

Research Article

Characterization of senegalese races of *Xanthomonas oryzae* PV. *oryzae* to identify resistance genes to use

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Abstract

Bacterial blight (BB), is a disease caused by *Xanthomonas oryzae* PV. *oryzae* (*Xoo*), was first reported in Senegal by Trinh in 1980. BB represents a severe threat to rice cultivation in West Africa. Characterizing the pathotypic diversity of bacterial populations is a key to the management of pathogen-resistant varieties. Pathogenicity tests show that all strains are virulent on the susceptible rice variety Azucena, and interact differentially with twelve near-isogenic rice lines, each carrying a single resistance gene. On this rice panel, six races were identified, two of which were previously reported in Mali (A3) and Burkina Faso (A1). Four races (S2, S4, S5, and S6) are described for the first time in Africa. Races A1, isolated in Ndiaye and Ndioum areas is the most prevalent in Senegal. The *Xa1* gene controls 100% of the isolates tested and *xa5* controls all isolates except S4 strains. The geographical distribution of *Xoo* races is contrasted. Four races are detected in the North and two in the South East of the country. Race S4 can be a major risk to rice cultivation because strains from this race are the most virulent and can only be controlled by *Xa1*. To identify local sources of resistance, we screened *Xoo* strains representative of the various races on twenty-three rice varieties grown by farmers in Senegal. Four rice varieties namely Sahel210, Sangangbye, Dansna2, and Sahel305 effectively control all the isolates tested. Our characterization of the first collection of Senegalese *Xoo* strains provided insight into the races present in the country and identified sources of resistance in local rice varieties. This information will help design effective breeding programs for resistance to bacterial leaf blight in Senegal.

Introduction

Rice is the second largest contributor to global human calorie intake [1] and its production continues to increase with the world's population. Over the past 20 years, West Africa has experienced a large surge in rice consumption. Rice is a massively cultivated cereal and is the number one cereal plant of agronomic interest in Senegal in terms of production. Its production has more than quadrupled, from 231,805 tons in 2003 to 1,011,269 tons in 2018. But this production does not meet Senegal's rice needs, which are estimated at 1,600,000 tons [2]. Although local production has progressed, through the expansion of planted areas and improvement of yields, Africa's rice production remains insufficient to cover the rising needs that are driven by demographic growth, the increase in per capita consumption, and urbanization [3]. Bacterial blight

(BB), caused by *Xanthomonas oryzae* PV. *oryzae* (*Xoo*), is one of the most widely distributed and devastating bacterial diseases on rice [4,5]. *Xoo* is a major constraint of rice production in almost all the paddy-growing regions [6]. Yield losses due to this disease generally vary from 20% to 30%, but sometimes these may go up to 50% [7,8]. The incidence of bacterial leaf blight was found to be aggravated by high dosages of nitrogen [9]. In addition to reducing yield, BB may also affect grain quality by interfering with maturation [7]. Although reported in Senegal as early as the 1980s [10], BB survey and sampling campaigns did not begin until 2014 and as a result, no rice breeding program for BB resistance has so far been developed in Senegal [11].

Xoo is a vascular pathogen that causes golden brown lesions along the leaf veins. Wounds and hydathodes are

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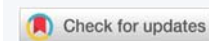
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Keywords: *Xanthomonas oryzae* PV; *Oryzae*; Races; IRBB; Rice; Senegal





the main entry sites for *Xoo*. It multiplies in the epidermis and subsequently enters into the xylem vessels where it starts active multiplication, leading to leaf wilting [12,13]. To colonize its host, *Xoo* has evolved a highly conserved type III secretion system (T3SS) that injects type III effectors into the plant cell. Type III effectors interfere with immune responses or facilitate nutritional or virulence processes to the benefit of the pathogen [14]. The Transcription Activator-Like Effectors (TALEs) type III effector family is composed of DNA-binding virulence proteins that modulate the expression of target genes. Some TALEs act as major virulence factors that are essential for BB to occur and that target rice susceptibility genes [15]. Type III secretion substrates also act as avirulence factors in incompatible interactions on resistant hosts and can trigger a defense reaction called the hypersensitive response (HR) [16]. The nine conserved groups of TALE identified in the genome of African *Xoo* [17,18] appear to be genetically distant from Asian ones [19].

BB control methods include agronomic practices, and chemical and biological control, all of which have proven ineffective in the long term [20-22]. Breeding and deployment of resistant cultivars carrying major resistance genes have been the most effective approach for BB management [23-27] and several resistance genes have been incorporated into elite rice cultivars [23]. However, the deployment of uniform, monogenic resistances over vast areas have decreased their effectiveness, because it provided a selection pressure for the emergence of virulent pathotypes [28,29]. It is therefore critical to monitor pathogen populations to detect new isolates that are able to overcome the resistance genes deployed in the field. A set of Near-Isogenic rice Lines (NILs), each carrying a single resistance gene and the recurrent parent IR24 are commonly used to characterize the race structure of the pathogen [30]. A *Xoo* race is a group of strains sharing the same virulence profile on a panel of these rice NILs. In West-Africa, three races have been identified with race A3 reported in Mali, race A1 and A2 in Burkina Faso, and A1 also reported in Niger [19]. In Mali, using twelve NILs, Tekete, et al. recently identified six new races (A4 to A9) in addition to race A3 [31].

Although BB was first reported in Senegal in the 1980s [10] and confirmed only recently [11], there has been no rigorous assessment of the incidence of this disease and no pathogen strain has been isolated. So far, in the absence of information on the pathogen and its impact on rice, these bacteria have not been considered threats to rice production. Thus no rice selection program for BB resistance has been developed in Senegal. The aim of this study was to update and expand our knowledge of *Xoo* in Senegal. To do this, surveys were conducted in the main rice-growing regions of the country and the race structure of the isolated strains of *Xoo* was determined. Finally, we assessed the susceptibility to BB of a set of Senegalese rice varieties NIL to identify local sources of resistance to this disease.

Materials and methods

Surveys and isolation of *X. oryzae* PV. *oryzae*

Surveys for rice BB disease were performed in 2015 and 2016 in major rice production areas in Senegal. Field visits were conducted from September to October of each year during the rainy season when symptoms were visible. Leaves with BB-like symptoms (translucent, yellow blight, sometimes with visible bacterial exudates on the leaf surface) were collected from *Oryza sativa* cultivars and wild rice species (*Oryza barthii*). The isolation of the bacteria was carried out from a fragment of leaf showing symptoms of BB. A 5 cm fragment of infected leaf including a seemingly healthy part was disinfected successively in 75% ethanol and 0.1% bleach, rinsed two times in sterilized distilled water, and dried on blotting paper. The leaf fragment was then cut into small pieces in a 2 ml Eppendorf tube containing 2 marbles. The tube was immersed in liquid nitrogen for 10 minutes by cryogrinding as described by Schoutten et al., 1986 and ground by shaking vigorously and 1 ml of sterilized distilled water is added to the leaf powder. The resulting solution was left to rest for at least 30 minutes and stirred periodically. A 50 µl aliquot of this solution is spread over the PSA culture medium (10 g Peptone, 10 g Sucrose, 16 g Agar and 1 g glutamic acid) supplemented with antibiotics (cephalexin 1ml (40 mg/ml), Kasugamicin 1 ml (20 mg/ml) and Actidione 1ml (50 mg/ml), for 1 l of culture medium). The Petri dishes were incubated in an inverted position for 72 hours at 28 °C. Colonies similar in appearance to that of *Xanthomonas oryzae* (light yellow, circular, mucous membrane, convex, bright, smooth) were purified on antibiotic-free PSA. The strains were stored at -80 °C in tubes containing liquid nutrient media (PSA 70% and glycerol 30%).

Multiplex PCR

All *X. oryzae* PV. *oryzae* strains were identified using a multiplex PCR specific for *X. oryzae* pathovars. Four primer pairs specific to *X. oryzae* (Xo3756F; CATCGTTAGGACTGCCAGAAG and Xo3756R: GTGAGAACCACCGCCATCT), to *X. oryzae* PV. *oryzae* (Xoo80F: GCCGCTAGGAATGAGCAAT and Xoo80R: GCGTCCTCGTCTAAGCGATA) to *X. oryzae* PV *oryzicola* (i.e. Xoc3866F: ATCTCCAGCATGTTGATCG and Xoc3866R: GCGTTCAATCTCCTCCATGT) and to universal for bacteria (Univ-0008-a-S-19F: GAGTTTGATCCTGGCTCAG [32]). For DNA extraction, bacteria were grown on a PSA medium at 28 °C. DNA extraction was carried out from a 48-hour bacterial culture in a solid PSA medium using the Gram-negative Bacteria-specific Promega Genomic DNA Purification Kit System (following the recommendations of the supplier). PCR with *X. oryzae*-specific multiplex primer was performed as previously described by Lang, et al. [32]. All isolates were tested at least twice.

Pathogenicity assays

The evaluation of the pathogenicity of *Xoo* strains was

carried out on the susceptible rice variety Azucena. The characterization of *Xoo* isolates in virulence groups (races) was carried out on a set of twelve Near Isogenic Lines (IRBB) each carrying a specific resistance gene (*Xa*) (Table 1) and the recurrent parent IR24 using a simplified scale developed by IRRI [33]. The seeds of the IRBB varieties were obtained from IRRI as part of an application by the Senegalese Institute for Agricultural Research (ISRA) for the search for a source of resistance against *Xoo* isolates. The resistance of twenty-three varieties of rice grown in Senegal was also assessed. The seeds were obtained from the selection department of the Saint Louis ISRA Center. Pathogenicity tests were conducted in a greenhouse at IRD, Montpellier, France (temperature and relative humidity: day 28 °C and 80%; at night 25 °C and 60%) and on field plots (temperature and relative humidity: maximum 35 °C and 90% minimum 22 °C and 60%) at the ISRA/ Kolda experimentation station, City, Senegal.

Rice seeds were sown in terrines filled with a "Siffy" substrate. For experiments with plants growing in the field, a Split-plot experimental setup was adopted. It included 3 repetitions with a large plot the variety within which the strains will be arranged randomly. Two factors were studied, a 'variety' factor with 12 modalities in race test and 23 in screening, and a 'strain' factor with 46 modalities. In field assays, each plot consisted of a line of 4 meters long and 2 meters wide with a 20-centimeter gap between the lines and between the pockets. A total of 200 plants per plot fertilized with NPK 15-15-15 due to 200 kg per hectare, and nitrogen fertilizer with 46% nitrogen due to 150 kg per hectare (in two fractions; half at the tillering stage and another half at the mounting stage). The inoculum was prepared with sterilized distilled water and bacterial colonies from a culture on a solid PSA medium incubated at 28 °C for 24h. Bacterial suspensions were adjusted to an optical density at 600nm of 0.2 (~10⁸ bacteria/mL) using a spectrophotometer. Forty-five days-old plants were inoculated by leaf-clipping using the Kauffman method [34]. A total of 9 plants and 3 leaves per plant were inoculated for each treatment.

Disease assessment

Lesion lengths from the cut leaf tip were measured in centimeters 15 days after inoculation [29]. Disease reactions were categorized according to lesion lengths, where plants with an average value of less than 5 cm were designated as resistant, and sensitive (S) otherwise [29,33,35].

Statistical analysis

Lesion length (LL) data for each rice line - *Xoo* isolates combination were used to evaluate the responses of rice lines to BB. A two-factor variance analysis (ANOVA) was performed following Fisher's Least Significant Difference (LSD). The means were separated by Fisher's least important tests ($p < 0.05$). Isolates were grouped into races or pathotypes when presenting the same virulence profile on NILs or

Senegalese varieties respectively. This data was analyzed with the R statistical software to perform Hierarchical Clustering (HC). Races are described according to their virulence profiles R (resistance) and/or S (sensitive) on IRBB varieties. The Complex Heatmap package was used to build and visualize the various heatmaps generated in this study [36].

Results

A survey of *Xoo* isolates in the main regions of rice production in Senegal

Field surveys were conducted during the rainy season in the main areas of rice production corresponding to the southern and northern regions of Senegal and part of the Tambacounda and Kedougou regions (Figure 1a,b). The distance between 2 consecutive sampling points (10 km to 20 km) depended on both rice field size and the diversity of the cultivated varieties. Samples were taken from infected rice leaves displaying symptoms of BB in the fields. For each sample, passport data was recorded (date and place of collection, code number, GPS coordinates, name of the site, and the name of the variety on which the sample was taken...). From September to October of the year 2015 and 2016, a total of 53 sites were visited and 103 samples were randomly collected from rice plants

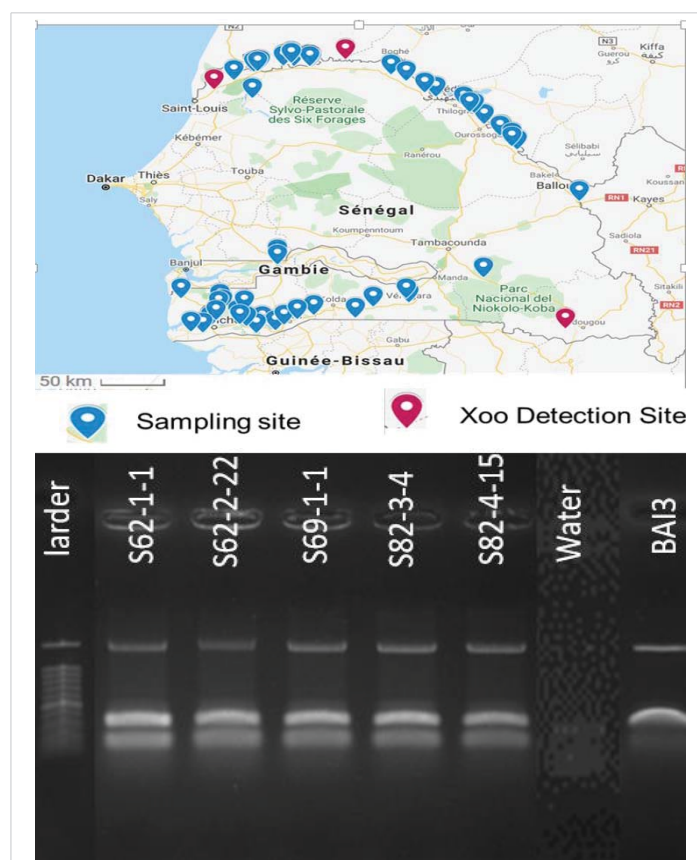


Figure 1: a: Sampling map and sites where *Xanthomonas oryzae* PV. *oryzae* strains were isolated. Diseased leaf samples were collected in the rice-growing fields of Senegal river valley in the North, Tambacounda, and Kedougou regions in the East and South East, and the Casamance region. The location of the surveyed sites is represented as blue pins on the map. Sites, where *Xoo* was isolated, are indicated on the map with red pins. b: Agarose gel of five Senegalese *Xoo* strains and two controls (BAI3 and water).



showing symptoms of BB. One leaf per sample was processed for bacterial isolation. In total, forty-four *Xoo* isolates forming pale yellow colonies on the PSA medium, were isolated and confirmed by multiplex PCR. Overall, *Xoo* strains were isolated from three sites (Ndiaye, Ndioum, and Bandafassi), corresponding to ten leaf samples that were collected on the most cultivated varieties in Senegal or on the wild species *Oryza barthii* (Table 1). To confirm their pathogenicity, all isolates were tested on the susceptible rice variety Azucena using a leaf-clipping assay. Typical symptoms of BB were visible for all isolates 14 days after inoculation. Lesion lengths

caused by the isolates varied from 6 cm to 37 cm (Figure 2). This indicates that all the isolates tested are virulent on Azucena, some of them being able to kill the plants after two months. One-way analysis of variance on lesion length revealed that there is a significant difference among isolates ($p < 0.001$). Compared to *Xoo* BAI3, the symptoms caused by strains from Senegal vary. Because some are comparable to BAI3 while others have caused shorter but significant lesions on Azucena. Altogether, our data (PCR and pathogenicity test) confirmed that the 44 isolates are indeed virulent *Xoo* bacteria (Table 1). Also, the complete genome of strain S62-2-

Table 1: List of *Xanthomonas oryzae* pv. *oryzae* strains characterized in this study.

Strain	Other names	Origin	Region	Site	Year	Host	MLSA	Virulence °	Race	Reference
S62-2-1	CIX2353	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S62-2-2	CIX2354	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S62-2-3	CIX2355	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S62-2-4	CIX2356	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	S2	This study
S62-2-5	CIX2357	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	S2	This study
S62-2-6	CIX2358	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S62-2-7	CIX2359	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S62-2-8	CIX2360	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S62-2-9	CIX2361	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S62-2-10	CIX2362	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S62-2-11	CIX2363	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S62-2-12	CIX2364	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S62-2-13	CIX2365	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S62-2-14	CIX2366	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S62-2-15	CIX2367	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S62-2-16	CIX2368	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S62-2-17	CIX2369	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S62-2-18	CIX2370	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S62-2-19	CIX2371	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S62-2-20	CIX2372	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	S5	This study
S62-2-21	CIX2373	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S62-2-22	CIX2374	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	S5	This study
S62-2-23	CIX2375	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S62-2-24	CIX2376	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	S5	This study
S62-2-25	CIX2377	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S62-2-26	CIX2378	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	S6	This study
S62-2-27	CIX2379	Senegal	Saint Louis	Ndiaye	2016	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S69-1-1	CIX2951	Senegal	Saint Louis	Ndioum	2017	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S69-1-2	CIX2952	Senegal	Saint Louis	Ndioum	2017	<i>Oryza sativa</i>	<i>Xoo</i> Africa	5	A1	This study
S82-3-4	CIX2964	Senegal	Kédougou	Bandafassi	2017	<i>Oryza bartei</i>	<i>Xoo</i> Africa	5	A3	This study
S82-3-5	CIX2965	Senegal	Kédougou	Bandafassi	2017	<i>Oryza bartei</i>	<i>Xoo</i> Africa	5	A3	This study
S82-3-6	CIX2966	Senegal	Kédougou	Bandafassi	2017	<i>Oryza bartei</i>	<i>Xoo</i> Africa	5	A3	This study
S82-3-7	CIX2967	Senegal	Kédougou	Bandafassi	2017	<i>Oryza bartei</i>	<i>Xoo</i> Africa	5	A3	This study
S82-3-8	CIX2968	Senegal	Kédougou	Bandafassi	2017	<i>Oryza bartei</i>	<i>Xoo</i> Africa	5	A3	This study
S82-3-9	CIX2969	Senegal	Kédougou	Bandafassi	2017	<i>Oryza bartei</i>	<i>Xoo</i> Africa	5	A3	This study
S82-3-10	CIX2970	Senegal	Kédougou	Bandafassi	2017	<i>Oryza bartei</i>	<i>Xoo</i> Africa	5	A3	This study
S82-3-11	CIX2971	Senegal	Kédougou	Bandafassi	2017	<i>Oryza bartei</i>	<i>Xoo</i> Africa	5	A3	This study
S82-3-13	CIX2972	Senegal	Kédougou	Bandafassi	2017	<i>Oryza bartei</i>	<i>Xoo</i> Africa	5	A3	This study
S82-3-14	CIX2973	Senegal	Kédougou	Bandafassi	2017	<i>Oryza bartei</i>	<i>Xoo</i> Africa	5	A3	This study
S82-4-1	CIX2974	Senegal	Kédougou	Bandafassi	2017	<i>Oryza bartei</i>	<i>Xoo</i> Africa	5	S4	This study
S82-4-2	CIX2975	Senegal	Kédougou	Bandafassi	2017	<i>Oryza bartei</i>	<i>Xoo</i> Africa	5	S4	This study
S82-4-3	CIX2976	Senegal	Kédougou	Bandafassi	2017	<i>Oryza bartei</i>	<i>Xoo</i> Africa	5	S4	This study
S82-4-4	CIX2977	Senegal	Kédougou	Bandafassi	2017	<i>Oryza bartei</i>	<i>Xoo</i> Africa	5	S4	This study
S82-4-15	CIX2978	Senegal	Kédougou	Bandafassi	2017	<i>Oryza bartei</i>	<i>Xoo</i> Africa	5	S4	This study
<i>Xanthomonas oryzae</i> PV. <i>Oryzae</i> (used as controls)										
MAI1	CFBP7325, LMG25985	Mali	O.Niger	Niono	2003	<i>O.sativa</i>	<i>Xoo</i> Africa	na	A3	Gonzalez et al, 2007
BAI3	CFBP7321, LMG25983	Burkina	East Center	Bagre	2003	<i>O.sativa</i>	<i>Xoo</i> Africa	na	A1	Gonzalez et al, 2007

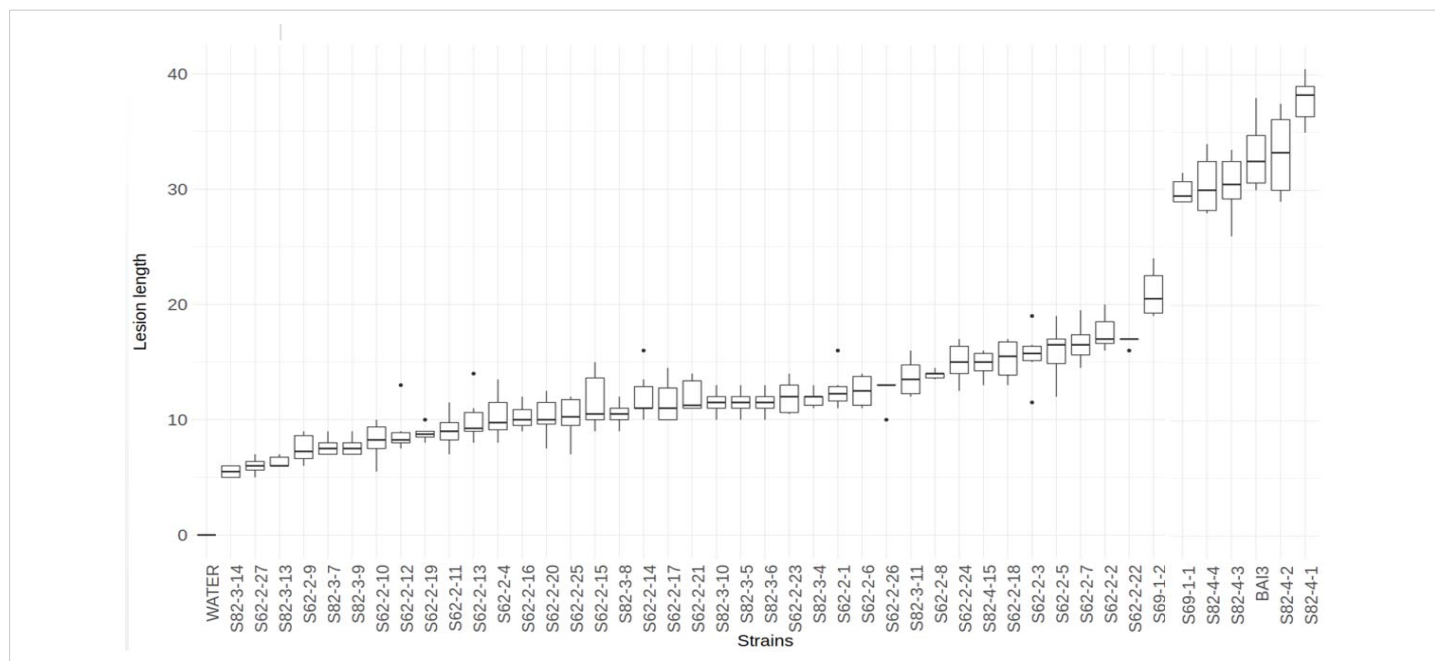


Figure 2: *Xanthomonas oryzae* PV. *oryzae* pathogenicity assay on the rice variety Azucena.

22/ CIX2374 (Table 1) was sequenced by Perez-Quintero, A.L. in 2019 and reflected at National Center for Biotechnology Information (NCBI) under the reference NZ_CP036377.1. The comparison of the genome of this strain to those of PXO99 (a strain of the Philippines) and BAI3 (a strain of Burkina Faso) confirms that the strains isolated are 100% African *Xoo*.

Box plots of the lesion length data by *Xoo* isolate. The X-axis indicates the *Xoo* isolates tested. Y-axis indicates the lesion length in cm measured 15 days after inoculation using the leaf-clipping method. The boxes represent the quartiles while the inside bars correspond to the median. Data from at least three independent replicates of the experiment with each 9 replicate lesion length values per treatment. The dots represent values that are out of the 5% and 95% quartiles delineated by the whiskers. *Xoo* BAI3 was used as a control.

Identification of Senegalese *Xoo* races from 2015 to 2016 surveys

The efficacy of *Xa* resistance genes was evaluated based on leaf clipping assay data of the 44 *Xoo* strains inoculated on 12 IRBB lines each containing a single *R* gene. IRBB lines exhibited different reaction profiles upon challenge with the 44 *Xoo* isolates (Figure 3). All IRBB lines were susceptible to one or more of our Senegalese *Xoo* strains except IRBB1 which was scored as resistant to all strains. Lesion length varied from 1 cm to 38 cm (Figure 3B). IRBB lines have different levels of resistance (Figure 3C). Similarly, variance analysis (ANOVA) shows that there is a significant difference between the IRBBs and the isolates tested ($p < 0.001$). IR24, the recurrent parent, is sensitive to 34 of the 46 isolates tested. Most IRBBs were sensitive to the majority of isolates (Figure 3A). Hierarchical clustering of lesion length data for this experiment shows that IRBB1 is resistant to all *Xoo* strains and that IRBB5 is sensitive

to only one group of isolates (Figure 3C, Table 2). Thus, *Xa1* and *xa5* provide broad resistance.

The same dataset was analyzed for the definition of *Xoo* races based on interactions between isolates and IRBB lines. The forty-four *Xoo* strain tested were classified into six races (named A1, S2, A3, S4, S5, and S6) following a categorical analysis of lesion length associated with each combination of IRBB line and *Xoo* isolate (Figure 3 A, Table 2). The A1 race is the most represented with almost 53.5% of the isolates tested and the S6 race, is the least represented with only 2.3% of the isolates (Table 1, Figure 4). The A3 race is avirulent on all IRBB lines and the recurrent parent IR24, while the S4 race is virulent on all IRBB lines except IRBB1 (Table 2, Figure 3C). Of the list of assayed resistance genes, *Xa1* is the only one that can control all the identified *Xoo* races (Table 2). The recessive gene *xa5* also controls all races except the S4 race (Table 2).

The geographical distribution of the different races of *Xoo* in Senegal is shown in Table 1. Isolates from Ndiaye (Figure 1) cover 4 out of 6 races and are the most diverse with regard to their virulence profile on the IRBB lines (Table 1, Figure 4). It should also be noted that no single region houses all described races. The A3 and S4 races are specifically present in the East of the country while the A1, S2, S5, and S6 races are confined to the Senegal River Valley in the North (Table 1, Figure 4). The A1 race is the most predominant with 23 out of 43 strains of *Xoo* and is present in two localities in the North while in the South East it is the A3 race (Table 3, Figure 4).

Virulence profile clustering of *Xoo* isolates on a set of twelve IRBB lines. On the heatmap, each interaction (IRBB lines x *Xoo*), (i.e individual cell colors) codes for the corresponding mean lesion length across three independent replicates experiment with every 9 values. Races are color-coded in the colored bar

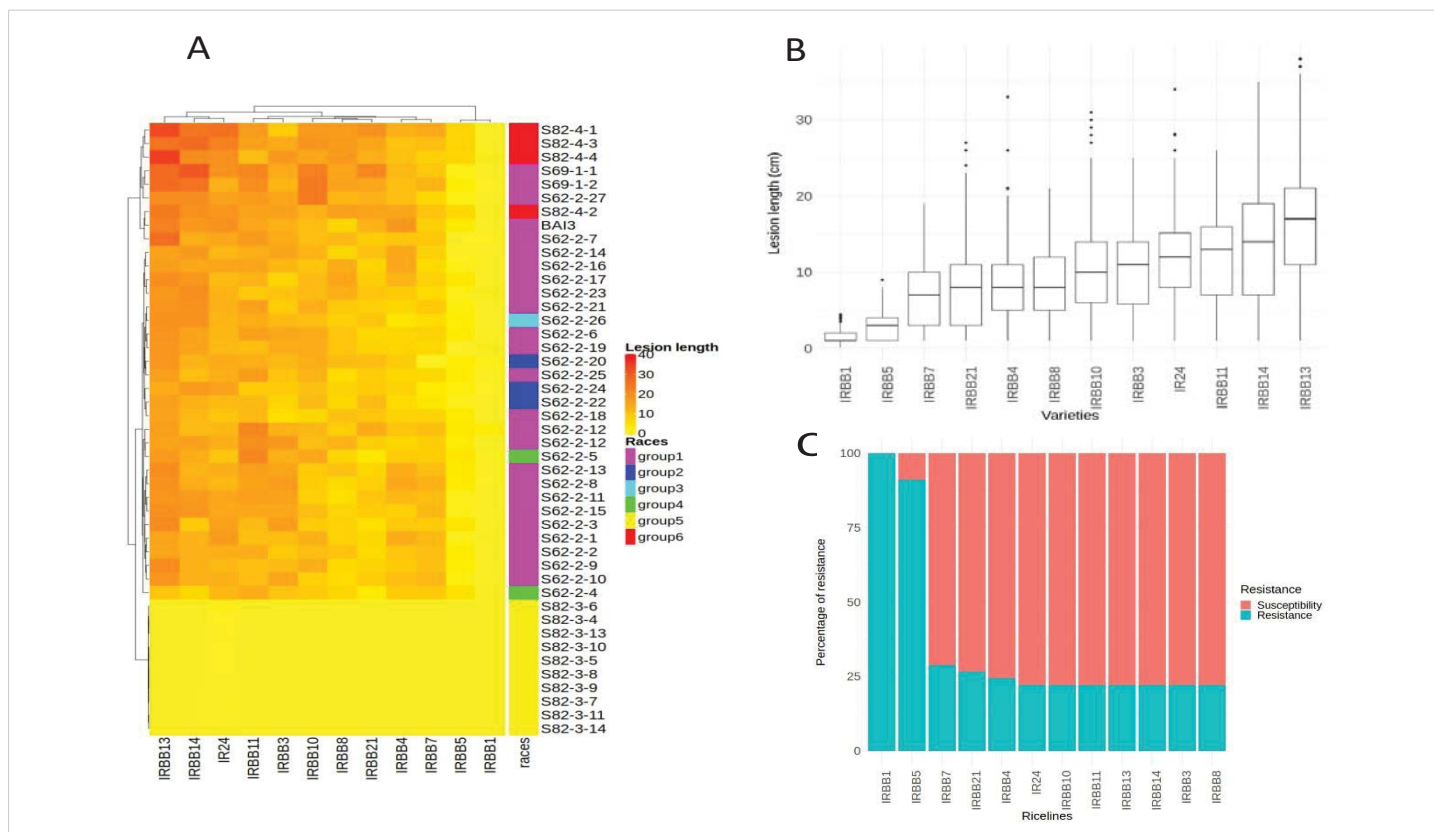


Figure 3: IRBB *Xa* resistance efficacy against forty-four *Xoo* strains.

NILs	R gene	<i>Xoo</i> Races					
		A1	S5	S2	S4	S6	A3
IR24	<i>Xa18</i>	S	S	S	S	S	R
IRBB1	<i>Xa1</i>	R	R	R	R	R	R
IRBB3	<i>Xa3</i>	S	S	S	S	S	R
IRBB4	<i>Xa4</i>	S	S	S	S	R	R
IRBB5	<i>xa5</i>	R	R	R	S	R	R
IRBB7	<i>Xa7</i>	S	R	S	S	S	R
IRBB8	<i>Xa8</i>	S	S	S	S	S	R
IRBB10	<i>Xa10</i>	S	S	S	S	S	R
IRBB11	<i>Xa11</i>	S	S	S	S	S	R
IRBB13	<i>Xa13</i>	S	S	S	S	S	R
IRBB14	<i>Xa14</i>	S	S	S	S	S	R
IRBB21	<i>Xa21</i>	S	S	R	S	S	R

Table 2: Virulence of Senegalese *Xanthomonas oryzae* PV. *oryzae* strain against NILs varieties containing a single *Xa* gene in IR24 genetic background: 44 *Xoo* strains (41 strains from this study and 4 reference strains) cluster into six races. Each race is characterized by its virulence profile on NILs. The ability of *Xa* genes to control different races is displayed. R = resistant (Lesion Length ≤ 5 cm) and S = susceptible (Lesions length > 5 cm).

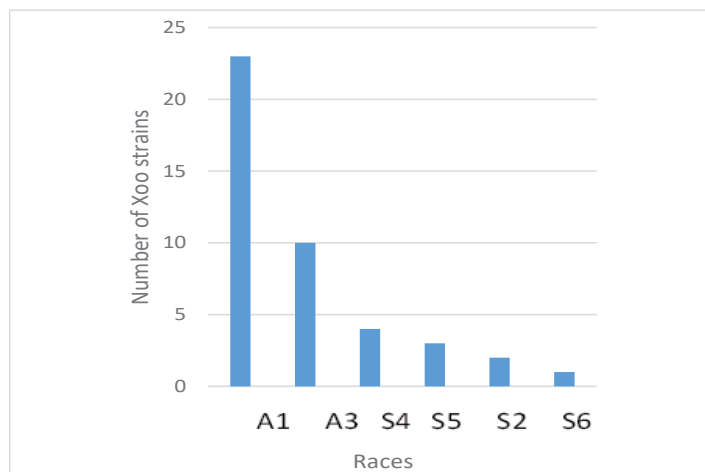


Figure 4: Number of strains collected in Senegal within the different races identified.

Table 3: Geographical distribution and race classification of *Xoo* isolated from 2015 to 2016 in Senegal. The number of strains belonging to each race is indicated.

Locality	Xoo Races						Total strains
	A1	S5	S2	S4	S6	A3	
Ndiaye	21	3	2	0	1	0	27
Ndioum	2	0	0	0	0	0	2
Bandafassi	0	0	0	4	0	10	14
Total strain	23	3	2	4	1	10	43

on the right. Isolates were also clustered according to their quantitative virulence profiles using a hierarchical clustering approach based on pairwise Euclidian distances as displayed in the tree on the left. The susceptibility profiles of the NILs were also clustered using the same method as displayed on the hierarchical tree above the heatmap. (B) The X-axis indicates the IRBB lines. Y-axis indicates the lesion lengths in cm observed 15 days after inoculation by leaf clipping. The displayed data (box plots) corresponds to lesion lengths of three repetitions per treatment (combination IRBBs x isolates). (C) Relative frequency of resistance (R in blue) or susceptibility (S in red) reactions against Senegalese isolates for individual IRBB lines. Reactions with an average lesion length < 5 cm were considered resistant, and ≥ 5 cm were considered susceptible.

Screening 23 rice varieties for resistance to BB

Given the limited number of *R* genes in the tested IRBB panel capable of controlling the different *Xoo* races encountered in Senegal (Table 2), we felt it necessary to identify additional resistance sources from 23 domestically grown varieties (Table 4) in order to diversify and adapt sources of resistance to BB. These varieties were released relatively recently compared to older varieties (such as DJ and ITA) and are most often cultivated in irrigated and low-land systems by farmers. Our Senegalese variety panel was challenged as before with 35 *Xoo* strains representing all previously identified races except race A3 strains because it is not virulent on any of the varieties except the Azucena variety. ANOVA indicates that there is a significant difference in virulence between the isolates ($p < 0,001$). Different

responses were also noted among the varieties (Figure 5). There is a large variation in the value of the median length of lesions per variety and these values range between 0 cm and 46.1 cm (Figure 5B). Average lesion length values per treatment were converted to a categorical scale and classified as 'susceptible' if the average length was equal or superior to 5 cm or 'resistant' otherwise. Similar to what is generally done for the classification of strains into races, we classified strains in the same pathotype if their categorical interaction profile on the Senegalese varieties were identical (Figure 5A). The hierarchical dendrogram of the average lesion length data induced by 35 strains on the 23 varieties was cut into 32 Clusters which correspond to different resistance profiles (Figure 5A). Nineteen varieties are found to be susceptible to at least one race of identified *Xoo* strain and four varieties (Dansan2; Sahel202, Sahel305, and Sahel328) are resistant to all strains tested (Figure 5C). Based on the percentage of strains these varieties are resistant to, they can be subdivided into five different groups (Figure 5C). The first group includes varieties resistant to 100% of the strains (4 varieties); the second is composed of varieties resistant to more than 75% of the strains (2 varieties); the third group corresponds to those varieties that are resistant to between 75% and 50% of strains (4 varieties); the fourth group includes those varieties that are resistant to between 50% and 25% of the strains (5 varieties). Finally, varieties classified in the fifth group are resistant to less than 25% of the strains (Figure 5 C).

(A) Aggressiveness profile of isolates on the rice varieties tested. Heatmap display of the aggregated lesion length data by isolate and by variety. For each interaction (rice varieties *Xoo* isolates), individual cell colors code for the corresponding mean lesion length across three independent replicates of the experiment with each six replicates. Strains are color-coded colored bar on the right. (B) Efficiency of rice varieties against the 35 isolates tested. X-axis indicates the rice varieties tested; Y-axis indicates the lesions lengths in cm observed 15 days after inoculation by leaf clipping. Overall lesion length data per rice varieties. The displayed data (box plots) corresponds to lesion lengths of three repetitions per treatment (rice varieties 35 isolates). (C) Frequency of resistance phenotypes among the rice varieties. Lesion lengths < 5 cm are considered as resistant (R in blue), and ≥ 5 cm susceptible (S in pink).

Discussion

Sampling for plant leaves with BB symptoms has been conducted at several sites but the isolation of bacteria was only successful in three sites despite the high number of sites surveyed. This may be due to the isolation protocol which has some limitations in extracting bacteria from the diseased tissues. For this reason it would be interesting to perform the multiplex PCR test directly on the leaf lysates since we cannot exclude that the selected portion no longer contains living *Xoo*. *Xoo* isolates from a collection of rice leaf samples from different regions of Senegal were characterized, revealing the

Table 4: List of Senegalese varieties used in the resistance screen.

Ecology	Variety name	Parents	species	origin	Cycle (days)	BB resistance	Creation year	Diffusion Year	Potential Yield (T/H)
Irrigated	Sahel 210	na	<i>O. Sativa (Indica)</i>	Latin America		sensitive	na	2007	12
Irrigated	Sahel 217	Sahel 201/4456	<i>O. sativa (Indica)</i>	AfricaRice	109-129	Tolerant	1997	2009	13
Irrigated	Sahel 209	TSY/MOROBERKAN//ITA306	<i>O. sativa (Indica)</i>	IITA/Nigeria	126-140	Tolerant	na	2007	12
Irrigated	Sahel 208	ITA 212/UPL RI 7	<i>O. sativa (Indica)</i>	IITA/Nigeria	125-145	Tolerant	na	2007	12
Irrigated	Sahel 201	IR 2071-586/BG 400-1	<i>O. sativa (Indica)</i>	Sri Lanka	121-142	Tolerant	na	1994	10
Irrigated	Sahel 222	Sahel 201/4456	<i>O. sativa (Indica)</i>	AfricaRice	103-129	Tolerant	1997	2009	13
Irrigated	Sahel 305	IR 64/4456	<i>O. sativa (Indica)</i>	AfricaRice	101-124	Tolerant	1995	2009	10
Irrigated	Sahel 317	4456/32 Xuan 5C	<i>O. sativa (Indica)</i>	AfricaRice	97-122	Tolerant	1995	2009	12
Irrigated	Sahel 328	IR 31851-96-2-3-2-1(Sahel 134)/IR 66231-37-1-2	<i>O. sativa (Indica)</i>	AfricaRice	87-116	Tolerant	1997	2009	10
Irrigated	Sahel 329	Jaya / Basmati 370	<i>O. sativa (Indica)</i>	AfricaRice	87-116	Tolerant	1997	2009	10
Irrigated	Sahel 108	IR 30 (BHP)/BABAWA//IR 36	<i>O. sativa (Indica)</i>	IRRI Philippines	110-117	Tolerant	na	1994	7,5
Irrigated	Sahel 134	IR 1791-5-4-3-3/IR 9129-209-2-2-2-1	<i>O. sativa (Indica)</i>	IRRI Philippines	110-131	Tolerant	na	2007	10
Irrigated	Sahel 159	IR 13240-108-2-2-3/IR 9129-209-2-2-2-1	<i>O. sativa (Indica)</i>	IRRI Philippines	110-130	Tolerant	na	2007	10
Irrigated	Sahel 177	IR 31851-96-2-3-2-1(Sahel 134)/IR 66231-37-1-2	<i>O. sativa (Indica)</i>	AfricaRice	110-122	Tolerant	1998	2009	10
Irrigated	NERICA S 19	Tog 5681/2*IR 64//IR 31785	<i>Oryza sativa x Oryza glaberrima</i>	AfricaRice	105-131	na	1997	2009	11
16	NERICA S 44	IR 64//Tog 5681/4*IR 64	<i>Oryza sativa x Oryza glaberrima</i>	AfricaRice	100-122	na	1998	2009	12
17	NERICA S 36	Tog 5681/2*IR 1529//IR 1529	<i>Oryza sativa x Oryza glaberrima</i>	AfricaRice	101-120	na	1998	2009	11
18	Dansan2	na	<i>O. sativa (Indica)</i>	na	na	Tolerant	na	na	na
19	MYRLANG 23	IR 1317-316-5-1/IR 24	<i>Tongil (japonica/ indica)</i>	South Korea	na	na	na	2017	13,5
20	TAEBAEGRYEO	IR 24*2/IR 747-B2-6-3	<i>Tongil (japonica/ indica)</i>	South Korea	na	na	na	2017	12,5
21	SAMGAMBYEO	na	<i>Tongil (japonica/ indica)</i>	South Korea	na	Tolerant	na	na	12,5
22	JINMIBYEO K9)	na	<i>Tongil (japonica/ indica)</i>	South Korea	na	na	na	na	-
Irrigated	Sahel 202	TOX 494-3696/TOX 711/BG 6812	<i>O. sativa (Indica)</i>	Nigeria	117-139	na		1994	11

Each variety is listed by pedigree, species and subspecies, origin (country or institution), cycle, year of diffusion, and potential yield. IRRI: International Rice Research Institute, Philippines, Africa Rice: Africa Rice Center, Senegal; IITA: International Institute of Tropical Agriculture, Ibadan, Niger, na: data not available

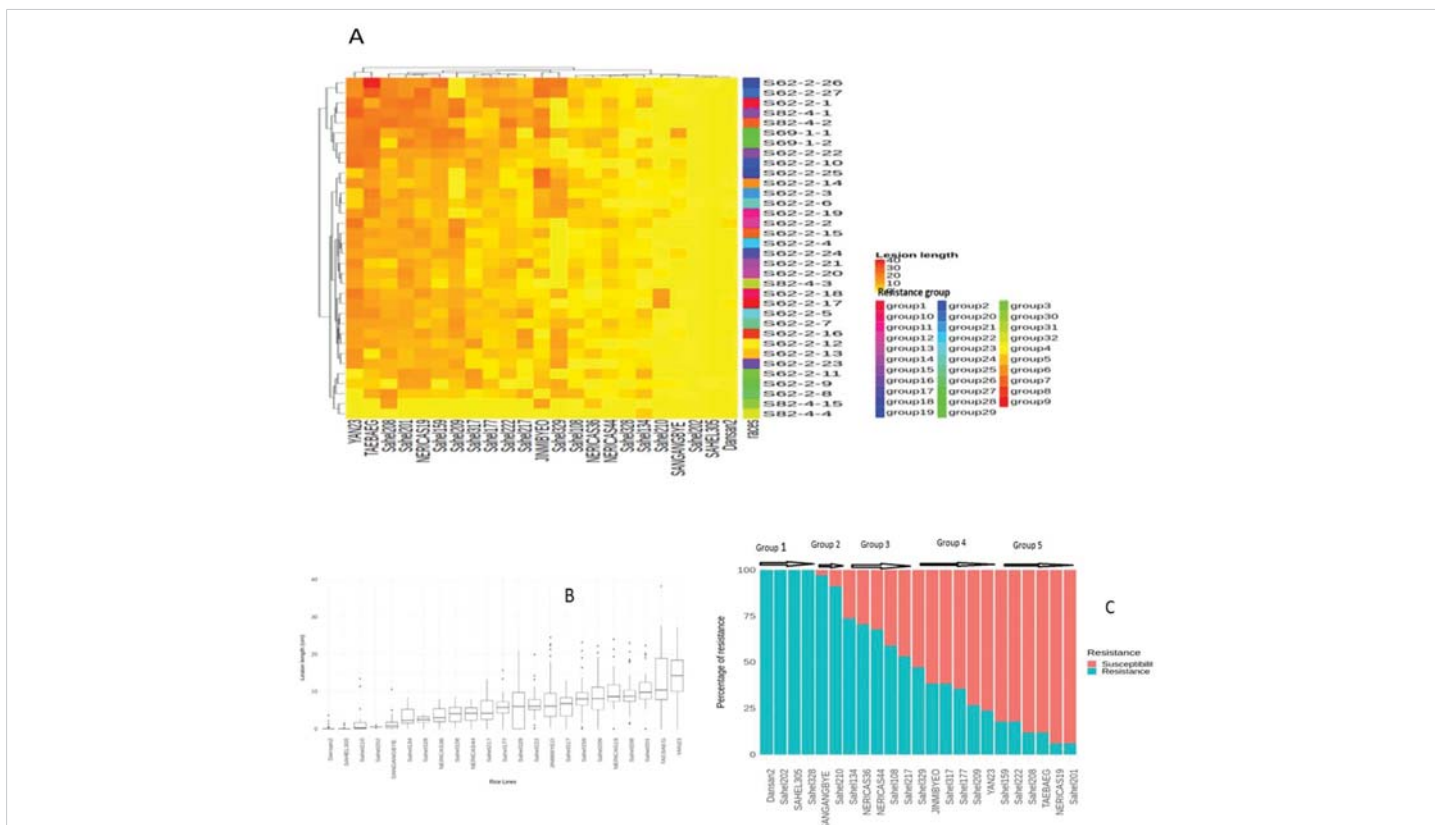


Figure 5: Senegalese rice varieties level resistance to 35 Xoo strains.



presence of six races in the set of 44 identified *Xoo* strains. These 6 races are the first *Xoo* characterized in Senegal following the confirmation of the presence of the pathogen by Tall, et al. [11]. *Xa1* stands out as the most effective *R*-gene capable of controlling all six Senegalese races. Furthermore, *xa5* also shows broad efficiency but remains ineffective against strains of the S4 race. Importantly, four rice varieties grown in Senegal, namely Dansan2, Sahel202, Sahel305 and Sahel328, were found to be resistant to all Senegalese *Xoo* strains tested.

The first characterization of *Xoo* races from Senegal points to *Xa1* and *xa5* as efficient *R*-genes against this pathogen population.

One of the major objectives of this work was to study the pathotypic diversity of *Xoo* strains isolated from different geographic areas in Senegal. In this study, all 12 IRBB nearly-isogenic lines showed different levels of resistance. Phenotypical diversity of *Xoo* exists throughout Senegal. The virulence profiles of *Xoo* collected on improved and wild rice were similar, suggesting that host diversity does not affect pathogenicity on the set of rice genotypes tested here (Table 1, Figure 2). Several authors have reported that the diversity of hosts does not affect the pathogenicity of *Xoo* populations [37]. However, other authors argue that host diversity has an effect on the pathogenicity of *Xanthomonas oryzae* pv. *oryzae* [38]. Our virulence analysis of 44 strains collected in 3 Senegalese rice-producing areas indicates that *Xoo* strains can be classified into 4 Senegalese-specific races (S2, S4, S5 and S6) that are different from those previously reported in West Africa [19,30,31,39]. However, our results confirmed the presence of race A3 that has been described in Mali and race A1 described in Burkina Faso [19,30,31,40]. None of the isolated *Xoo* strains from Senegal were able to overcome the *Xa1* resistance gene. Similarly, the *xa5* gene controls all *Xoo* races except S4 (Figure 3A and 3C). Thus, the *xa5* and *Xa1* genes have good potential for breeding BB resistant rice varieties in Senegal. Two *Xoo* races (A1 and S4) showed similar reactions on the 12 NILs, except on IRBB5, harboring the R *xa5* gene. The S4 race was the only one virulent on IRBB5. Several studies have shown that the degree of resistance conferred by *xa5* varies for *Xoo* strains from different regions of West Africa [31,41,42]. A majority of *Xoo* races (83%) was virulent on IRBB3, IRBB8, IRBB10, IRBB11, IRBB13 and IRBB14 suggesting that the genes *Xa3*, *xa8*, *Xa10*, *Xa11*, *xa13* and *Xa14* cannot provide rice resistance against most *Xoo* strains in Senegal. The *Xa21* gene controls only 2 races out of the 6 identified races. This indicates that *Xa21* action spectrum is limited against Senegalese *Xoo* races. This results is in line with previous reports [31,40]. Altogether, these studies conclude on a limited practical value of *Xa21* to globally control African *Xoo* races. This contrasts with other studies that have reported the broad efficiency of the *Xa21* gene against Asian races of *Xoo* [43,44]. In conclusion, we believe that pyramiding *Xa1* and *xa5* *R*-genes would be most effective in providing broad and potentially durable BB resistance to Senegalese rice varieties.

These two *R*-genes could be transferred to susceptible elite cultivars via marker-assisted selection. On the other hand, it will be difficult to understand the evolutionary trajectories of Senegalese *Xoo* because there are no isolates prior to H. Tall sampling in 2019 [11].

We also examined if the geographical distribution of Senegalese *Xoo* races displayed any detectable pattern. When cross-referencing race test results with geographic sampling data (Table 1), we observed that races A1, S2, S5 and S6 are exclusively detected in the Senegal River Valley in the North of the country, while the A3 and S4 races were only found in the South-East. We therefore conclude that Senegalese races segregate along a North/South pattern. The probable hypothesis at this origin of differentiation may be due to the fact that two different agro-ecologies can be distinguished with intensive irrigated system in the North and low-land rainfed system in the South-East with specific rice varieties adapted to each ecology. Our results highlight the importance of continuously monitoring Senegalese *Xoo* population using IRBB lines as a discrimination tool.

Varieties Sahel202, Sahel305, Sahel328 and Dansan2 possess broad resistance against Senegalese *Xoo*

The rice varieties used in this screening study are listed in Table 2. A total of 46 strains were tested, including the BAI3 strain [19] as a positive control and distilled sterilize water as a negative control for leaf inoculations in all trials. In the set of 23 rice varieties widely cultivated in Senegal and screened for bacterial blight (BB) resistance, accessions have various levels of resistance to BB (Figure 5C). Six of them are highly and broadly resistant to all tested strains, with Sahel202, Sahel305, Sahel328 and Dansan2 being the most promising. These 4 varieties may carry new genes for BB resistance with broad resistance spectrum against *Xoo* populations in Senegal. Similar approaches have been carried out in West Africa, notably in Mali and Burkina Faso [31,45] which also identified domestic varieties with good potential as BB resistance donors in breeding programs. In this respect, the characterization of the genetic determinants of the resistance of Sahel202, Sahel305, Sahel328 and Dansan2 needs further investigation.

In conclusion, this study on Senegalese *Xoo* virulence diversity and host resistance profiles is a pioneering work in the country and parallels recent progresses on BB pathology in the sub-region of West Africa. We studied isolates collected over two years to investigate the nature and diversity of pathogenic races, their geographic distribution and the *R*-genes resistance needed to control BB. The collected *Xoo* strains were subdivided into 6 races displaying a geographic distribution pattern with 4 races in the North and 2 in the South-East of Senegal. Compared to other studies in West Africa, the composition of Senegalese *Xoo* races indicates the presence of races already described in Mali and Burkina Faso, and 4 new races described for the first time in Africa.



The *Xa1* and *xa5* *R*-genes are the most promising candidate genes for a broad control of *Xoo* races detected to date. The study also identified several varieties grown in Senegal that are highly resistant to different races detected in the country. However, further analysis is needed if we want to understand and identify the *R*-genes that are involved in this resistance. The transfer of this resistance to other high-yielding varieties grown in Senegal is expected to significantly improve rice productivity and BB control.

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Author contributions

H.T., K.N. and V.V. designed the research; H.T., K.N.; S.C. and V.V. performed the research; H.T., S.C., A.C and V.V. analysed the data; H.T., K.N., S.C., B.S, A.C., M.H. and V.V wrote the manuscript.

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