

# Characterization of bacterial leaf blight epidemic in the Office du Niger (Mali) and search for a sustainable resistance against the pathogen

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## Abstract

Since 2002, plant health monitoring has allowed us to observe the appearance of a new disease whose symptoms are whitish to yellowish lesions on the leaves and partial or complete sterility of spikelets, giving a blackish aspect to the panicle. These symptoms suggest bacterial infection. The objectives of this study were:

- To characterize disease epidemic through plant health surveys in order to identify the level and condition of epidemic development and impact on rice production;
- To test the resistance potential of rice germplasm, mainly varieties bearing known resistant genes, in order to identify those that can protect rice against bacterial disease.

Twenty-nine farmers were surveyed throughout the Office du Niger. The disease incidence and severity were monitored through observations every 10 days with all of these farmers. The disease impact on production was determined through survey plots in attacked and unattacked zones. The first symptoms of the disease appeared between 45 and 147 days after sowing. Disease incidence varied from one area to another and also evolved over time. It increased from the first to the third observation (10–30 days after first appearance of symptoms) on all plots. From the third to the fourth observation (30–40 days after first appearance of symptoms), the incidence decreased in the zones of N'Debougou (28.5 to 16%) and Kouroumari (23.25 to 20%). Highest incidences were recorded during the fourth observation in Niono (45.7%) and Molodo (40.5%). Yield losses up to 80% were recorded. Twenty-four varieties and near-isogenic lines (NILs) were tested for their reaction to infections of bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* in five sites in an experimental design in randomized blocks with three replications on small plots of three lines of 1 m long.

Reactions of the different NILs and varieties showed a zonal effect on the severity of the disease. Strains in Kayo seemed to be more virulent than those of the other sites. Those in Niono 1 appeared to be less aggressive rarely reaching incidences above 20%. Lines Xa-4/xa-5 and Xa-7 performed better than other lines under conditions of BLB infections in all sites with 16 and 19% incidence on average, respectively.

## Introduction

Bacterial leaf blight was identified for the first time in Africa in the late 1970s in Mali (Buddenhagen *et al.*, 1979) and thereafter in many other African countries (Notteghem and Baudin, 1981; Reckhaus, 1983; Buddenhagen, 1985; Awoderu *et al.*, 1991; Ashura *et al.*, 1999). Since 2002, plant health monitoring has permitted the observation of increasing epidemics of bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), and bacterial leaf stripe (BLS) caused by *X. oryzae* pv. *oryzicola* (Xoc) in Mali (unpublished). These two diseases are a major constraint to rice intensification in Mali. *Xanthomonas oryzae* pv. *oryzae* is a vascular pathogen, while Xoc is nonvascular (Nino-Liu *et al.*, 2006). They can both cause important yield losses in irrigated and lowland rice.

Bacterial leaf blight is devastating and can cause yield losses from 20 to 74% in southeast Asia and India (Ahmed and Singh, 1975; Srivastava, 1967; Ou, 1985). Studies on the variability of the pathogen have revealed a difference between pathotypes in Japan and those in the Philippines, and the existence of two biotypes of the bacterium (Mew, 1978; Horino *et al.*, 1980, 1981; Ou, 1985). Molecular and pathological studies have shown that African strains of Xoo are generally different from the Asian ones: three new races of the pathogen have been identified by screening isogenic lines (Gonzalez *et al.*, 2007).

Using resistant varieties is the most efficient means to control these diseases. To control vascular bacterial disease (BLB), 30 major resistance genes (*R*) have been identified against different races of Asian Xoo, comprising 21 dominant *R* genes (*Xa*) and 9 recessive ones (*xa*). However, there is a high risk that this resistance will be avoided by the pathogen, because there is a lack of information on the distribution and occurrence of the races and haplotypes of Xoo and Xoc in Mali, as well as the population structure of the pathogen at the national level. It is therefore important to characterize the incidence and distribution of the pathogen.

The objectives of the study were:

- To characterize the epidemics of BLB in the Office du Niger in order to identify control measures including varietal resistance in an integrated control approach;
- To characterize the pathogen population structure.

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## Material and methods

### *Characterization of epidemics through plant health survey*

Twenty-nine farmers were surveyed in the Office du Niger to determine the agronomic practices in the perimeters (varieties, cultural practices, fertilizers, etc.), as well as the level and condition of epidemic development and its impact on rice production. After the first appearance of BLB symptoms, the incidence (percentage of plants infected) and the severity (dimensions of lesions on attacked leaves) of the disease were monitored through observation at 10-day intervals in all infected fields of farmers. The disease's impact on production was determined using survey plots of 4 m<sup>2</sup> in diseased and disease-free zones.

### *Reaction of varieties within the trapping nurseries*

Twenty-four varieties and near-isogenic lines (NILs) with known resistance genes (Table 1) were tested against the natural inoculum of BLB in a randomized complete block design, with three replications of small plots of three lines of 1 m length. The trials were established in five sites (Kayo, Kouroumari, N'Debougou, Niono 1 and Niono 2) in farmers' plots, which were mostly contaminated.

Mineral fertilizer of 100 kg DAP (18% N, 46% P<sub>2</sub>O<sub>5</sub>)/ha and 100 kg KCl (60% K<sub>2</sub>O)/ha at transplanting or sowing, 200 kg urea/ha at tillering and 200 kg urea/ha at panicle initiation was applied to all trials.

Two hand weeding were done as necessary.

When the first symptoms of BLB appeared, the severity of the attacks on the leaves of the central line of each entry was marked every week following a scale of 0 to 9 (0 = no traces; 1 = traces; 3 = 1/4 of the leaf; 5 = 1/2 of the leaf; 7 = 3/4 of the leaf and 9 = all the leaf). These severity marks were used to classify lines and varieties as immune (0 = IM); resistant (1 = R); moderately resistant (3 = MR); moderately susceptible (5 = MS); susceptible (7 = S) and highly susceptible (9 = TS).

The incidence was calculated using the following formula: I% = (n × 100)/N where I% = percentage of diseased plants, n = number of diseased plants, N = total number of plants.

### *Study of the pathogenicity of isolates collected in Office du Niger*

Eighty-seven isolates of Xoo were isolated from the cultivated rice (*O. sativa*) and from two wild species (*O. longistaminata* and *O. barthii*) using the technique described by Adhikari *et al.* (1999). They were characterized biochemically by the absence of growth on a medium where L-alanine was the only source of carbon and by the growth on a medium with 0.001% copper nitrate (Xie and Mew, 1998).

Bacterial cultures 72-h old were suspended in sterile distilled water. Using a spectrophotometer, the absorbance (*A*) of the inoculum was adjusted to *A* = 0.05 (620 nm) corresponding to a concentration of 10<sup>8</sup> cells per ml (Lee *et al.*, 2003). Four NILs from IRRI (IRBB4, IRBB7, IRBB5 and IRBB13) each carrying a resistance gene (*Xa-4*, *Xa-7*, *xa-5* and *xa-13*, respectively) and one susceptible variety (FKR14) from Burkina Faso (Ouédraogo *et al.*, 2007) were cultivated in 2-L pots with four plants per pots. Thirty-day-old plants were inoculated using the method described by Dardick *et al.* (2003). To increase the moisture and therefore promote the infection, the inoculated plants were covered with plastic cages for 48 hours.

The length of lesions on about 20 inoculated leaves was evaluated 14 days after inoculation and the average length of the lesions was calculated.

**Table 1.** List of varieties and NILs† tested in trapping nurseries for their resistance to natural strains of Xoo in the Office du Niger

Line/variety	Resistance gene(s)‡	Line/variety	Resistance gene(s)
IRBB1	<i>Xa-1</i>	IRBB21	<i>Xa-21</i>
IRBB2	<i>Xa-2</i>	IRBB50	( <i>Xa-4/xa-5</i> )
IRBB3	<i>Xa-3</i>	IRBB51	( <i>Xa-4/xa-13</i> )
IRBB4	<i>Xa-4</i>	IRBB52	<i>Xa-4/xa-5</i>
IRBB5	<i>xa-5</i>	IRBB53	<i>Xa-4/xa-13</i>
IRBB7	<i>Xa-7</i>	IRBB54	<i>Xa-4/Xa-21</i>
IRBB8	<i>xa-8</i>	IRBB55	<i>xa-5/xa-13</i>
IRBB10	<i>Xa-10</i>	IRBB59	( <i>xa-5/xa-13/Xa-21</i> )
IRBB11	<i>Xa-11</i>	IRBB60	( <i>Xa-4/xa-5/xa-13/Xa-21</i> )
IRBB13	<i>xa-13</i>	Giganté (Tete)	Undetermined
IRBB14	<i>Xa-14</i>	TCS 10	Undetermined
IR24	<i>Xa-18</i>	PNA	Undetermined

† The NILs were from International Rice Research Institute (IRRI).

‡ *Xa*- genes are dominant; *xa*- genes recessive.

## Results

### *Characterization of epidemics through plant health survey*

#### *Description of the perimeter*

The plant health survey showed similarity among the different sites regarding date of commissioning, gravity irrigation method and major soil types (clayey and clay loam soils).

Since 2000, the cropped area of all zones has increased during both the rainy and the dry seasons (results not shown).

Generally, farmers established nurseries in January and June for off-season and main season crops, respectively.

The major varieties cultivated during the dry season were IR 32307, Adny11 and Kogoni 1. IR 32307 was the most widely grown variety from 2000 to 2003. During the last dry season, the most widely grown varieties were Adny 11 followed by Kogoni 91-1. During the rainy season, Kogoni 91-1 was the most widely cultivated variety throughout the zones, followed by Adny 11.

Over 90% of the farmers obtained supplies of selected R1 and R2 post-certified seeds from seed farms in Niegue and Molodo and at the agricultural research station in Niono. These seeds were renewed after four or five campaigns.

Quantities of fertilizers used did not change over the years: 100–150 kg diammonium phosphate/ha and 200–250 kg urea/ha.

#### *Monitoring epidemics on farmers' plots*

A first basic diagnosis revealed the importance of bacterial infections that are becoming a real threat to rice production in the Office du Niger, as all varieties commonly grown (Adny11, Kogoni 91-1, BG 90-2, Nionoka, Sahelika) are attacked with incidences of between 40 and 80%.

In 2007, the survey did not properly enable identification and characterization of BLB because most followed plots did not develop important epidemic. This suggest that our protocol of selecting farmers' fields before the onset of disease symptoms was inefficient — it would be better to select fields after the first appearance of symptoms. Only one of the monitored plots experienced bacterial epidemics: the first symptoms of BLB appeared 45 days after seeding with a 10% incidence. Twenty days later (65 days after seeding), the infection reached 100% of the plot. From this date, a decrease of the infection was observed over time. The one field infected with BLB suffered yield losses of 75–80% (zone of Molodo). A low milling yield with poor grain quality was observed throughout the Office du Niger.

In 2008, the first symptoms of the disease appeared between 57 and 119 days after seeding. The disease incidence varied among zones, and also evolved over time (Table 2). It was progressive from the first to the third observation throughout the plots. From the third to the fourth observation, the incidence decreased in the zones of N'Debougou (28.5 to 16%) and Kouroumari (23.25 to 20%). The highest incidences were recorded during the fourth observation in Niono (45.7%) and Molodo (40.5%). The severity varied from 1 to 7.

**Table 2.** Incidence of bacterial infection (%) in 2008

Zone	1st observation†	2nd observation	3rd observation	4th observation
Macina	11.5	18	19.5	24.5
N'Debougou	17.5	19.5	28.5	16
Niono	20	29.5	37	45.7
Molodo	23.5	31.5	34.5	40.5
Kouroumari	14.5	19	23.25	20

† Observations made at 10-day intervals after detection of first symptoms, i.e. at 10, 20, 30 and 40 days after first appearance of symptoms.

The impact of bacterial infections on the number of panicles was not significant, because differences between uninfected areas and infected ones were very small (Table 3). In Kouroumari, the number of panicles/m<sup>2</sup> in infected areas was slightly higher than that in healthy areas.

The impact of BLB infections on the weight of paddy differed among the zones of the Office du Niger (0.14 to 4.35%; Table 4). In N'Debougou and Niono, the depressive effect of the disease on the weight of paddy rice was significant (3.88 to 4.35%). There was also a qualitative loss of rice grains, which affected the market value of the product.

**Table 3.** Impact of BLB on the number of panicles

Zone	No. panicles/m <sup>2</sup>		
	Healthy areas	Infested areas	Difference between areas
Macina	412	410	2
N'Debougou	413	412	1
Niono	436	429	7
Molodo	405	399	6
Kouroumari	397	403	-6

**Table 4.** Impact of bacterial infections on paddy weight of plots in the Office du Niger (Mali)

Zone	Paddy weight (g/m <sup>2</sup> )		Reduction in paddy weight (%)
	Healthy areas	Infested areas	
Macina	698	697	0.14
N'Debougou	722	694	3.88
Niono	759	726	4.35
Molodo	704	689	2.13
Kouroumari	641	635	0.94

*Reaction of varieties within the trapping design*

The analysis of variance (Table 5) of the data related to severity (expressed in percentage of injured leaf area) showed a highly significant difference between the varieties and between the sites. There was also a variety × site interaction, indicating that the sites differ in terms of the virulence of their pathogenic population and the varieties in terms of their vertical resistance.

**Table 5.** Analysis of variance of the severity of BLS on 24 lines and varieties at five sites in the Office du Niger

Source of variation	Degrees of freedom	F calculated	P
Replication (R)	2	1.25	ns
Treatment	119	10.03	**
Varieties (V)	23	15.20 **	**
Sites (S)	4	145.62 **	**
V×S	92	2.84 **	**
Error	238		
Total	359		

ns = not significant; \*\* = significant at 1% level.

Table 6 gives the results of the means comparison. Based on the results of various authors (mainly Liu *et al.*, 2007), we considered the severity of 25% as the threshold below which the reaction of varieties was considered incompatible (i.e. resistant).

The bacterial disease was more important in Kayo (Macina) than in the other sites, in terms of disease severity as well as in terms of virulence spectrum of the pathogenic population (Table 6): 15 of the 24 genetic structures tested were susceptible, while in Niono1 and Niono2 only Giganté was susceptible; in N'Débougou, in addition to Giganté, the combination *Xa4/xa5* showed a compatibility reaction. No susceptibility reaction was noted in Kogoni, thus confirming farmers' field observations that the bacterial disease pressure was relatively low.

The most affected materials in Kayo were the NILs carrying *Xa-1*, *Xa-2*, the variety Gigante and the susceptible check TCS10 (Table 6). Since these are NILs, we can rightly think that these severe attacks show a preponderance of races able to overcome the resistances *Xa-1* and *Xa-2*.

The races able to overcome the resistances *xa-5*, *Xa-7*, *Xa-14*, *Xa-18*, *Xa-21*, and the associations *Xa-4/xa-5*, *xa-5/Xa-21*, *xa-13/Xa-21* were not detected in the bacterial population in Kayo. These genes could be used to protect rice in Macina, even in all five sites of the Office du Niger. We know that in Asia *Xa-4*, *Xa-21* and *Xa-7* are considered to provide resistance to a wide range of Xoo races (Vera Cruz *et al.*, 2000; Leach *et al.*, 2001). Markers associated with genes *Xa-21*, *xa-5*, *Xa-4*, *Xa-7* and *xa-13* (Iyer-Pascuzzi *et al.* 2008) should facilitate such transfers via marker assisted selection.

**Table 6.** Comparison of severity averages of BLS on 24 lines and varieties in five sites in Office du Niger

Variety	Gene(s)	Sites									
		Kayo		Kogoni		N'Débougou		Niono1		Niono2	
		Sev	React	Sev	React	Sev	React	Sev	React	Sev	React
IRBB1	<i>Xa-1</i>	44.4 bc	S	5.0 c-g	R	5.8 bcd	R	0.0 b	R	7.4 b	R
IRBB2	<i>Xa-2</i>	39.8 bcd	S	0.5 fg	R	8.5 b	R	0.9 b	R	5.7 bc	R
IRBB3	<i>Xa-3</i>	27.9 c-f	S	8.7 a-f	R	5.7 bcd	R	0.0 b	R	5.8 bc	R
IRBB4	<i>Xa-4</i>	32.6 b-e	S	5.8 b-g	R	4.8 bcd	R	1.0 b	R	1.1 bc	R
IRBB5	<i>xa-5</i>	5.6 hi	R	2.4 d-g	R	4.7 bcd	R	0.1 b	R	3.7 bc	R
IRBB7	<i>Xa-7</i>	11.1 fgh	R	5.8 a-g	R	3.8 bcd	R	0.0 b	R	0.1 c	R
IRBB8	<i>xa-8</i>	22.4 efg	S	1.2 efg	R	1.0 cd	R	0.1 b	R	4.7 bc	R
IRBB10	<i>Xa-10</i>	24.3 def	S	9.7 a-d	R	1.9 bcd	R	0.0 b	R	0.0 c	R
IRBB11	<i>Xa-11</i>	22.2 d-g	S	12.5 abc	R	0.1 d	R	0.0 b	R	0.0 c	R
IRBB13	<i>xa-13</i>	29.7 b-e	S	0.1 g	R	7.5 bcd	R	1.9 b	R	3.9 bc	R
IRBB14	<i>Xa-14</i>	11.2 fgh	R	1.3 efg	R	0.1 d	R	2.0 b	R	2.9 bc	R
IRBB18	<i>Xa-18</i> (IR24)	8.6 ghi	R	5.1 b-g	R	6.5 bc	R	0.0 b	R	0.1 c	R
IRBB21	<i>Xa-21</i>	19.7 efg	R	5.0 b-g	R	1.0 cd	R	1.0 b	R	3.8 bc	R
IRBB50	<i>Xa-4/xa-5</i>	2.1 i	R	2.0 d-g	R	1.0 cd	R	0.1 b	R	2.0 bc	R
IRBB51	<i>Xa-4/xa-13</i>	23.2 d-g	S	6.9 a-g	R	31.6 a	S	1.0 b	R	5.7 bc	R
IRBB52	<i>Xa-4/Xa-21</i>	22.3 d-g	S	0.2 g	R	5.7 bcd	R	0.1 b	R	0.1 c	R
IRBB53	<i>xa-5/xa-13</i>	22.3 d-g	S	4.9 c-g	R	0.1 d	R	0.0 b	R	1.1 bc	R
IRBB54	<i>xa-5/Xa-21</i>	13.1 fgh	R	12.3 abc	R	0.0 d	R	0.0 b	R	0.0 c	R
IRBB55	<i>xa-13/Xa-21</i>	16.0 efg	R	7.8 a-e	R	3.9 bcd	R	1.0 b	R	2.0 bc	R
IRBB59	<i>xa-5/xa-13/ Xa-21</i>	21.4 d-g	S	18.8 abc	R	1.9 bcd	R	2.0 b	R	2.8 bc	R
IRBB60	<i>Xa-4/xa-5/ xa-13/Xa-21</i>	25.3 d-g	S	1.1 efg	R	1.1 bcd	R	0.1 b	R	3.8 bc	R
Giganté	Und	75.0 a	S	14.3 ab	R	33.4 a	S	36.2 a	S	44.5 a	S
TCS10	Und	49.1 b	S	18.9 a	R	7.5 bc	R	1.0 b	R	1.0 bc	R
PNA647F4	Und	21.7 d-g	R	0.1 g	R	0.1 d	R	2.0 b	R	3.8 bc	R

Average severities followed by the same letter are not significantly different at the threshold of 5%.  
Sev, severity; React, reaction; R, resistance; S, susceptibility; Und, undetermined.

Variety Gigante was susceptible to the isolates of all sites except that of Kouroumari (Kogoni). Such information is extremely important in sustainable rice protection, because Gigante is used in crosses for resistance to *Rice yellow mottle virus* (RYMV), another major rice disease in the Office du Niger. Particular attention should be given to the material resistant to this viral disease to make sure that it does not carry the susceptibility of Gigante to bacterial disease.

The 'parental' variety of the NILs, IR24, which carries the gene *Xa-18*, was resistant to the disease. This variety was selected in Asia as the recurrent parent for developing NILs because of his susceptibility to all the Asian strains. Thus, the reaction of IR24 to Malian isolate indicated a difference between Asian and Africa strains as concluded by molecular studies (Gonzales *et al.*, 2007). Moreover it means that IR24 must carry other resistance genes that were not present in the susceptible NILs.

#### **Pathogenicity of *Xoo* isolates**

It is estimated that an average length of lesions less than 3 cm indicates incompatibility relationship — that is, resistance of the variety and the avirulence of the isolate (Adhikari *et al.*, 1999). Based on the gene-for-gene concept, the compatibility of the host-pathogen interaction between a variety carrying a resistance gene results from the absence or nonactivation of the corresponding avirulence gene (Lee *et al.*, 1999). However, we use the term 'virulence' instead of 'non-functional avirulence'.

Our results showed great diversity in reaction of the 88 isolates, indicated by 26 profiles (Table 7). About 16% of the isolates were virulent on all the genetic structures tested, while 4% were not virulent on any of the material tested. Such avirulent isolates could still be of interest. They should be tested against other known resistance genes: if their avirulence is confirmed, they could be used in the search for new resistance genes in the African germplasm.

The virulence genes able to overcome *Xa-4*, *xa-5*, *Xa-7* and *xa-13* are frequent in the Malian Xoo population: the 88 isolates showed, respectively, 45, 47, 50 and 56 compatibility reactions with these resistance genes.

The eight isolates collected from wild rice were pathogenic on at least one of the varieties tested (Table 8). These host species and, in particular, their ratoons are a reservoir of perpetuation and diffusion of the inoculum between and through rice campaigns.

Isolates collected from rice stalks (volunteers) were also pathogenic, confirming the vascular and systemic nature of the bacterial infection. Volunteers that remain in the fields could also be reservoirs if the prolonged viability of the bacteria they host is proved.

**Table 7.** Reaction profile of the 88 isolates of Mali on four isogenic lines and the variety FKR14

Profile number	Virulence spectrum†	Reaction profile on four genotypes					Number of R reactions	%
		FKR14	IRBB7	IRBB4	IRBB13	IRBB5		
1	0	R	R	R	R	R	4	4.5
2	1	R	R	R	R	S	4	4.5
3	1	R	R	R	S	R	3	3.4
4	1	R	R	S	R	R	2	2.3
5	1	R	S	R	R	R	3	3.4
6	1	S	R	R	R	R	4	4.5
7	2	R	R	R	S	S	2	2.3
8	2	R	R	S	S	R	2	2.3
9	2	S	R	R	S	R	3	3.4
10	2	S	R	R	R	S	3	3.4
11	2	S	R	S	R	R	1	1.1
12	3	R	R	S	S	S	2	2.3
13	3	R	S	S	S	R	1	1.1
14	3	R	S	S	R	S	2	2.3
15	3	S	R	R	S	S	3	3.4
16	3	S	R	S	R	S	2	2.3
17	3	S	R	S	S	R	1	1.1
18	3	S	S	R	S	R	5	5.7
19	3	S	S	S	R	R	4	4.5
20	3	S	S	R	R	S	2	2.3
21	4	R	S	S	S	S	3	3.4
22	4	S	S	S	S	R	8	9.1
23	4	S	S	R	S	S	7	8.0
24	4	S	R	S	S	S	2	2.3
25	4	S	S	S	R	S	1	1.1
26	5	S	S	S	S	S	14	15.9
Number of compatible reactions per line /variety tested		60	50	45	56	47		

† The virulence spectrum of an isolate is defined by the number of compatibility reactions that it provokes on the genetic structures tested. R, resistant; S, susceptible

**Table 8.** Pathogenicity of Xoo isolates from wild rice of the Office du Niger

Isolate code	Host species	Organ	FKR14	IRBB7	IRBB4	IRBB13	IRBB5
MX120	<i>O. barthii</i>	Ratoons	R	R	R	R	S
MX040	<i>O. barthii</i>	Ratoons	R	R	S	R	R
MX115	<i>O. barthii</i>	Ratoons	S	R	R	R	R
MX156	<i>O. longistaminata</i>	Leaves	S	R	R	R	S
MX181	<i>O. longistaminata</i>	Leaves	S	R	S	R	R
MX158	<i>O. longistaminata</i>	Leaves	S	S	S	S	R
MX027	<i>O. longistaminata</i>	Leaves	S	S	S	S	R
MX204	<i>O. longistaminata</i>	Leaves	S	S	S	S	S

## Discussion and conclusion

The plant health survey has showed the significance of bacterial infections that are becoming a serious threat to rice production in the Office du Niger, where all varieties commonly grown (Adny11, Kogoni 91-1, BG 90-2, Nionoka, Sahelika) are attacked with incidences varying between 40 and 80%. The appearance of the first symptoms of BLB between 45 and 119 days after seeding in farmers' plots and between 81 and 147 days after seeding in the trials suggest contamination through leaves. An infection through seeds would have a greater impact on yield. The disease incidence varied from one zone to another. This could be due to pathogen variability. This incidence also evolved over time: it was progressive from the first to the third observation on all plots; from the third to the fourth observation, incidence decreased in some cases. The decrease in the incidence of the disease observed over time may be attributed to a varietal reaction to the infection. Infected varieties react by growing new leaves that do not have the symptoms of the disease. This could explain the low

paddy yield losses (4.35%) generally observed in farmers' plots, except for the few cases where production was destroyed at 80%. The latter could be attributed to early infection from seeds. Poor grain quality with low milling yield of the paddy were recorded.

Reactions of the different lines and varieties showed a zonal effect on the extent of the disease. The sites of Kayo, N'Debougou and Niono 2 seemed to be the most infected. Infections were noted earlier in Kayo than in the other two locations. The absence of epidemics in Kouroumari where seeding was done on the same date as in N'Debougou shows that the seeding date effect is not significant between the zones. On the contrary, in the zone of Niono, an epidemic of the disease was observed on late seeded rice (Niono 2), while plants sown earlier did not develop infection symptoms (Niono 1). The site of Niono 1 experienced incidences rarely above 20%. This could be due to weak aggressiveness of the races in this site. Molecular and pathogenic characterization of the recovered strains could confirm this hypothesis.

Most of the NILs and varieties were infected by BLB with a severity of less than 5. These results confirm those obtained by Gonzalez *et al.* (2007). Gigante, a cultivar resistant to RYMV, turned out to be susceptible to BLB in the five sites of the Office du Niger. This result should be taken into account in future breeding programs.

The pathological test indicated diversity within the Malian BLB pathogen population and a difference between Malian and Asian strains that needs to be investigated further. Pathotyping should be performed again on many other NILs and varieties.

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