Basmati 217 conserved in JIRCAS, was also included.

Moreover, Nipponbare, Azucena, Akihikari, Aichi-Asahi, Koshihikari and F3-11, were included as representative cultivars of Japonica Groups. Kasalath, Surjamkuhi and four International Rice Research Institute (IRRI) bred cultivars (IR 8, IR 24, IR 36 and IR 64) were used as Indica Group control cultivars. Also included as controls for blast resistance were Japonica Group accessions harboring partial-resistance genes, such as Owarihatamochi (*pi21*; Fukuoka and Okuno 2001), Hokkai 188 (*Pi38*(t); Nguyen *et al.* 2006), WIL23 (*Pi35*t); https://agriknowledge.affrc.go.jp/RN/2039017308.pdf), Chugoku 40 (*Pi34*; Washio *et al.* 1968), Kahei (*Pi63* = *Pikahei-1*(t); Xu *et al.* 2014) and Hokkai-PL9 (*PiPHL9*; Unpublished). Hokkai-PL9, which was bred as a parental line with cold tolerance (Kuroki *et al.* 2007), has

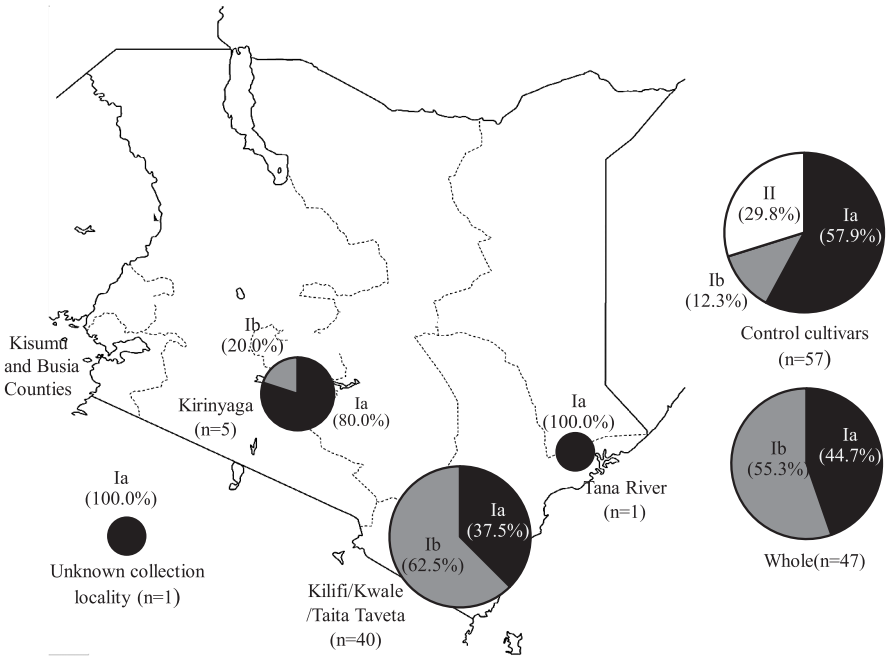

Fig. 1. Distributions of rice accessions classified into three cluster groups in Kenya.

low amylose content (Ando *et al.* 2010) and harbors a partial resistance gene in its genetic background (personal communication with Dr. Ando). An African rice accession, Moroberekan, which has shown high resistance and is a source of several resistance genes, such as *Pi5*(t) and *Pi7*(t), was added. A total of four upland NERICA cultivars; NERICA 1, NERICA 4, NEICA10 and NERICA18, and CG 14 (*Oryza glaberrima*), which was one of the parents of the upland NERICA cultivars, was also investigated.

DNA analysis

To clarify the genetic variation among the rice accessions on the basis of genomic chromosome components, whole-genome DNA was extracted from a young leaf of a rice plant in each accession following the simple DNA extraction method described by Wang *et al.* (1992). Leaf tissue (around 0.25 cm^2) was ground in 100 µl of 0.25 N sodium hydroxide (NaOH) with zirconium beads in 2.0 ml tubes. A volume of 400 µl of 100 mM Tris-HCl (pH 7.5) was added to the grinding tubes. The sample was then mixed and centrifuged for 10 min at 10,000 rpm. The supernatant was recovered by pouring off into fresh 1.5 ml tubes.

The genetic variation was investigated by the polymorphisms of 67 SSR markers (McCouch *et al.* 2002) which were distributed across the 12 rice chromosomes (**Supplemental Table 1**). All SSR markers were selected from a public database (http://www.gramene.org) and was the same set of SSR markers that was used for the classification of Japanese germplasm by Kawasaki-Tanaka and Fukuta (2014). PCR was performed in a 10 µl PCR mixture containing 1 µl sterile H_2O, 1.5 µl of forward (2 µM) and reverse (2 µM) primers of SSR markers, 7.5 µl of 2× Quick Taq TM HS DyeMix (Toyobo Co., Ltd., Japan), and 5 µl DNA concentrated to around 5–10 ng/µl. Polymerase chain reaction (PCR) amplification was carried out with the following profile: 94°C for 2 min, followed by 40 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 68°C. For polymorphism detection, amplified products were separated by electrophoresis on 2% agarose gels in 1× TAE buffer at 150 V for 90–120 min and DNA fragments were detected with ethidium bromide. The polymorphic bands of each line were recorded and compared with the banding patterns of control cultivars Nipponbare, Kasalath, and the others.

Resistance of rice accessions

A total of 15 SDBIs, including ten from Japan (Hayashi 2005), one from the Philippines (Telebanco-Yanoria *et al.* 2008), and four from Kenya (unpublished), were used to characterize the variations of blast resistance in the accessions.

Stock blast isolates were re-cultured from storage on an oatmeal agar plate with streptomycin and allowed to grow for 12 to 13 days at about 25°C. The culture plates were scraped with a toothbrush and then put on a tray covered with wrapping film pitted with several holes and left under a fluorescent light for 4 to 5 days to induce sporulation. Conidia were dislodged from the surface of sporulated plates with a paintbrush into 10 to 20 ml of sterilized distilled water. Spore suspensions were filtered through 4 layers of cheesecloth and spore concentration was adjusted to 1×10^5 conidia per ml using a hemacytometer. Tween 20 was added to 0.01% just before inoculation (Hayashi *et al.* 2009). Plants were grown for 2 weeks in a greenhouse (until the 5- to 6-leaf stage) and were then inoculated by spraying with a

Table 1. Relationship of rice accessions in Kenya and controls between two classifications of polymorpshim of SSR makres and reaction to standard differential blast isolates

Group by polymorphism of SSR markers	No. of rice accessions in Kenya (Control cultivar)			Total
	Resistance group			
	Ia	Ib	II	
A	Basmati 370, Basmati 217 (Kenya) Basmati217 (JIRCAS), Sindano, Matako nyeusi Kanja, NERICA1, NERICA4, NERICA10 NERICA18, Moroberekan Azusena*, Akihikari*, Aichi-asahi* Owarihatamochi (*pi21*)* F3-11*, WIL23 (*Pi35*(t))*, Chugoku40 (*Pi34*)* HokaiPL9 (*PiHPL9*)*, Kahei (*Pi63* = *PiKahei*-1(t))*	Hokkai188 (*Pi38*(t))*	Koshihikari*, Nipponbare*, LTH (None)*	
Sum	20	1	3	24
B	Sigae tune, Mtumbatu, Kichana chawa, Pumba ya muwa, Bibi wa muhaka, Sifara, Moshi, Supa, Ringa, Japan 54, Japan 64, Supa Saro, IR 2793-80-1, ITA 310, Sindano Bahari, Msambweni CG 14 (*O. glaberrima*)* Surjamkuhi*, IR 8*, IR 24*, IR 36*, IR 64*	Abedi, Riziki, Pamba, BW196, Anguri, Chijego, Sigaya, Sigaye, Kitumbo, Kibawa (Bawa la inzi), Pachanga (Supa ya pa), Usinicheleweshe, Uzungu, Macho ya wanda, Sigae nyeupe 2, Sigae nyeupe 1, Sigae nyekundu, Chinga cha mosi, Kigamti, Kijero, Mzungu, Niwahi, Cushe, Pachanga, Pishori, Madevu Kasalath*	US-2 (None)*	
Sum	22	27	1	50
Others	IRBLi-F5 (*Pii*)*, IRBL5-M (*Pi5*(t))*, IRBL9-W (*Pi9*)*, IRBLz-Fu (*Piz*)*, IRBLz5-CA-1 (*Piz-5*)*, IRBLzt-T (*Piz-t*)*, IRBLta2-Pi (*Pita-2*)*, IRBLta2-Re (*Pita-2*)*, IRBLta-K1 (*Pita*)*, IRBLta-CP1 (*Pita*)*, IRBLta-CT2 (*Pita*)*, IRBLsh-S (*Pish*)*	IRBLb-B (*Pib*)*, IRBLt-K59 (*Pit*)*, IRBL3-CP4 (*Pi3*)* IRBL12-M (*Pi12*(t))*, IRBL20-IR24 (*Pi20*(t))*	IRBLsh-B (*Pish*)*, IRBLa-A (*Pia*)*, IRBLks-F5 (*Pik-s*)*, IRBLks-S (*Pik-s*)*, IRBL1-CL (*Pi1*)*, IRBLkh-K3[LT] (*Pik-h*)*, IRBLk-Ka[LT] (*Pik*)*, IRBKkp-K60 (*Pik-p*)*, IRBLkm-Ts (*Pik-m*)*, IRBL7-M (*Pi7*(t))*, US2NIL*Pi12*(t)-M (*Pi12*(t))*, IRBL19-A (*Pi19*(t))* US2NIL*Pi5*(t)-M (*Pi5*(t))*	
Sum	12	5	13	30
Total	54	33	17	104

* Control cultivars.
Under line: Landrace in Kenya.

fresh preparation of conidial suspension. Inoculated plants were incubated for 1 day in an incubator at 25°C and >90% relative humidity and were then transferred to a greenhouse with humidity of approximately 60% and temperature of 25°C to 30°C for 7 days.

At 7 days after inoculation, disease reactions of the inoculated plants were scored from 0 (resistant) to 5 (susceptible) using the 6-scale rating system of Hayashi *et al.* (2009).

Statistical analysis

Cluster analyses were performed by Ward's hierarchical method (Ward 1963) with the software JMP version 11.2 for Windows (2014; SAS Institute, Inc., Cary, NC, USA), based on the polymorphism data of the SSR markers and the reactions to 15 SDBIs among the rice accessions and controls.

Results

Classification of rice accessions based on the polymorphism data of SSR markers

A total of 67 SSR markers were used, and 65 of them showed polymorphisms (**Table 2**). The number of SSR markers used ranged from two to nine and total alleles of SSR markers varied from five to 31 among 12 rice chromosomes. Alleles per SSR marker varied from 2.0 to 4.7 among them, and 202 alleles were detected, finally.

A total of 74 rice accessions including control cultivars were classified into two cluster groups, A and B, including 24 and 50 accessions, respectively, based on the polymorphism data of SSR markers (**Table 1**, **Supplemental Table 2**, **Supplemental Fig. 1**). Cluster A included Japonica

Table 2. SSR markers used for polymophism analysis and No. of alleles detected in each chromosome

Chromosome	1	2	3	4	5	6	7	8	9	10	11	12	Total or Mean
NO. of SSR markers used	7	9	6	6	7	5	4	8	2	4	3	4	65
No. of alleles detected	22	31	19	23	21	15	8	23	5	9	14	12	202
Range of alleles	(2–5)	(2–4)	(2–4)	(3–5)	(2–6)	(2–4)	(2)	(2–4)	(2–3)	(2–3)	(4–5)	(3)	(2–6)
Mean numbers of alleles per SSR marker	3.1	3.4	3.2	3.8	3.0	3.0	2.0	2.9	2.5	2.3	4.7	3.0	3.1

Table 3. Reaction of rice groups classified by reaction patterns to satndard differential blast isolates

Cluster group (No. of accessions)	Infection score																	
	Standard Differential Blast isolate																	
	Country																	
	Japan											Philippines	Kenya					Over all mean
	Ai79-142	Mu-95	GFOS8-1-1	Sasamori121	IW81-04	Ai74-134	Ina93-3	Kyu92-22	31-4-151-11-1	Ken53-33	Mean	M64-1-3-9-1	Ke842-4(a)	Ke873-1a	Ke937(b)	Ke936(a)	Mean	
Ia (54)	1.5	0.8	0.9	1.2	1.1	1.9	1.5	1.5	0.7	1.3	1.2	1.1	1.3	1.6	1.4	1.3	1.4	1.3
Ib (33)	1.9	1.1	1.0	1.2	1.2	2.1	1.3	1.7	1.6	1.4	1.5	1.7	3.3	1.7	4.2	3.7	3.2	1.9
II (17)	4.6	2.6	2.3	4.3	3.9	4.2	2.9	4.0	2.8	3.7	3.5	3.2	3.7	4.0	2.7	2.7	3.3	3.4
Total (104)	2.2	1.2	1.2	1.8	1.6	2.4	1.7	2.0	1.4	1.7	1.7	1.8	2.3	2.1	2.5	2.3	2.2	1.9

Group control cultivars, such as Nipponbare, Koshihikari, LTH and four upland NERICAs. An African cultivar, Moroberekan, three Basmati cultivars (Basmati 217 [Kenya], Basmati 217 [JIRCAS] and Basmati 370), and three other accessions collected in Kenya were also classified into this cluster. Cluster group B included Indica Group control cultivars, such as Kasalath, IR 8, IR 24, IR 36 and IR 64. The Indica Group cultivars IR 2793-80-1, ITA310 and US-2 (susceptible line for blast resistance, unpublished material), and an *O. glaberrima*, CG 14, were also classified into this cluster. These results indicate that clusters A and B corresponded respectively to Japonica and Indica Groups.

Resistance of rice accessions to blast isolates

A wide variation in resistance to 15 standard differential blast isolates (SDBIs) was found in the rice accessions from Kenya, control cultivars, and differential varieties. Based on their patterns of reaction to these SDBIs, these accessions were classified into three cluster groups, Ia, Ib and II (**Tables 1**, **3**, **Supplemental Table 2**, **Supplemental Fig. 2**).

The degrees of resistance were different among the three groups (**Table 3**, **Supplemental Table 2**). The accessions of Group Ia showed resistance to almost all SDBIs. The mean infection scores with respect to SDBIs from Japan, the Philippines and Kenya were 1.2, 1.1 and 1.4, respectively; the overall mean was only 1.3. Group Ib also showed low mean infection scores with respect to the isolates from Japan and the Philippines (1.5 and 1.7, respectively), but the mean infection scores with respect to the isolates from Kenya was 3.2. The overall mean was 1.9. The reaction to SDBIs from Kenya was remarkably different from Group Ia. The accessions in Group II were susceptible to almost all SDBIs and showed high mean infection scores. The mean scores with respect to isolates from Japan, the Philippines and Kenya were 3.5, 3.2 and 3.3, respectively, and the overall mean was 3.4. These results indicate that Clusters Ia and II indicated resistant and susceptible groups, respectively, and Group Ib was intermediate between Ia and II with specific susceptible reactions with respect to SDBIs from Kenya. The mean infection scores among all accessions were 1.7 with respect to Japanese SDBIs, 1.8 with respect to the Philippine SDBI, and 2.2 with respect to the Kenyan SDBIs. The results indicate that the virulence of SDBIs from Kenya was higher than the virulence of SDBIs from Japan and the Philippines.

Rice accessions from Kenya were categorized into Groups Ia and Ib, but not Group II. Group II included only 17 accessions: 13 DVs for *Pish* (IRBLsh-B), *Pia*, *Pik-s* (two lines), *Pi1*, *Pik-h*, *Pik*, *Pik-p*, *Pik-m*, *Pi7*(t), *Pi12*(t) (US2NIL*Pi12*(t)-M, *Pi19*(t) and *Pi5*(t) (US2NIL*Pi5*(t)-M); two susceptible cultivars, LTH and US-2; and two control cultivars, Koshihikari and Nipponbare (**Table 1**). A total of

54 accessions were classified into high-resistance Group Ia including 15 control cultivars, 27 accessions from Kenya, and 12 DVs for *Pii*, *Pi5*(t) (IRBL5-M), *Pi9*(t), *Piz*, *Piz-5*, *Piz-t*, *Pita-2* (two lines), *Pita* (three lines) and *Pish* (IRBLsh-S). The controls included four cultivars harboring partial resistance genes *pi21*, *Pi34*, *Pi38*(t) and Hokkai-PL9 (*PiPHL9*); four IRRI bred cultivars (IR 8, IR 24, IR 36 and IR 64); and *O. glaberrima* (CG 14). Basmati 217 and Basmati 370, four upland NERICAs, IR 2793-80-1 and ITA 310 were also included as the rice accessions from Kenya. Group Ib included a total of 27 accessions from Kenya; five DVs for *Pib*, *Pit*, *Pi3*, *Pi12*(t) (IRBL12-M), and *Pi20*(t); and two control cultivars, Hokkai 188 (harboring *Pi35*(t)) and Kasalath. These results indicate that DVs for blast resistance genes and the other control cultivars were classified into three groups and showed a wide variation, but rice accessions from Kenya were limited to the high-resistance (Ia) and intermediate-resistance groups (Ib). Landraces in Kenya were included in cluster groups Ia and Ib, but improved type's cultivars were mainly categorized into cluster Ia. IRBL5-M and IRBLsh-S were classified into Group Ia, and IRBL12-M was classified into Group Ib. The other DVs for these resistance genes—IRBL*sh*-B, US2NIL*Pi12*(t)-M, and US2NIL*Pi5*(t)-M—were categorized into Group II and showed different reactions from the former three DVs. These results suggested that IRBL5-M, IRBLsh-S, and IRBL12-M might harbor additional resistance gene(s) in their genetic backgrounds.

We tried to estimate the genotypes of resistance in four major cultivars in Kenya—Basmati 217, Basmati 370, IR 2793-80-1 and ITA 310—based on their reaction patterns to 15 SDBIs from Japan, the Philippines and Kenya. Basmati 217 and Basmati 370 were estimated to harbor *Pi20*(t) and one of the *Pik* allele genes or *Pi3*. ITA 310 was estimated to harbor one of *Pib* or *Pi12*(t), one of *Pik-m* or *Pi1*, and one or more unknown genes. IR 2793-80-1 showed resistance to all SDBIs and could not be estimated. In the other accessions, *Pia*, *Pish*, *Pit*, one of *Pi3* or *Pi5*(t), one of *Pik* allele genes, and one of *Pita-2*, *Pi12*(t), *Pi19*(t), or *Pi20*(t) were also estimated (**Supplemental Table 2**). These results indicate that there were only limited kinds of resistance genes harbored in the rice accessions.

Relationships between cluster groups of DNA marker polymorphism and resistance

Based on the classifications of SSR marker polymorphisms and resistance to blast isolates, the rice accessions from Kenya were classified into only categories A-Ia, B-Ia and B-Ib, and there was no accession in the other six categories (**Table 1**, **Supplemental Table 2**). Cluster group A corresponded to Japonica Group rice, and Group A-Ia comprised 20 accessions including four upland NERICAs, Moroberekan, two Basmati 217 (Kenya and JIRCAS), Basmati 370 and five control cultivars harboring partial resistance genes: Owarihatamochi (*pi21*), WIL23 (*Pi35*(t)), Chugoku 40 (*Pi34*), HokaiPL9 (*PiPHL9*(t)), and Kahei (*Pi63*). Cluster group B corresponded to Indica Group rice. Group B-Ia included 16 accessions from Kenya including two improved accessions, IR 2793-80-1 and ITA 310, and CG 14 (*O. glaberrima*); Surjamkuhi and four IRRI bred cultivars (IR 8, IR 24, IR 36 and IR 64) were also included as Indica Group control cultivars. A total of 26 accessions from Kenya were classified into Group B-Ib; the control cultivar Kasalath was also included.

Hokkai 188 harboring *Pi35*(t) was categorized into Group A-Ib, and three Japonica Group controls, Koshihikari, Nipponbare and LTH, were categorized into Group A-II. US-2 as the Indica Group's susceptible control was categorized into Group B-II.

These results indicate that the genetic variation of blast resistance in the Indica Group was wider than that of the Japonica Group in Kenya.

Discussion

A total of 47 rice accessions from Kenya were classified into two cluster groups, A and B, corresponding to Japonica and Indica Groups, respectively. Only nine Kenyan accessions were categorized into Group A, and the other 38 were categorized into Group B. The nine accessions in Group A were all categorized into the high-resistance cluster group Ia, and the other 38 accessions in Group B were divided into Groups Ia (high resistance) and Ib (intermediate resistance) which showed susceptible reactions to blast isolates from Kenya (**Table 1**). These results indicate that although the numbers and variations of Japonica Group cultivars were limited, and that Indica Group cultivars were basically cultivated with a wider genetic variation of blast resistance in comparison with the Japonica Group in Kenya.

In Kenya, four major cultivars—Basmati 217, Basmati 370, IR 2793-80-1 and ITA 310—have been cultivated continuously over wide areas of irrigated lowland, such as Kirinyaga County, Kisumu and Busia Counties and Tana River County. And high-resistance cultivars categorized into cluster group Ia were dominantly used in Kirinyaga County and Tana River County. In the contrast, intermediate resistance, cluster group Ib was dominant in Kilifi, Kwale, Taita Taveta Counties where small irrigated or rainfed paddy fields are prevalent (**Fig. 1**). Nevertheless, serious infections had already been reported in Basmati 217, Basmati 370 and ITA 310, and had become a big problem for rice cultivation in Kenya (Kariaga *et al.* 2016, Kihoro *et al.* 2013). The genotypes of resistance in these cultivars were estimated based on the reaction patterns to SDBIs. Basmati 217 and Basmati 370 harbored *Pi20*(t) and one of *Pik* allele genes or *Pi3*, and ITA 310 harbored one of *Pib* or *Pi12*(t), one of *Pik-m* or *Pi1*, and one or more unknown genes (**Supplemental Table 2**). Even if Basmati 217, Basmati 370 and ITA 310, were categorized into high resistance cluster group Ia, virulent blast races were outbroken against these resistance genes in these rice cultivars in Kiringaya County and Tana River County, where the extensive irrigation schemes were

constructed and monoculture using a few numbers of rice cultivars have been conducted.

Nyongesa *et al.* (2016) suggested that the continuous cultivation of limited kinds of rice cultivars induced the outbreaks of virulent blast fungus. The dominant blast race(s) which are virulent with respect to these resistance genes might be distributed in the irrigated lowlands of Kenya. Clarifying the resistance gene(s) in the genetic backgrounds of these cultivars by genetic analysis and clarifying the distributions of dominant blast races in the rice cultivation areas will be necessary to understand the differentiation and relationships between blast races and resistance in rice cultivars. IR 2793-80-1 and several other accessions showed resistance to all SDBIs, and these are of interest as a source of genetic material for breeding rice varieties resistant to blast disease.

Kariaga *et al.* (2016) reported the susceptibility or resistance to blast disease in several rice cultivars based on field observations in Kenya. However, the numbers of rice cultivars in those studies were quite limited. This study is the first to differentiate rice accessions collected from across a wide area of Kenya into Japonica and Indica Group cultivars based on the polymorphism data of DNA markers and the first to use SDBIs to clarify their resistance to blast. The findings of this study will be used as basic information for building up a stable system of protection against blast disease in Kenya.

Author Contribution Statement

Y. Fukuta, R. Osawa, S. Yanagihara and D. Makihara conducted the planning of study and writing for the manuscript together. D. Makihara, Y Fukuta, M. Obara and N. Hayashi collected the rice samples in Kenya, and T. Suzuki and A. Tomita did the research works and data arrangements.

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