Evaluation of African Cultivated Rice *Oryza glaberrima* for Resistance to Bacterial Blight

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Abstract

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Xanthomonas oryzae pv. *oryzae* is the causal agent of bacterial blight in rice, one of the most devastating diseases of rice worldwide. African *X. oryzae* pv. *oryzae* strains belong to a clear genetic group distinct from those of Asia. Three new races of the pathogen were characterized among strains from West Africa. We evaluated 107 *Oryza glaberrima* accessions for resistance to bacterial blight under greenhouse conditions. Six-week-old seedlings were inoculated with five different African *X. oryzae* pv. *oryzae* strains originating from the West African nations of Burkina and Mali and representing different races (A1, A2, and A3). Philippine *X. oryzae* pv. *oryzae* strain PXO86 (race 2) was also used. Most (48%) of the accessions of *O. glaberrima* were highly susceptible to *X. oryzae* pv. *oryzae* strains from Burkina, while 20 and 36 were resistant to *X. oryzae* pv. *oryzae* strains from Mali and the Philippines, respectively. CAPS markers and dot blot assays were used for detection of resistance genes *xa5* and *Xa21* from a selected set of *O. glaberrima* accessions. Our results suggest that the *O. glaberrima* germplasm contains a narrow genetic base for resistance to *X. oryzae* pv. *oryzae*. Sources of resistance identified among *O. glaberrima* are recommended for rice breeding programs to develop bacterial blight-resistant cultivars for West Africa.

One of the most important diseases that affects rice is bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae*. This disease, which occurs as a vascular wilt at the early stages of crop growth and as a leaf blight at later stages, severely affects rice production worldwide (24,25). It is especially prevalent in irrigated and rain-fed lowland rice-growing areas. Yield losses caused by *X. oryzae* pv. *oryzae* typically range from 20 to 30% and can be as high as 50% in some areas of Asia (1). The disease was reported in 1980 from most African rice-growing countries (29,35). The recent intensification and expansion of rice areas in the absence of known resistant cultivars contributes to the increase of the disease in Africa. In 2003 and, more recently, in 2009, extensive surveys in three West African countries indicated a high incidence of BB (9,39). Because host resistance is still the most effective way to control the disease in Asia, breeding efforts to develop rice cultivars with resistance to BB are urgently needed in West Africa. To date, over 30 *Xa* genes have been identified (21). So far, six resistance genes (*Xa1, xa5, xa13, Xa21, Xa26*, and *Xa27*) have been cloned and characterized as encoding different types of proteins, suggesting multiple mechanisms of resistance-gene-mediated *X. oryzae* pv. *oryzae* resistance (46,47). *Xa3/Xa26* and *Xa21* encode leucine-rich repeat (LRR) receptor kinase type proteins (42,49) which are the only two characterized plant LRR receptor kinase resistance proteins that mediate race-specific resistance. *Xa1* encodes a nucleotide binding–LRR protein (50) and *Xa27* a novel protein (10). The recessive genes *xa5* and *xa13* encode a gamma subunit of transcription factor IIA (13,15) and a novel plasma membrane protein (7), respectively. *Xa21* has been widely exploited in breeding programs and remains effective in many rice-growing regions (18).

Marker-assisted selection (MAS) for recessive resistance genes is not always efficient because it involves the use of markers that are only indirectly linked to the target genes and there is the risk of the markers being separated from the trait by recombination. Recently, functional markers were developed for *xa13*, *xa5*, and *Xa21* (14). To facilitate the application of MAS, low-cost applications such as dot blot assays for single-nucleotide protein (SNP) detection have been developed (40). Recent applications for targeted introgression of *xa5*, *xa13*, and *Xa21* genes into different rice cultivars using MAS have been successfully reported (19,43).

The genetic characterization of a collection of *X. oryzae* pv. *oryzae* strains revealed unique features of African *X. oryzae* pv. *oryzae* strains compared with Asian ones (9). African *X. oryzae* pv. *oryzae* strains have a smaller number of transcription activator-like (TAL) effector genes and insertion sequence (IS) elements in their genome (9). A race is a group of strains sharing a common phenotype of virulence or avirulence to a set of host cultivars. Three new races (A1, A2, and A3) were characterized (9). Race A1 is present in Niger, Burkina, and Cameroon, and is virulent on near-isogenic lines (NILs) carrying resistance genes *Xa3*, *xa8*, *Xa10*, *Xa11*, *xa13*, *Xa14*, and *Xa21* and avirulent to those carrying *Xa4*, *xa5*, and *Xa7*. Race A2 is reported from Burkina and race A3 is reported from Mali. Differences between races A2 and A3 are based on their reaction to the parental line IR24; race A3 is avirulent and race A2 is virulent (9).

Oryza glaberrima (2n = 24, AA) is the African cultivated species of rice that was domesticated in the Niger River delta of West Africa in approximately 1500 B.C.E. from the wild relative *O. barthii* (syn. *O. breviligulata*). *O. glaberrima* is isolated from *O. sativa* by reproductive barriers and has lower genetic diversity than *O. sativa* (31,37). After the introduction of Asian rice (*O. sativa*) to Africa,

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O. glaberrima was progressively abandoned in favor of *O. sativa*, which has higher yields (16). Nevertheless, *O. glaberrima* is still grown in marginal production areas (Mali, Guinea, and Burkina), where local adaptation to adverse conditions is recognized by farmers. Indeed, *O. glaberrima* has many useful traits such as weed competitiveness, drought tolerance (23), submergence tolerance, and tolerance to soil acidity, salt, iron toxicity (28,36), and aluminum toxicity (4). *O. glaberrima* is also reported as highly resistant to several diseases and pests, such as *Rice yellow mottle virus* (3,26,44), blast (41), sheath blight, nematodes (22,33), stem borer and hispa, stalked eye fly (2), and African gall midge (30,48). Recently, sources of resistance to different Indian *X. oryzae* pv. *oryzae* pathotypes have been identified among *O. glaberrima* accessions (45). Some of the interesting traits of *O. glaberrima* are being combined with high-yielding traits of *O. sativa* to develop NERICA, the "New Rice for Africa" (16).

Although there are few *Xa* resistance genes that are detectable or desirable to work with (9), sources of resistance to African *X. oryzae* pv. *oryzae* strains are still lacking and, therefore, highly desirable for the development of resistant rice cultivars for sustainable crop production in West Africa. The objective of this study was to evaluate a representative collection of *O. glaberrima* accessions that originated from Africa for its resistance to African *X. oryzae* pv. *oryzae* strains.

Materials and Methods

Germplasm evaluated. In total, 107 accessions of *O. glaberrima* from the Africa Rice Center gene bank (Cotonou, Benin) were selected according to specific geographical origin characteristics such as drought tolerance and resistance to other rice diseases (Fig. 1). Also included were *O. glaberrima* accessions CG14 (accession IRGC 96717) and MG12 (accession IRGC 103544) that are being used to develop new genetic resources at the Africa Rice Center (12). The population structure of *O. glaberrima* using simple sequence repeat markers was conducted on some of the accessions selected in our study (38). Eight *O. sativa* cultivars (IR24, IR64, ITA212, ITA306, BG90-2, Kogoni [subspecies *indica*], Azucena, and WAB165 [subspecies *japonica*]) were also included in this study. Azucena is known to be highly susceptible to African *X. oryzae* pv. *oryzae* strains and was used as a susceptible control in each of our screenings. Plants were grown under controlled conditions (28°C, 80% humidity, and 12-h day length) in the greenhouse at IRD Montpellier.

Inoculation and disease assessment. Five strains of *X. oryzae* pv. *oryzae* were used in this study. *X. oryzae* pv. *oryzae* strains BAI3 and BAI4 from Burkina correspond to race A1 and A2, respectively. *X. oryzae* pv. *oryzae* strain MAI1 from Mali belongs to race A3 (Table 1). Two other African *X. oryzae* pv. *oryzae* strains, NAI8 from Niger (race A1) and CFBP1949 from Mali (race A3), were tested on a subset of 10 *O. glaberrima* accessions. We also included Philippine *X. oryzae* pv. *oryzae* strain PXO86, which belongs to Philippine race 2. All strains were stored in 15% glycerol at –80°C. Before inoculation, strains were streaked on PSA medium (10 g of peptone, 10 g of sucrose, 1 g of glutamic acid, and 16 g of Bacto agar per liter of H_2O, pH 7.0). The inoculum was prepared by suspending bacterial cells from PSA in sterile, distilled

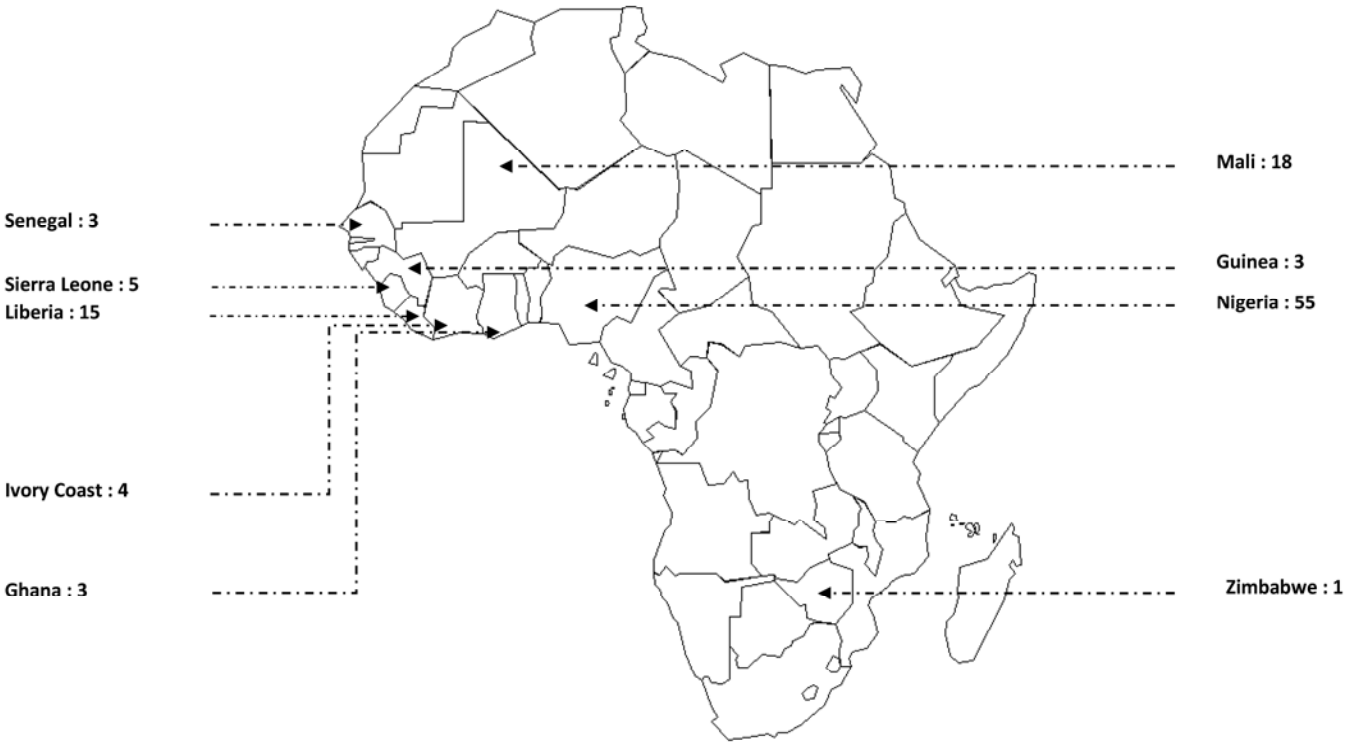

Fig. 1. Geographical location of the *Oryza glaberrima* origin and number of accessions tested per country.

Table 1. Characteristics of *Xanthomonas orzyzae* pv. *oryzae* strains used and their average reactions on 107 *Oryza glaberrima* accessions

			No. of different reactions induced[a]				Lesion length (cm)[b]			Virulence level[b]		
Strains	**Origin**	**Race**	**R**	**MR**	**MS**	**S**	**Min**	**Mean**	**Max**	**Min**	**Mean**	**Max**
BAI3	Burkina	A1	0	2	15	90	5.68	17.75	22.9	2	4.27	5
BAI4	Burkina	A2	0	3	17	87	6.15	18.21	31.4	2	4.36	5
MAI1	Mali	A3	20	51	20	16	0.93	9.04	24.18	1	2.54	5
PXO86	Philippines	Phil2	36	41	10	20	1.25	8.36	22.45	1	2.33	5

[a] Resistant (R), moderately resistant (MR), moderately susceptible (MS), and susceptible (S) as described in the text.
[b] Min = minimum and Max = maximum; virulence level as described in the text.

water at an optical density at 600 nm of 0.2, bearing approximately 10^8 CFU/ml. Inoculations were conducted under greenhouse conditions at 28°C and 80% relative humidity. Plants were inoculated at 45 days after sowing using the leaf-clipping method (17). Lesion length (in centimeters) was measured 21 days post inoculation. The disease reaction of each accession was classified according to the mean lesion length, as follows: resistant if lesion length was <5 cm, moderately resistant if between 5 and 10 cm, moderately susceptible if between 10 and 15 cm, and susceptible if >15 cm. Virulence was defined as the capacity of the bacteria to infect the plants and was based on lesion length using the following scale, as described previously (45): 0 = no obvious lesion, 1 = a lesion length of 1 to 3 cm, 2 = 4 to 7 cm, 3 = 8 to 12 cm, 4 = 13 to 18 cm, and 5 = >19 cm.

Experimental design and data analysis. Two replicate trials for disease assays were done. Each trial consisted of an assessment of 115 rice accessions (107 *O. glaberrima* and 8 *O. sativa*) against each of the four respective strains. Per strain, 10 seeds were sown for each accession and inoculated. Two fully expanded leaves were inoculated per plant. The 10 replicate plants per accession were sown on row in a small seed tray, each containing eight randomly selected accessions. The seed trays were arranged in a completely randomized design in the greenhouse. For some accessions, germination was not 100%; such plants were considered as missing data in the analysis. In this way, the percentage of missing data (i.e., nongerminated seed) in each trail (per 2,300 total seeds planted) was as follows: 1.3, 2.26, 4.43, and 2.43% for the *X. oryzae* pv. *oryzae* strains BAI3, BAI4, MAI1, and PXO86, respectively, in trial 1, and 0.69, 1.04, 1.91, and 0.86% in trial 2.

A combined trial data analysis was performed by strain based on a linear mixed model using the nlme package of R V.2.7.2 (32,34). The model was defined as $y_{ijk} \approx \mu + \alpha_j + \beta_k + \varepsilon_{ijk}$, where y_{ijk} is the disease reaction (leaf lesion length) of individual i for accession j in trial k, μ is the intercept, α_j is the effect of the accession j, β_k is the effect of the trial k, and ε is the residual of the model. Accession was considered as a fixed effect and trial was considered as a random effect. The existence of significant differences ($P < 0.001$) in lesion lengths among accessions was assessed using the F test. Comparisons of accessions was based on best linear unbiased estimators for each accession j with respect to each strain, as $y'_g = \mu + \alpha_j$, where μ is the intercept and α_j is the fixed effect of the accession j (fixed effect coefficients for the mixed model fitted object).

Detection of resistance alleles *xa5* and *Xa21*. Twelve *O. glaberrima* accessions that originated from different geographical areas and had resistant, moderately resistant, moderately susceptible, or susceptible reactions to *X. oryzae* pv. *oryzae* strains were selected and genotyped to identify the presence or absence of *xa5* and *Xa21* resistance alleles. The NILs IRBB5 and IRBB21, which are known to carry *xa5* and *Xa21*, respectively, were used as positive controls. The primer pairs used for amplification of DNA fragment covering the SNP regions of *xa5* and *Xa21* were xa5-6F: 5′GATAGCAGCATTTCCAAGAG3′, xa5-4R: 5′GATTCCTTT AGCAAGGTGTG3′, Xa21F: 5′ATAGCAACTGATTGCTTGG3′, and Xa21R: 5′CGATCGGTATAACAGCAAAAC3′.

For *xa5* detection, DNA was extracted as described previously (13). Approximately 10 to 20 ng of genomic DNA were used in a 25-µl polymerase chain reaction (PCR), performed in an automated thermal cycler according to the following cycles: initial denaturation at 94°C for 5 min; 33 cycles of denaturation at 94°C for 30 s, annealing at 51°C for 30 s, and extension at 72°C for 1 min; and a final extension step at 72°C for 5 min. Restriction endonuclease digestion was done directly on the PCR products using *Bsr*I or *Sml*I enzymes (14). Digest products were run on a 2.5% agarose gel. The CAPS marker size of *xa5* was 949 bp.

Xa21 detection was done by dot-blot-SNP analysis (40; International Rice Research Institute, *unpublished*). DNA fragments covering the SNP sites were amplified by PCR using the *Xa21* primers as indicated above and with the following conditions: 94°C for 4 min; 34 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min; and a final extension step at 72°C for 8 min. The PCR target size of *Xa21* was 1,400 and 1,250 bp for the resistance and susceptible alleles, respectively.

The diluted PCR products (1:20) were denatured in a solution of 0.4 N NaOH and 10 mM EDTA, and dot blotted onto a nylon membrane (Nytran; Schleicher & Schuell, Germany). DNA was fixed to the membrane using a UV-cross linker. Blots were prehybridized at 42°C as recommended by the manufacturer using prewarmed Hyb buffer (Roche DIG Easy Hyb), for at least 30 min. The probes (10 µM) prepared by resuspending digoxigenin (DIG)-labeled oligo probes in Tris-EDTA, pH 8, were denatured at 68°C and immediately added to prewarmed hybridization buffer. Blots were then hybridized at 60°C for at least 6 h. Filters were washed with three solutions: the first of 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 0.1% sodium dodecyl sulfate (SDS) for 20 min, followed by two washings with 1× SSC and 0.1% SDS for 10 min each, and a final wash with 0.1× SSC and 0.1% SDS for 20 min. Signals were detected with a DIG Nucleic Acid Detection Kit, NBT/BCIP (Roche, Switzerland).

Results

Identification of resistant accessions among *O. glaberrima*. The *X. oryzae* pv. *oryzae* strains tested by the leaf clipping method had different reactions on the *O. glaberrima* accessions (Fig. 2; Table 1). For all strains, accessions had significantly different lesion lengths and the relative differences were not consistent among strains ($P < 0.0001$). The *O. glaberrima* accessions that were resistant to African *X. oryzae* pv. *oryzae* strains originated from different geographical areas (Supplementary Table 1). Among the 20 accessions of *O. glaberrima* that are highly resistant to *X. oryzae* pv. *oryzae* Malian strains (race A3), 4 originated from Mali and the 16 others from Guinea, Ivory Coast, Liberia, Nigeria, Senegal, Sierra Leone, and Zimbabwe. No correlation was observed between the resistance levels and the geographical origin of the *O. glaberrima* accessions and that of the *X. oryzae* pv. *oryzae* strains used (Supplementary Table 1).

For *O. glaberrima*, 20 and 36 accessions showed a high level of resistance to *X. oryzae* pv. *oryzae* strains MAI1 (race A3) and PXO86 (Phil race 2), respectively (Fig. 2; Table 1), with 10 accessions highly resistant to both strains (Table 2). Nineteen accessions were moderately resistant to both MAI1 and PXO86 (Table 2). Six accessions that had a high level of resistance to MAI1 were moderately resistant to PXO86 (Table 2). Conversely, 17 accessions among the 51 found as moderately resistant to MAI1 showed a high level of resistance to PXO86 (Table 2). Only two accessions were moderately resistant to BAI3 (race A1) while BAI4 (race A2) induced a moderate resistance reaction in three accessions (Table 2). Fifteen accessions were highly susceptible to all African *X. oryzae* pv. *oryzae* strains (Table 2). Although strains NAI8 (race A1 from Niger) and CFBP1949 (race A3 from Mali) were tested on only 10 accessions, the reactions matched the results obtained with strains BAI3 (race A1) and MAI1 (race A3). Strain CFBP1949 (race A3) induced resistant, moderately resistant, and moderately susceptible reactions, whereas strain NAI8 (race A1) induced only susceptible reactions in all accessions.

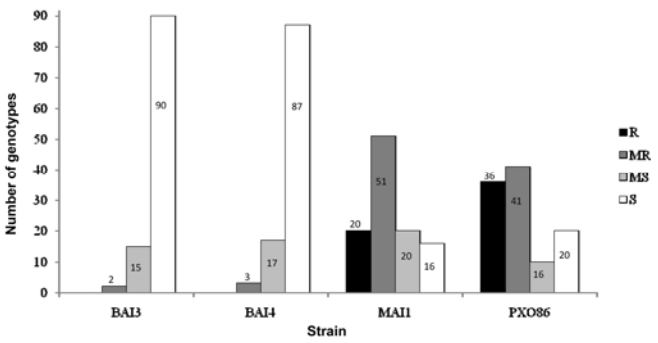

Fig. 2. Number of different types of reactions induced by *Xanthomonas oryzae* pv. *oryzae*.

For *O. sativa*, five of eight accessions were highly resistant to the African *X. oryzae* pv. *oryzae* strains (Table 3). Two other accessions (IR24 and ITA212) had variable reactions. Azucena was highly susceptible to all the strains tested. All the *O. sativa* accessions were highly susceptible to the Phil *X. oryzae* pv. *oryzae* strain PXO86.

Virulence of *X. oryzae* pv. *oryzae* strains. The mean lesion length (± standard error) induced by African *X. oryzae* pv. *oryzae* strains MAI1 (race A3), BAI3 (race A1), and BAI4 (race A2) on 107 *O. glaberrima* accessions was 9.04 ± 0.23, 17.25 ± 0.28, and 18.21 ± 0.28 cm, respectively (Table 1). The mean lesion length induced by CFBP1949 (race A3) and NAI8 (race A1) was 8 and 19 cm, respectively (*data not shown*). Differences in virulence were observed among African strains and Philippine strain PXO86, with an average ranging from 2.33 ± 0.12 to 4.36 ± 0.07 (Table 1). Particularly, strains BAI3 and BAI4 from Burkina caused remarkable symptoms on all tested *O. glaberrima* accessions, with an average virulence level (VL) of 4.27 and 4.36, respectively (Table 1) and up to 5 on *O. sativa* IR24 and Azucena (Table 3). Strain MAI1 (race A3) induced significantly less severe symptoms on *O. glaberrima* accessions, with an average VL = 2.54 (Fig. 2; Table 1). Strain MAI1 exhibited also a low VL on almost all *O. sati*va cultivars (VL = 1) except Azucena (VL = 4) (Table 3). Strain PXO86 (Phil race 2) exhibited a similar VL on *O. glaberrima* accessions (VL = 2.33) but was highly virulent on all *O. sativa* cultivars (Fig 2; Tables 1 and 3). Strains MAI1 and PXO86 were very similar in terms of virulence on *O. glaberrima*. Strain NAI8 (race A1) showed high virulence (VL = 4.4), whereas CFBP 1949 (race A3) showed low virulence (VL = 2.3) on 10 accessions of *O. glaberrima* (*data not shown*).

xa5* and *Xa21* resistance allele detection among *O. glaberrima. Twelve *O. glaberrima* accessions originating from diverse geographical areas (Table 4) and three *O. sativa* accessions (IR24 and the derived NILs IRBB5 and IRBB21) were genotyped for the presence or absence of *xa5* and *Xa21* alleles (Table 4). As expected, our results revealed that the NILs IRBB5 and IRBB21

Table 2. Reactions of *Oryza glaberrima* accessions for resistance to *Xanthomonas oryzae* pv. *oryzae* strains[a]

	***X. oryzae* pv. *oryzae* strains**			
	African			**Asian**
Reactions	**BAI3 (A1)**	**BAI4 (A2)**	**MAI1(A3)**	**PXO86 (Phil race 2)**
R	…	…	CG14, TOG5284, TOG5314, TOG5437, TOG5439, TOG5453, TOG5803, TOG6195, TOG6710, TOG7020	
	…	…	**TOG6202, TOG6206, TOG6238, TOG6356, TOG6767, RAM98,** RAM69, RAM77, RAM94, TOG6308	***IG02*, *TOG5293*,** TOG5406, ***TOG5447*, *TOG5458*, *TOG5464*, *TOG5473*,** TOG5491, TOG5514, ***TOG5523***, TOG5533, ***TOG5566*,** TOG5591, TOG5602, ***TOG5620*, *TOG5650*, *TOG5675*, *TOG5810*,** TOG5882, ***TOG5953*, *TOG5989*,** TOG5997, TOG6000, ***TOG6007*, *TOG6231*, *TOG7173***
MR	TOG5672, TOG6767	RAM63, RAM94, TOG5832	CG17, RAM24, RAM90, TOG5283, TOG5420, TOG5486, TOG5540, TOG5672, TOG5680, TOG5681, TOG5747, TOG5775, TOG5820, TOG5832, TOG6038, TOG6080, TOG6165, TOG6208, TOG6211	
	…	…	***IG02, TOG5293, TOG5447, TOG5458, TOG5464, TOG5473, TOG5523, TOG5566, TOG5620, TOG5650, TOG5675, TOG5810, TOG5953, TOG5989, TOG6007, TOG6231, TOG7173*,** TOG5286, TOG5287, TOG5324, TOG5390, TOG5400, TOG5404, TOG5500, TOG5556, TOG5641, TOG6208, TOG6221, TOG7420, RAM63, RAM116, RAM123, MG12	**TOG6202, TOG6206, TOG6238, TOG6356, TOG6767, RAM98,** TOG5307, TOG5429, TOG5639, TOG5649, TOG5687, TOG5695, TOG5885, TOG5923, TOG5980, TOG6181, TOG6334, TOG7106, TOG7345, RAM55, RAM101, Saliforeh
S	RAM55, RAM112, TOG5307, TOG5639, TOG5649, TOG5666, TOG5687, TOG5885, TOG5997, TOG6334, TOG7106, TOG7345, Saliforeh, Dckono, PaDckono			RAM2, RAM59, RAM63, RAM77, RAM94, RAM95, RAM112, RAM116, RAM123, Dckono, PaDckono, TOG5286, TOG5287, TOG5324, TOG5326, TOG5400, TOG5404, TOG5556, TOG6221, TOG6308

[a] Reactions induced by *X. oryzae* pv. *oryzae* strains on *O. glaberrima* accessions: R = resistant, MR = moderately resistant, and S = susceptible. In bold: accessions that were both resistant to African *X. oryzae* pv. *oryzae* strain MAI1 (race A3) and moderately resistant to Asian strain PXO86 (Phil race 2). In bold and italic: accessions that were both resistant to Asian *X. oryzae* pv. *oryzae* strain PXO86 (Phil race 2) and moderately resistant to African *X. oryzae* pv. *oryzae* strain MAI1 (race A3).

Table 3. Disease reaction induced by *Xanthomonas orzyzae* pv. *oryzae* on *Oryza sativa* accessions

		Strains used[a]											
		BAI3 (race A1)			**BAI4 (race A2)**			**MAI1 (race A3)**			**PXO86 (Phil race 2)**		
Acc.[b]	**Subspecies**	**LL ± SE**	**T**	**VL**	**LL ± SE**	**T**	**VL**	**LL ± SE**	**T**	**VL**	**LL ± SE**	**T**	**VL**
IR24	*Indica*	20.78 ± 0.28	S	5	21.00 ± 0.28	S	5	0.30 ± 0.24	R	1	15.33 ± 0.24	S	4
IR64	*Indica*	3.88 ± 0.28	R	1	0.98 ± 0.28	R	1	0.27 ± 0.24	R	1	17.33 ± 0.24	S	4
WAB165	*Japonica*	0.27 ± 0.24	R	1	3.20 ± 0.28	R	1	0.32 ± 0.24	R	1	17.08 ± 0.24	S	4
BG90-2	*Indica*	0.24 ± 0.28	R	1	0.38 ± 0.28	R	1	0.41 ± 0.24	R	1	19.58 ± 0.24	S	5
ITA212	*Indica*	7.10 ± 0.28	MR	2	7.28 ± 0.28	MR	2	2.73 ± 0.24	R	1	20.05 ± 0.24	S	5
ITA306	*Indica*	3.07 ± 0.29	R	1	2.73 ± 0.28	R	1	2.77 ± 0.24	R	1	20.43 ± 0.24	S	5
Kogoni	*Indica*	2.05 ± 0.28	R	1	2.32 ± 0.29	R	1	2.40 ± 0.24	R	1	20.13 ± 0.24	S	5
Azucena	*Japonica*	22.48 ± 0.23	S	5	22.48 ± 0.23	S	5	16.45 ± 0.17	S	4	25.58 ± 0.19	S	5

[a] LL = lesion length (mean, in centimeters) induced by the different *X. oryzae* pv. *oryzae* strains used and calculated with $Pr > |t| < 0.0001$ using software R; SE indicates the standard error; T = disease reaction as described in the text with R = resistant, MR = moderately resistant, MS = moderately susceptible, and S = susceptible; VL = virulence level as described in the text, where 0 = no obvious lesion, 1 = LL of 1 to 3 cm, 2 = LL of 4 to 7 cm, 3 = LL of 8 to 12 cm, 4 = LL of 13 to 18 cm, and 5 = LL >19 cm according to Vikal et al. (45).

[b] Accession name.

carried the resistance alleles *xa5* and *Xa21*, respectively, and both were absent from IR24 (Table 4; *data not shown*). None of the 12 *O. glaberrima* accessions carried either allele, with the exception of RAM 95, which was heterozygous for *xa5* (Table 4; *data not shown*).

Discussion

In summary, 107 different *O. glaberrima* accessions were tested for their resistance to *X. oryzae* pv. *oryzae*. Of those, 20 were found to be highly resistant to Mali strain MAI1 and 36 to Philippine strain PXO86. Only five moderately resistant accessions were identified against strains from Burkina. The 20 *O. glaberrima* accessions that were highly resistant to the *X. oryzae* pv. *oryzae* strain from Mali were highly susceptible to *X. oryzae* pv. *oryzae* strains from Burkina and Niger, suggesting that there are complex interactions between these African *X. oryzae* pv. *oryzae* strains and their respective resistance genes in *O. glaberrima*. There was no clear correlation between the resistance level of *O. glaberrima* accessions and their geographical origin or that of the *X. oryzae* pv. *oryzae* strains tested. The *O. glaberrima* accessions identified as resistant might be useful for developing a breeding program in Mali. *X. oryzae* pv. *oryzae* strains originating from Mali belong exclusively to race A3, suggesting that this race is endemic to this country (9), but it is also possible that race A3 is widespread in parts of West Africa not yet surveyed. Further analyses are needed to determine the range of race A3.

Results from this study suggest that the Philippines *X. oryzae* pv. *oryzae* strain PXO86 (race 2) is highly similar to the African *X. oryzae* pv. *oryzae* strain MAI1 (race A3), specifically in terms of virulence on *O. glaberrima*. However, it should be noted that these two strains are genetically very distant (9). Interestingly, the different African *X. oryzae* pv. *oryzae* strains exhibited differential virulence and disease reactions on *O. glaberrima* accessions, despite the fact that they have conserved TALs (9), which are known to play a critical role in the rice–*X. oryzae* pv. *oryzae* interaction. *X. oryzae* pv. *oryzae* strains from the Philippines and Mali were not as virulent as strains from Burkina or Niger on *O. glaberrima,* suggesting that *O. glaberrima* may not be their natural host. In contrast, *X. oryzae* pv. *oryzae* strains from Burkina and Niger were highly virulent on *O. glaberrima* inducing predominantly susceptible reactions, suggesting a strong adaptation of such strains to *O. glaberrima.*

Based on the race pattern of African *X. oryzae* pv. *oryzae* strains on the set of NILs (9), we hypothesize that the few moderately resistant to resistant *O. glaberrima* accessions that were characterized in this study carry some resistance genes (i.e., *Xa4, xa5, Xa7*, and *Xa21*). However, none of the *O. glaberrima* accessions tested had the *xa5* or *Xa21* resistance alleles. Accession RAM 95 that was found to be heterozygous for *xa5* was found to have some parentage originating from *O. sativa* (A. Ghesquiere, *personal communication*). Conversely, when no *O. glaberrima* was found to be resistant in our study, such as is the case with BAI4, we can tentatively conclude that these resistance genes do not play a major role in resistance of *O. glaberrima*.

Of the different *indica* and *japonica* cultivars we evaluated, IR64 and Azucena had different disease reactions with the African *X. oryzae* pv. *oryzae* strains. Further research is needed, using comparative mapping of bacterial blight resistance to African *X. oryzae* pv. *oryzae* strains in the reference mapping population (IR64 × Azucena) (5), to determine if there are specific genes or quantitative trait loci (QTL) shared in common. Identification of new genes or QTL can then be studied in *O. glaberrima* by using interspecific bridges between the two cultivated species with improved crossability toward *O. sativa* (12).

Of the 30 known *Xa* genes, 4 (*Xa21* [18], *Xa23* [51], *Xa27* [10,11], and *Xa30* [t] [6]) have been identified from wild rice species *O. longistaminata, O. rufipogon, O. minuta*, and *O. nivara*, respectively. Special attention needs to be given to the screening of other African wild rice species (e.g., *O. longistaminata*) for resistance to African *X. oryzae* pv. *oryzae* strains. Interestingly, various *O. glaberrima* accessions had a good level of resistance to *X. oryzae* pv. *oryzae* PXO86 (race 2) and may be good sources of resistance for breeding materials in the Philippines as well as for other Asian countries. Pathogen populations of *X. oryzae* pv. *oryzae* in the Philippines are highly variable, as revealed based on virulence and DNA fingerprinting analysis, and more than 10 *X. oryzae* pv. *oryzae* races have been reported here(20,27). Also, new races have emerged to overcome resistance (8). Therefore, although *X. oryzae* pv. *oryzae* strains belonging to race 2 are widespread throughout the lowland areas of the Philippines (27), it will be necessary to screen the *O. glaberrima* accessions with other *X. oryzae* pv. *oryzae* Philippines strains representative of the different races.

Knowledge of pathogen population structure, particularly related to race, is needed to guide predictions of which resistant genes are most likely to be successful. Accordingly, we need to increase our *X. oryzae* pv. *oryzae* collections in West Africa. The *X. oryzae* pv. *oryzae*-resistant *O. glaberrima* accessions identified in our study should be evaluated with other *X. oryzae* pv. *oryzae* races, both in the greenhouse and the field. Indeed, the resistant *O. glaberrima*

Table 4. Reaction of a selected set of *Oryza glaberrima* accessions to *Xanthomonas oryzae* pv. *oryzae* strains and their genotypes using markers for *xa5* and *Xa21*

		***X. oryzae* pv. *oryzae* strains**[a]									
		African			**Asian**	**Genotypes of the accessions at loci *xa5* and *Xa21*[b]**					
Accession	**Origin**	**BAI3 (A1)**	**BAI4 (A2)**	**MAI1 (A3)**	**PXO86 (Phil race 2)**	***xa5* (R)**	***Xa5* (S)**	***xa5* genotype**	***Xa21* (R)**	***xa21* (S)**	**Xa21 genotype**
TOG6208	Guinea	S	S	MR	MR	–	+	*Xa5/Xa5*	–	+	*xa21/xa21*
TOG6308	Liberia	S	S	R	S	–	+	*Xa5/Xa5*	–	+	*xa21/xa21*
RAM24	Mali	S	MS	MR	MR	–	+	*Xa5/Xa5*	–	+	*xa21/xa21*
RAM55	Mali	S	S	S	MR	–	+	*Xa5/Xa5*	–	+	*xa21/xa21*
RAM63	Mali	MS	MR	MR	S	–	+	*Xa5/Xa5*	–	+	*xa21/xa21*
RAM90	Mali	MS	S	MR	MR	–	+	*Xa5/Xa5*	–	+	*xa21/xa21*
RAM95	Mali	MS	S	MS	S	+	+	*xa5/Xa5*	–	+	*xa21/xa21*
TOG5307	Nigeria	S	S	S	MR	–	+	*Xa5/Xa5*	–	+	*xa21/xa21*
TOG5500	Nigeria	S	S	MR	MS	–	+	*Xa5/Xa5*	–	+	*xa21/xa21*
TOG6211	Nigeria	S	S	MR	S	–	+	*Xa5/Xa5*	–	+	*xa21/xa21*
CG14	Senegal	MS	S	R	R	–	+	*Xa5/Xa5*	–	+	*xa21/xa21*
CG17	Senegal	S	S	MR	MR	–	+	*Xa5/Xa5*	–	+	*xa21/xa21*
IRBB5	Philippines	R	R	R	R	+	–	*xa5/xa5*	–	+	*xa21/xa21*
IRBB21	Philippines	MS	MR	R	R	–	+	*Xa5/Xa5*	+	–	*Xa21/Xa21*
IR24	Philippines	S	S	R	MS	–	+	*Xa5/Xa5*	–	+	*xa21/xa21*

[a] Reaction as defined in the text: resistant (R), moderately resistant (MR), moderately susceptible (MS), and susceptible (S).

[b] Symbols + and – indicate presence or absence, respectively, of allele as detected by Shirasawa et al (40), the International Rice Research Institute (*unpublished data*), and Iyer and McCouch (14).

accessions identified here may be used either in cross combinations with accessions carrying other genes or for deployment in specific geographical areas.

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