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Molecular Analysis of *Xa4* and *xa5* Resistance Genes Involved in the Response to Bacterial Lieaf Blight in Conventional Rice Lines in Burkina Faso

Soumana Kone
Centre Nationale de Recherches Scientifiques et Technologiques (CNRST) / Institut de l'Environnement et de Recherches Agricoles (INERA), Burkina Faso
Université Daniel OUEZZIN COULIBALY / Ecole Doctorale Sciences et technologies, Laboratoire Biosciences, Burkina Faso

Abdourasmane Kadougoudiou Konate
Centre Nationale de Recherches Scientifiques et Technologiques (CNRST) / Institut de l'Environnement et de Recherches Agricoles (INERA), Burkina Faso

Antoine Barro
Université Daniel OUEZZIN COULIBALY / Ecole Doctorale Sciences et technologies, Laboratoire Biosciences, Burkina Faso

Issa Wonni
Centre Nationale de Recherches Scientifiques et Technologiques (CNRST) / Institut de l'Environnement et de Recherches Agricoles (INERA), Burkina Faso

ABSTRACT

Rice is a staple crop in Burkina Faso, but its production is threatened by bacterial leaf blight, which caused yield losses of up to 50% between 1998 and 2004. To address this issue, new rice varieties have been developed through crosses with IRBB lines carrying multiple resistance genes, particularly Xa genes. This study aimed to identify the presence or absence of Xa4 and xa5 resistance genes in these new varieties and their parental lines. The plant material consisted of 12 rice lines, including five new varieties and their respective parents. Molecular detection was performed using PCR-based markers; MP1 for the *Xa4* gene and RM122 for the *xa5* gene. Genomic DNA was extracted using a modified Permingeat protocol, and amplification products were analyzed by electrophoresis on 3.5% agarose gel in 0.5X TAE buffer. Results showed that six lines carried the xa5 gene: the parents CT21376-F3-9-1, IRBB60, and the new varieties AR19L018-F4-27, AR19L018-F4-22, and AR19L025-F4-117. The Xa4 gene was detected in seven lines: the parents IRBB50, IRBB60, CT21376-F3-9-1, and the new varieties AR19L018-F4-22, AR19L018-F4-27, AR19L021-F4-99, and AR19L025-F4-117. Notably, three new varieties AR19L018-F4-22, AR19L018-F4-27, and AR19L025-F4-117 possess both Xa4 and xa5, suggesting a pyramiding of resistance genes that may provide durable protection against multiple *Xanthomonas oryzae* pv. *oryzae* strains. Field trials in disease-endemic areas are recommended to validate their resistance and agronomic performance.

Keywords: Rice, Bacterial leaf blight, gène *Xa*4 et *xa*5

INTRODUCTION

Rice is the staple food for over half the world's population. By 2050, its production will need to increase by 42% to meet the food needs of a steadily growing population [1]. However, without the implementation of concrete and sustainable measures, global rice yields could fall by 15-20% by 2050, due to the combined impact of biotic and abiotic factors. Biotic factors alone are responsible for almost 52% of productivity losses, with diseases, particularly vascular bacterial blight, playing a predominant role.

Bacterial leaf blight (BLB) is an emerging disease of rice, caused by the bacterium *Xanthomonas oryzae* pv*. oryzae* (Xoo), responsible for yield losses ranging from 20% to 80% depending on varietal susceptibility [2]. In Burkina Faso, this disease has caused significant losses of up to 50%, particularly after the introduction of the particularly susceptible Chinese variety TCS10 [3].

Faced with this threat, the development of high-yielding, BLB blight-resistant rice varieties is a key strategy. This approach has the advantage of reducing the use of synthetic pesticides, thus helping to protect the environment and human and animal health. Genetic control of bacterial blight is mainly based on the introduction of single-gene resistance genes. However, this form of resistance can be circumvented by the evolution of the pathogen, Xoo, which is capable of adapting to the selective pressure exerted by resistance genes [4].

To enhance the durability of resistance, the gene pyramiding strategy is recommended. This consists of combining several resistance genes in a single genotype, offering broader protection against different strains of *Xoo* [5]. This combination of genes confers more stable resistance and reduces the likelihood of the pathogen overcoming the plant's genetic defense [6].

Molecular markers have been developed to identify genotypes carrying multiple resistance genes. Among these, SSR markers specific to Xa genes have been extensively studied [7]. In particular, the Xa4 and xa5 genes were introduced into the IR24 variety using backcrossing techniques, giving rise to quasi-isogenic lines such as IRBB50 and IRBB60. These lines were then crossed with local varieties to generate new varieties likely to carry the same resistance genes.

Molecular markers MP1 (for the Xa4 gene), [8] and RM122 (for the xa5 gene), [9], based on polymerase chain reaction (PCR), are used to detect the presence of these resistance genes in rice lines.

Some of these new varieties have shown good resistance to BLB under artificial inoculation conditions in greenhouses. It is therefore essential to confirm the presence of Xa4 and xa5 genes in these promising genotypes, which are both agronomically efficient and tolerant to the disease.

The aim of the present study is to identify the presence or absence of the Xa4 and xa5 resistance genes in the new varieties developed, as well as in their respective parental lines.

MATERIALS AND METHODS

Experimental Site

The study was carried out at the molecular biology laboratory of the Centre d'Excellence en Fruits et Légumes, based at the INERA/Farako-Bâ research station. This station is located in the western region of Burkina Faso, about ten kilometers southwest of the town of Bobo-Dioulasso, on the Bobo-Banfora road.

It covers a total area of 475 hectares, 375 hectares of which are set aside as experimental plots for agricultural research. The station's geographical coordinates are 04°20' west longitude, 11°60' north latitude, at an altitude of 450 meters (Figure 1).

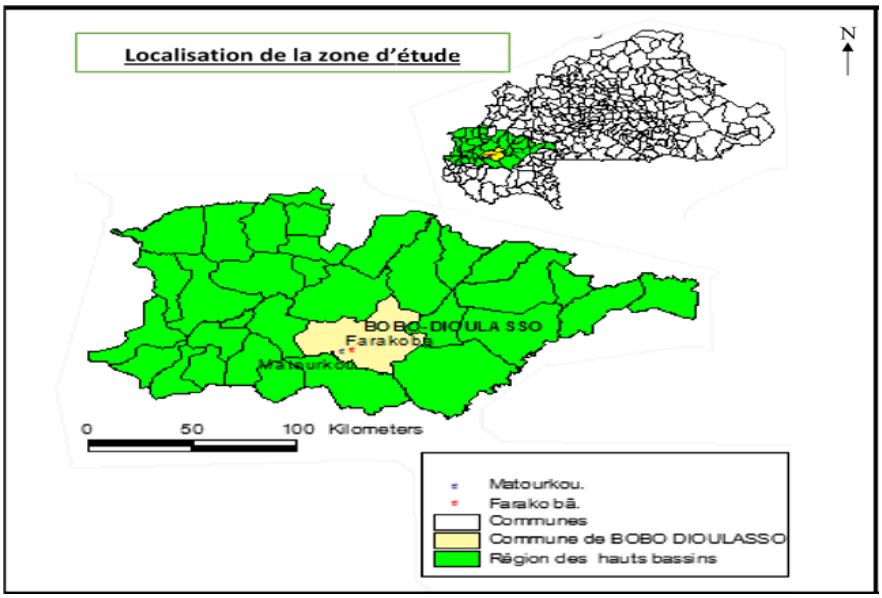

Figure 1: Map of the study zone

Plant Material

The plant material consists of twelve (12) varieties, including five (05) new varieties, five parental varieties and two (02) local varieties. Table 1 presents the characteristics of these different varieties. The five (05) new varieties were developed by the Africa Rice Center (AfricaRice). The IRBBs are isogenic lines containing the *Xa* pyramid genes. IRBB50 contains the *Xa4* + *xa5* genes and IRBB60 the *Xa4* + *xa5* + *xa13* + *Xa21* genes [10;11]). The FKR64 (TS2) and FKR19 controls are the most widely produced local varieties in Burkina Faso.

Strain of *Xanthomonas oryzae* pv. *oryzae*

The Xoo BAI3 bacterial strain belongs to race A1 and originates from Burkina Faso, more specifically from the Bagré plain. It was isolated in 2003 from leaf samples of *Oryza longistaminata* by Gonzalez et al. (2007). Resistance tests carried out by [12] Gonzalez *et al.* revealed that the *Xa3*, *Xa8*, *Xa10*, *Xa11*, *Xa13*, *Xa14* and *Xa21* genes did not confer any efficacy against the BAI3 strain, unlike the *Xa4*, *xa5* and *Xa7* genes, which significantly reduced the aggressiveness of this strain. In addition, the analysis of TAL (Transcription Activator-Like Effector) effector profiles carried out by Diallo *et al*. identified eight TAL effectors in the BAI3

strain, among which TALC plays a major role as a virulence factor [13] . It should also be noted that the TALE sequences of BAI3 are available and have been described by.

Table 1: Characteristics of the varieties evaluated

Variéty	Parents	Reaction to BLB	Nature
ARICA 3	-	S	Parent
IRBB50	-	R	Parent
CT21376-F3-9-1	-	Nd	Parent
SAHEL 177	-	Nd	Parent
IRBB60	-	R	Parent
FKR64 (TS2)		S	Control
FKR19		R	Control
AR19L016-F4-174	ARICA 3/IRBB50	Nd	New variety
AR19L018-F4-22	CT21376-F3-9-1/IRBB50	Nd	New variety
AR19L018-F4-27	CT21376-F3-9-1/IRBB50	Nd	New variety
AR19L021-F4-99	SAHEL 177/IRBB50	Nd	New variety
AR19L025-F4-117	ARICA 3/IRBB60	Nd	New variety

Nd : Not determined ; R : Resistant; S : Susceptible

DNA Extraction and PCR Amplification of Xa Genes

Rice seeds of all genotypes were grown in germination pots under a controlled environment. Young leaves were collected for DNA extraction at the seedling stage, following the protocol described by Permingeat *et al*. with a few modifications [14] .

DNA fragments corresponding to the *Xa4* and *xa5* genes were amplified by polymerase chain reaction (PCR) using closely related markers: “MP1” for *Xa4* ([8] and “RM122” for xa5 ([9] (Table 2). The sequences of the primers (sense and antisense) used are shown in Table 3. PCR was performed in a total volume of 10 µL, comprising 2 µL of genomic DNA (30 ng/µL), 0.75 µL of each primer (10 pmoles/µL), 1.5 µL of ultrapure water (doubly distilled) and 5 µL of master mix (Thermo Scientific, USA). Reactions were run in a Biometra Tone thermocycler according to the following program: initial denaturation at 95°C for 5 minutes, followed by 35 cycles comprising denaturation at 95°C for 1 minute, annealing at 54°C for 1 minute and elongation at 72°C for 2 minutes. A final extension at 72°C for 5 minutes completed amplification. PCR products of the *Xa4* and *xa5* genes were separated by electrophoresis on 3.5% agarose gel prepared with 0.5X TAE solution. The gel was stained with ethidium bromide (10 µg/mL), then visualized under ultraviolet light using a Tech UV system (USA). The presence or absence of amplified DNA fragments was noted by “++” and “--” respectively.

Table 2: Sequences of primers specific to the Xa4 and xa5 genesI

Gene	Located on chromosome	Marker	Type of marker	Primer sequence (5’-3’)	Resistance band (bp)	Susceptible band (bp)
Xa4	4	MP1	STS	ATCGATCGATCTTCACGAGG TCGTATAAAAGGCATTCGGG	150	120
xa5	5	RM122	STS	GAGTCGATGTAATGTCATCAGTGC GAAGGAGGTATCGCTTTGTTGGAC	240	230

RESULT AND DISCUSSION

Molecular analysis of the rice lines and their parents detected the presence or absence of the Xa4 and xa5 BLB resistance genes (Table 3). The results revealed that seven (07) lines carried the Xa4 gene, namely CT21376-F3-9-1, AR19L018-F4-22, AR19L018-F4-27, AR19L021-F4-99, AR19L025-F4-117, as well as the near-isogenic lines IRBB50 and IRBB60. The MP1 microsatellite marker used to screen for this gene proved effective in discriminating resistant lines.

With regard to the xa5 gene, the results showed that six (06) lines were carriers: parents CT21376-F3-9-1, IRBB50, IRBB60, and varieties AR19L018-F4-22, AR19L018-F4-27, and AR19L025-F4-117, identified using the RM122 marker. Among these lines, three (03) new varieties (AR19L018-F4-22, AR19L018-F4-27 and AR19L025-F4-117) combine both Xa4 and xa5 genes, indicating possible enhanced resistance through gene pyramiding.

Table 3: Identification of Xa genes in tested varietiess

Code	Line	Parent	*Xa4*	*xa5*
6	ARICA 3		--	--
22	IRBB50		++	++
4	CT213 76-F3-9-1		++	++
30	SAHEL 177		--	--
8	IRBB60		++	++
9	AR19L016-F4-174	ARICA3/IRBB50	--	--
10	AR19L018-F4-22	CT213 76-F3-9-1/IRBB50	++	++
14	AR19L018-F4-27	CT213 76-F3-9-1/IRBB50	++	++
15	AR19L021-F4-99	SAHEL 177/IRBB50	++	--
16	AR19L025-F4-117	ARICA3/IRBB60	++	++
36	FKR64	Control	--	--

--: absence ofgene; ++: presence of gene

The presence of Xa4 or xa5 confers partial or complete resistance to different Xoo strains. These results are in line with those of Sabar et al. who identified the Xa4 gene in 41 lines and the xa5 gene in 14 lines out of 80 lines tested [15] . The Xa4 gene, widely used in breeding, is known to offer broad-spectrum resistance at all growth stages *(Petpist et al., 1997)*, while the xa5 gene, recessive and located on chromosome 5, is renowned for its efficacy against many Xoo strains and its usefulness in breeding programs [10].

To date, over 30 major Xoo resistance genes (from Xa1 to Xa38) have been identified, from cultivated varieties (indica, japonica) and wild species (*Oryza longistaminata*, *O. rufipogon*, *O. minuta*, *O. officinalis*) [16,17]). Some genes have also been obtained by mutagenesis using N-methyl-N-nitrosourea or thermal neutron irradiation (*Nakai et al., 1988*; *Gao et al., 2002*).

In addition, several of these genes have been introgressed into the IR24 variety via repeated backcrossing, enabling the development of pyramided quasi-isogenic lines such as IRBB50 and IRBB60, whose multiple resistance has been confirmed by Nino-Liu *et al*. and *Cheema et al.* [10,11].

Pyramiding several resistance genes in a single variety is a sustainable strategy for controlling BLB disease. It limits the risk of pathogen escape, unlike the use of a single gene. This strategy was validated by the work of Singh et al. who showed that triple pyramiding of the xa5, xa13 and Xa21 genes in the PR106 variety resulted in stable long-term resistance to several strains of Xoo in Punjab, India [18].

Similarly, Khanna et al. achieved long-lasting resistance to blast (a rice disease caused by Magnaporthe oryzae) in Basmati and Supa Basmati varieties by introgressing seven Pi genes from different donor lines, demonstrating the effectiveness of genetic accumulation in disease management [19].

In the context of Burkina Faso, these results are particularly promising. The new varieties carrying the Xa4 and xa5 genes could represent an effective solution for stabilizing rice yields in areas endemic to BLB disease. In addition, they could help reduce the use of phytosanitary products, thereby enhancing the sustainability of production systems.

CONCLUSION

The results revealed that six (06) lines carried the xa5 gene, including three (03) new varieties (AR19L018-F4-22, AR19L018-F4-27 and AR19L025-F4-117) and their respective parents (IRBB50, IRBB60 and CT21376-F3-9-1). In addition, seven (07) lines have been identified as carrying the Xa4 gene, including the three new varieties mentioned above, as well as the parents IRBB50, IRBB60, CT21376-F3-9-1, and AR19L021-F4-99.

Notably, lines AR19L018-F4-22, AR19L018-F4-27 and AR19L025-F4-117 display pyramiding of the Xa4 and xa5 genes, indicating a potentially more durable and effective dual resistance against several strains of Xanthomonas oryzae pv. oryzae.

These results are promising for breeding programs, providing breeders with genotypes carrying resistance genes that can be used in crosses with local varieties that perform well but are susceptible to the disease. However, although the results obtained under semi-controlled conditions are encouraging, multi-location field trials on sites endemic to BLB are needed to confirm the agronomic performance and durability of the resistance of these lines in real environments.

Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this article.

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