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Evaluation of Elite Rice Varieties Unmasks New Sources of Bacterial Blight and Leaf Streak Resistance for Africa

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Abstract

Background: Bacterial Leaf Blight (BLB) and Bacterial Leaf Streak (BLS) diseases respectively caused by *Xanthomonas oryzae* pv *oryzae (Xoo)* and *X. oryzae* pv *oryzicola* (*Xoc*) have become a real threat to rice production in West Africa. Genetic and pathogenicity studies revealed African *X. oryzae* (*Xo*) pathovars to be both fairly distant from their Asian counterparts. It is therefore mandatory to evaluate whether available major resistance genes are adapted for an efficient and durable control of BLB in Africa. As to BLS no resistance sources have been identified in rice so far.

Results: We screened eleven upland and lowland elite rice varieties cultivated in Burkina Faso for resistance to bacterial blight and bacterial leaf streak. We show that all five upland varieties including three NERICAs are highly resistant to each of the three races of *Xoo* identified in Africa and generally susceptible to several strains from Asia. We next demonstrated that resistance is occurring independently of the plant developmental stage. Strikingly, phenotyping and in planta growth curves assays showed that the five varieties are also highly resistant to a set of *Xoc* strains representative of the diversity in West Africa. Finally, we show that resistance of the NERICA varieties to both *Xoo* and *Xoc* is likely to be inherited from the *O. sativa* ssp. *japonica* rather than the *O. glaberrima* parental lines.

Conclusion: We identified and characterized rice accessions exhibiting new sources of resistance against African *Xoo* and *Xoc* strains specifically. These results provide important perspectives for the control of BLB and BLS in West Africa.

Keywords: Disease resistance; Bacterial blight; Bacterial leaf streak; Upland varieties; NERICA rice cultivars

Background

The cultivation of rice has drastically increased in Africa over the last decade. Bacterial Blight (BLB) and Bacterial Leaf Streak (BLS) respectively caused by *Xanthomonas oryzae* pv *oryzae* (*Xoo*) and *Xanthomonas oryzae* pv *oryzicola (Xoc),* impose significant disease pressure on cultivated rice (*Oryza sativa* L), most particularly in irrigated and upland rice growing regions. While BLB was first reported in the 1980s in several West African rice growing countries. BLS is definitely considered a new emerging disease in West as well as in East Africa and Madagascar. Both diseases are expanding to new rice production areas and are therefore potential sources of serious threaten for food security in West Africa. BLB and BLS can cause severe yield losses of up to respectively 70% and 20% in rice irrigated areas, depending on the rice variety, host growth stage during epidemics, geographic location and seasonal conditions. In Burkina Faso, the intensification and expansion of rice areas with *X. oryzae* susceptible lines, may lead to an increase of BLB and BLS incidence in the country. For instance, BLB epidemics were reported in 1998 in Bagre area, leading to severe yield losses in fields planted with the Chinese rice variety TCS10. Moreover, in 2003 and more recently in 2009 to 2014, extensive surveys in Burkina Faso, Mali and Niger indicated a higher incidence of BLB and BLS diseases [1-9].

During infection, *Xoo* enters the leaf through hydathodes or wounds and spreads systemically by multiplying in the xylem vessels. In contrast, *Xoc* which is a mesophyll colonizer, enters through leaf stomata or by injury. Both pathovars rely on a set of diverse molecular tools to overcome the host defense system and establish a favorable niche, thus resulting in BLB and BLS diseases. *Xoo* is able to infect adult plants and seedlings, respectively leading to leaf blight and wilting of young plants, a syndrome referred to as kresek. Kresek usually occurs during transplantation of seedlings from nurseries to the field, mainly because of leaf cuttings which occur prior transplantation and serve as major routes of infection. Employing cultivars with efficient resistance occurring at all developmental stages is therefore crucial and the most economical way to control the disease. Nowadays, at least 41 BLB resistance genes have been identified, of which eight have been isolated. The effectiveness of resistance at different developmental stages has been previously tested for many of these genes [10-17].

African *Xoo* strains are genetically distant from Asian one and are notably characterized by a reduced set of transcription activator-like (*TAL*) effector genes in their genome [2,9,6]. Moreover, three new races of the pathogen where identified among strains collected in West Africa. Race A1 is present in Niger, Burkina Faso and Cameroon, and is a virulent on near-isogenic lines (NILs) carrying *Xa4*, *xa5* and

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Xa7. In contrast to *Xoo*, no races have been reported for *Xoc*, though quantitative differences in virulence of strains exist [18,19]. Despite some effort, no major genes for resistance to BLS have been identified in *Oryza sativa* so far. The only available source of genetic resistance is the *Rxo1 R* gene which was cloned from maize and transferred to rice, conferring resistance to *Xoc* strains carrying the effector gene *xopAJ* [20-22]. Yet, while *xopAJ* is highly conserved in *Xoc* strains from Asia, it is absent from the majority of African strains [2,14]. Deployment of *Rxo1* gene-containing varieties is therefore not an appropriate breeding strategy for controlling BLS disease in Africa. More recently, [23] reported on the identification of the recessive gene *bls1* from the wild species *Oryza rufipogon* but its spectrum of action is unknown.

In West Africa many improved varieties have been developed including NEw RICe for Africa (NERICA), which were developed with the aim of combining the high yields from Asian rice species *Oryza sativa* with the ability of the African species *O. glaberrima* (CG14) to resist local stresses. *O. glaberrima* is resistant to a number of major african diseases and pest such as blast, Rice yellow mottle virus, sheath blight, nematodes, stem borer and African gall midge [24-28]. Yet, [29] recently evaluated a subset of 107 *O. glaberrima* accessions for resistance to BLB and reported that only a few of them were resistant to African *Xoo* strains. Despite of the importance of the areas cultivated with improved varieties and NERICA lines in Africa, little is known about their interaction with *X. oryzae.* Understanding the resistance level of these accessions towards the diversity of African *Xoo* and *Xoc* strains and the influence of plant developmental on their function is essential for optimal disease control. With the objective of evaluating the resistance status of most popular rice elite varieties cultivated in Burkina Faso against African *X. oryzae* pathovars, our study unraveled unexpected sources of resistance in rice that are mostly effective against a wide range of *Xoo* and *Xoc* strains from Africa (Figure S1 and Table 1).

Results

Evaluation of rice elite varieties cultivated in Burkina Faso for resistance to African *Xanthomonas oryzae* pv. *oryzae*

In an attempt to examine the potential of rice elite varieties cultivated in Burkina Faso to control BLB disease, we performed leaf-clip inoculation of the eleven most popular varieties. As detailed in Table 1, four *O. sativa* improved rice varieties and seven NERICA cultivars were inoculated with *Xoo* strains BAI3, BAI4 and MAI1 representing the three races identified so far in Africa and the Asian *Xoo* reference strain PXO99^A. To our surprise, a high level of resistance against the three African races could be evidenced in five varieties, including three NERICA cultivars (NERICA 12, NERICA 13 and NERICA 17) and two improved rice varieties (FKR19 and FKR43). Remarkably, all these accessions are adapted to upland ecosystems Table 2. Conversely, lowland varieties (FKR14, FKR28, NERICA-L-20 and NERICA-L-19) were susceptible to all strains, except for NERICA-L-41 and NERICA-L-60, which resisted against races A2 and A3 but not race A1. Noteworthy, upland resistant accessions derived from an *O. sativa* ssp *japonica* background or, in the case of NERICAs, from interspecific crosses involving *O. sativa* ssp *japonica* as recurrent parent. Reciprocally, varieties made from intra- or interspecific crosses deriving from *O. sativa* ssp. *indica* parents were generally susceptible (Figure 1A and Table 3). Finally, a strong HR-like response was observed five days-post infiltration of leaves of each of the five upland resistant varieties with each of the three African *Xoo* strains, as exemplified for NERICA 12 cultivar when challenged with strain BAI3 (Tables 2 and 3) (Figure 1 and Figure S2).

Upland Burkinabe rice varieties exhibit resistance against African but not Asian strains of *X. oryzae* pv. *oryzae*

To confirm the resistance phenotypes of upland varieties, we next performed *in planta* growth curves analysis of each of the three African *Xoo* races and strain PXO99^A in the five resistance accessions. As illustrated in Figure 2, quantification of bacterial populations within the top 10 cm at 4, 8 and 12 days after leaf-clip inoculation clearly confirmed the capacity of the resistant varieties to prevent the growth of the three African strains. In line with the observation that African *Xoo* strains elicited an HR-like response in resistant plants, growth of these strains were significantly reduced starting 4 days after inoculation. In contrast, inoculation of each of the five resistant accessions with Asian *Xoo* strain PXO99^A led to full bacterial growth, as expected from the pathogenicity assays. Thus, we next addressed the question whether

[a]Accession	B NERICA ref	Ecosystem	Subspecies	Backcross / comment	Source
FKR14		Irrigated	*O. sativa* sp. *indica*		INERA
FKR19		Upland, irrigated	*O. sativa* sp. *japonica*	Mashuri x IET 1444	INERA
FKR28		Irrigated	*O. sativa* sp. *indica*	Traditional variety	INERA
FKR43		Upland	*O. sativa* sp. *japonica*	IREM 293-B x IAC 81-176	INERA
FKR45N	NERICA 12	Upland	japonica/*O. glaberrima*	WAB56-50/CG14//WAB56-50	Africa Rice
FKR47N	NERICA 17	Upland	japonica/*O. glaberrima*	CG14/WAB181-18//WAB181-18	Africa Rice
FKR49N	NERICA 13	Upland	japonica/*O. glaberrima*	WAB56-50/CG14//WAB56-50	Africa Rice
FKR56N	NERICA-L-41	Irrigated, lowland	indica/*O. glaberrima*	TOG5681/4*IR64	Africa Rice
FKR58N	NERICA-L-60	Irrigated, lowland	indica/*O. glaberrima*	IR64/TOG5681//4*IR64	Africa Rice
FKR60N	NERICA-L-20	Irrigated, lowland	indica/*O. glaberrima*	TOG5681/3*IR64	Africa Rice
FKR62N	NERICA-L-19	Irrigated, lowland	indica/*O. glaberrima*	TOG5681/3*IR64	Africa Rice
CG14		Upland, lowland	*O. glaberrima*	Donor parent for upland NERICAs	Africa Rice
WAB50-56		Upland	*O. sativa* sp. *japonica*	Recurrent parent for upland NERICA	Africa Rice
WAB181-18		Upland	*O. sativa* sp. *japonica*	Recurrent parent for upland NERICA	Africa Rice
IR24		Irrigated	*O. sativa* sp. *indica*	Recurrent line for IRBBs	IRRI
IRBB4		Irrigated	*O. sativa* sp. *indica*	IR24 line introgressed with *Xa4*	IRRI
IRBB5		Irrigated	*O. sativa* sp. *indica*	IR24 line introgressed with *xa5*	IRRI
IRBB7		Irrigated	*O. sativa* sp. *indica*	IR24 line introgressed with *Xa7*	IRRI

Table 1: Characteristics of Burkinabe elite rice varieties and other accessions investigated in this study [a]Nomenclature of rice accessions developed by INERA (Burkina Faso) [b]New Rice for Africa (NERICA) varieties nomenclature.

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Strains	Origin	Race/ pathotype	[a]*xopAJ*	Reference
Xanthomonas oryzae* pv. *oryzae				
BAI3	Burkina Faso	A1		Gonzalez et al. [2]
BAI4	Burkina Faso	A2		Gonzalez et al.[2]
MAI1	Mali	A3		Gonzalez et al. [2]
PXO86	Philippines	Race 2		Gonzalez et al. [2]
PXO99^A	Philippine	Race 6		Choi and Leach [15]
NXO662	Nepal	na		Gonzalez et al. [2]
IXOO57	Indonesia	na		Gonzalez et al. [2]
XOO212	Korea	na		Gonzalez et al. [2]
GXO357	China	na		Gonzalez et al. [2]
Xanthomonas oryzae* pv. *orizycola				
BLS256	Philippines	na	+	Gonzalez et al. [2]
BAI6	Burkina Faso	5	+	Wonni et al. [3]
BAI11	Burkina Faso	5	+	Wonni et al. [3]
BAI20	Burkina Faso	3	-	Wonni et al. [3]
BAI43	Burkina Faso	4	+	Wonni et al.[3]
BAI62	Burkina Faso	3	-	Wonni et al. [3]
BAI65	Burkina Faso	3	-	Wonni et al. [3]
MAI3	Mali	4		Gonzalez et al. [2]
MAI10	Mali	na	-	Gonzalez et al. [2]
MAI16	Mali	na	+	Gonzalez et al. [2]

Table 2: *Xanthomonas oryzae* strains used in this study [a]Presence/absence of the *Rxo1*-matching a virulence gene *xopAJ* from *Xoc*; na: not annotated.

additional *Xoo* strains representative of the Asian population diversity would also be virulent on Burkinabe rice resistant varieties. We selected five strains originating from five different geographic locations in Asia, therefore corresponding to five genetic lineages. Interestingly, each of the five Asian *Xoo* strains were virulent on upland Burkinabe rice accessions with the exception of NERICA 17. Interestingly the *O. glaberrima* donor parent accession CG14 turned out to control four out of six of the tested *Xoo* strains. Altogether these results suggest that the identified sources of resistance within Burkinabe upland elite rice varieties preferentially act against African *Xoo* races (Figure 2 and Figure S2).

The BLB resistance phenotype of upland varieties is not influenced by plant development

Understanding the range of developmental phases in which a given resistance is effective is particularly important for the deployment of the variety carrying this resistance. To that end, we evaluated the effect

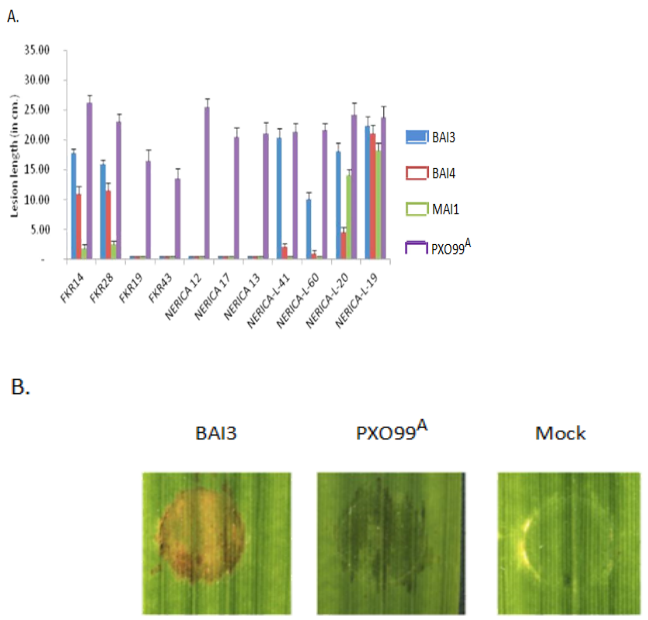

Figure 1: Phenotyping of Burkinabe rice elite varieties by leaf-clipping inoculation. (A) Three-week-old Burkinabe improved rice (FKR) varieties, and NERICA upland (NERICA 12, NERICA 13 and NERICA 17) or lowland (NERICA-L-41, NERICA-L-60, NERICA-L-20 and NERICA-L-19) varieties were challenged with African *Xoo* strains BAI3 (race A1), BAI4 (race A2), MAI1 (race A3) and Asian *Xoo* strain PXO99^A. Disease symptoms (lesion length) were analyzed 14 days after leaf-clip inoculation. This experiment was reproduced three times with similar results. (B) Leaves of three-week-old NERICA 12 were infiltrated, using a needleless syringe, with *Xoo* strains BAI3, PXO99^A or water as control. Leaves were photographed 7 days after inoculation (upper panel).

		[a]Phenotypes					
Rice	NERICA	Xoo			Xoc		
Accessions	nomenclature	BAI3	BAI4	MAI1	[b]Asian	[c]African9	Asian strain
		(race A1)	(race A2)	(race A3)	strains	strains	BLS256
FKR14		S	S	S	S	R	S
FKR19		R	R	R	S	R	S
FKR28		S	S	S	S	R	S
FKR43		R	R	R	S	R	S
FKR45N	NERICA 12	R	R	R	S	R	S
FKR47N	NERICA 17	R	R	R	dS/R	R	S
FKR49N	NERICA 13	R	R	R	S	R	S
WAB50-56		R	R	R	S	R	S
WAB181-18		R	R	R	S	R	S
CG14		S	S	r	dS/R	S	S
FKR56N	NERICA-L-41	S	r	r	S	S	S
FKR58N	NERICA-L-60	S	r	r	S	S	S
FKR60N	NERICA-L-20	S	S	S	S	S	S

Table 3: Reactions of Burkinabe rice varieties against African *X. oryzae* strains a Plants challenged with Xoo were leaf-clip inoculated and lesion length was measured 14 DPI. R, resistance with lesion length < 5 cm. and dark browning reaction; r, resistance with lesion length < 5 cm without dark browning reaction; S, Susceptible with lesion length > 5 cm. Plants challenged with Xoc were leaf-infiltrated and lesion length was measured at 8 DPI. R, resistance with lesion length < 0,2 cm and dark browning reaction; S, susceptibility with lesion length > 0,2 cm bXoo Asian strains that were inoculated are referenced in Table 2. cXoc African strains that were inoculated are referenced in Table 2 dCultivars NERICA 17 and CG14 are susceptible to Xoo strains PXO99A and NXO622, and resistant to the four other asian Xoo strains.

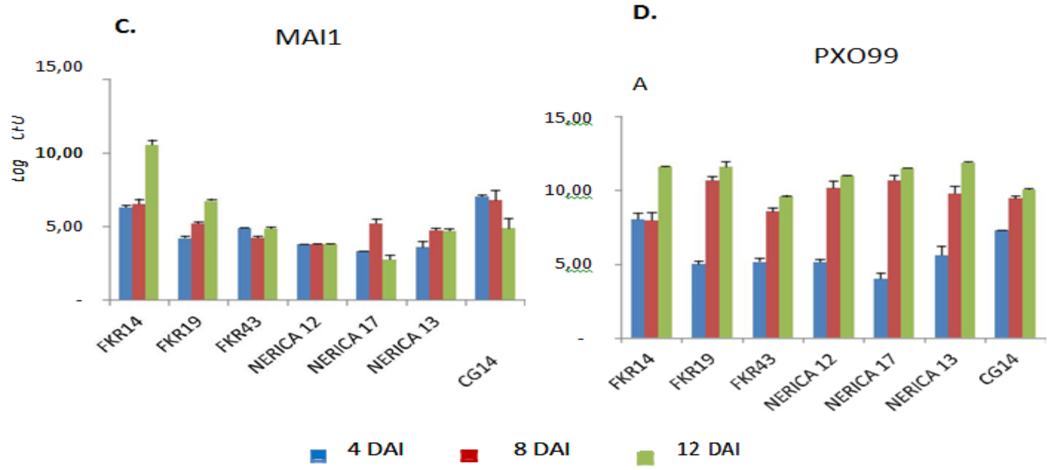

Figure 2: The upland rice varieties exhibit resistance to African *X. oryzae* pv *oryzae*. In plant growth curves analysis of *Xoo* strains BAI3 (A), BAI4 (B), MAI1 (C) and PXO99^A (D) in three-week-old rice leaves of upland resistant rice varieties FKR19, FKR43, NERICA 12, NERICA 17 and NERICA 13, and FKR14 and CG14 control accessions. A 10-centimeter leaf segment (proximal inoculation site) was scored at 4, 8 and 12 days after leaf-clip inoculation, as indicated in blue, red and green panels, respectively. The experiment was repeated three times, using three leaves per tested condition. Error bars represent standard deviation.

of different growth stages on the resistance level of several Burkinabe rice varieties. We compared them to IRBLB lines containing either *Xa4*, *xa5* or *Xa7*, for which a similar study had been conducted previously [17]. As shown in Figure 3, the *Xoo* strain BAI3 which is virulent on the susceptible lines IR24 and FKR14, accordingly produced disease symptoms at all growth stages. In contrast, leaf-clip inoculation of the five resistant upland varieties with BAI3, but also *Xoo* strains BAI4 and MAI1, led to the appearance of a typical dark brown phenotype suggesting of an HR response (not shown). This resistance response was evidenced at all developmental stages, from seedlings to adult plants, and in all tested upland varieties. In conclusion, these results indicate that the resistance mechanisms unmasked in Burkinabe upland rice is not affected by plant development. Moreover, while not a single lesion could be detected on upland varieties upon inoculation of African *Xoo* strains, they still were able to maintain some levels of virulence on IRBLB lines, with the exception of MAI1 which is anyway avirulent on IR24 (Figure 3).

Burkinabe upland varieties confer resistance against bacterial leaf streak

We next investigated the potential of Burkinabe upland rice varieties to control BLS. Nine African *Xoc* strains representative of at least three pathotypes and of different geographic origins Table 2 [9] were leaf-infiltrated on our set of eleven Burkinabe elite rice varieties and scored for disease development 8 days-post infiltration. To our surprise, eight out of 12 tested varieties were able to confer resistance against BLS Table 3, including the four improved rice varieties FKR14, FKR19, FKR28 and FKR43, the three upland varieties NERICA 12, NERICA 13 and NERICA 17, as well as one lowland NERICA variety (NERICA-L-19). A follow-up of symptoms appearance dynamic over a 12-days period was next operated on six resistant varieties, including the five upland accessions that were resistant to BLB. Plants were inoculated with *Xoc* strains BAI6, BAI11 and BLS256 and lesions were scored at 4, 8 and 12 days post inoculation. Strikingly, no disease symptoms could be observed over the time course on any of the Burkinabe elite varieties inoculated with African *Xoc* strains BAI6 and BAI11 (Figures 4A and 4B). In contrast, the Asian *Xoc* strain BLS256 was able to produce variable disease symptoms on the six resistant varieties (Figure 4C). To confirm these results, we performed *in planta* bacterial growth curves based on the same experimental setup as for pathogenicity assays. While *Xoc* strains BAI6 and BAI11 were able to grow on control susceptible line Kitaake, no bacterial multiplication could be evidenced in Burkinabe varieties (Figures 5A and 5B). As expected, the observed growth phenotype was reminiscent of what we observed upon inoculation of the rice accession Kitaake-*Rxo1*, which is resistant to *Xoc* strains carrying the avirulence gene *xopAJ*, such as BAI6 or BLS256 but not BAI11. In conclusion, among eleven rice elite varieties cultivated in Burkina, we showed that eight of them are able to confer resistance to bacterial leaf streak (Figures 4 and 5).

The *O. sativa* ssp. *japonica* parental lines of upland NERICA varieties confer resistance to African strains of *Xoo* and *Xoc*

NERICA varieties result from interspecific crosses between *O. glaberrima* and *O. sativa* ssp *japonica* or *indica* accessions, with the aim of combining specific agro-ecological adaptation of local varieties to the high yielding potential of *O. sativa* subspecies. *O. glaberrima* germplasm, domesticated in West Africa, possesses useful genetic traits, such as good levels of resilience against abiotic and biotic stresses but generally has a low yield potential and unfavourable agronomic characteristics. Upland NERICA cultivars were developed from crosses between the *O. glaberrima* cultivar CG14 as donor and *japonica* subspecies [25]. NERICA 12/13 and NERICA 17 respectively derive from crosses with the recurrent lines WAB56-50 and WAB181-18. Thus, we evaluated the resistance level exhibited by these two recurrent *japonica* subsp. parental lines and the *O. glaberrima* donor. Leaf-infiltration of African but not Asian *Xoo* strains led to a resistance and disease symptoms in respectively both *O. sativa* ssp. japonica recurrent and *O. glaberrima* donor parental lines, similarly to what was observed upon infiltarion of NERICA 17. In the same line of idea, inoculation of the African *Xoc* strain BAI6 respectively conducted to a resistance phenoptype and BLS symptoms on *japonica* ssp and *O. glaberrima* accessions. Altogether, our results show that the resistance phenotype uncovered in the three upland NERICA varieties were probably inherited from the *japonica* subspecies genomes.

Materials and Methods

Plant material and *Xanthomonas oryzae* strains

Seeds of rice cultivars and near isogenic lines were obtained from

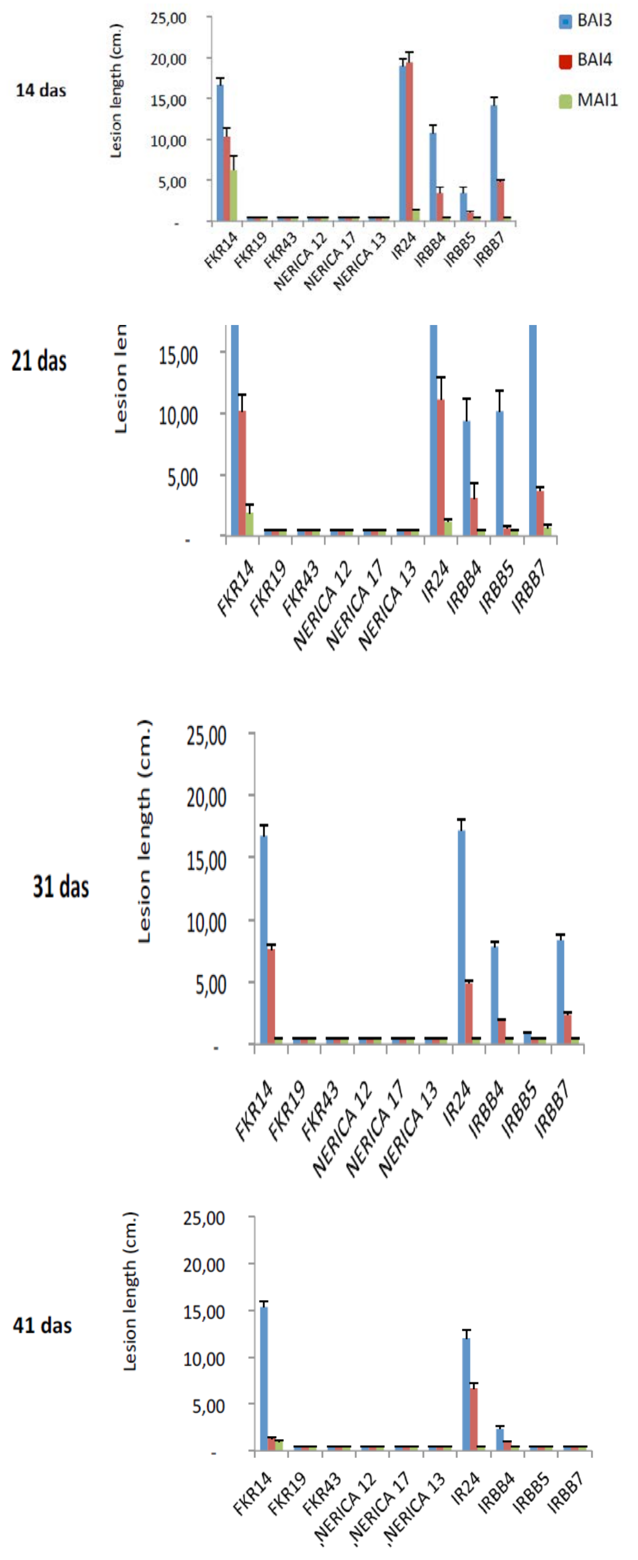

Figure 3: Upland Burkinabe rice varieties control BLB at all growth stages. Lesion lengths included by Xoo strains BA13, BA14 and MAI1, as indicated in blue, red and green panels respectively, on upland rice varieties (FKR19, FKR43,NERICA 12, NERICA 13 and NERICA 13), lowland variety FKR14, and on IRBB rice lines IR24, IRBB4 (Xa4), IRBB5 (xa5), and IRBB7 (Xa7) at different plant growth stages as days after sowing (das). This experiment was reproduced three times with similar results.

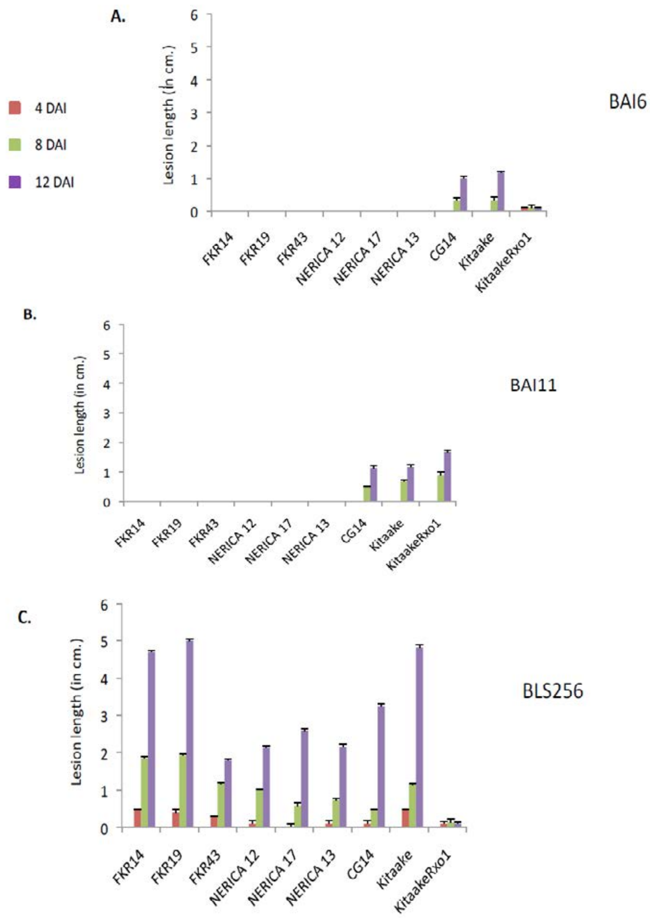

Figure 4: Phenotypic evaluation of Burkinabe rice elite varieties for resistance against BLS. Three-week-old rice leaves of upland resistant rice varieties FKR19, FKR43, NERICA 12, NERICA 17 and NERICA 13, and FKR14 and CG14 control accessions were infiltrated with African *Xoc* strains BAI6 (A) and BAI11 (B), and the reference Asian *Xoc* strain BLS256 (C). Kitaake lines carrying or not the BLS resistance gene *Rxo1* were used as control for incompatible interactions against *xopAJ*, which is present in *Xoc* strains BAI6 and BLS256 and absent from strain BAI11. Disease symptoms (lesion length) were evaluated at 4, 8 and 12 days after leaf infiltration. This experiment was reproduced three times with similar results. Error bars represent standard deviation.

Agricultural and Environmental Research Institute (INERA-Burkina Faso), IRD-Montpellier and Africa-Rice-Benin. Upland NERICAs were developed from the crosses between *O. sativa* ssp *japonica* WAB56-50 or WAB181-18, and the *O. glaberrima* CG14 accession. Irrigated-lowland NERICAs were developed from the crosses between the *O. sativa* ssp. *indica* IR64 accession and the *O. glaberrima* TOG5681 accession. Kitaake and Kitaake transgenic line containing the *Rxo1* gene were used as control to characterize resistance phenotypes against *Xoc*. Plants were grown under controlled conditions (28°C, 80% humidity and 12-h day length) in the greenhouse at IRD Montpellier. *X. oryzae* strains used in this study are described in Table 2. *X. oryzae* cells were cultivated at 28°C in PSA medium (10 g of peptone, 10 g of sucrose, 1 g of glutamic acid, 16 g of agar per liter of H_2O) as already described [2].

Pathogenicity assays

For the evaluation of resistance, plants were inoculated at 21 days after sowing by leaf-clipping inoculation, as described previously [30]. Prior inoculation, strains were streaked on PSA medium and the

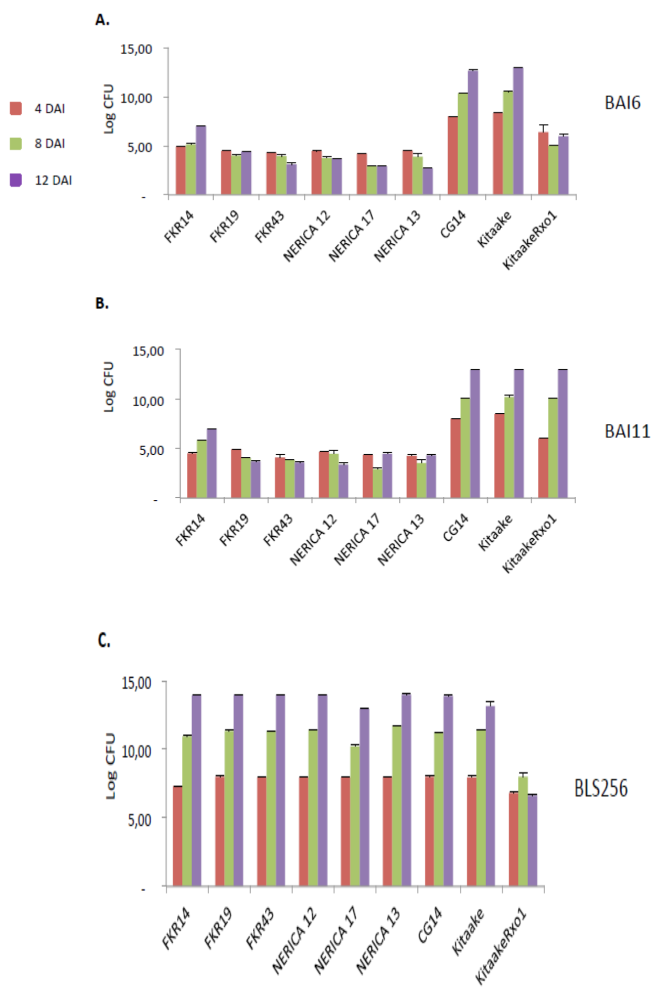

Figure 5: Growth cure analysis show efficient resistance of several Burkinabe rice elite varieties against a diverse set of X. oryzae pv. oryzicola strains from Africa. Three-week-old leaves of rice varieties FKR14, FKR19, FKR43,NERICA 12, NERICA 17, NERICA13, CG14, kitaake and kitaake-Rxo1 were infiltrated using a needleless syringe, with African, Xoc strains BA16 (A), BAI1 (B), and the references Asian Xoc strain BLS256 (C), and scored for bacterial populations at 4,8 and 12 days after inoculation, as indicated in red, green and violet panels, respectively. The experiment was repeated three times, using three leaves per tested condition. Error bars represent standard deviation.

inoculum was prepared by resuspending bacterial cells in sterile distilled water at an optical density of 0.2 (λ = 600 nm), bearing approximately 10^8 CFU/ml. Ten leaves of ten different individuals were inoculated per variety. At 14 days after inoculation (dai), we assessed leaf lesion length (in cm). For leaf infiltration assays, *X. oryzae* strains were introduced with a needleless syringe into the intercellular spaces of rice leaves from 3-weeks-old plants at concentrations of 10^8 cfu ml^{-1} as described previously [31]. The HR was scored at 72-96h after inoculation. All these experiments were conducted three times independently.

Assessment of bacterial numbers

Ability of bacterium to multiply in the plant tissues was assessed at 4, 8 and 12 days after inoculation of three weeks old plants. For *Xoo* the number of bacterial cells in the leaves was determined within the top 10cm of each leaf. *Xoc* cells number was assessed from the portion of leaf where symptoms developed, including the site of inoculation and around. Leaf sections were surface-sterilized in 75% ethanol for 10 s, followed by submersion in sterile water for 30 s, and crushed and resuspended in 1ml sterile distilled water. Leaf homogenates were serially diluted (up to 10^{-12}) and plated onto PSA medium amended with cephalexin (40 ug/ml), kasugamicyn (20 ug/ml) and cycloheximide (50 ug/ml) to avoid contamination. The plates were incubated at 28°C until single colonies could be counted. The experiment was repeated independently three times.

Discussion

Upon the phenotypic evaluation of eleven elite rice varieties from Burkina Faso for resistance against *X. oryzae* pv *oryzae* and *X. oryzae* pv *oryzicola*, we demonstrated that five of them hold the full potential to controling both BLB and BLS diseases in sub-Saharan Africa. Noteworthy, all five resistant accessions were equally efficient towards a range of African *Xoo* and *Xoc* strains representative of the race and pathotype diversity in the region, respectively. In contrast, typical BLB symptoms developed when plants were challenged with a set of five *Xoo* strains originating from diverse areas in Asia and corresponding to different lineages. In the same line of idea, inoculation of the plants with the Asian *Xoc* strain BLS256 also led to typical BLS symptoms. This is suggesting that the unveiled resistance is targeting African strains of *Xoo* and also potentially *Xoc*, specifically. Finally, in all accessions, the resistance response was visualized as an early browning at the inoculation site, which is reminiscent of an HR-like phenotype. To our knowledge, this is the first report on the characterization of rice varieties exhibiting resistance against both pathovars of *Xanthomonas oryzae*. Given no resistance sources against BLS was ever reported in rice so far, these results are of major interest towards the development of potential novel genetic mechanisms.

From an applied point of view, the evaluation of the resistance level towards biotic stresses executed by popular elite varieties is crucial as it enables to predict epidemics, provided data on pathogen populations structure are available. While NERICA varieties and other improved varieties developed by national and international programs are nowadays used wide-spread in Burkina Faso and other countries in Africa, a picture as to their ability to control or not local strains of *Xoo* and *Xoc* was crucially missing until this study. As a matter of fact, one major output of our work will be to assist regional and national programs responsible for the selection of rice varieties to be cultivated in Burkina on a yearly base. In tight conjunction with annual monitoring of local pathogen populations diversity, our results should concretely help to reduce the incidence of BLB and BLS diseases, by guiding the ce of appropriate rice genotypes in Burkina and surrounding countries.

Altogether, our results enabled to identify a potentially new source of resistance against BLB in several upland rice varieties including two improved rice varieties (FKR19 and FKR43) and three NERICA varieties (NERICA 12, NERICA 13 and NERICA 17). Strikingly the associated resistance phenotype is typically characterized by an HR-like response at the site of inoculation appearing within 3-4 days post-infection, and conducing to the absolute arrest of bacterial growth observed as early as 4 days post infiltration. This feature differs from what was reported previously for the *xa5* resistance gene, which controls disease by restricting bacterial movement down the leaf, but still allows bacterial multiplication to high levels at the site of inoculation [32]. Surprisingly, despite of different genetic backgrounds, the five upland varieties, all displayed a similar resistance spectrum which was always associated with the very same typical HR-like macroscopic response. While, it is impossible to infer from our data whether the genetic basis underlying this phenotype is the same among all the lines, examination of their pedigree indicate that they all inherited from at least one *O. sativa* ssp. *japonica* accession (Table 3). This is suggesting that at least some genetic determinants required for resistance might be deriving from *O. sativa* ssp *japonica*. In addition for NERICA varieties, our analysis of the donor (*O. glaberrima* CG14) and recurrent parental lines (japonica WAB56-50 and WAB181-18) indicates that the resistance phenotype observed in their progenies is probably inherited from the japonica subspecies genomes. Indeed, both of the two japonica recurrent parents exhibited the typical HR-like response. Because the genome sequence of many NERICA varieties including NERICA 12, NERICA 13 and NERICA 17, is being processed (Sabot F and Ghesquière A), and since the one of the donor parental line CG14 is already available [32], our prospect of isolating the genetic regions and candidate genes underlying the resistance against African *X. oryzae* strains might become a reality in a much sooner time period than expected (Figure 6).

Many of the isolated crop *R* genes reported so far confer resistance to a narrow spectrum of races relying on gene-for-gene types of interactions. Our results show that while upland varieties are susceptible to Asian *Xoo*, they are highly resistant to African *Xoo* and *Xoc* strains. This strongly suggests that African *X. oryzae* strains are targeted preferentially as a potential result of some rice plants adaptation to local aggressive strains. Noteworthy, African *X. oryzae* strains are genetically distant from those from Asia and are characterized by a comparatively reduced number of transcription activator-like (TAL) effector genes [2]. Interestingly, a mutation in *hrcC* which disrupts the functionality of the type three secretion system of *Xoo* strain BAI3, prevented induction of the typical HR-like response on the NERICA 12 variety (data not shown). This is suggesting that the elicitor of this response could be a type three effector. As in the Rice/*Xoo* pathosystem, the only examples reported so far of effectors holding avr activity are TAL effectors, we hypothesize that they might be good Avr candidates for the elicitation of HR on upland varieties. Future experiments requiring the isolation of the full TAL effectors repertoire of African *Xoo* strains might help to identify the potential elicitor triggering the resistance response we unraveled in African upland varieties [33,34].

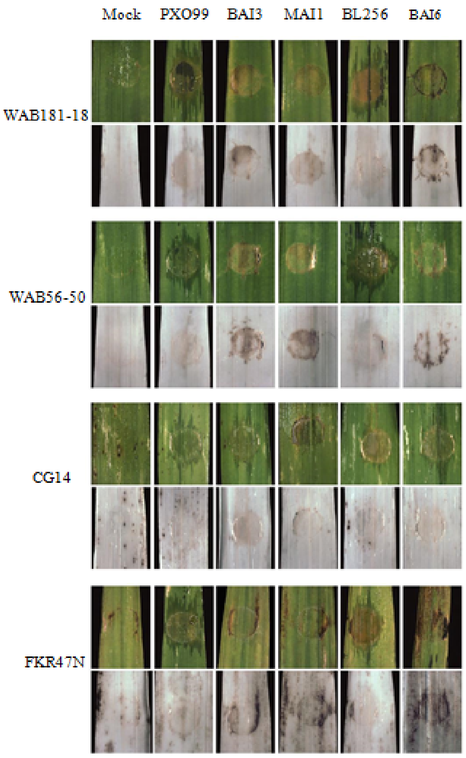

Figure 6: Phenotyping of upland NERICA varieties parental lines reveals that resistance is inherited from *O sativa*. Three-week-old *O. sativa* ssp. *japonica* (WAB181-18 and WAB56-50) and *O. glaberrima* (CG14) parental lines, as well as the FKR47N (NERICA 17) variety which was used as control, were leaf-infiltrated using a needleless syringe with Mock, avirulent *Xoo* strains MAI1 (race A3) and BAI3 (race A1), or PXO99^{A} as virulent control, as well as virulent (BLS256) and avirulent (BAI6) strains of *Xoc*. Leaves were photographed 5 days after inoculation (upper panel) and cleared with ethanol to better visualize HR-like responses. This experiment was reproduced three times with similar results.

In conclusion host-plant resistance is a cost effective and environmentally safe approach to reduce yield loss caused by pathogenic bacteria. Our study showed that all tested African *Xoo* and *Xoc* strains are avirulent on upland cultivars from Burkina Faso. Based on the effectiveness of these accessions to resist to the African *Xo* at all stages of growth under greenhouse conditions, it is next imperious to assess this potential in field conditions. In a near future, our major prospect will be the elucidation of the molecular genetic basis of the resistance mechanisms we identified and characterized in African upland rice varieties, as such achievement is a prerequisite for the development of new locally-adapted and pathogen-informed cultivars with multiple and durable resistance suitable for use in farmers fields.

Competing Interest

The authors declare that they have no competing interest.

Author's Contribution

IW and MH carried out the experiments and data analysis. LO, IS and VV provided scientific comments. IW and BS designed the study design, and wrote the manuscript. All authors read and approved the final manuscript.

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