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Evaluation of Rice Germplasm against Bacterial Leaf Streak Disease Reveals Sources of Resistance in African Varieties

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Abstract

Bacterial leaf streak caused by *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) is a rice disease emerging in West Africa. Its emergence is correlated with the recent expansion of rice cultivation and the introduction of new rice varieties. Our goal is to identify resistance sources to control BLS in rice. We evaluated six *Oryza sativa* and two *Oryza glaberrima* accessions for resistance to bacterial leaf streak under greenhouse conditions. Three week-old plants were inoculated with different *Xoc* strains originated from Mali and the Philippines. Two *Oryza sativa* accessions (FKR14 and ITA306) show a high level of resistance to African *Xoc* while are susceptible to the Philippines one. The others accessions tested are susceptible to all *Xoc* strains tested. We identify new resistance sources to *X. oryzae* pv. *oryzicola* that could be used by breeders, thus improving yield of rice crops in West Africa.

Keywords: *Xanthomonas oryzae* pv. Oryzicola; Resistance; Rice; Africa

Introduction

Bacterial leaf streak (BLS) is an important disease of rice and is caused by *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*). *Xoc* is occuring in the tropical and subtropical areas of Asia, and Australia [1]. In Africa the disease was reported in Madagascar, Senegal and Nigeria in the 1980's [2]. More recently BLS was observed in Mali and Burkina Faso [3,4]. Recently increase of BLS disease was observed in Asia and Africa likely due to the planting of susceptible varieties [3-5]. The increase of BLS disease may also be related to climate changes occuring in Sub-Saharan Africa [6].

BLS develops in the field at any growth stage of rice. Initial symptoms are water-soaked, interveinal streaks along the leaf. *Xoc* is an intercellular pathogen that enters plants through wounds or by invading open stomata [1]. *Xoc* multiplies in the substomatal chamber and colonizes the apoplast of the mesophyll cells [7,8]. *Xoc* oozes from natural openings in strands or strings on the leaf surface and exudates can spread the disease directly from plant to plant by contact, or indirectly via irrigation water and by windblown rain [9]. *Xoc* is a seedborne and a seed-transmitted pathogen [10]. Yield losses due to this disease depend on the rice variety cultivated and climatic conditions but typically range from 10-20% [1]. Effective quarantine and deployment of resistant germplasm are key factors to control BLS disease. Accurate detection of *Xoc* is critical for diagnostic and regulatory purposes. A multiplex PCR assay was developed to simultaneously detect and distinguish the different pathovars of *X. oryzae* infecting rice [11]. The multiplex is currently used to identify *Xoc* strains in West Africa [4,12].

Planting of resistant cultivars is the most effective method for controlling BLS. However, most rice germplasm cultivated in Asia is susceptible to BLS. Raymundo et al. [13] reported that the African variety Morobereckan (upland japonica) is one of the most resistant to Asian *Xoc* strains. The wild rice *O. meyeriana* and *O. officinalis* showed a high level of resistance to Chinese *Xoc* strains [14].

Single rice resistant genes have not been found to control BLS resistance and no strategy has been pursued for controlling the disease in Africa so far. *Rxo1*, a gene from maize, confers resistance to *Xoc* strains in transgenic rice when the corresponding effector gene *avrRxo1* (also named *xopAJ*) is present in the pathogen [15]. *avrRxo1* is present in all the Philippines *Xoc* strains [10] while absent in most African *Xoc* strains isolated in 2003 and 2009 [3,4]. More recently it has been shown that a larger number of *Xoc* strains isolated in Mali and Burkina Faso harbor a functional *avrRxo1* [12]; Dao et al. personal communication). According to Han et al. [16], *AvrRxo1*-ORF1 is adjacent to *AvrRxo1*-ORF2 gene, which was predicted to encode a molecular chaperone of *AvrRxo1*-ORF1. They found that *AvrRxo1*-ORF1 contributes to *X. oryzae* proliferation on rice plants, and strongly suppresses bacterial growth in the absence of *AvrRxo1*-ORF2.

While *Rxo1* may be a useful gene for combatting BLS in Asia, it is not clear yet if that will be the case in West Africa. Also the genetic diversity observed among *Xoc* strains require to identify an efficient breeding strategy for BLS in Africa [3]. Comparative mapping of BLS resistance to Asian *Xoc* strains has led to the identification of Quantitative Trait Loci (QTL) [17]. Recently a recessive *R* gene, *bls1*, conferring resistance on *Xoc* was localized to chromosome 6 from a rice line DP3 derived from *Oryza rufipogon* [18].

Given the severity and extent of BLS epidemics in recent years in West Africa, identifying resistance against *Xoc* is an important goal for breeding programs. The objective of this study was to evaluate rice varieties for resistance to *Xoc* strains.

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Materials and Methods

Germplasm evaluated

Six *O. sativa.* susbp. *indica* accessions Curinga, FKR14, ITA306, PaDckono, TN1, and IR64; two accessions of *O. glaberrima* TOG6767 and TOG5672 and the transgenic line Kitaake-*Rxo1* were evaluated for resistance to *Xoc* strains (Table 1). Most of these accessions are grown in West Africa and possess good agronomic characteristics such as drought tolerance and resistance to other diseases. TN1 is known to be susceptible to BLS [19] and was used in this study as a susceptible control. IR64 and ITA306 were identified as highly resistant to African *Xanthomonas oryzae* pv. *oryzae*, the causal agent of Bacterial Blight (BLB) while TOG6767 and TOG5672 are moderately resistant [20]. FKR14 is grown in main rice areas in Burkina Faso. Originated from India, FKR14 was introduced in Burkina Faso in 1976 [21]. It is a highly productive, plastic and suitable variety for upland and irrigated rice growing regions. It is sensitive to iron toxicity and susceptible to Rice Yellow Mottle Virus and Bacterial Blight diseases [21,5]. All plants were grown in the greenhouse under controlled conditions at 28°C, 12h/12 day/night with 80% humidity in IRD Montpellier.

Bacterial strains and plant inoculation

Three *Xoc* strains were used in this study (Table 2). *Xoc* MAI3 (CFBP7326) and MAI10 (CFBP7331) were isolated in Mali in 2003 [3]. Both strains exhibit different virulence level on the rice cultivar Nipponbare and possess different Transcription Activator like (TAL) genes [3,12]. *Xoc* BLS256 originated from the Philippines was isolated in 1985. Except *xopAJ* and *xopW* genes, African *Xoc* strains share the same type III effectors genes with *Xoc* strain BLS256 [12]. Additional 50 *Xoc* strains collected in Mali and Burkina Faso in 2009 representative of the genetic diversity of *Xoc* in West Africa [12] were tested on the FKR14 variety. All strains were stored in 15% glycerol at-80°C. *X. oryzae* strains were grown on PSA medium (peptone 10 g, sucrose 10 g, glutamic acid 1 g, agar 8 g, pH 7.0) for 24 h at 30°C, then resuspended in sterile water at 0,2 OD_{600} (approximately 10^8 cfu ml^{-1}).

Accessions	Subspecies	IRGC accession number-ID	*R* gene to BB or BLS	Origin
Curinga	*indica*	-	ND	Brazil
FKR14	*indica*	-	ND	Burkina Faso
IR64	*indica*	66970	ND	IRRI
ITA306	*indica*	-	ND	Nigeria
PaDcKono	*indica*	-	ND	Sierra Leone
TN1	*indica*	105	*Xa14*	Taiwan
TOG 6767	*glaberrima*	-	ND	Liberia
TOG5672	*glaberrima*		ND	Nigeria
Kitaake *Rxo1*	*Transgenic line*	-	*Rxo1*	IRRI

Table 1: Accessions used in this study.

Strains	MAI3	MAI10	BLS256
Origin	Mali	Mali	Philippines
virulence classe	5	**na**	5
[a]**xopAJ**	+	-	+
[b]**xopW**	+	>	+
Reference	Wonni et al. [20]	Wonni et al. [20]	Wonni et al. [20]

[a]All the *Xoc* strains that are positive for xopAJ induced a hypersensitive response 72 hours after inoculation while the strains that are negative for xopAJ induce watersoaking lesions on Kitaake-*Rxo1*.
[b]Presence or absence of xopW; > indicates that a DNA arrangement (insertion element) was identified within the *xopW* gene.

Table 2: Strains used in this study to screen cultivars for resistance to *Xoc*.

Rice leaves from 3-week-old plants were inoculated by leaf infiltration as described previously [22]. Leaf reactions were observed 72 hr after inoculation (hai), and lesions were measured 12 days after inoculation (dai). At least six infiltrations were done per leaf with two leaves per plant and two to three plants per strain. A scale was established for BLS disease according to the size of the lesion length (LL) induced by *Xoc* strains: Resistant (R), 0<LL ≤ 1 mm; Moderately Resistant (MR), 1<LL ≤ 10 mm; Moderately Susceptible (MS), 10<LL ≤ 30 mm; Susceptible (S), LL>30 mm. The entire experiment was repeated three times.

Colonization of leaf tissue by *Xoc*

Four rice varieties TOG5672, TOG6767, FKR14 and TN1 were selected and further inoculated as described above. Multiplication of *Xoc* strains was measured *in planta* at three time points (0, 7 and 15 dai). Each leaf was cut into 5 cm section below and above the leaf-infiltrated area. The leaf pieces were ground in 1 ml of sterile water. Bacterial numbers were assessed in serial dilutions that were spread onto PSA agar plates. The plates were incubated at 28°C until single colonies could be counted. The experiment was repeated four times.

Results

Xoc virulence vary depending on rice variety

To determine whether *Xoc* strains vary in their virulence, eight rice varieties were leaf infiltrated with *Xoc* strains BLS256, MAI3 and MAI10. *Xoc* BLS256 caused leaf streak symptoms on all varieties (unless Kitaake*Rxo1*) with lesion length varying from 7,6 to 59,5 mm depending the variety (Table 2). Lesion length induced by MAI3 and MAI10 vary from 0 to 30,8 mm.

The reaction induced by *Xoc* strains varied according to the variety. For example, on varieties TN1, ITA306, *Xoc* strain BLS256 caused large lesions, while on others (TOG5672, TOG6767) lesions were small (Figure 1). Both strains MAI3 and MAI10 induced very similar lesions on all the varieties tested but one.

While BLS256 induces small and large lesions on FKR14 and ITA306 respectively, MAI3 and MAI10 induced a resistant reaction. On FKR14, a hypersensitive reaction (HR) was observed 48 h to 72 h post inoculation with strains MAI3 and MAI10 (Figure 2). FKR14 was then tested with 50 African *Xoc* with an HR consistently observed (data not shown). Out of eight varieties, two (FRK14 and ITA306) showed a strong resistance response to African *Xoc* strains. The HR response observed with FKR14, suggests the resistance to be mediated by African *Xoc* effectors. It is noted that MAI3 and BLS256 harboring *xopAJ* gene induce HR on Kitaake-*Rxo1*. ITA306 is susceptible when challenged with strain BLS256. On the opposite, ITA306 is immune to African *Xoc* strains (Table 3). Variety TN1 is highly susceptible to all *Xoc* strains tested.

Resistance to BLS is associated with low multiplication of *Xoc* strains

Xoc strain BLS256 caused substantial lesions on rice varieties FKR14 and TN1 when compared to MAI3 and MAI10. To determine the effect of plant on bacterial population growth, bacterial numbers were determined in infiltrated leaves.

There was no significant difference in population growth between MAI3 and MAI10 on all varieties tested except Kitaake-*Rxo1* (Figure 3). In TOG6767 and TOG5672, no difference in population growth

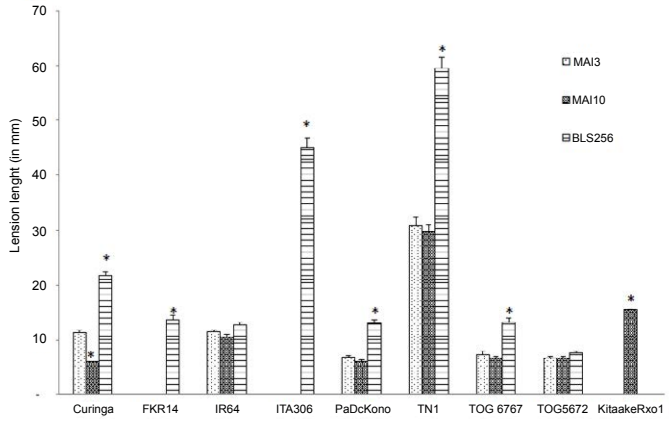

Figure 1: Lengths of lesions caused by *X. oryzae* pv. *oryzicola* strains BLS256, MAI10 and MAI3 on six rice accessions of *O. sativa* sp. *Indica*, two accessions of *O. glaberrima* (TOG6767, TOG5672) and trangnic indica line Kitaake-*Rxo1*. Lesions were measured 15 days after infiltration. An asterisk denotes a significant difference between BLS256 and African *Xoc* (*, P<0.05). The experiment was repeated three times with similar results.

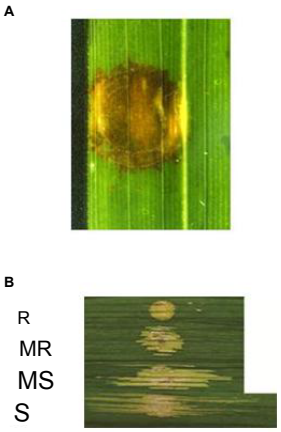

Figure 2: Reactions in leaves of rice accessions at 15 days after infiltration with MAI3, MAI10 and BLS256. Phenotypes were photographed on a light box. (A) Hypersensitive reaction (HR), visible as browning in response to MAI3 and MAI10 on FKR14 leaves; (B) Scale used to assess disease level: Resistant (R), 0<LL ≤ 1mm; Moderately Resistant (MR), 1<LL ≤ 10 mm; Moderately susceptible (MS), 10<LL ≤ 30 mm; Susceptible (S), LL>30 mm.

was observed between plants infected with BLS256, MAI3 and MAI10. Unlike FRK14 no significant increase of bacterial growth of *Xoc* strains was observed with MAI3 and MAI10 between 7 and 15 days after inoculation (Figure 3).

On TN1 although *Xoc* BLS256 induced larger lesions compared to that of MAI3 and MAI10 (Figure 1), there was no significant difference between the population growth of *Xoc* strains (Figure 3). A significant increase of the bacterial population was observed 15 days after inoculation with all the strains tested (Figure 3).

In FKR14, BLS256 numbers were significantly greater than MAI3 and MAI10 at 7 and 15 days after inoculation. Both African strains were detected at low levels in leaf tissues. Together these results show that bacterial population increases in a susceptible cultivar (TN1) and remains stable in resistant accessions FKR14.

Discussion

Eight rice accessions were tested for their resistance to African and Asian *Xoc* strains. Among these, two *O. sativa* FKR14 and ITA306 show a resistance response when challenged with African *Xoc* strains while are moderately to highly susceptible to the Philippines strain *Xoc* BLS256. The reaction induced by African *Xoc* strains on FKR14 corresponds to a hypersensitive reaction with a dark browning area appearing 48 to 72 h after inoculation. Resistance and susceptibility to *Xoc* in rice are correlated with quantitative differences in the bacterial population. The hypersensitive reaction is associated with lower levels of bacterial population in leaves when compared to a susceptible reaction.

No single resistance gene to BLS disease has been characterized in rice. The transgenic rice lines with *Rxo1* gene exhibit an HR symptom when inoculated with *Xoc* harboring the corresponding effector gene *avrRxo1* [3,4]. The HR phenotype observed in African cultivars (FKR14) occurs independently of the presence or absence of the *avrRxo1* gene in *Xoc* strains. This suggests that the resistance observed in FKR14 to African *Xoc* strains differs from the one induced by *Rxo1*.

Zhou et al. [23] showed that upon infection with *Xoc* a larger number of Differentially Regulated Genes (DRG) are expressed in transgenic rice line expressing *Rxo1* gene when compared to wild type. We need to characterize genes that are induced in resistant cultivars such as FKR14 upon infection with African *Xoc* strains.

Also, TOG5672 a glaberrima accession exhibits a moderately resistant reaction correlated with low bacterial numbers when challenged with African and Asian *Xoc*. To confirm TOG5672 as a broad source of resistance to *Xoc*, we need to test a larger number of strains.

We identify novel and broad-spectrum resistance sources to contsrol BLS in rice in Africa. Characterization of genes and markers underlying BLS resistance mechanisms will be mandatory for future use in breeding program.

Crossing of FKR14 with TN1 that is highly susceptible to *Xoc* strains and/or the use of Multi-Parent Advanced Generation Inter-Cross lines (MAGIC) can be achieved to identify markers associated to BLS resistance [24]. Meanwhile, resistance in FKR may be introgressed in others rice accessions by classical breeding to further manage BLS disease in Africa.

	Strains used					
	MAI3		**MAI10**		**BLS256**	
Accession	LL + SE	DR	LL+ SE	DR	LL + SE	DR
Curinga	11.3 ± 0.4	MS	6	MR	21.7 ± 0.8	S
FKR14	0	R	0	R	13.67 ± 0.9	MS
IR64	11.4 ± 0.4	MS	10.5 ± 0.5	MS	12.7 ± 0.5	MS
ITA306	0	R	0	R	45 ± 1.8	S
PaDcKono	6.8 ± 0.3	MR	6 ± 0.4	MR	13 ± 0.6	MS
TN1	30.8 ± 1.5	S	29.71 ± 1.2	S	59.4 ± 2	S
TOG 6767	7.3 ± 0.5	MR	6.7 ± 0.3	MR	13.2 ± 0.7	MS
TOG5672	6.7 ± 0.2	MR	6.7 ± 0.2	MR	7.6 ± 0.3	MR
Kitaake-*Rxo1*	0	R	15.5 ± 0.11	MS	0	R

LL: Length lesion induces by *Xoc* strains upon infiltration 15 days after inoculation, P <0.005; SD: Standard Deviation, DR: Disease Reaction, Resistant (R), 0 < LL ≤ 1mm; Moderately Resistant (MR), 1 < LL ≤ 10mm; Moderately susceptible (MS), 10 < LL ≤ 30mm; Susceptible (S), LL > 30mm.

Table 3: Length lesion induced by *Xoc* strains on rice varieties.

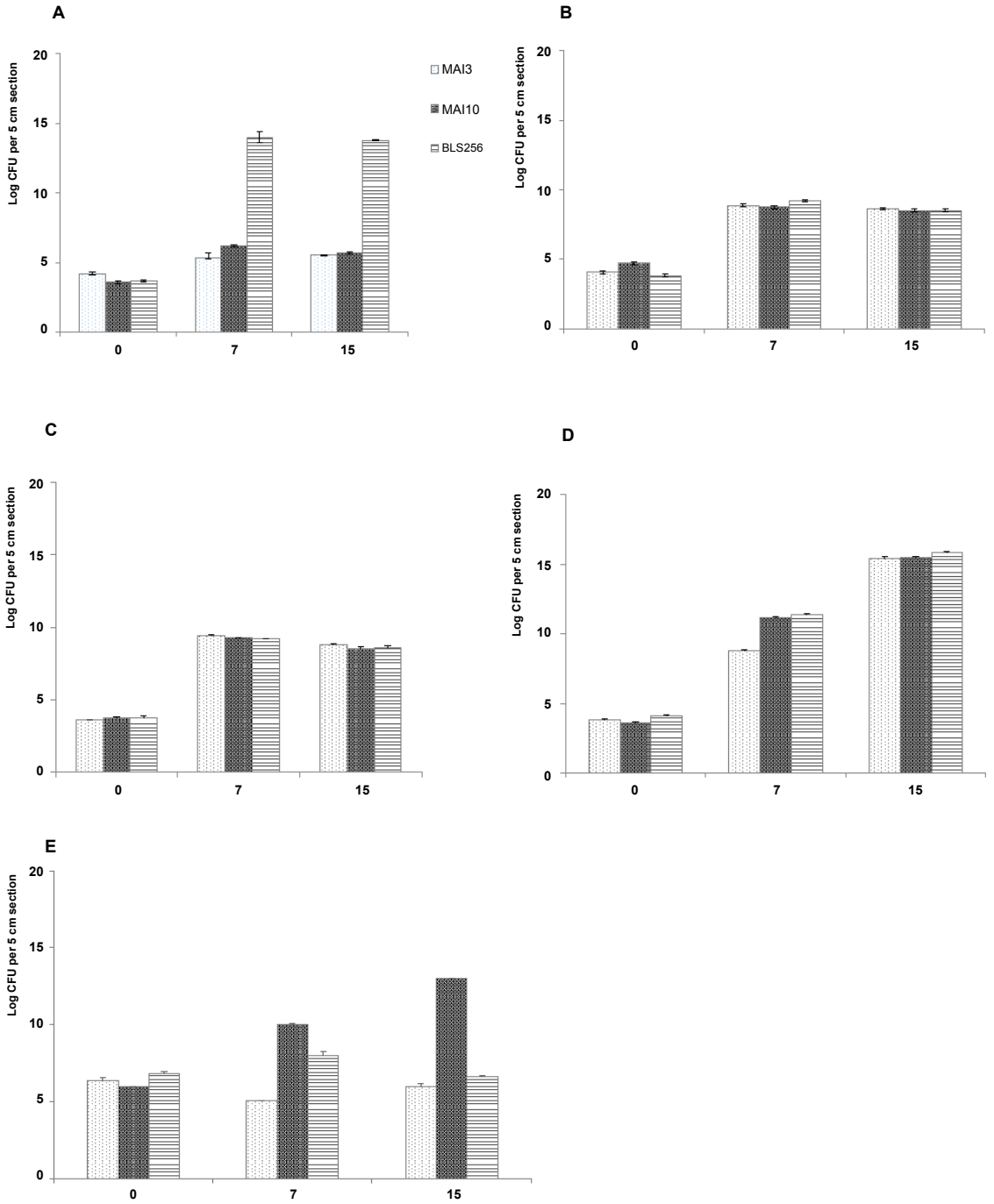

Figure 3: Bacterial growth in 3-week-old leaves of FKR14 (A), TOG5672 (B), TOG6767 (C), TN1 (D) rice accessions and trangenic line Kitaake-*Rxo1* (E). Bacterial population was measured in a 5 cm leaf segment around the infiltration point at 0, 7 and 15 days after inoculation with *Xoc* strains MAI3, MAI10 and BLS256. The experiment was repeated three times. Error bars represent standard error.

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