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 ooze 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 is critical for control BLS disease. Accurate detection of Xoc is key 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 Moroberekan (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 *xopA*) 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*. subsp. *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 *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 Blast 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.

Bacterial strains and plant inoculation

Three *Xoc* strains were used in this study (Table 2). *Xoc* MAI3 (CF87326) 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, *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 OD600 (approximately 10⁸ cfu ml⁻¹).

| Accessions       | Subspecies | IRGC accession number-ID | R gene to BB or BLS | Origin       |
|------------------|------------|--------------------------|---------------------|--------------|
| Curinga          | indica     |                          |                     | Brazil       |
| FKR14            | indica     |                          |                     | ND           |
| IR64             | indica     | 66970                    | ND                  | Burkina Faso |
| ITA306           | indica     |                          | ND                  | ND           |
| PaDcKono         | indica     |                          | ND                  | ND           |
| TN1              | indica     | 105                      | Xa14                | Taiwan       |
| TOG6767          | glaberrina |                          | ND                  | Liberia      |
| TOG5672          | glaberrina |                          | ND                  | Nigeria      |
| Kitaake Rxo1     | Transgenic |                          | Rxo1                | IRRI         |

Table 1: Accessions used in this study.

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-infected 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 (FKR14 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...
was observed between plants infected with BLS256, MAI3 and MAI10. Unlike FKR14 no significant increase of bacterial growth of Xoc strains was observed with MAI3 and MAI10 between 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 control BLS in rice in Africa. Characterization of genes and markers underlying BLS resistance mechanisms will be mandatory for future use in breeding programs.

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 other rice accessions by classical breeding to further manage BLS disease in Africa.

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

| 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 |  |
| PaDeKono     | 6.8±0.3 MR | 6±0.4 MR | 13±0.6 MS |  |
| TOG 6767     | 30.8±1.5 S | 29.7±1.2 S | 59.4±2 S |  |
| TOG5672      | 7.3±0.5 MR | 6.7±0.3 MR | 13.2±0.7 MS |  |
| Kitaake-Rxo1 | 0 R  | 15.5±0.11 S | 0 S | R |

LL: Length lesion induced by Xoc strains upon infiltration 15 days after inoculation, P<0.005; SD: Standard Deviation, DR: Disease Reaction, 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.
Figure 3: Bacterial growth in 3-week-old leaves of FKR14 (A), TOG5672 (B), TOG6767 (C), TN1 (D) rice accessions and transgenic 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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