Molecular Identification of Xa4 resistance gene to *Xanthomonas oryzae* pv. *oryzae* in cultivated rice in Western Benin

ABSTRACT

Aims: The aim of this study was to identify the cultivated rice varieties that were resistant to *Xanthomonas oryzae* pv. *oryzae (Xoo)* by molecular screening. *Where, Xanthomonas oryzae* pv. *oryzae (Xoo)* was the causal agent of bacterial blight, in the department of Atacora in Benin.

Place and Duration of Study: Laboratory of Molecular Biology and Bioinformatics Applied to Genomics, between July 2021 and November 2021.

Methodology: Thirty-two rice accessions were collected in the department of Atacora and the IRBB4 isogenic line carrying Xa4 resistance gene, as a positive control, were screened using SSR marker. Genomic DNA was extracted from plants leaves. PCR using a pair of MP12 primers linked to the Xa4 gene were performed and amplified products were analyzed by electrophoresis in a 2 % agarose gel.

Results: Our results showed that a significant number of rice varieties grown in northern Benin were resistant to Xoo. 62.5% were resistant of which 70% were local varieties. Some of these resistant varieties (35%) were heterozygous (*Xa4/xa4)* and others were homozygous *(Xa4/Xa4)*. 10% had specific genotypes other than those expected, which showed the probability of existence new resistance alleles that need to be characterized.

Conclusion: This is the first time that a bacterial blight resistance gene has been identified in Beninese rice cultivation. This result will be very useful to rice breeders for developing elites resistant varieties through markers assisted selection programs.

Keywords: Rice, Bénin Xanthomonas oryzae pv oryzae, Bacterial blight, Xa4 resistance gene

1. INTRODUCTION

Rice is a cereal crop and one of the most important food in the world. With a production of 755,473 million tons and a yield of 4661.8 kg/ha in 2019 (1), rice becomes the staple food of about 60% of the world's population. According to the most recent FAO forecast, global rice consumption is expected to increase by 69 million tons by 2029 (1). In Africa, because of its significant contribution to food security and balance, as well as for its important source of income and employment for the poorest populations, it constitutes an essential component of sustainable development. Its total consumption in sub-Saharan Africa should increase from 20 to 48 million tons in 2050 assuming an increase of 1.5% per year (2). In Benin, statistics revealed that rice production is constantly increasing and increased to 406,000 tons of paddy rice in 2019 (1). It is therefore urgent to ensure food security while satisfying the growing demand for rice by the world's population (which is constantly increasing to reach 9.7 billion by 2050). Efforts are being made by many governments and agricultural research structures to boost rice production. However, one of the major problems remains the impact of pathogens on rice. Indeed, rice production is limited by many biotic and abiotic constraints. Among the biotic constraints are bacterial diseases such as bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) causing huge crop losses of up to 50% (3, 4,5,6) In West Africa, the incidence of the disease ranged from 70-85% with a yield loss of 50-90%, indicating wide spread of bacterial wilt in farmers' fields (5). In Benin, Xoo was first described in 2013 on wild related species *Oryza longistaminata* (7). So far, none study has been done to cheek the incidence of this disease in cultivated rice. Since the use of resistant varieties, carrying resistance genes, is the only highly effective and environmentally friendly way to control this disease (8,9), we have undertaken the identification of rice resistant varieties in Benin. To date, approximately 45 genes and QTLs conferring resistance to bacterial blight in rice have been identified using various rice sources globally (10, 11). Xa4, one of the resistance genes with a broad spectrum of action against *Xoo*, is one of the most widely exploited in many rice breeding programs and it confers durable resistance in many commercial rice cultivars (12). Furthermore, *Xa4* is associated with shorter stature, a beneficial agronomic trait that helps prevent lodging. The simultaneous improvement of multiple agronomic traits conferred by Xa4 may account for its widespread and lasting utilization in rice breeding programs globally (13). However, none information is available on these *Xoo* resistance genes in rice varieties grown in Benin. Therefore, this study aimed to identify the cultivated rice varieties, carrying Xa4 gene that are resistant to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) by molecular screening in the department of Atacora in Benin. Identification of *Xoo* resistance genes is a prerequisite for the development of suitable local varieties.

2. MATERIAL AND METHODS

2.1 Plant material

The biological material consisted of IRBB4, the near isogenic line carrying Xa4 resistance gene used as positive control and 32 samples of paddy rice were collected from 7 municipalities in the department of Atacora in Benin (figure 1). These samples were listed in the table 1.

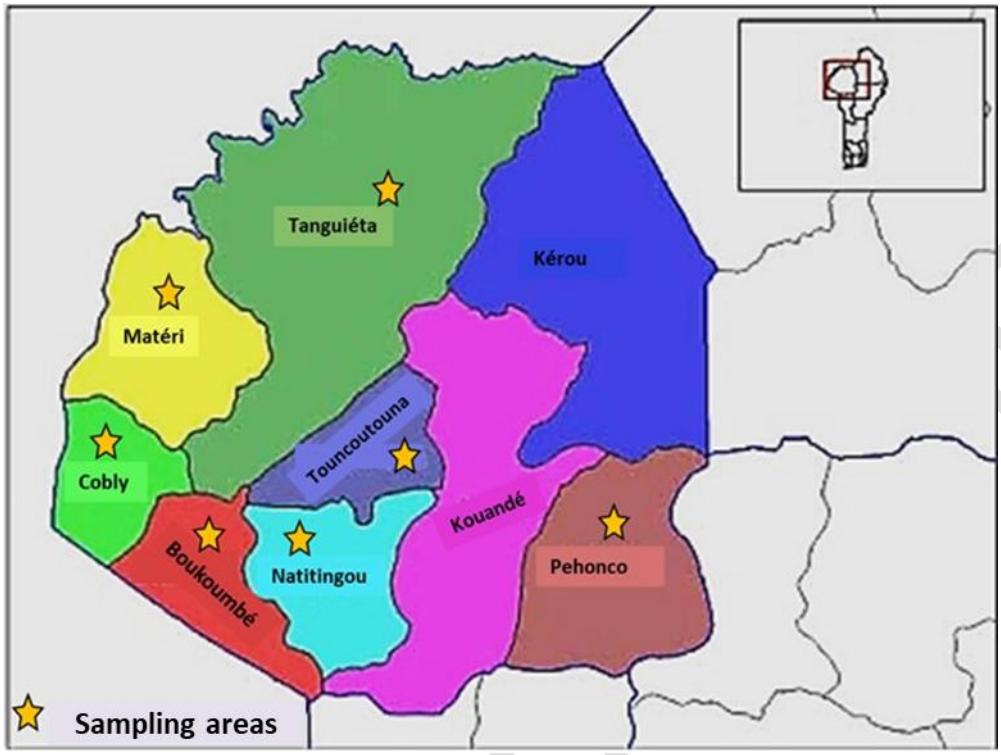

Figure 1: Distribution area of collected rice samples

Table 1: List of collected accessions per villages and per municipalities of Atacora

Municipalities	Villages or localities	Accession Codes	Number of samples per municipality
Cobly	Nanagade	Nana29 Nana30 Nana32	3
Touncoutouna	Tchakalakou	Tchaka33 Tchaka34 Tchaka36 Tchaka38 Tchaka39 Tchaka41	6
Natitingou	Koudengou	Koud42 Koud43 Koud44 Koud45 Koud46	5

Boukoumbe	Koumadogou	Koum47 Koum49 Koum50 Koum51 Koum53 Koum54 Koum55	7
Material	Kankini-Seri	Kan58 Kan59 Kan60 Kan61	4
Wassa Pehonco	Koungarou	Koung65 Koung67 Koung68	3
Tanguieta	Kochessi	Kotch70 Kotch71 Kotch72 Kotch73	4

2.2 Methods

2.2.1 Sampling of young rice leaves for genomic DNA extraction

Paddy rice grains from all collected accessions were grown in germination pots under a greenhouse, a controlled environment that promotes growth. Watering was done as needed for 10 to 15 days. The youngest leaves at the seedling stage were removed using scissors then they were wrapped in aluminum foil on which the references of the pot (the name and code of the sample and the date of collection) are marked (figure 2). These samples were kept cool in a cooler containing ice. Upon arrival at the laboratory, they were kept in a fridge at 4 ^{o}C.

A B C

Figure 2: Sampling of young leaves of rice 21 days after sowing.
A: Sowing of paddy rice grains;
B: Obtaining young seedlings;
C: Sampling of young leaves for DNA extraction

2.2.2: Extraction of total genomic DNA

Total genomic DNA was extracted according to (14) using MATAB (Mixed Alkyl Trimethyl Ammonium Bromide) and which was modified by (15). 0, 2g of young leaves were weighed and then ground in porcelain mortars with 2ml of Tris HCl EDTA Sorbitol (TES). Then, the ground material was transferred to a new 2ml eppendorf tube and then centrifuged at 10,000 rpm for 10 minutes, at 4° C. The supernatant was drained and 750µL of 4% MATAB buffer was added to the pellet. The whole is homogenized and incubated at 65°C for 1h in a water bath (and homogenized after each 10min) in order to facilitate the lysis of the cell membranes. After this incubation period, the tubes were removed and then left to cool, after which 750 µl of the Chloroform Isoamyl Alcohol (CIA) buffer in the proportions 24:1 were added. After homogenization by inversion for 10 minutes, centrifugation was performed at 10,000 rpm for 15 min at 4° C. Then the supernatant is collected in a new 1.5 ml eppendorf tube bearing the same label. Precipitation of the DNA pellet was made by adding refrigerated isopropanol (-20° C) equal volume with slight homogenization by inversion. This is followed by centrifugation at 10,000 rpm for 10 minutes at 4°C. The aqueous solution was carefully removed so that the pellet was not damaged or lost. The pellet was then washed with 70% ethanol followed by centrifugation at 10,000 rpm for 10 minutes; this is repeated three times successively in order to properly purify the DNA pellet. The tubes were opened and left to dry on blotting paper for the right time (overnight). Finally, a suspension of the dried DNA pellet was made by adding 100 µL of ultra-pure sterile water and then the latter is stored at -20° C.

2.2.3: Verification of the quality of total genomic DNA by electrophoresis

This check was made by electrophoresis on a 1% agarose gel (1g of agarose powder dissolved in 100mL of TBE (diluted 0.5 times, pH 8.5). Indeed, a mixture of 3µL of extract of Total DNA and 8µL of 2X loading blue was migrated at 100V for 30 min in Tris Bromate EDTA buffer (TBE). After migration, the gel was put in ethidium bromide (0.1%BET) solution for 15min then rinsed with distilled water for five minutes. Then, the gel was visualized on a UV trans-illuminator. Finally, dilution of the DNA was carried out and stored at 4°C for subsequent tests of DNA.

2.2.4: PCR amplification

The target sequence of Xa4 resistance gene was amplified using primers selected on the basis of a bibliographic study. Details of primers, sequences ('sense' and ' antisense '), chromosomal location, and references are given in Table 2. IRBB4 control DNA was also amplified using these primers.

Table 2: Sequences of primers used for PCR amplification

Gene	Located on the chromosome	Marker	Marker type	Sequence of primers (5'-3 ')	Resistance allele (pb)	susceptible allele	Reference
Xa4	11	MP12	STS	ATCGATCGATCTTCACGAGG TGCTATAAAAGGCATTCGGG	150	120	(16)

The reaction mixture brought to a volume of 20 µl was used for the PCR amplification. It consists of: 2.5µl of PCR Buffer; 0.75µl of dNTP; 0.5 µM of MgCl2; 2.5 µM of each primer, 0.1 µM of Taq polymerase; 3µl of 25ng/µl DNA and 8.15µl of ultrapure distilled water

The amplification was carried out in a thermocycler according to the following program: pre-denaturation at 94° C for 5 min followed by 35 cycles consisted of a denaturation at 94°C for 1 min; a hybridization at 55°C for 1min an elongation at 72°C for 2min and a final extension at 72°C for 5min and this with 35 cycles

2.2.5: PCR products revelation

The amplicons and a molecular weight marker of 100 bp were subjected to 2.5% agarose gel electrophoresis carried out in a solution of TBE (diluted 0.5 times) at 150 V for 45 min. The gel was put in 0.1% BET for 15 minutes then rinsed with distilled water for 5 minutes. Then, the gel was visualized on a UV trans-illuminator.

2.2.6: Data analysis

A comparison between the bands of the different amplicons and that of the positive control IRBB4 was made. The data was noted using " 1" and "0 " which mean respectively presence and absence of the target gene. The Excel 2019 workbook was used to make graphs to better analyze data.

3. RESULTS AND DISCUSSION

The STS marker used in this study was polymorphic (figures 3; 4 and 5). DNA analysis of the near isogenic line IRBB4, the positive control, and the 32 accessions collected from Atacora revealed two types of Xa4 gene alleles. These were respectively the susceptible allele (xa4) to Xoo with 120bp band and the resistant allele (Xa4) to Xoo with a band of 150bp (figure 3). The same results were obtained by (16, 17) who used the same marker MP12 to search for the Xa4 gene encoding resistance to bacterial leaf blight caused by *Xoo*. This confirmed the effectiveness of the molecular marker used in this study.

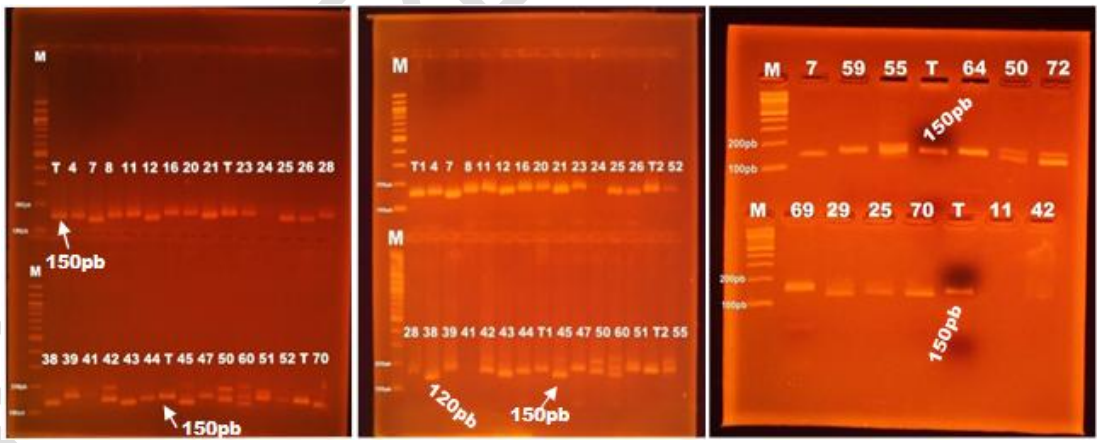

Figure 3: Electrophoresis profiles showing the presence (150 bp) or absence (120 bp) of the Xa4 resistance allele in different individuals. Line M: Molecular weight marker, T line: IRBB4 used as a positive control possessing the Xa4 resistance allele (150bp).

According to the table 3, it appeared that twenty out of thirty-two accessions, i.e. 62.5%, present a band of 150 bp as confirmed by the positive control IRBB4. This means that these

varieties were resistant to *Xoo* (Xa4/Xa4 or Xa4/xa4 genotypes since the Xa4 was a dominant gene). On the other hand, ten varieties out of the thirty-two (31.25%) showed a band of 120 bp, which corresponds to the susceptible allele marking the susceptibility of these varieties to the pathogen (xa4/xa4 genotypes). The local varieties Koum54 (Su Itaré Kpika) and Koung67 (Darou Morri) from the villages of Koumadogou and Koungarou respectively did not carry either of the two alleles of the Xa4 gene. Our results were approximately close to those of (18), who, following a similar study using the same pair of primers, reported a polymorphism between the individuals tested. This polymorphism was asserted with reference to the positive control (IRBB4 and IR64) and the negative control (IR24) with DNA fragments of 150 bp and 120 bp respectively. Likewise, results were closed to those of (19) with 51.15% of accessions carrying the resistant allele and 48.75% carrying the susceptible allele of Xa4. It should be noted that of the 20 resistant varieties, 14 (70%) are local and the 6 others are improved varieties. This would be explained by the fact that northern Benin has a great diversity of rice with a large number of traditional varieties, which makes this region a better place for an in situ conservation program (20).

Table 3: Summary table on the state of individuals in relation to the Xa4 gene after electrophoresis

Samples code	**Local name**	**Type of Variety**	***Xa4* gene**
Tchaka33	Bakilafema	LV	**1**
Nana30	IR841	IV	**0**
Tchaka34	Kpantcho tèro	LV	**1**
Koum47	IR841	IV	**1**
Koud44	Timonwonti (Gambiaka rouge)	LV	**0**
Kan60	Moï Nihoun	LV	**1**
Kotch70	Moï Poga	LV	**1**
Tchaka36	Nérica	IV	**0**
Koum55	Yamaboba	LV	**1**
Koum54	Su Itaré Kpika	LV	∅
Kotch71	Moï Touanga	LV	**0**
Kan61	Moï Lague	LV	**0**
Koung65	Takamorri	LV	**1**
Koud46	IR841	IV	**0**
Koud43	Pointinini	LV	**0**
Koung68	Moï Lopiro	LV	**1**
Koung67	Darou Morri	LV	∅
Tchaka38	Inaris	IV	**1**

Koud42	Nérica L19	IV	**0**
Kan59	Moï Lague	LV	**0**
Koum53	IR841	IV	**1**
Kotch73	Moï Nihoun	LV	**1**
Koum49	Béris 21 (Toukounchèti)	IV	**1**
Koum50	Tomonsoti	LV	**1**
Koum51	Gambiaka	LV	**1**
Kotch72	Unknown	Unknown	**1**
Nana29	Common Kounkounga	LV	**1**
Koud45	Nérica L20	IV	**1**
Tchaka39	Bakikrouma	LV	**1**
Tchaka41	IR841	IV	**1**
Nana32	Gambiaka	LV	**0**
Kan58	Moï Poua	LV	**1**
Positive control	IRBB4	IV	**1**

IV = Improved variety; LV = Local variety; 0 = absence of Xa4; 1 = Presence of Xa4; □: no band

The analysis also revealed particular bands in certain varieties (figures 4 and 5). It can therefore be seen that the varieties Tchaka38; Koum49; Koum50; Kotch72 and Kan58 showed two bands, one of which is greater than 150bp and very close to 165bp (figure 4). According to the literature, this band of 165 Pb also corresponds to a resistance allele of the Xa4 gene (12). On this basis, we therefore considered these varieties as homozygotes resistant. So 65% of resistant varieties are homozygous (Xa4/Xa4) and 35% were heterozygous (Xa4/xa4).

Varieties with bands of 120 bp and 165 bp could be considered in our study as heterozygotes resistant to *Xoo*. Indeed, very recent studies revealed that the 165bp DNA fragment corresponds to a resistant allele. Varieties carrying this allele showed proven resistance to *Xoo* after the pathogenicity test (12).

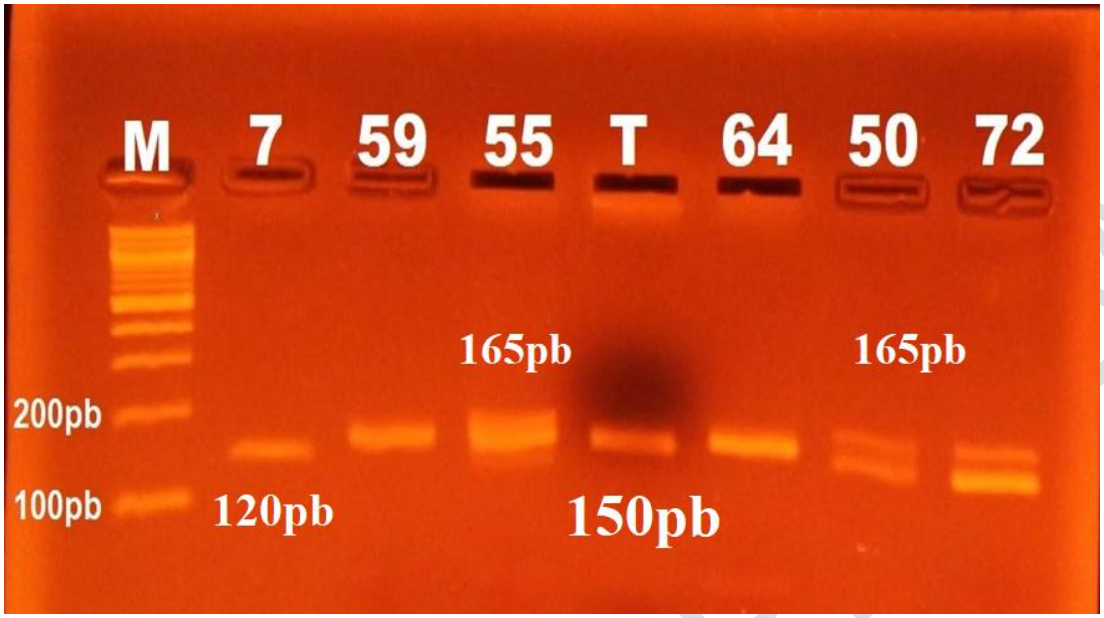

Figure 4: Electrophoresis gel showing individuals having 165bp band

In addition, we identified 2 resistant varieties (Koum53 and Koud45, corresponding to the accessions 45 and 60 respectively), presenting a particular genotype due to the presence in the latter of an additional band of 190bp, close to 200bp (figure 5). Similar results were obtained by (12) who reported that out of 14 resistant varieties three varieties namely NWGR2014, Pankhali 203 and Ratna revealed an additional band of 190 bp which was absent both in the control positive (IRBB4) and the negative control (IR24). These results suggested the existence of several alleles of the Xa4 gene. Moreover, these 2 varieties being improved, this third band could correspond to another resistance gene or not which would be introgressed into the plant by the breeders during their varietal improvement.

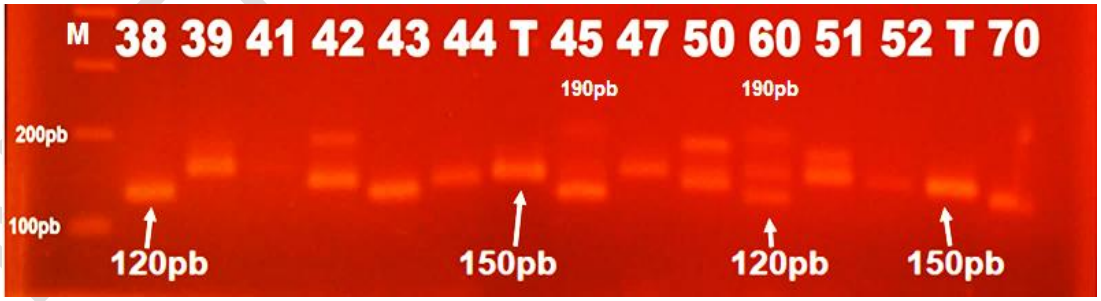

Figure 5: Electrophoresis gel showing individuals having three bands

In total, there is a broad genetic diversity of resistance to bacterial blight in Atacora as summarized in the table 4.

Table 4: Types, genotypes and phenotypes of rice varieties analyzed

Samples code	**Type of Variety**	***Xa4* (R)**	***xa4* (S)**	**Genotypes**	**Phenotypes**
Tchaka33	LV	+	-	*Xa4/Xa4*	*R*
Nana30	IV	-	+	*xa4 /xa4*	*S*
Tchaka34	LV	+	-	*Xa4/Xa4*	*R*
Koum47	IV	+	-	*Xa4/Xa4*	*R*
Koud44	LV	-	+	*xa4 /xa4*	*S*
Kan60	LV	+	-	*Xa4/Xa4*	*R*
Kotch70	LV	+	-	*Xa4/Xa4*	*R*
Tchaka36	IV	-	+	*xa4 /xa4*	*S*
Koum55	LV	+	-	*Xa4/Xa4*	*R*
Koum54	LV	-	-	∅	?
Kotch71	LV	-	+	*xa4 /xa4*	*S*
Kan61	LV	-	+	*xa4 /xa4*	*S*
Koung65	LV	+	-	*Xa4/Xa4*	*R*
Koud46	IV	-	+	*xa4 /xa4*	*S*
Koud43	LV	-	+	*xa4 /xa4*	*S*
Koung68	LV	+	-	*Xa4/Xa4*	*R*
Koung67	LV	-	-	∅	?
Tchaka38	IV	+	+	*Xa4/xa4*	*R*
Koud42	IV	-	+	*xa4 /xa4*	*S*
Kan59	LV	-	+	*xa4 /xa4*	*S*
Koum53	IV	++	+	*Xa4/xa4*	*R*

Kotch73	LV	+	-	*Xa4/Xa4*	*R*
Koum49	IV	+	+	*Xa4/xa4*	*R*
Koum50	LV	++	-	*Xa4/Xa4*	*R*
Koum51	LV	+	-	*Xa4/Xa4*	*R*
Kotch72	-----	++	-	*Xa4/Xa4*	*R*
Nana29	LV	+	-	*Xa4/Xa4*	*R*
Koud45	IV	++	+	*Xa4/xa4*	*R*
Tchaka39	LV	+	-	*Xa4/Xa4*	*R*
Tchaka41	IV	+	-	*Xa4/Xa4*	*R*
Nana32	LV	-	+	*xa4 /xa4*	*S*
Kan58	LV	+	+	*Xa4/xa4*	*R*
IRBB4	IV	+	-	*Xa4/Xa4*	*R*

(IV) Improved variety; (LV) Local variety; (-) absence of allele; (+) presence of allele; (S) susceptible; (R) Resistant; (++) presence of two alleles, varieties with three alleles; □ : no allele

In view of all these results obtained in this study, the pathogenicity test of *Xoo* strains from Benin on the resistant accessions identification is necessary to evaluate the expression of this resistance gene in real environment and especially the behavior of heterozygous individuals to analyze their level of resistance to the pathogen. For this achievement, prospecting efforts in the rice-growing areas of Benin must be made to collect infected leaves and identify and characterize *Xoo* strains. Accessions confirmed to be resistant may be used by breeders in crosses involving accessions carrying other genes of agronomic interest, or directly deployed in specific geographical areas of Benin according to the geographical distribution of the *Xoo* strains that will be identified. We will also evaluate the contribution of the allele of 190bp in the expression of resistance to *Xoo*. Thus, by backcross assisted selection, we will generate isogenic lines carrying this allele. This material will be screened using Beninese *Xoo*. Also, the entire genome of these varieties deserves to be sequenced in order to better understand the genetic events that led to an additional band of 190 bp.

4. CONCLUSION

The present study was carried out in Atacora in Northern Benin to search for the Xa4 resistance gene to *Xoo*. At the end, out of 32 varieties analyzed, 20 carried three different

resistance alleles thus resistant to *Xoo*. Among the resistant varieties, 14 were local, 6 were improved and 2 presented particular genotypes with three alleles. These results will be very useful to rice breeders to develop elites resistant varieties. In total, there is a broad genetic diversity of resistance to bacterial blight in Atacora. There is therefore a very urgent need to extend the study to whole country for further research of bacterial blight resistance genes in rice in Benin.

REFERENCES

1. FAO. 2020.www.fao.org com. Accessed on 05/12/2021

2. Agrimonde. World agriculture and food in 2050: scenarios and challenges for sustainable development. Briefing note, 2nd ed. INRA/CIRAD 2009 Montpellier: Cirad. www.cirad.fr/publications-ressources/edition/etudes-et-documents/agrimonde.

3. Adhikari TB, Vera Cruz CM, Zhang Q, Nelson RJ, Skinner DZ, Mew TW, Leach JE. Genetic Diversity of *Xanthomonas oryzae* pv. *oryzae* in Asia. Applied and environmental microbiology. 1995; 61:6.

4. Djedatin G, Ndjiondjop MN, Mathieu T, Vera Cruz CM, Sanni A, Ghesquière A, Verdier V. Evaluation of African cultivated rice *Oryza glaberrima* for resistance to bacterial blight. Plant Disease. 2011; 95:441-447

5. Onasanya A, Ekperigin M M, Nwilene FE, Séré Y, Onasanya R O. Two pathotypes of *Xanthomonas oryzae* pv. *oryzae* virulence identified in West Africa. Current Research in Bacteriology. 2009; 2: 22-35.

6. Sere Y, Onasanya A, Verdier V, Akator K, Ouedraogo LS, Segda Z, Mbare MM, Sido AY, Baso A. 2005. Rice Bacterial Leaf Blight in West Africa: Preliminary studies on disease in farmer's field and screening. Asian Journal of Plant Sciences. 2005; 4:577-579.

7. Afolabi O, Amoussa R, Bilé M, Oludare A, Gbogbo V, Poulin L, Koebnik R, Szurek B, Silué D. First report of bacterial leaf blight in Benin. 2015. https: //doi.org/10.1094/PDIS-07-15-0821-PDN

8. Pandey MK, Rani NS, Sundaram RM, Laha GS, Madhav MS, Rao KS. Improvement of two traditional Basmati rice varieties for bacterial blight resistance and plant stature through morphological and marker-assisted selection. Molecular Breeding. 2013; (31): 239–246. doi: 978-3-319-45021-6_20/s11032-012-9779-7

9. Priya LB, Ujjal KN, Sharmistha G, Gayatri G, Shalim U, Omar MA, Arafat AH, Alison ML, Yong-Ming G and Akbar H. Introgression of Bacterial Blight Resistance Genes in the Rice Cultivar Ciherang: Response against Xanthomonas oryzae pv. oryzae in the F6 Generation. Plants 2021; 10, 2048. https://doi.org/10.3390/plants10102048

10. Neelam K, Mahajan R, Gupta V, Bhatia D, Gill BK and Komal, R. High-resolution genetic mapping of a novel bacterial blight resistance gene xa45(t) identified from Oryza glaberrima and transferred to *Oryza sativa*. Theor. Appl. Broom. 2020;133, 689–705.

11. Djedatin G, Ndjiondjop MN, Sanni A, Lorieux M, Verdier V, Ghesquiere A. Identification of novel major and minor QTLs associated with *Xanthomonas oryzae* pv. *oryzae* (African

Strains) resistance in Rice (*Oryza Sativa L*.). Rice. 2016; 9:18. https://doi.org/10.1186/s12284-016-0090-9.

12. Bhupendra SP, Ruchi T, Rallapalli R, Chet R and Subhash N. Molecular Marker-based Screening for Bacterial Leaf Blight Resistance Genes in Landraces and Cultivars of Rice in Gujarat. Indian J. Plant Genet. Resour 2018; 31(1): 51-56 DOI 10.5958/0976-1926.2018.00008.6

13. Hu K, Cao J, Zhang J, Xia F, Ke Y, Zhang H, Xie W, Liu H, Cui Y, Cao Y, Sun X, Xiao J, Li X, Zhang Q and Wang S. Improvement of multiple agronomic traits by a disease resistance gene via cell wall reinforcement. Nature Plants 2017 3, 17009 | DOI: 10.1038/nplants.2017.9 |

14. Gawel NJ and Jarret RL. A modified CTAB DNA extraction procedure for Musa and Ipomoea. Plant Molecular Biology Reporter. 1991 (9):262–266

15. Adje C, Achigan-Dako E and Agbangla C. Optimizing genomic DNA isolation in Pineapple (Ananas Comosus L.) J. Plant Breed. Genet. 2016 (01): 11-18

16. Ma B, Wang W, Zhao B, Zhou Y, Zhu L, and Zhai W. Studies of PCR marker for the rice bacterial blight resistance gene Xa-4. Hereditas. 1999; 21(3): 9-12.

17. Wang S, Liu W, Lu D, Lu Z, Wang X, Xue J and He X. Distribution of Bacterial Blight Resistance Genes in the Main Cultivars and Application of Xa23 in Rice Breeding. Frontiers in Plant Science. 2020; 11:555228. doi:10.3389/fpls.2020.555228

18. Arif M, Jaffar M, Babar M, Sheikh MA, Kousar S, Arif A, Zafar Y. Identification of bacterial blight resistance genes Xa4 in Pakistani rice germplasm using PCR. Afr. J. Biotechnol. 2008; (7): 541-545.

19. Muhammad S, Tahira B, Hafiz UF, Zulqarnain H, Imad N, Abid M, Muhammad A. Molecular screening of rice (*Oryza sativa L*.) germplasm for Xa4, xa5 and Xa21 bacterial leaf blight (BLB) resistant genes using linked marker approach African Journal of Biotechnology. 2016;15(41): 2317-2324

20. Loko YLE, Ewedje E, Orobiyi A, Djedatin G, Toffa J, Gbemavo CDSJ, Tchakpa C, Gavoedo D, Sedah P, Sabot F. On-farm management of rice diversity, varietal preference criteria, and farmers' perceptions of the African (*Oryza glaberrima* Steud.) versus Asian rice (*Oryza sativa* L.) in the Republic of Benin (West Africa): implications for breeding and conservation. Economic Botany 2021; 75 (1): 1-29