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Research Paper

Genetic variation in blast resistance in rice germplasm from West Africa

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The genetic variation in resistance to blast (*Pyricularia oryzae* Cavara) in 195 rice accessions comprising 3 species of the AA genome complex (Asian rice [*Oryza sativa* L.], African rice [*Oryza glaberrima* Steud.] and wild rice [*Oryza barthii*]) was investigated based on their patterns of reaction to standard differential blast isolates (SDBIs) and SSR marker polymorphism data. Cluster analysis of the polymorphism data of 61 SSR markers identified 3 major clusters: cluster A (mainly Japonica Group or upland accessions), cluster B (mainly Indica Group or lowland accessions) and cluster C (*O. glaberrima* and *O. barthii*). The accessions were classified again into 3 resistance groups based on reactions to SDBIs: group Ia (susceptible), group Ib (middle resistance) and group II (high resistance). Group Ia included only a few differential varieties, susceptible controls and the Japonica Group cultivar Nipponbare. Accessions in clusters A and B included all 3 resistance groups and showed a wide variation in blast resistance, but cluster C contained only group Ib. These results demonstrated that variations in Asian rice (*O. sativa*) accessions in West Africa were skewed toward high resistance and that variations in *O. glaberrima* and *O. barthii* were limited and lower than the Asian rice accessions.

Key Words: blast (*Pyricularia oryzae* Cavara), genetic variation, resistance, rice, West Africa.

Introduction

Blast disease caused by the fungal pathogen *Pyricularia oryzae* Cavara is one of the most serious rice diseases worldwide (Valent *et al*. 1991), significantly damaging rice production. Development of cultivars resistant to blast is considered the most effective strategy for protecting the crop, and in West Africa, it is the most economical and effective way of controlling rice blast in the fields of resource-poor farmers (Séré *et al*. 2007). Unfortunately, effective and durable use of blast resistance is limited, because of breakdowns in resistance due to the occurrence of virulent races (Koizumi 2008, Zhou *et al*. 2007).

The interaction between host resistance and fungus virulence can be explained by the gene-for-gene theory: i.e., for every resistance gene in the host, there is a corresponding avirulence gene in the pathogen (Flor 1971, Silue *et al*. 1992). On the basis of the gene-for gene theory, Tsunematsu *et al*. (2000) developed 23 monogenic lines and Telebanco-Yanoria *et al*. (2010) developed 2 near-isogenic lines (NILs), each with the genetic background of a susceptible Japonica Group cultivar, Lijiangxintuanheigu (LTH), as a new set of international differential varieties (DVs). This set of DVs targets 23 different resistance genes as follows: monogenic lines IRBLsh-B for *Pish*, IRBLt-K59 for *Pit*, IRBLb-B for *Pib*, IRBLa-A for *Pia*, IRBLi-F5 for *Pii*, IRBL3-CP4 for *Pi3*, IRBL5-M for *Pi5*(t), IRBLks-F5 for *Pik-s*, IRBLkm-Ts for *Pik-m*, IRBL1-CL for *Pi1*, IRBLkp-K60 for *Pik-p*, IRBL7-M for *Pi7*(t), IRBL9-W for *Pi9*(t), IRBLz-Fu for *Piz*, IRBLz5-CA-1 for *Piz-5*, IRBLzt-T for *Piz-t*, IRBLta2-Re and IRBLta2-Pi for *Pita-2*, IRBL12-M for *Pi12*(t), IRBLta-K1 and IRBLta-CP1 for *Pita*, IRBl19-A for *Pi19*(t) and IRBL20-IR24 for *Pi20*(t) (Tsunematsu *et al*. 2000); and 2 NILs IRBLkh-K3[LT] for *Pik-h* and IRBLk-K[LT] for *Pik* (Telebanco-Yanoria *et al*. 2010). This set of DVs has made it possible to efficiently characterize the pathogenicity of blast isolates. In Japan, for example, the monogenic lines have been used to clarify the pathogenicities of standard differential blast isolates (SDBIs) selected by Hayashi (2005). In West Africa, on the other hand, pathological studies of blast fungus published so far

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have only been performed by using Asian DVs harboring few known resistance genes or by using NILs with a CO39 genetic background (Mackill and Bonman 1992). Those studies focused mainly on nursery trap analysis in the screening of sites for durable resistance (Nutsugah *et al*. 2008, Séré *et al*. 2004) and did not select any SDBIs. We also collected blast isolates from West Africa and used the monogenic lines and LTH NILs to clarify the diversity of blast races (Odjo *et al.* 2014) and to select SDBIs (unpublished).

Two species of cultivated rice have been independently domesticated: Asian rice (*Oryza sativa* L.) in Asia and African rice (*Oryza glaberrima* Steud.) in Africa (Second 1982). Asian cultivated rice is widely distributed from tropical to temperate zones and grows under various conditions of water availability (Morishima *et al*. 1992). It contains a number of groups, including the Japonica Group and the Indica Group, and may have been domesticated from the wild progenitor *Oryza rufipogon* approximately 9000 years ago (Chang 1976). African rice, grown primarily in tropical West Africa (Mohapatra 2010), was domesticated in West Africa more than 3500 years ago (Angladette 1966, Portères 1956). Asian rice cultivars were introduced into West Africa by the Portuguese as early as the middle of the 16th century (Linares 2002). Thus, in comparison with Asian rice in Asia, the history of Asian and African rice cultivated in West Africa is short and the areas under cultivation are limited.

However, African rice is different from its Asian counterpart in many qualitative and quantitative aspects (Vaughan *et al*. 2008), and importantly, African rice has many unique and useful traits such as weed competitiveness, tolerance to various abiotic stresses (acidity, salinity and drought) and resistance to diseases/pests. It has also been established that related rice species, such as *Oryza barthii* and *Oryza longistaminata*, belonging to the *Oryza* AA genome complex contain a high level of diversity (Sarla and Swamy 2005). Indigenous African rice species have acquired adaptive or protective mechanisms against many of the major biotic and abiotic stresses during their evolution and would be a rich reservoir of useful genes for resistance and/or tolerance to many of these stresses (Linares 2002). In this regard, assessment of the genetic diversity and population structure of germplasm collections, mainly *O. glaberrima* germplasm, is an essential component for crop improvement in this tropical area. Rice breeders have combined these features with the high yields of Asian rice to develop a series of popular hybrid varieties known as New Rice for Africa (NERICA) that are high-yielding, drought- and pest-resistant, and adapted to the growing conditions of West Africa (Sarla and Swamy 2005). Jones *et al*. (1997) bred 18 rainfed upland NERICA cultivars, and Sie *et al*. (2008) released 61 lowland NERICA cultivars as interspecific cross progenies between Asian rice and African rice. However, there is little detailed information on the genetic diversity and variation in blast resistance among rice of the AA genome complex, such as Asian rice (*O. sativa*), African rice (*O. glaberrima)*, its wild relative (*O. barthii)*, and inter-species hybrid cultivars (NERICAs) grown or bred in West Africa.

Molecular markers can be used to reveal differences between accessions at the DNA level and thus provide a more direct, reliable, and efficient tool for analysis of genetic diversity, and many studies have been conducted to evaluate genetic diversity among rice cultivars by using microsatellite (SSR) markers (e.g., Agnoun *et al*. 2012, Chen *et al*. 1997, Ndjiondjop *et al*. 2010, Semagn *et al*. 2007, Temnykh *et al*. 2000).

In this study, we used SSR markers to investigate the genetic diversity in rice accessions of the AA genome complex, such as *O. sativa* including inter-species hybrids, NERICA varieties, *O. glaberrima* and *O. barthii*, in West Africa. We then evaluated the blast resistance of these accessions and clarified the genetic variation using SDBIs from Japan and West Africa. Moreover, we elucidate the relationships between the blast resistance and the genome chromosome components of the rice accessions and discuss the diversity of germplasm of the rice AA genome complex.

Materials and Methods

Plant materials

A total of 164 West African accessions were used. These included 56 Asian rice cultivars (*O. sativa* L, AA) (34 from lowland areas and 22 from upland areas), 45 African rice accessions (*O. glaberrima*, AA) (37 from lowland areas and 8 from upland areas), 58 NERICA cultivars (18 for upland ecosystems [Jones *et al*. 1997] and 40 for lowland ecosystems [Sie *et al.* 2008]), and 5 wild rice accessions (*O. barthii*, AA) from upland areas. These accessions were investigated together with 23 monogenic lines (Tsunematsu *et al*. 2000) for 21 resistance genes and 2 NILs for *Pik* and *Pik-h*, each with a Chinese Japonica Group cultivar, Lijiangxintuanheigu (LTH), genetic background (Telebanco-Yanoria *et al*. 2010) and 2 NILs for *Pi5*(t) and *Pi12*(t) with a blast-susceptible Indica Group US-2 genetic background (unpublished) targeting a total of 23 resistance genes as the differential varieties (DVs); Nipponbare as representative of the Japonica group and Kasalath as representative of the Indica Group; and LTH and US-2 as the blast-susceptible control cultivars (**Table 1**).

DNA analysis

To clarify the genetic variation among the 164 accessions and the 27 DVs, 2 susceptible controls and 2 Indica and Japonica Group controls, on the basis of genomic chromosomes, whole-genome DNA was extracted from a young leaf of a rice plant in each accession. DNA extraction followed the simple method described by Wang *et al*. (1992), and the genetic variation in chromosome components was investigated by the polymorphisms of 64 SSR markers distributed across the 12 rice chromosomes (McCouch *et al.* 2002). All SSR markers were selected from a public

Table 1. Number of rice accessions from West Africa

Ecosystem for rice cultivation	No. of accessions					
	Species					
	O. sativa			*O. glaberrima*	*O. barthii*	Total
	Cultivar	NERICA	Sum			
Lowland	34	40	74	37	0	111
Upland	22	18	40	8	5	53
Sum	56	58	114	45	5	164
Differential varieties	27	0	27	0	0	27
Susceptible controls (LTH and US-2)	2	0	2	0	0	2
Indica and Japonica Group controls (Kasalath and Nipponbare)	2	0	2	0	0	2
Total	87	58	145	45	5	195

NERICA: Cultivar with chromosome segments from *O. glaberrima* introduced into *O. sativa* genetic background.
Differential varieties: 23 monogenic lines (Tsunematsu *et al.* 2000), 2 LTH NILs (Telebanco-Yanoria *et al.* 2010) and 2 US-2 NILs (unpublished) targeting 23 resistance genes were included.

database (http://www.gramene.org). We used the same set of SSR markers that was used for the classification of Japanese germplasm by Kawasaki-Tanaka and Fukuta (2014).

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. 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. 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 and Kasalath. The numbers of alleles were counted as the different polymorphism patterns in each SSR marker among rice accessions.

Resistance of rice accessions

A total of 32 SDBIs, including 20 from Japan (Hayashi 2005) and 12 from West Africa (unpublished), were used to characterize the diversity of blast resistance in the accessions.

Stock blast isolates were re-cultured from storage on an oatmeal agar plate with streptomycin, and were allowed to grow at about 25°C for about 12–13 days. To induce sporulation, 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 4–5 days. 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). Rice plants were grown for 2 weeks in a greenhouse (until 5- to 6-leaf stage) and were then inoculated by spraying them with a 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 7.02 for Windows, on the basis of the polymorphism data of the SSR markers and the reactions to 32 standard differential blast isolates among the rice accessions and controls.

Results

Classification by SSR markers

In total, 67 SSR markers were used, and 61 of them showed polymorphisms among the rice accessions and check cultivars. A total of 133 alleles were detected by the SSR markers. The numbers of alleles per locus varied from 1.2 to 1.8 among the 12 chromosomes, with an average of 1.6 per locus.

The cluster analysis showed significant genetic variation among rice accessions, and the genetic distance ranged from 0.0 to 26.7. At a genetic distance of 13.6, the cluster analysis revealed 3 major groups, A, B and C, in the West African rice accessions (**Fig. 1**, **Table 3**). Group A was divided again into 2 subgroups, A1 and A2. Subgroup A1 contained 2 accessions of wild rice (*O. barthii*), 25 DVs and 2 controls (LTH and Nipponbare); and subgroup A2 contained 37

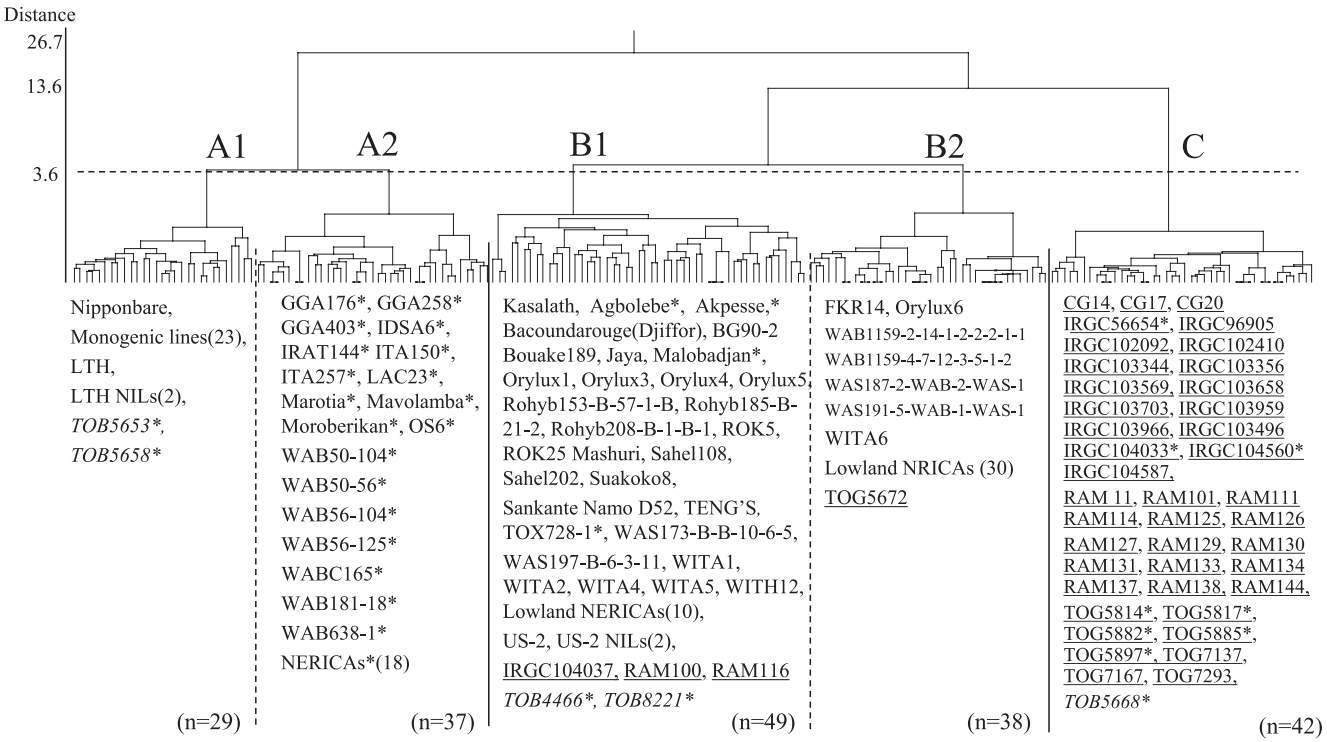

Fig. 1. Classification of rice accessions from West Africa based on the polymorphism data of DNA markers. A total of 164 rice accessions from West Africa, differential varieties for blast resistance genes (including 23 monogenic lines [Tsunematsu *et al.* 2000], 2 LTH NILs [Telebanco-Yanoria *et al.* 2010] and 2 US-2 NILs [unpublished materials]) and 2 blast-susceptible controls (US-2 [unpublished material] and LTH), were used. Nipponbare and Kasalath were also used as controls for the Japonica and Indica Group cultivars, respectively. *, Upland accessions; Underline, *O. glaberrima*; Italic, *O. barthii*. ( ): No. of accessions.

accessions including 18 upland NERICAs and 19 upland cultivars including parents of upland NERICAs. The accessions in group A consisted mainly of upland type cultivars and wild rice and corresponded to the Japonica Group.

Group B was the largest cluster, comprising 87 accessions and containing the Indica Group control Kasalath, the Indica Group susceptible control US-2, 2 NILs for *Pi5*(t) and *Pi12*(t) with US-2 genetic background and 40 lowland NERICAs with an Indica Group cultivar IR 64 genetic background. This group was further divided into 2 subgroups, B1 and B2. Subgroup B1 contained 49 accessions consisting of 27 lowland cultivars, 3 upland cultivars, 10 lowland NERICAs, 3 lowland *O. glaberrima* accessions, 2 upland *O. barthii* accessions, Kasalath, US-2 and the 2 DVs with US-2 genetic background. Subgroup B2 contained 38 accessions including 7 lowland cultivars, 30 lowland NERICAs and 1 lowland *O. glaberrima*. Thus, group B corresponded to the Indica Group, and contained mainly lowland accessions.

The third group, group C, contained 42 accessions, and almost all were *O. glaberrima* (41), and the single remaining accession was an upland *O. barthii*.

These results indicated that groups A, B and C corresponded respectively to the Japonica Group, Indica Group and to African rice (*O glaberrima/O. barthii*). The average number of alleles per locus of SSR markers in groups A, B, and C were 1.6, 1.8 and 1.3, respectively, among rice accessions.

Genetic variation of resistance

Rice accessions and check cultivars were classified again into 3 groups, Ia, Ib and II, based on the patterns of reaction to 20 SDBIs from Japan and 12 SDBIs from West Africa (**Fig. 2**, **Tables 2**, **3**).

The plants classified into group Ia were the most susceptible, and included only the 5 DVs for *Pia*, *Pi19*(t), *Pik-s*, *Pish* and *Pi12*(t) (IRBL12-M[US]) and 3 controls (US-2, LTH and Nipponbare). The mean infection scores of the 8 members of group Ia varied from 1.3 to 4.5 with respect to all 32 blast isolates, and the mean score for isolates from West Africa was 3.4, the average for isolates from Japan was 3.8, and the overall mean was 3.7. The reaction scores for the DV for *Pish* and Nipponbare (which harbors *Pish* in its genetic background) with respect to blast isolates from Japan were remarkably higher than with isolates from West Africa.

The average values in group Ib varied from 0.7 to 3.3 with respect to all SDBIs, and the overall average was 1.7. A total of 80 members fell into this group, including 20 DVs for resistance genes *Pib*, *Pit*, *Piz*, *Piz-5*, *Pii*, *Pi3*, *Pi5*(t) (2 lines), *Pik-h*, *Pik*, *Pik-m*, *Pik-p*, *Pi1*, *Pi7*(t), *Pi12*(t), *Pi20*(t), *Pita* (2 lines) and *Pita-2* (2 lines), the Indica Group control

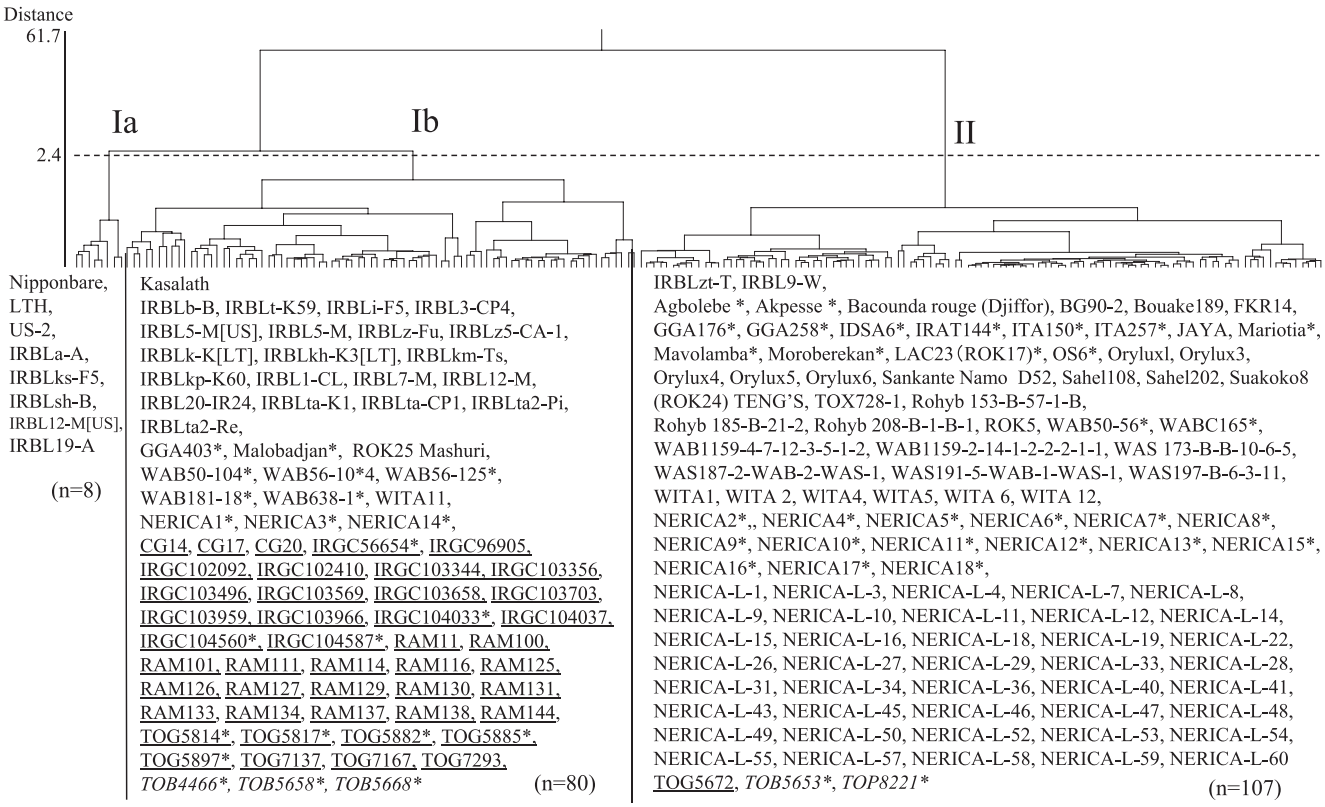

Fig. 2. Classification of rice accessions from West Africa based on the reactions to standard differential blast isolates. A total of 164 rice accessions from West Africa, differential varieties for blast resistance genes (including 23 monogenic lines [Tsunematsu *et al.* 2000], 2 LTH NILs [Telebanco-Yanoria *et al.* 2010] and 2 US-2 NILs [unpublished materials]); and 2 blast-susceptible controls (US-2 [unpublished material] and LTH), were used. Nipponbare and Kasalath were also used as controls for the Japonica and Indica Group cultivars, respectively. *, Upland accessions; Underline, *O. glaberrima*; Italic: *O. barthii.*

Kasalath, 4 lowland cultivars, 5 upland cultivars, 3 upland NERICAs (NERICA1, NERICA3 and NERICA14), 44 *O. glaberrima* and 3 *O. barthii*. Among these, the reaction scores of Kasalath and DVs for *Pib*, *Pit*, *Pi5*(t), *Pi12*(t) and *Pi20*(t) with respect to blast isolates from Japan were remarkably lower than with respect to those from West Africa.

The members of group II showed resistance to almost all SDBIs, and this group showed the highest resistance among the three cluster groups. A total of 107 accessions including 47 cultivars, 15 upland NERICAs, 40 lowland NERICAs, 1 *O. glaberrima*, 2 *O. barthii* and 2 DVs for *Pi9* and *Piz-t* were classified into this group. The mean infection score varied from 0.0 to 2.5 with respect to all blast isolates, and the overall mean was 0.4.

Group Ia was the most susceptible, group II was most resistant among all 3 groups, and group Ib was of intermediate resistance between Ia and II and had specific reactions which showed resistant and susceptible to SDBLs.

Relationships between resistance groups and polymorphism groups

The relationships between the 5 polymorphism cluster groups and the 3 resistance cluster groups of the accessions were evaluated. Of the 15 possible combinations of subclusters, no accessions fell into the 4 subcluster combinations A2–Ia, B2–Ia, C–Ia or C–II (**Table 3**).

Subcluster A1–Ia included Nipponbare, LTH, and 4 DVs for *Pia*, *Pik-s*, *Pish* and *Pi19*(t), and was classified as the most susceptible group. Subcluster A1–Ib included 1 *O. barthii* and 19 DVs for *Pib*, *Pit*, *Pii*, *Pi3*, *Pi5*(t), *Piz*, *Piz-5*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pi1*, *Pi7*, *Pi12*(t), *Pi20*(t), *Pita* (2 lines) and *Pita-2* (2 lines). Subcluster A1–II included 1 *O. barthii* and 2 DVs for *Piz-t* and *Pi9*. Subclusters A2–Ib and A2–II comprised 7 and 30 accessions, respectively, including upland NERICAs. Subcluster B1–Ia contained only 2 members, US-2 and the NS-2 NIL for *Pi12*(t). Subcluster B1–Ib included Kasalath, 2 lowland accessions, 1 upland accession, US-2 NIL for *Pi5*(t), 3 *O. glaberrima* and 1 *O. barthii.* Subcluster B1–II had 35 lowland accessions including 10 lowland NERICAs, in addition to 2 upland accessions and 1 *O. barthii*. Subcluster B2–Ib had only 2 lowland accessions. Subcluster B2–II included in 5 lowland accessions, 30 lowland NERICAs and 1 *O. glaberrima*. Subcluster C–Ib contained a total of 41 *O. glaberrima* and 1 *O. barthii*.

Oryza sativa accessions including NERICAs were categorized into both groups A and B; almost all were included in group II and the numbers in Ib were limited. For example, upland NERICA1, NERICA3 and NERICA14, were

Table 2. Reactions (infection scores) of rice accessions from West Africa and controls with respect to standard differential blast isolates from Japan and West Africa

Resistant group	Accession name	Reaction to standard diffrential blast isolates																																			
		Japan																					West Africa														
		Ai79-142	Mu95	GFOS8-H	Kyu89-246	Sasamori121	IW81-04	Ai74-134	24-22-1-1	TH68-140	Ina93-3	Kyu92-22	TH68-126	Ina86-137	31-4-151-11-1	Ken53-33	1804-4	P-2b	0528-2	Ao92-06-2	Mu183	Mean	Ma56	Ni2	CI9	Ma37	CI14	Ma29	60	43	32	53	9	Bn45	Mean	Overall mean
Ia (n = 8)	Nipppbare	4.5	4.0	3.7	4.5	4.2	3.5	3.7	4.0	4.3	4.5	4.8	4.2	4.0	4.2	4.2	4.7	1.3	3.5	4.2	3.2	4.0	0.2	3.8	0.8	0.7	0.3	1.5	1.2	0.3	1.5	3.2	0.8	1.3	2.0	3.0
	IRBL19-A	3.5	3.0	4.5	3.0	3.5	3.0	3.5	3.0	3.0	4.0	5.0	3.0	4.0	4.5	3.5	3.0	0.0	4.0	5.0	3.0	3.5	5.0	3.5	3.0	3.0	3.0	3.0	4.0	5.0	5.0	3.0	3.0	3.5	3.7	3.5
	IRBLa-A	3.0	0.5	3.0	5.0	3.5	3.5	3.0	5.0	1.0	2.0	5.0	3.5	4.0	3.5	5.0	0.0	0.5	3.5	3.0	3.5	3.1	5.0	4.5	4.5	3.0	4.5	3.0	3.0	2.0	5.0	5.0	4.0	4.0	3.4	3.4
	IRBLks-F5	3.0	4.0	5.0	3.0	3.0	4.5	5.0	3.5	5.0	5.0	4.0	4.5	4.5	4.0	5.0	4.5	1.5	3.0	3.0	3.0	3.9	4.0	4.0	5.0	3.0	3.5	3.0	4.5	3.0	5.0	4.5	3.0	3.0	3.6	3.9
	IRBLsh-B	5.0	4.0	4.0	4.0	3.0	3.0	3.5	3.0	3.5	3.5	4.0	3.5	5.0	5.0	5.0	5.0	1.0	3.0	4.0	3.0	3.8	0.5	5.0	1.0	2.0	0.0	2.0	2.0	0.0	1.5	2.0	0.0	0.5	2.0	2.9
	IRBL12-M[US]	4.5	5.0	4.0	4.0	5.0	4.5	4.0	5.0	4.0	4.5	5.0	4.0	5.0	5.0	5.0	5.0	2.0	4.5	5.0	4.5	4.5	5.0	5.0	4.5	4.5	5.0	2.5	5.0	4.5	5.0	5.0	5.0	5.0	4.5	4.5
	LTH	3.3	3.3	3.7	4.7	3.7	3.8	3.2	4.2	4.2	4.3	3.7	3.8	3.8	4.0	3.5	4.8	0.5	3.3	3.7	2.0	3.6	4.8	4.7	5.0	3.7	4.3	3.2	5.0	3.0	3.7	3.7	4.7	3.7	3.7	3.8
	US-2	4.3	4.3	4.0	4.3	4.8	4.5	4.3	4.5	4.3	3.8	4.8	4.3	4.2	5.0	5.0	5.0	3.5	4.7	5.0	4.2	4.5	4.8	5.0	4.7	4.2	4.7	4.2	5.0	5.0	5.0	5.0	5.0	5.0	4.7	4.6
	Mean	3.9	3.5	4.0	4.1	3.8	3.8	3.8	4.0	3.7	4.0	4.5	3.9	4.3	4.4	4.5	4.0	1.3	3.7	4.1	3.3	3.8	3.7	4.4	3.6	3.0	3.2	2.8	3.7	2.9	4.0	3.9	3.2	3.3	3.4	3.7
Ib (n = 80)	Kasalath	1.3	1.2	0.8	0.7	0.3	2.0	1.5	1.0	1.3	0.0	0.8	1.0	0.8	1.0	1.8	1.3	0.5	1.2	1.2	1.0	1.0	4.7	3.0	2.8	2.5	1.8	1.8	4.8	4.8	4.7	4.5	4.2	4.3	2.8	2.0
	IRBLb-B	4.0	4.5	1.0	2.0	0.0	0.5	1.0	0.5	2.0	0.0	0.5	0.0	0.0	5.0	1.0	0.0	0.0	0.5	1.5	3.0	1.4	0.0	5.0	1.0	5.0	5.0	1.0	5.0	3.0	1.5	4.0	4.0	3.0	2.4	2.0
	IRBLt-K59	1.5	1.0	2.0	3.0	3.0	3.0	1.5	2.5	3.0	4.5	2.0	1.0	1.5	2.0	4.0	1.0	0.0	1.5	1.0	0.5	2.0	5.0	4.0	4.5	2.0	4.0	3.0	3.5	3.5	2.0	1.5	4.0	4.0	2.6	2.5
	IRBLz-Fu	1.5	1.5	1.0	1.0	5.0	2.0	4.5	1.5	2.0	1.5	1.0	1.5	1.0	1.0	1.5	1.5	1.5	2.0	1.0	1.0	1.7	2.0	2.0	1.5	1.0	1.5	2.0	3.0	2.0	2.0	3.5	2.0	2.0	1.8	1.8
	IRBLz5-CA-1	1.5	0.0	0.5	1.5	0.5	3.5	2.0	1.0	2.0	0.5	0.5	0.5	4.0	0.0	3.0	1.0	1.0	1.0	0.5	0.0	1.2	0.5	0.0	1.0	2.0	1.0	1.0	1.0	0.0	3.5	1.0	1.5	3.5	1.2	1.3
	IRBL3-CP4	3.0	0.5	1.0	1.5	4.5	3.5	4.0	5.0	5.0	1.0	3.5	0.0	3.5	3.5	3.0	0.0	0.0	0.5	4.0	3.0	2.5	4.5	4.0	1.0	3.0	0.0	3.0	4.5	3.0	3.0	4.0	3.0	3.0	2.6	2.7
	IRBL5-M	3.5	0.0	0.5	0.0	2.0	3.5	3.0	2.0	3.5	0.0	3.5	0.0	3.0	2.0	4.0	0.0	0.5	1.0	2.0	2.0	1.8	4.5	4.5	1.0	3.0	0.0	3.0	5.0	3.0	5.0	5.0	0.5	4.0	2.5	2.3
	IRBL5-M[US]	4.0	0.5	1.0	1.0	4.5	4.5	4.0	4.5	3.0	1.0	5.0	0.5	4.5	5.0	5.0	0.5	1.0	0.5	4.5	3.0	2.9	4.0	4.5	0.0	2.0	1.0	1.0	4.5	5.0	5.0	5.0	0.5	5.0	2.8	3.0
	IRBLi-F5	3.0	0.5	1.0	1.5	4.5	4.5	3.0	4.5	5.0	1.5	4.5	0.0	5.0	4.0	4.0	0.5	1.0	0.5	5.0	3.0	2.8	4.5	5.0	1.5	3.5	1.0	3.0	4.0	2.0	5.0	5.0	1.0	4.0	2.9	3.0
	IRBLkh-K3[LT]	3.0	0.0	0.0	0.5	4.5	3.0	4.0	3.5	3.5	1.5	0.5	4.5	0.0	0.0	3.5	4.5	0.5	1.0	3.0	3.0	2.2	0.5	0.0	0.0	1.0	0.0	0.5	5.0	0.0	3.0	5.0	0.0	3.0	1.8	1.9
	IRBLk-K[LT]	3.5	0.0	0.0	0.0	3.5	3.0	3.5	4.0	5.0	0.0	3.5	4.0	0.0	0.0	2.0	3.5	0.0	3.0	3.0	3.0	2.2	0.5	2.0	0.0	1.0	0.0	1.5	5.0	0.0	4.0	4.0	0.0	4.0	2.0	2.1
	IRBLkm-Ts	4.0	0.0	0.0	0.0	4.0	4.0	4.5	4.5	4.5	0.0	1.5	4.5	0.0	0.0	5.0	5.0	0.0	2.0	3.5	4.5	2.6	0.0	0.5	0.0	0.5	0.0	2.0	5.0	1.5	5.0	5.0	0.0	5.0	2.3	2.4
	IRBLkp-K60	5.0	0.0	0.0	0.5	3.5	3.5	3.5	3.5	3.5	0.0	4.5	3.5	0.0	0.0	5.0	5.0	1.0	4.0	5.0	3.5	2.7	0.0	4.0	0.0	1.5	0.0	2.0	3.0	3.5	3.0	5.0	0.0	5.0	2.7	2.5
	IRBL1-CL	3.0	0.0	0.5	0.5	3.5	3.5	4.0	3.5	4.0	0.0	1.0	4.0	0.0	0.0	4.0	4.5	0.0	1.0	3.0	3.0	2.2	0.0	2.0	0.5	0.0	0.0	1.5	5.0	3.0	4.0	5.0	0.0	5.0	2.2	2.2
	IRBL7-M	3.5	0.0	0.0	0.0	3.0	3.0	3.5	4.5	3.5	0.0	5.0	4.0	0.0	0.5	1.0	5.0	0.5	3.0	4.5	3.0	2.4	0.0	5.0	0.0	4.0	0.0	3.0	3.0	3.0	4.5	5.0	0.0	4.5	2.8	2.5
	IRBL12-M	4.0	3.0	3.0	2.0	1.5	0.0	1.5	2.0	2.0	2.0	0.0	0.0	0.0	1.5	1.5	0.0	0.0	2.0	4.0	3.5	1.7	3.0	5.0	2.0	2.0	4.0	2.0	5.0	1.5	1.5	5.0	4.0	0.0	2.6	2.1
	IRBL20-IR24	1.5	0.0	4.0	0.5	0.0	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	1.0	3.0	0.0	0.0	0.5	0.0	0.5	0.6	0.5	3.0	0.0	0.5	3.0	0.0	5.0	4.5	1.0	4.5	4.0	4.5	1.8	1.3
	IRBLta2-Pi	1.0	0.0	4.5	0.0	0.5	0.0	0.5	0.0	0.0	5.0	0.0	0.0	0.0	0.5	3.0	0.0	1.5	3.0	3.0	1.0	1.2	0.0	0.0	0.5	0.0	0.0	0.5	5.0	0.0	0.0	0.0	0.0	0.0	0.9	0.9
	IRBLta2-Re	0.5	0.0	3.5	0.5	0.5	0.5	0.5	0.0	0.0	3.5	0.0	0.0	0.0	0.0	2.0	0.0	1.0	3.0	3.0	0.5	1.0	0.0	0.0	1.0	0.5	0.0	0.0	5.0	0.0	0.0	0.5	0.0	0.0	0.9	0.8
	IRBLta-CP1	2.0	1.5	4.0	2.0	0.5	1.0	1.5	2.0	2.0	3.0	1.5	0.0	0.5	0.5	5.0	2.0	2.0	3.0	4.0	3.0	2.1	4.0	0.5	3.5	3.0	0.0	3.0	4.5	0.0	1.0	1.0	0.0	0.0	2.0	1.9
	IRBLta-K1	1.0	1.0	4.5	2.0	0.5	1.5	2.0	1.5	0.5	3.5	0.5	0.0	0.5	2.0	5.0	1.0	1.0	4.0	5.0	3.0	2.0	3.0	0.0	4.5	1.0	0.0	3.0	3.5	0.0	2.0	1.0	0.0	0.5	1.9	1.8
	O. sativa (9)	1.7	1.9	1.3	2.1	1.4	1.3	2.0	1.9	2.3	1.2	2.0	1.2	1.9	1.3	1.2	1.2	0.5	1.6	1.7	1.2	1.5	1.4	1.8	1.8	1.5	0.9	1.3	1.8	0.8	1.1	1.6	1.2	1.4	1.4	1.5
	NERICA (3)	2.2	0.8	1.2	1.2	1.3	1.8	2.2	1.0	1.8	0.7	1.8	1.0	1.8	1.2	2.0	0.8	0.5	1.0	1.8	0.7	1.3	1.0	1.2	1.0	1.3	0.3	1.3	1.5	0.5	1.3	2.2	0.2	0.5	1.1	1.2
	O. glaberrima (44)	1.7	0.9	0.9	1.0	1.5	1.6	1.7	1.4	1.5	0.3	2.0	0.8	1.4	1.7	1.5	0.9	0.8	1.2	2.0	1.5	1.3	1.6	1.3	1.1	1.9	0.4	1.6	2.6	2.5	4.1	2.7	0.9	2.2	1.7	1.5
	O. barthii (3)	1.8	0.7	1.0	1.3	1.7	1.2	2.0	2.3	2.0	1.0	1.3	1.0	1.3	1.0	1.5	0.8	0.7	0.2	1.5	1.2	1.3	0.7	2.7	1.5	1.8	2.2	0.8	3.2	3.0	2.8	2.5	2.5	2.8	1.8	1.6
	Mean	2.0	0.9	1.1	1.1	1.7	1.8	2.0	1.8	1.9	0.7	1.9	1.0	1.4	1.5	1.9	1.2	0.7	1.3	2.2	1.7	1.5	1.6	1.8	1.2	1.8	0.7	1.6	2.9	2.1	3.3	2.8	1.1	2.3	1.8	1.7
II (n = 107)	IRBL9-W	3.5	3.0	4.5	3.0	3.5	3.0	3.5	3.0	3.0	4.0	5.0	3.0	4.0	4.5	3.5	3.0	0.0	4.0	5.0	3.0	3.5	5.0	3.5	3.0	3.0	3.0	3.0	4.0	5.0	5.0	3.0	3.0	3.5	3.5	3.5
	IRBLzt-T	3.0	0.5	3.0	5.0	3.5	3.5	3.0	5.0	1.0	2.0	5.0	3.5	4.0	3.5	5.0	0.0	0.5	3.5	3.0	3.5	3.1	5.0	4.5	4.5	3.0	4.5	3.0	3.0	2.0	5.0	5.0	4.0	4.0	3.4	3.4
	O. sativa (47)	0.6	0.3	0.4	0.4	0.2	0.3	0.5	0.1	0.5	0.2	0.1	0.2	0.3	0.3	0.2	0.1	0.1	0.2	0.2	0.4	0.3	0.1	0.5	0.6	0.7	0.5	0.4	2.7	0.2	0.4	1.3	0.6	0.2	0.5	0.4
	NERICA (15)	0.8	0.3	0.4	0.8	0.6	0.6	1.0	0.4	0.9	0.2	0.6	0.1	0.7	0.3	0.5	0.0	0.3	0.6	0.9	0.5	0.5	0.4	0.5	0.9	0.7	0.3	0.9	1.1	0.3	0.8	1.0	0.5	0.4	0.6	0.6
	Lowland NERICA (40)	0.3	0.2	0.2	0.1	0.2	0.1	0.3	0.2	0.0	0.2	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.2	0.1	0.2	0.1	0.0	0.1	0.1	0.1	0.4	0.1	2.8	0.0	0.2	0.4	0.5	0.1	0.3	0.2
	O. glaberrima (1)	0.5	0.5	0.0	0.5	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.5	0.0	1.0	1.0	1.0	0.0	4.0	0.0	0.0	1.0	3.5	0.0	0.7	0.4
	O. barthii (2)	0.5	1.0	0.5	0.5	0.8	0.5	1.0	0.8	0.3	0.0	0.3	0.3	0.5	0.0	0.5	0.3	0.3	0.3	0.3	0.0	0.4	0.0	0.0	1.0	0.8	0.3	0.0	2.0	0.3	1.0	1.3	0.0	0.3	0.5	0.5
	Mean	0.5	0.3	0.3	0.3	0.3	0.3	0.5	0.2	0.4	0.2	0.2	0.1	0.2	0.2	0.2	0.0	0.1	0.3	0.2	0.3	0.3	0.1	0.3	0.4	0.5	0.4	0.3	2.5	0.2	0.4	0.9	0.6	0.2	0.5	0.4
	Overall mean	1.2	0.7	0.8	0.8	1.0	1.0	1.3	1.0	1.1	0.6	1.0	0.6	0.9	0.9	1.1	0.7	0.4	0.8	1.2	1.0	0.9	0.9	1.1	0.9	1.1	0.6	0.9	2.8	1.1	1.7	1.8	0.9	1.2	1.1	1.0

Table 3. Relationships between rice accessions in cluster groups classified by reactions to standard differential blast isolates and classified by polymorphism data of DNA markers

Groups classified by data of DNA markers' polymorphism	No. of accessions						
	Groups classified by reactions to standard differential blast isolates						
	I				II		Total
	a		b				
A1	1: Nipponbare (Lowland) 1: LTH (Lowland) 4: Monogenic lines for *Pia*, *Pik-s*, *Pish* and *Pi19*(t)	6	1: *O. barthii* (upland) 2: LTH NILs for *Pik* and *Pik-h* 17: Monogenic lines for *Pib*, *Pit*, *Pii*, *Pi3*, *Pi5*(t), *Piz*, *Piz-5*, *Pik-m*, *Pik-p*, *Pi1*, *Pi7*(t), *Pi12*(t), *Pi20*(t), *Pita*(2) and *Pita-2*(2)	20	1: *O. barthii* (upland) 2: Monogenic lines for *Piz-t* and *Pi9*	3	29
A2	—	0	4: *O. sativa* (Upland) 3: *O. sativa* (Upland NERICAs)	7	15: *O. sativa* (Upland) 15: *O. sativa* (Upland NERICAs)	30	37
B1	1: US-2 1: US-2 NIL for *Pi12*(t)	2	1: Kasalath (lowland) 2: *O. sativa* (Lowland) 1: *O. sativa* (Upland) 1: US-2 NIL for *Pi5*(t) 3: *O. glaberrima* (lowland) 1: *O. barthii* (upland)	9	25: *O. sativa* (Lowland) 2: *O. sativa* (Upland) 10: *O. sativa* (Lowland NERICAs) 1: *O. barthii* (upland)	38	49
B2	—	0	2: *O. sativa* (Lowland)	2	5: *O. sativa* (Lowland) 30: *O. sativa* (Lowland NERICAs) 1: *O. glaberrima* (lowland)	36	38
C	—	0	8: *O. glaberrima* (upland) 33: *O. glaberrima* (lowland) 1: *O. barthii* (upland)	42	—	0	42
Total		8		80		107	195

categorized into A2–Ib, whereas all of the other upland and lowland NERICAs were in A2–II, B1–II or B2–II. In contrast, almost all *O. glaberrima* and *O. barthii* accessions were categorized into subcluster C–Ib, except for 1 *O. glaberrima* and 2 *O. barthii* in group II. The accessions of *O. glaberrima* were classified into the three subclusters of B1–Ib, C–Ib and B2–II, and accessions of *O. barthii* were distributed widely into the 5 subcluster groups of A1–Ib, A1–II, B1–Ib, B1–II and C–Ib.

Discussion

The 61 SSR markers used detected 133 alleles in the 195 rice varieties (164 West African accessions, 27 DVs and 4 control cultivars). The average number of alleles per locus was 2.2 over the entire 195 varieties, but was only 1.6 over the 164 West African accessions. On the basis of polymorphisms of SSR markers, the 195 rice varieties fell into 3 major clusters: group A, which included mainly accessions of the Japonica Group; group B, which included mainly accessions of the Indica Group; and group C, which included mainly accessions of the 2 other species *O. glaberrima* and *O. barthii* (**Table 3**). The average number of alleles per locus in groups A, B and C, were 1.6, 1.8 and 1.3, respectively. These results suggested that the accessions with an Indica Group genetic background had high diversity, those of Japonica Group were of intermediate diversity, and *O. glaberrima* and *O. barthii* were of low diversity. Studies into the genetic diversity in *O. glaberrima* estimated based on RFLP (Wang *et al.* 1992) and isozyme markers (Second 1982, 1986) indicate that diversity in *O. glaberrima* is significantly lower than in cultivated Asian rice (*O. sativa*). Li *et al*. (2011) reported that *O. glaberrima* had about 70% less diversity in comparison with its progenitor *O. barthii.* The low value of diversity found for *O. glaberrima* in this study agreed with the results of those previous studies. Ebana *et al*. (2008) reported an average of 7.7 alleles per locus using 32 SSR markers in 236 accessions from Japan. Thomson *et al*. (2007) reported an average of 13.0 alleles per locus using 30 fluorescently-labeled microsatellite markers in 330 Indonesian rice varieties. In comparison with these results of previous reports, the value for rice accessions from West Africa was very low, suggesting that the rice accessions from West Africa do not have particularly high diversity. *O. glaberrima* is unique to Africa (Mohapatra 2010) and was domesticated in West Africa only around 3500 years ago (Angladette 1966, Portères 1956), and Asian rice (*O. sativa*) was introduced into West Africa by the Portuguese in the middle of the 16th century (Linares 2002). Thus, the low diversity of rice accessions of *O. glaberrima* in West Africa might be due to the relatively short history and limited area of cultivation in Africa compared with the history and area of cultivation of Asian rice. However, the number of rice accessions used in this study was limited, and the genetic diversity of rice will need to be confirmed by using a much greater amount of germplasm from West Africa.

The West African rice accessions, the 27 DVs and the 4 control cultivars were classified into 3 groups (Ia, Ib and II) based on their patterns of reaction with respect to 20 SDBIs from Japan and 12 from West Africa. Each group showed unique reactions and were different from each other. Group Ia included the 5 DVs for *Pik-s*, *Pi19*(t), *Pia*, *Pish* and

Pi12(t) (IRBL12-M[US]), and the 3 control cultivars US-2, LTH and Nipponbare. This group was the most susceptible. Group Ib showed intermediate reactions to blast isolates, and almost all accessions of *O. glaberrima* and 20 DVs were included. Conversely, group II was the most resistant among the three groups, and included the 2 DVs for *Pi9* and *Piz-t*, all lowland NERICAs and most upland NERICAS, and several landraces. The findings in this study indicated that rice accessions of West Africa mostly varied from intermediate to high resistance with few susceptible types. Many NERICAs showed high resistance, and these will be a useful source of breeding materials. However, 3 upland NERICAs (NERICA1, NERICA3 and NERICA14) were categorized into group Ib, and they were found to be susceptible to some blast isolates. Virulent blast outbreaks may occur more easily in these 3 NERICAs than in the other NERICAs.

Nipponbare and the DV for *Pish* in group Ia showed differences between their reactions to blast isolates from Japan and their reactions to blast isolates from West Africa. Yaegashi *et al*. (1983) and Imbe and Matsumoto (1985) reported that Nipponbare also harbored *Pish* in its genetic background. These results suggested that the blast races from Japan (temperate region) and West Africa (tropical region) used in this study were differentiated by their virulence to *Pish*. In group Ib, the Indica Group cultivar Kasalath and the 4 DVs for *Pi20*(t), *Pib*, *Pit* and *Pi5*(t) also showed remarkable differences between their reactions to blast isolates from West Africa and those from Japan. These were more resistant to isolates from Japan than to those from West Africa. These results suggested that there were differentiations in the virulence of blast races to these genes and that Kasalath harbored in its genetic background specific resistance gene(s) to isolates from Japan. The NIL IRBL12-M[US] and the monogenic line IRBL12-M, which were both developed as DVs for *Pi12*(t), were categorized into different groups, Ia and Ib, respectively, and remarkable differences in their reactions to blast isolates were found. These results suggested that additional resistance gene(s) were harbored in the genetic background of the monogenic line.

Group A (Japonica Group) and B ((Indica Group) included accessions of the 3 groups Ia, Ib and II, but group C, consisting of *O. glaberrima* and *O. barthii*, only included accessions of group Ib. These results indicated that the accessions of *O. sativa* varied more in blast resistance compared with that of *O. glaberrima* and *O. barthii*. Only 5 accessions of *O. barthii* were used in this study, and these were categorized into the 5 subclusters A1–Ib, B1–Ib, C–Ib, A1–II and B1–II. These results suggest that *O. barthii* has a wide genetic variation in comparison with *O. glaberrima*. Li *et al.* (2011) found less diversity in *O. glaberrima* than in *O. barthii*; coalescent simulation indicated that the dramatic reduction in nucleotide diversity in African rice could be explained by a severe bottleneck during its domestication. The wide variation of *O. barthii* found in this study is in agreement with that report. Much more evaluation of accessions of *O. glaberrima* and *O. barthii* will be needed to clarify their genetic diversity and to understand their utility as gene sources.

The genetic variation of blast resistance in rice germplasm from West Africa, including Asian rice (*O. sativa*), African rice (*O. glaberrima*) and wild rice (*O. barthii*), was demonstrated well by using SDBIs and polymorphism data of SSR markers in this study. This is the first step for understanding the genetic diversity of blast resistance in rice germplasm from Africa and for genetic studies including gene identification and analysis of their mechanisms of resistance. To build up a durable system of protection against blast disease, the high-resistance varieties found in this study will be useful materials as gene sources for blast resistance. The genetic mechanisms of resistance are very interesting and will need to be clarified. Yan *et al*. (2017) tried screening 11 resistance genes (*Pid2*, *Piz*, *Piz-t*, *Pi9*, *Pi36*, *Pi37*, *Pi5*, *Pib*, *Pik-p*, *Pik-h* and *Pita-2*) by using molecular markers closely linked to them. Based on the molecular information, the relationships between genetic variation of resistance to SDBIs and genotypes of resistance gene(s) will also need to be clarified.

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Literature Cited

Agnoun, Y., M. Sié, G. Djedatin, K.N. Dramé, B. Toulou, S.A. Ogunbayo, K.A. Sanni, D. Tia, A. Ahanchédé, R.S. Vodouhè *et al.* (2012) Molecular profiling of interspecific lowland rice progenies resulting from crosses between TOG5681 and TOG5674 (*Oryza glaberrima*) and IR64 (*Oryza sativa*). Int. J. Biol. 4: 19–28.

Angladette, A. (1966) Le riz, Paris, Maisonneuve et Larose, p. 930.

Chang, T.T. (1976) The origin, evolution, cultivation, dissemination, and diversification of Asian and African rices. Euphytica 25: 425–441.

Chen, X., S. Temnykh, Y. Xu, Y.G. Cho and S.R. McCouch (1997) Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). Theor. Appl. Genet. 95: 553–567.

Ebana, K., Y. Kojima, S. Fukuoka, T. Nagamine and M. Kawase (2008) Development of mini core collection of Japanese rice landrace. Breed. Sci. 58: 281–291.

Flor, H.H. (1971) Current status of the gene-for gene concept. Annu. Rev. Phytopathol. 9: 275–296.

Hayashi, N. (2005) Rice blast fungus, MAFF Microorganism Genetic

Resources Manual No. 18. National Institute of Agrobiological Sciences, Ibaraki, Japan.

Hayashi, N., N. Kobayashi, M.C.M. Vera Cruz and Y. Fukuta (2009) Protocols for the sampling of diseased specimens and evaluation of blast disease in rice. JIRCAS Working Report No. 63. Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki, pp. 17–33.

Imbe, T. and S. Matsumoto (1985) Inheritance of resistance of rice varieties to the blast fungus strains virulent to the variety "Reiho". Japan. J. Breed. 35: 332–339.

Jones, M.P., M. Dingkuhn, G.K. Aluko and M. Semon (1997) Interspecific *Oryza sativa* L. × *O. glaberrima* Steud. progenies in upland rice improvement. Euphytica 94: 237–246.

Kawasaki-Tanaka, A. and Y. Fukuta (2014) Genetic variation in resistance to blast disease (*Pyricularia oryzae* Cavara) in Japanese rice (*Oryza sativa* L.), as determined using a differential system. Breed. Sci. 64: 183–192.

Koizumi, S. (2008) Durability of resistance to rice blast disease. *In*: Fukuta, Y., C.M. Vera and N. Kobayashi (eds.) A Differential System for Blast Resistance for Stable Rice Production Environment. JIRCAS Working Report No 53. Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki, Japan, pp. 1–10.

Li, Z.M., X.M. Zheng and S. Ge (2011) Genetic diversity and domestication history of African rice (*Oryza glaberrima*) as inferred from multiple gene sequences. Theor. Appl. Genet. 123: 21–31.

Linares, O.F. (2002) African rice (*Oryza glaberrima*): History and future potential. Proc. Natl. Acad. Sci. USA 99: 16360–16365.

Mackill, D.J. and J.M. Bonman (1992) Inheritance of blast resistance in near-isogenic lines of rice. Phytopathology 82: 746–749.

McCouch, S.R., L. Teytelman, Y. Xu, K.B. Lobos, K. Clare, M. Walton, B. Fu, R. Maghirang, Z. Li, Y. Xing *et al.* (2002) Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res. 9: 199–207.

Mohapatra, S. (2010) Pockets of gold. Rice Today 9: 32–33.

Morishima, H., Y. Sano and H.-I. Oka (1992) Evolutionary studies in cultivated rice and its wild relatives. *In*: Futuyma, D. and J. Antonovics (eds.) Oxford Surveys in Evolutionary Biology. Oxford University Press, Oxford, pp. 135–184.

Ndjiondjop, M.N., F. Cisse, G. Girma, M. Sow, R. Bocco, G. Djedatin and B. Fatondji (2010) Morpho-agronomic and molecular characterisation of *Oryza glaberrima* germplasm from Mali. Afr. J. Biotechnol. 9: 7409–7417.

Nutsugah, S.K., J.K. Twumasi, J. Chipili, Y. Sere and S. Sreenivasaprasad (2008) Diversity of the rice blast pathogen populations in Ghana and strategies for resistance management. Plant Pathol. J. 7: 109–113.

Odjo, T., A. Kawasaki-Tanaka, T. Noda, B.C. Ahohuendo, Y. Sere, T. Kumashiro, S. Yanagihara and Y. Fukuta (2014) Pathogenicity analysis of blast (*Pyricularia oryzae* Cavara) isolates from West Africa. JARQ 48: 403–412.

Portères, R. (1956) Taxonomie agrobotanique des riz cultivés *O. sativa* Linné et *O. glaberrima*. Steudel. J. Agric. Trop. Bot. Appl. 3: 341–384.

Sarla, N.M. and B.P.M. Swamy (2005) *Oryza glaberrima*: A source for the improvement of *Oryza sativa*. Curr. Sci. 89: 955–963.

Second, G. (1982) Origin of the genic diversity of cultivated rice (*Oryza* spp.): Study of the polymorphism scored at 40 isozyme loci. Jpn. J. Genet. 57: 25–57.

Second, G. (1986) Isozymes and phylogenetic relationship in *Oryza*. *In*: Rice Genetics: Proceedings of the International Rice Genetics Symposium, May 27–31, 1985. International Rice Research Institute, Manila, pp. 27–39.

Semagn, K., M.N. Ndjiondjop, M. Lorieux, M. Cissoko, M. Jones and S. McCouch (2007) Molecular profiling of an interspecific rice population derived from a cross between WAB 56-104 (*Oryza sativa*) and CG 14 (*Oryza glaberrima*). Afr. J. Biotechnol. 6: 2014–2022.

Séré, Y., A.A. Sy, S.K. Akator, A. Onasanya and K. Zai (2004) Screening strategy for durable resistance to rice blast disease at WARDA. Rice Blast in West Africa: Characterisation of Pathogen Diversity, Key Screening Sites and Host Resistance. WARDA Proc. Ser. 3: 4–6.

Séré, Y., A. Onasanya, A. Afolabi, H.D. Mignouna and K. Akator (2007) Genetic diversity of the blast fungus, *Magnaporthe grisea* (Hebert) Barr, in Burkina Faso. Afr. J. Biotechnol. 6: 2568–2577.

Sie, M., Y. Sere, S. Sanyang, L.T. Narteh, S. Dogbe, M.M. Coulibaly, A. Sido, F. Cisse, E. Drammeh, S.A. Ogunbayo *et al.* (2008) Regional yield evaluation of the interspecific hybrids (*O. glaberrima* × *O. sativa*) and intraspecific (*O. sativa* × *O. sativa*) lowland rice. Asian J. Plant Sci. 7: 130–139.

Silue, D., J.L. Nottegherm and D. Tharreau (1992) Evidence of a gene-for-gene relationship in the *Oryza sativa–Magnaporthe grisea* pathosystem. Phytopathology 82: 577–580.

Telebanco-Yanoria, M.J., Y. Koide, Y. Fukuta, T. Imbe, H. Kato, H. Tsunematsu and N. Kobayashi (2010) Development of near-isogenic lines of Japonica-type rice variety Lijiangxintuanheigu as differentials for blast resistance. Breed. Sci. 60: 629–638.

Temnykh, S., W.D. Park, N. Ayres, S. Cartinhour, N. Hauck, L. Lipovich, Y.G. Cho, T. Ishii and S.R. McCouch (2000) Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). Theor. Appl. Genet. 100: 697–712.

Thomson, M.J., E.M. Septiningsih, F. Suwardjo, T.J. Santoso, T.S. Silitonga and S.R. McCouch (2007) Genetic diversity analysis of traditional and improved Indonesian rice (*Oryza sativa* L.) germplasm using microsatellite markers. Theor. Appl. Genet. 114: 559–568.

Tsunematsu, H., M.J.T. Yanoria, L.A. Ebron, N. Hayashi, I. Ando, H. Kato, T. Imbe and G.S. Khush (2000) Development of monogenic lines of rice for blast resistance. Breed. Sci. 50: 229–234.

Valent, B., L. Farrall and F.G. Chumley (1991) *Magnaporthe grisea* genes for pathogenicity and virulence identified through a series of back crosses. Genetics 127: 87–101.

Vaughan, D.A., B.R. Lu and N. Tomooka (2008) The evolving story of rice evolution. Plant Sci. 174: 394–408.

Wang, Z.Y., G. Second and S.D. Tanksley (1992) Polymorphism and phylogenetic relationships among species in the genus *Oryza* as determined by analysis of nuclear RFLPs. Theor. Appl. Genet. 83: 565–581.

Ward, J.H. (1963) Hierarchical grouping to optimize an objective function. J. Am. Stat. Assoc. 58: 236–244.

Yaegashi, H., K. Asaga and M. Yamada (1983) Presumed genotypes for true resistance of recommended rice varieties to rice blast. Bull. Tohoku Natl. Agric. Exp. Stn. 68: 1–19.

Zhou, E., Y. Jia, P. Singh, J.C. Correll and F.N. Lee (2007) Instability of the *Magnaporthe oryzae* avirulence gene *AVR-Pita* alters virulence. Fungal Genet. Biol. 44: 1024–1034.