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New Rice Lines Resistant to *Xanthomonas oryzae* pv. *oryzae*: a Promising Pathway To Food Security in Burkina Faso

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Abstract

Rice is one of the main food crops in Burkina Faso, but its production is seriously threatened by rice bacterial leaf blight, a disease responsible for yield losses of over 50% in 1998 and 2004. The aim of this study was to evaluate the phenotypic response of new rice lines to this disease caused by *Xanthomonas oryzae* pv. *oryzae*. A trial was carried out under semi-controlled conditions with 12 rice lines, five of which were derived from crosses between various parental varieties, and two elite local varieties included as controls. The BAI3 strain of *Xanthomonas oryzae* pv. *oryzae*, identified as the most virulent in Burkina Faso, was used for inoculation. Analysis of variance revealed significant differences in susceptibility between the lines, their parents and the controls. Lines AR19L025-F4-117 and AR19L018-F4-22, as well as their respective parents IRBB60 and CT21376-F3-9-1, showed strong resistance, with leaf lesions of less than 10 cm. Conversely, the FKR64 and ARICA3 lines suffered a total loss of yield, with complete wilting 45 days after inoculation. Correlation analysis revealed a significant negative relationship between disease severity and several yield components. For example, the average sterility rate was 28.47% in inoculated plants, compared with 18.57% in unifected plants. The average number of panicles per plant fell from seven (07) to five (05) after inoculation. The AR19L018-F4-22 and AR19L025-F4-117 lines, combining productivity and resistance, showed promise, with the confirmed presence of the *Xa4* and *xa5* resistance genes. Further research is needed to assess their ecological adaptation in endemic areas.

Keywords: Rice; bacterial Leaf Blight; *Xanthomonas oryzae* pv. *oryzae*; Agronomic Performance; Phenotype

Introduction

In Burkina Faso, rice is the fourth most important crop after maize, sorghum and millet. The crop has a strategic role in the country's food security because of its importance to the economy and its role in food security [1]. During the 2023/2024 season, national production reached a record level of 504.254 tonnes, while average national yields remained low at 1948 kg/ha [2]. This low yield is partly due to biotic and abiotic constraints that have a negative

impact on the yields of the varieties developed. These biotic constraints include bacterial leaf blight (BB), disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*).

It was first reported in 1884 in the province of Fukuoka in southern Japan [3]. However, it became important from the 1960s onwards with the adoption of new high-yielding varieties. The pathogen enters the plant through the stomata and wounds on the roots or leaves [4]. As a result, leaves wilt and photosynthesis is reduced, leading to yield losses of 20-50 % [5].

In Burkina Faso, the disease first appeared in the 1980s. However, it was in 1998 and 2004 that major epidemics occurred on the Bagre rice plain, potentially following the introduction of the Chinese rice variety TCS10, which proved to be very susceptible, causing yield losses of over 50% [6]. This variety was quickly replaced by others that also proved susceptible to the disease. Diallo A, et al. [7] have shown that most of the irrigated rice varieties grown in Burkina Faso are susceptible to bacterial leaf blight disease. However, this form of rice growing accounts for more than 90 % of national rice production.

Indeed, the results of Wonni I [8] showed that rainfed rice varieties with a japonica background are resistant against the diversity of *Xoo* strains in Burkina Faso. However, these varieties do not perform well in irrigated rice. In addition, the evaluation of certain lines with pyramided genes, in particular IRBB50 and IRBB60 developed by IRRI, showed good resistance to all the *Xoo* races described in Burkina Faso [9]. Studies by Ogawa T [10] and Yoshimura S [11] have shown that the combination of several genes (gene pyramiding) gives varieties durable resistance compared with the action of a single gene. In addition, the presence of two or more genes for resistance to *Xoo* in a rice variety has been shown to give it greater resistance. Thus, the possibility of the pathogen mutating to overcome this resistance is much lower than in the case of a single gene. These pyramid-gene rice lines would be good genotypes for improving the resistance of other varieties.

These two (02) lines (IRBB50 and IRBB60) were therefore used by AfricaRice to make crosses with other varieties. The lines obtained from these crosses were introduced into Burkina Faso. The general objective of the study was to evaluate the performance of these new varieties inoculated with the BAI3 strain of *Xoo*, considered to be the most virulent strain of *Xoo* originating in Burkina Faso.

Materials and Methods

Materials

Experimental site: The experiment was conducted in the greenhouse of the former Plant Protection Department of the Direction Régionale de la Recherche Agricole de l'Ouest. It is located in western Burkina Faso, in the Hauts-Bassins region, Houet province and Bobo-Dioulasso commune. This site housed the plant protection laboratories. Its geographical coordinates are 11°10 north latitude, 04°17 west longitude and an average altitude of 423 m (Figure 1).

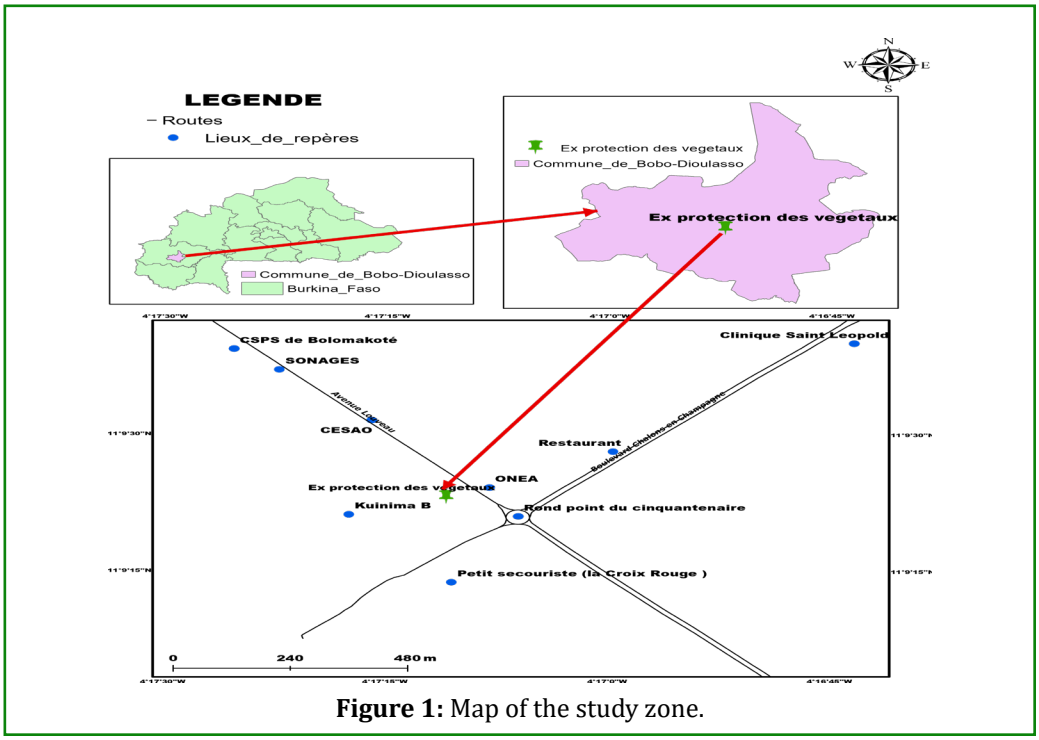


Figure 1: Map of the study zone.

Study 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 [4,12]. The FKR64 (TS2) and FKR19 controls are the most widely produced local varieties in Burkina Faso.

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 C [13]. Resistance tests carried out by [13] 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 A [14] identified eight TAL effectors in the BAI3 strain, among which TALC plays a major role as a virulence factor. It should also be noted that the TALE sequences of BAI3 are available and have been described by Tran TT [15].

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* Source: Sié M [16] and Wopereis MCS [17].

Table 1: Characteristics of the varieties evaluated.

Methods

Experimental design: The trial was conducted using a split-plot design with two factors and three replications. The primary factor consisted of 12 rice varieties. The secondary factor consisted of two inoculation treatments: the pathogenic strain *Xanthomonas oryzae* pv. *oryzae* (strain BAI3) and distilled water used as a control (negative control). The experimental unit was represented by pots. For each replicate, a total of 24 pots were used, i.e. 12 pots inoculated with strain BAI3 and 12 pots treated with distilled water. The complete set-up thus comprised 72 pots for all three replicates. Each pot, with a volume of five (5) liters, was placed on a tablar. Three (3) rice plants were transplanted per pot, and observations were made on these plants. Climatic conditions during the experimental period were characterized by (i) a minimum temperature of 22.17°C and a maximum temperature of 39.54°C; (ii) relative humidity ranging from 59.22% (minimum) to 88.60% (maximum).

Agronomic management: Sowing was carried out in pots filled with potting soil that had been sterilised beforehand and placed in a tank containing permanent water after germination. The seeds were pre-germinated in Petri dishes containing moistened blotting paper for 72 hours before sowing. The pre-germinated seeds were transferred to the pots at a rate of three (03) seeds per pot. Weeding and watering were carried out regularly as required. Fertilisation consisted of applying small quantities of NPK fertiliser at a rate of 1.2 g/pot three (03) days after transplanting. Urea was then applied at a rate of 0.17 g/pot at 15 days after transplanting and 0.31 g/pot at 55 days after transplanting. Harvesting took place when 2/3 of the panicles in a pot had turned straw-yellow.

Preparation of culture media: The culture medium used was LPGA (for 1 L, 7 g yeast extract, 7 g peptone, 7 g glucose and 18 g agar). This solution was sterilised in an autoclave at 121°C for 30 min, then 25 ml of it was poured into Petri dishes.

Bacterial inoculum: The bacterial inoculum was prepared from a 48 h bacterial culture suspended in a tube containing 50 ml of distilled water, then vortexed. The optical density (OD) of the suspension was measured and adjusted to 0.2 using an Amersham Biosciences spectrophotometer. Finally, 200 ml of this bacterial suspension corresponding to a concentration of 108 bacteria/ml was used to inoculate the plants. Sterile distilled water was used as a negative control.

Inoculation of varieties: At 21 days after sowing, the last two leaves of each plant were inoculated using the 'leaf clipping' method. This method involves dipping the blades of a pair of scissors into the bacterial suspension, then cutting off the end of the leaf.

Assessment of varietal resistance: BLB symptoms were assessed by measuring leaf lesions at 14- and 21-days post-inoculation (DPI) using a graduated ruler. The adapted IRRI scale IRRI [18] was used to assess the BB phenotype of the new varieties (Table 2).

Scale (cm)	Phenotype	Adapted scale
0-5	Resistant (R)	Resistant (R)
05-10	Moderately resistant (MR)	Resistant (R)
10-15	Moderately sensitive (MS)	Susceptible (S)
15 and overs	Sensitive (S)	Susceptible (S)

Table 2: Phenotype assessment scale for BLB varieties

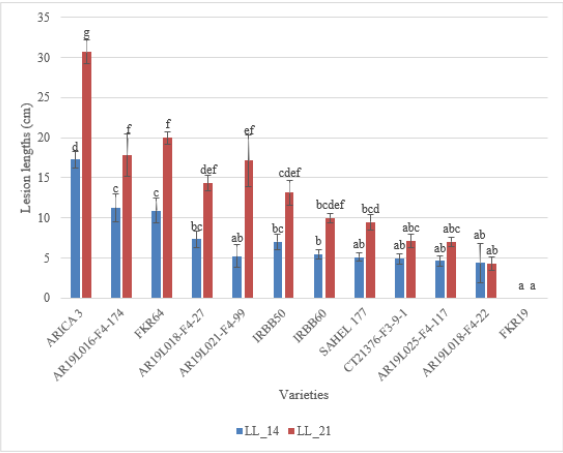
Evaluation of agro-morphological traits: Observations of agro-morphological data focused on 10 traits. These were:
Number of tillers on the 60th day after sowing (T60) determined by manual counting of tillers per bunch;
Plant height at maturity (cm) (PH) measured at plant maturity on the longest tillers using a graduated ruler from the surface of the soil in the pots to the tip of the tallest panicle;
the sowing-heading cycle (SHC) and sowing-maturity cycle (SMC), representing respectively the number of days between sowing and 50% heading and the maturity of the plants when ¾ of the panicles have a straw-yellow colour. They were evaluated in days;
Number of panicles (Npan), determined at maturity before harvest by manually counting the number of panicles per bunch;
Panicles length (cm) (Lpan), measured at maturity before harvest;
Panicle weight (Pw), assessed after harvest using a laboratory balance and expressed in grams (g);
Thousand-grain weight (TGW)(g) assessed after counting 1000 grains at a moisture content of 14%;
Sterility rate (SR): Evaluated by counting the number of empty and filled grains on five (05) panicles. The sterility rate was calculated using the following formula : SR=NGV/(NGP+NGV) x 100. Where NGV is the number of empty grains per panicle, and NGP is the number of filled grains per panicle;
Weight of grains per plant (g) (Wg/p) assessed per plant and expressed in grams per plant (g/plant) at 14% moisture content.

Data analysis: Excel 2016 was used for data entry. Analysis of variance was performed using XLSTAT version 2016 software. Means were separated using the Fisher test at the 5% probability level. The severity of the disease on the varieties was assessed using histograms. The Pearson correlation matrix was used to study the relationships between the agronomic characteristics and between these characteristics and the severity of the disease. Hierarchical ascending classification was also carried out by grouping varieties according to their performance and behaviour in relation to BLB.

Results and Discussion

Behaviour of Varieties against Rice Bacterial Leaf Blight

Leaf lesion lengths induced by the BAI3 strain: The lesion lengths due to bacterial leaf blight (BB) disease on the lines at 14 and 21 days after inoculation (DAI) are summarised in Figure 2. Analysis of variance showed a significant difference at both day 14 ($Pr<0.001$) and day 21 ($Pr<0.001$). On day 14 after inoculation, the ARICA3 variety had the longest lesion at 17.2 cm. The control variety FKR19 had no symptomatic lesions. The new varieties AR19L025-F4-117 and AR19L018-F4-22 and their respective parents IRBB60 and CT21376-F3-9-1 had lesion lengths of less than 5 cm. However, by the 21st DAI, lesion lengths were greater. The parent variety ARICA3 had large lesions reaching 31 cm. The variety FKR19 remained free of symptoms. On the other hand, lesions did not develop on the new variety AR19L018-F4-22 (5 cm). The sensitive control FKR64 recorded lengths of 11 and 20 cm at 14 and 21 days respectively.



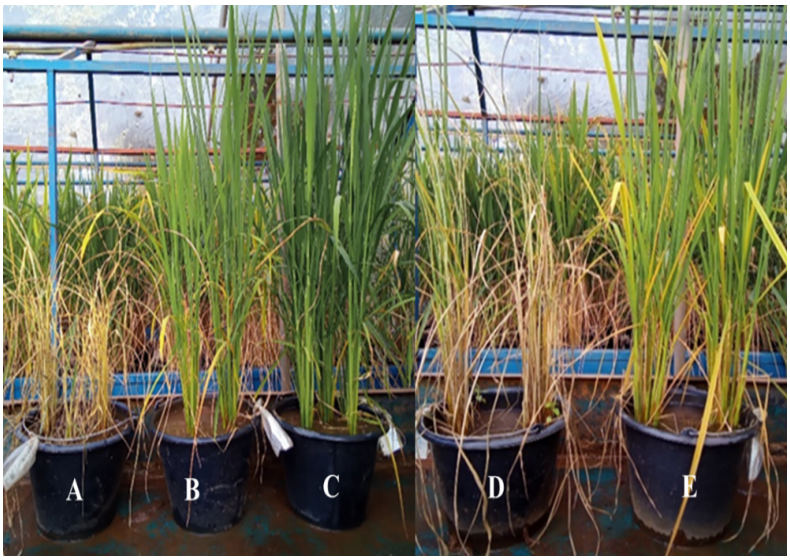
LL_14: Leaf lesion lengths at 14 days; LL_21: Leaf lesion lengths at 21 days.
Figure 2: Leaf lesion lengths induced by strain BAI3 at 14 and 21 days of age.

Phenotype of varieties to bacterial leaf blight disease: Table 3 shows the phenotype of the different varieties tested according to the IRRI (2002) scale adapted into two components to assess susceptibility or resistance to the disease at 14 and 21 DAI. The varieties differ in their response to the BAI3 strain. The control FKR19 and the new varieties

AR19L018-F4-22 and AR19L025-F4-117 proved to be resistant at both 14 and 21 DAI. The parent variety ARICA3, the control FKR64 and the new variety AR19L016-F4-174 remained susceptible at both 14 and 21 DAI (Figure 3). The parents IRBB60 and IRBB50 were resistant regardless of the evaluation date.

Phenotypes		
Varieties	**LL_14 JAI**	**LL_21 JAI**
ARICA 3	S	S
AR19L016-F4-174	S	S
FKR64 (TS2)	S	S
AR19L018-F4-27	R	S
AR19L021-F4-99	R	S
IRBB50	R	S
IRBB60	R	R
SAHEL 177	R	R
CT21376-F3-9-1	R	R
AR19L025-F4-117	R	R
AR19L018-F4-22	R	R
FKR19	R	R

***R**: resistant; **S**: Susceptible; **LL_14J DAI**: Leaf lesion length at 14 days; **LL_21 DAI**: Leaf lesion length at 21 days.*
Table 3: Phenotype of varieties at 14 and 21 DAI.



***A** : ARICA3 ; **B** : IRBB60 ;**C** : AR19L025-F4-117; **D** : FKR64*
Figure 3: Illustration of the reaction of some lines 45 days after inoculation.

Performance of Agro-Morphological Traits

Agronomic performance of traits: Table 4 summarises the agronomic trait results for inoculated lines. For the traits sowing- heading cycle, sowing-maturity cycle, number of tillers at day 60 and panicle length, analysis of variance showed no significant difference between inoculated and non-inoculated lines. However, panicle number, plant height, panicle weight, sterility rate, thousand-grain weight and grain weight per plant discriminated between inoculated and non-inoculated lines. The non-inoculated lines performed better, with a number of panicles of eight (08) per plant compared with five (05) for the non-inoculated lines. Their height was shorter (96.03 cm) than that of the inoculated

lines (103.9 cm). In addition, panicle and thousand-grain weights were very high in non-inoculated lines (2.78 g and 22.02 g respectively) compared with inoculated lines (2.06 g and 19.45 g respectively). The sterility rate was low in non-inoculated lines (18.42%) compared with 28.41% in inoculated lines. Finally, the weight of grains per plant was remarkable in the non-inoculated lines, at 14.15 g. The inoculated lines, on the other hand, had a grain weight of 10.84 g per plant.

Traits	SHC	SMC	T60	Npan	PH	Lpan	Pw	SR	TGW	Wg/p
Control	84	104	9	8b	103.29b	24.4	2.78b	18.42a	22.02b	14.15b
inocu-lated	79	107	8	5a	95.91a	22.3	2.06a	28.41b	19.45a	10.47a
Pr > F	0.2	0.1	0.1	1E-04	0.003	0.1	1E-04	0.007	0.001	0.002
Signif-icant	Ns	Ns	Ns	***	**	Ns	***	**	**	**

SHC: *Sowing-heading cycle,* ***SMC:*** *Sowing-maturity cycle,* ***T60:*** *Number of tillers at 60 days after sowing;* ***Npan****: Number of panicles per plant;* ***Hp:*** *Height of plants at maturity;* ***Lpan:*** *Panicle length;* ***Pw:*** *Panicle weight;* ***SR****: Sterility rate;* ***TGW:*** *Thousand grain weight;* ***Wg/p****: Weight of grains per plant;* ***Ns:*** *Not significant;* ****:*** *Significant;* *****:*** *Highly significant;* **********: very highly significant*

Table 4: Agronomic trait of varieties tested.

Agronomic performance of control lines: Analysis of variance showed that all the characters discriminated between the lines, their parents and the two (02) local varieties, except for the number of panicles (Table 5). The sowing, heading and maturity cycles varied from 74 to 97 days and from 94 to 118 days respectively. On average, the lines headed at 85 days before reaching maturity at 104 days. The FKR19 variety was the earliest with a heading of 74 days and maturity of 95 days. The parent ARICA3 was the latest. It headed at 94 days and reached maturity at 116 days. The average tillering performance was nine (09) tillers per plant with ranges from 4 to 15 tillers per plant. Line AR19L016-F4-174 performed less well with five (05) tillers while SAHEL 177 produced 11 tillers. The variation in height was fairly wide (87 - 119.75 cm) with an average of 103.28 cm. The parent IRBB60 was shorter at 87 cm. The control FKR64 was the tallest at 110.88 cm. Panicle length also varied between 19.3 and 29.6 cm with an average of 24.36 cm. The parental line CT21376-F3-9-1 had the longest panicles at 28.2 cm. However, line AR19L021-F4-99 had the shortest panicles (20.66 cm). Panicle weight ranged from 1.71 to 4.22 g. The average weight of the varieties was 2.78 g. The AR19L018-F4-27 line recorded the highest weight at 3.02 g, while CT21376-F3-9-1 had the lowest weight (1.83 g). The sterility rate varied from 6.54 to 31.61%, with an average of 18.42%. The FKR19 variety had a very low sterility rate of 7.5%, while the IRBB60 parental line was the least fertile, with a sterility rate of 22.6%. The weight of 1000 grains also varied between lines, ranging from 15.68 to 28.32 g. The average for all lines was 22.01 g. Line AR19L018-F4-27 weighed the most at 27.2 g. The parent CT21376-F3-9-1 weighed less with only 18.2 g. Lastly, the weight of grains per plant varied between 9 and 22.36 g, with an average of 14.15 g. The AR19L018-F4-22 line performed better with 19.9 g compared with 10 g per plant for the CT21376-F3-9-1 parent.

Lines	SHC	SMC	T60	Npan	HP	Lpan	Pw	SR	TGW	Wg/p
SAHEL 177	82abc	102abc	11ab	9	106.5ab	24.2abc	2.83ab	16.86abc	26.48b	17.5bc
AR19L018-F4-22	83abcd	99ab	14b	10	108.72ab	22.3ab	2.75ab	20.9bc	23.62ab	19.9 c
AR19L021-F4-99	87bcd	109bcd	10ab	8	96.27ab	20.66a	2.96ab	19.93abc	22.54ab	15.1abc
IRBB50	87bcd	106abcd	10ab	7	102.7ab	25.01abc	2.68ab	22.83bc	21.09a	14.9abc
ARICA 3	94cd	116d	7a	6	114.69b	24.16abc	2.9ab	12.29ab	20.46a	11.96ab
FKR64	82abc	101abc	7a	6	110.88ab	26.86bc	2.9ab	17.8abc	20.68a	13.73ab
AR19L025-F4-117	88bcd	109bcd	7ab	6	105.47ab	23.9abc	2.69ab	19.16abc	23.12ab	12.5ab
AR19L018-F4-27	82abc	100ab	7a	4	103.16ab	25.45abc	3.9b	16.24abc	27.2b	12.9ab
IRBB60	95d	114cd	6a	5	87a	22.5ab	1.91ab	22.6bc	22.24ab	12.6ab
AR19L016-F4-174	86abcd	106abcd	5a	6	104.16ab	23.95abc	2.8ab	17.17abc	21.5ab	12.58ab
CT21376-F3-9-1	81ab	101abc	9ab	7	88.88a	28.2c	1.83a	25.24c	18.2a	10a

FKR19	74a	95a	8ab	6	101.16ab	23.3abc	3.02ab	7.56a	19.92a	12.72ab
Minimum	74	94	4	4	87	19.3	1.71	6.54	15.68	9
Maximum	97	118	15	14	119.75	29.6	4.22	31.61	28.32	22.36
Mean	85	104	9	7	103.28	24.36	2.78	18.57	22.01	14.15
Standard deviation	5.97	6.6	2.86	2.28	9.6	2.38	0.63	5.83	2.93	3.03
CV (%)	6	6	32	31	8	9	20	32	13	21
Pr > F	0.0001	0.0001	0.01	0.1	0.025	0.002	0.04	0.001	0.001	0.0001
Significant	***	***	*	Ns	*	*	*	**	**	***

SHC: *Sowing-heading cycle,* ***SMC****: Sowing-maturity cycle,* ***T60****: Number of tillers at 60 days after sowing;* ***Npan:*** *Number of panicles per plant;* ***Hp****: Height of plants at maturity;* ***Lpan:*** *Panicle length;* ***Pw:*** *Panicle weight;* ***SR:*** *Sterility rate;* ***TGW****: Thousand grain weight;* ***Wg/p****: Weight of grains per plant;* ***Ns:*** *Not significant;* ****:*** *Significant;* *****:*** *Highly significant;* ******:*** *very highly significant*

Table 5: Characteristic analysis of control lines.

Agronomic performance of inoculated lines: Table 6 summarises the results of the analysis of the traits of the lines inoculated with the BAI3 strain. The analysis of variance showed a significant difference for all the agronomic traits assessed. The control variety FKR64 and the parent ARICA3 withered completely without reaching maturity. With respective variations of 75 to 96 days and 94 to 120 days, the average sowing-heading and maturity cycles were 84 days and 105 days. FKR19 was early with a heading of 75 days and maturity of 96 days. IRBB60 was later. It headed at 86 days and reached maturity at 119 days. The average numbers of tillers and panicles were respectively eight (08) and five (05) per plant with variations ranging from three (03) to 15 and two (02) to nine (09) per plant. The parent IRBB50 performed better with 14 tillers that produced eight (08) panicles, whereas line AR19L018-F4-27 produced less with only six (06) tillers and three (03) panicles per plant. In addition, the height of the inoculated lines varied from 81 to 110 cm with an average of 95.91 cm. Panicle length and weight ranged from 15.4 to 28.7 cm and 1 to 3.8 g respectively, with averages of 22.26 cm and 2.06 g respectively. Parent line CT21376-F3-9-1 had the longest panicles (27.23 cm), while variety FKR19 had the heaviest panicles (3.48 g). Lines AR19L021-F4-99 and AR19L018-F4-27 had the shortest panicles at 18.66 cm and 18.96 cm respectively. The variety SAHELL 177 and AR19L025-F4-117 had panicles that weighed less. In general, the lines were less fertile with an average sterility of 28.47 % and variations ranging from 10.78 to 58 %. Variety FKR19 was more fertile with a sterility of 11.31 %. Line CT21376-F3-9-1 was less fertile with a sterility of 55 %. With regard to the weight of 1000 grains, the inoculated lines varied from 13.9 to 26.32 g. The average was 19.45 g. With the exception of the varieties ARICA3 and FKR64, which wilted completely, line IRBB50 had a very low 1000-grain weight of 14.32 g. The SAHELL 177 variety performed better with 24.08 g. The weight of grains per plant also varied from 3.5 to 20.09 g, with an average of 10.47 g per plant. The variety SAHELL 177 was productive under inoculation conditions with 14.9 g per plant. The variety AR19L025-F4-117 was less productive (4.42 g). Varieties ARICA3 and FKR64 wilted and were unable to produce grain.

Lines	SHC	SMC	T60	Npan	HP	Lpan	Pw	SR	TGW	Wg/p
SAHEL 177	82cd	101b	12cd	5ab	99.66bcd	19.26a	1.43a	35.83d	24.08f	14.9fg
AR19L018-F4-22	82bc	99b	10bc	5ab	101cd	23.88bc	2.45bc	17.89b	22.96ef	12.69ef
AR19L021-F4-99	84cd	101b	7ab	4ab	96.08bcd	18.66a	1.99abc	24.31c	20.74de	8.31bcd
IRBB50	86d	119e	14d	8d	104d	26.16cd	1.96abc	20.05bc	14.32a	10.03cde
ARICA3	withered	withered	7ab	withered	withered	withered	withered	withered	withered	withered
FKR64	withered	withered	8ab	withered	withered	withered	withered	withered	withered	withered
AR19L025 -F4-117	91e	109cd	7ab	4ab	94.08bc	21.4ab	1.42a	18.97bc	18.7bcd	8.92bcd
AR19L018-F4-27	85cd	101b	6a	3a	94.58bcd	18.96a	1.69ab	44.74e	18.92cd	6.4abc
IRBB60	95f	113d	8ab	7cd	98.25bcd	24.28c	2.7cd	18.05b	22.49ef	13.24ef

AR19L016 -F4-174	86d	100b	8ab	3ab	91.16ab	19a	1.91abc	38.47d	17.16bc	4.42a
FKR19	75a	95a	8ab	7c	98.33bcd	23.76bc	3.48d	11.31a	19.06cd	18.2g
CT21376-F3-9-1	78ab	109c	10bc	4ab	82a	27.23d	1.58ab	55.11f	16.1ab	11.6def
Minimum	75	94	3	2	81	15.4	1	10.78	13.96	3.5
Maximum	96	120	15	9	110	28.7	3.8	58	26.32	20.09
Mean	79	107	8	5	95.91	22.26	2.06	28.47	19.45	10.47
Standard deviation	5.86	7.42	3.25	1.89	7.55	3.38	0.77	13.99	3.28	4.71
CV (%)	6	7	31	32	7	15	36	35	16	34
Pr > F	0.0001	0.0001	0	0.0001	0.009	0.0001	0.004	0.0001	0.0001	0.0001
Significant	***	***	**	***	**	***	**	***	***	***

SHC: *Sowing-heading cycle,* ***SMC:*** *Sowing-maturity cycle,* ***T60:*** *Number of tillers at 60 days after sowing; Npan: Number of panicles per plant;* ***Hp:*** *Height of plants at maturity;* ***Lpan:*** *Panicle length;* ***Pw:*** *Panicle weight;* ***SR:*** *Sterility rate;* ***TGW:*** *Thousand grain weight;* ***Wg/p****: Weight of grains per plant;* ***:** *very highly significant*

Table 6: Characteristic analysis of inoculated lines.

Relationship between characteristics of control lines: Analysis of the relationships between the characteristics of uninfected lines show the existence of significant positive and negative correlations (Table 7). In fact, there is a positive and significant correlation between the number of tillers and the number of panicles (r=0.89) on the one hand, and on the other hand with the weight of grains per plant (r=0.75). Grain weight per cluster was also positively and significantly correlated with panicle number (r=0.76). Finally, plant height at maturity was positively and significantly correlated with panicle weight. However, a negative and significant correlation (r=-0.59) was observed between panicle weight and sterility rate.

Traits	SHC	SMC	T60	Npan	HP	Lpan	Pw	SR	TGW	Wg/P
SHC	1									
SMC	0.969	1								
T60	-0.304	-0.397	1							
Npan	-0.131	-0.178	0.89	1						
HP	-0.05	-0.056	0.109	0.181	1					
Lpan	-0.299	-0.29	-0.227	-0.269	0.026	1				
Pw	-0.291	-0.283	-0.063	-0.181	0.598	-0.121	1			
SR	0.332	0.208	0.255	0.252	-0.51	0.176	-0.597	1		
TGW	0.007	-0.102	0.157	0.114	0.217	-0.288	0.569	-0.054	1	
Wg/P	-0.116	-0.258	0.785	0.764	0.354	-0.478	0.209	0.084	0.52	1

Values in bold are different from 0 at significance level alpha=0.05.

SHC: *Sowing-heading cycle,* ***SMC:*** *Sowing-maturity cycle,* ***T60:*** *Number of tillers at 60 days after sowing;* ***Npan:*** *Number of panicles per plant;* ***Hp:*** *Height of plants at maturity;* ***Lpan:*** *Panicle length;* ***Pw:*** *Panicle weight;* ***SR:*** *Sterility rate;* ***TGW:*** *Thousand grain weight;* ***Wg/p:*** *Weight of grains*

Table 7: Two-way correlation between traits in non-inoculated lines.

Relationship between the characteristics of the inoculated lines: Table 8 shows the relationships between the characteristics of the inoculated lines and between the characteristics and the severity of vascular bacterial disease 21 days after inoculation. The analysis showed significant positive and negative correlations. There was a significant positive correlation (r=0.58) between the weight of grains per plant and the number of panicles. The number of

panicles was also positively and significantly correlated with panicle length (r=0.63) and plant height at maturity (r=0.57). However, a negative and significant correlation was observed between the sterility rate and the height of plants at maturity (r=-0.74); also between the sterility rate and panicle weight (r=-0.63). The same was true between disease severity and grain weight per plant (r=-0.63), number of tillers per cluster (r=-0.53), panicle length (r=-0.53) and panicle weight (r-0.50).

Traits	SHC	SMC	T60	Npan	HP	Lpan	Pw	SR	TGW	Wg/P	LL_21
SHC	1										
SMC	0.564	1									
T60	-0.201	0.397	1								
Npan	0.081	0.509	0.558	1							
HP	0.208	0.062	0.348	0.578	1						
Lpan	-0.169	0.54	0.485	0.639	-0.064	1					
Pw	-0.23	-0.3	-0.102	0.531	0.355	0.305	1				
SR	-0.211	-0.02	-0.018	-0.562	-0.743	-0.121	-0.636	1			
TGW	0.122	-0.437	-0.107	-0.142	0.345	-0.375	0.158	-0.259	1		
Wg/P	-0.479	-0.199	0.413	0.582	0.313	0.439	0.626	-0.618	0.391	1	
LL_21	0.271	0.118	-0.535	-0.19	-0.052	-0.535	-0.504	0.232	-0.134	-0.635	1

Values in bold are different from 0 at significance level alpha=0.05

SHC: *Sowing-heading cycle,* ***SMC:*** *Sowing-maturity cycle,* ***T60:*** *Number of tillers at 60 days after sowing;* ***Npan:*** *Number of panicles per plant;* ***Hp:*** *Height of plants at maturity;* ***Lpan:*** *Panicle length;* ***Pw:*** *Panicle weight;* ***SR:*** *Sterility rate;* ***TGW:*** *Thousand grain weight;* ***Wg/p:*** *Weight of grains,* ***LL_21****: Leaf lesion length at 21 days after inoculation*

Table 8: Pairwise correlation between traits and expression of disease.

Grouping of control lines according to their agronomic performance: The grouping of lines using discriminating and poorly correlated traits (sowing maturity cycle, height at maturity, panicle length, sterility rate, thousand grain weight and grain weight per panicle) (Table 9) enabled the lines to be structured into three (03) groups (Figure 4). Group 1, the best performing group, includes almost all the lines, including all the new varieties, the FKR19 and FKR64 controls and the IRBB50 and SAHELL 177 parents. This group reached maturity at 102 days with a height at maturity of 104.34 cm and a panicle length of 23.96 cm. This group also had an average sterility rate of 17.61 % and a higher 1000-grain weight of 22.91 g. Grain weight per plant was also high at 14.69 g. This was followed by group 2, which consisted solely of the ARICA3 variety. It was the latest variety with a cycle of 116 days and a greater height (114.69 cm). Panicle length was average at 24.16 cm. However, its sterility rate was low at 12.29 %. The thousand-kernel weight was also low (20.46 %). The weight of seeds per bunch was intermediate at 11.96 g. Group 3 was the least successful. It includes two (02) varieties, namely IRBB60 and CT21376-F3-9-1. These varieties completed their cycle at 107 days. Their height at maturity was semi-dwarf at 87.94 cm. However, the panicles were the longest at 25.35 cm. The sterility rate was the highest at 23.93 %. The weight of 1000 grains was the lowest at 20.22 g. Similarly, the weight of grains per plant was low at 11.33 g.

Group	SMC	HP	Lpan	SR	TWG	Wg/P
1	103	104.34	23.965	17.611	22.91	14.69
2	116	114.69	24.167	12.297	20.467	11.965
3	108	87.94	25.35	23.939	20.22	11.338

SMC: *Sowing-maturity cycle,* ***Hp:*** *Height of plants at maturity;* ***Lpan:*** *Panicle length;* ***SR:*** *Sterility rate;* ***TGW:*** *Thousand grain weight;* ***Wg/p:*** *Weight of grains*

Table 9: Average performance of groups of uninfected lines.

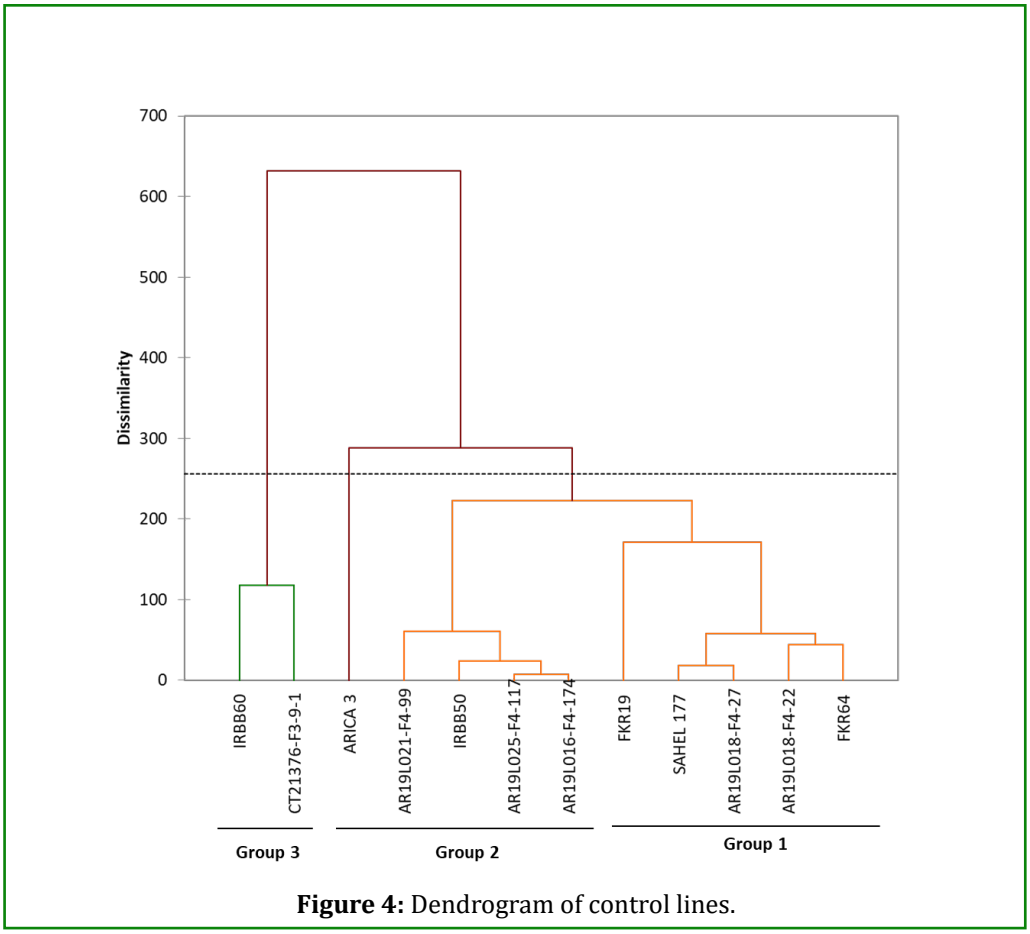


Figure 4: Dendrogram of control lines.

Grouping of inoculated lines according to agronomic performance and BLB behaviour: Poorly correlated and discriminating traits (Table 10) such as the sowing-maturity cycle, number of tillers, panicle length, sterility rate, thousand-kernel weight and weight of kernels per plant made it possible to structure the inoculated lines into three (03) distinct groups (Figure 5). Group 1 consists of lines ARIC3 and FKR64, which wilted completely. The only difference was the number of tillers, which was eight (08) per plant. The length of leaf lesions on these varieties was 25.33 cm. Group 2 includes the new lines AR19L018-F4-22, AR19L025-F4-117, the parents IRBB60 and IRBB50 and the variety FKR19. These lines recorded the best agronomic performances, with good resistance to bacterial leaf blight disease (lesion length of 6.93 cm). These lines reached maturity at 107 days with a number of tillers of 10 per plant. Their panicle length was longer at 23.90 cm. These lines were more fertile, with a sterility rate of only 17.26%. In addition, the weight of 1000 grains and the weight of grains per bunch were 19.50 g and 11.82 g respectively. Group 3 was an intermediate performer. It includes lines AR19L016-F4-174, AR19L018-F4-27, AR19L021-F4-99, SAHEL 177 and CT21376-F3-9-1. These lines completed their cycle at 102 days. They produced nine (09) tillers per plant with a panicle length of 20.62 cm. In addition, these lines were less fertile with a sterility rate of 39.69%. Their thousand kernel weight and kernel weight per cluster were 19.40 g and 9.13 g respectively.

Group	SMC	T60	Lpan	SR	TGW	Wg/P	LL_21
1	withered	8	withered	withered	withered	withered	25.33
2	107	10	23.9	17.26	19.5	11.82	6.93
3	102	9	20.62	39.69	19.4	9.13	13.17

SMC: *Sowing-maturity cycle,* ***T60:*** *Number of tillers at 60 days after sowing;* ***Lpan:*** *Panicle length;* ***SR:*** *Sterility rate;* ***TGW:*** *Thousand grain weight;* ***Wg/p:*** *Weight of grains,* ***LL_21****: Leaf lesion length at 21 days after inoculation*

Table 10: Average performance of groups of inoculated lines.

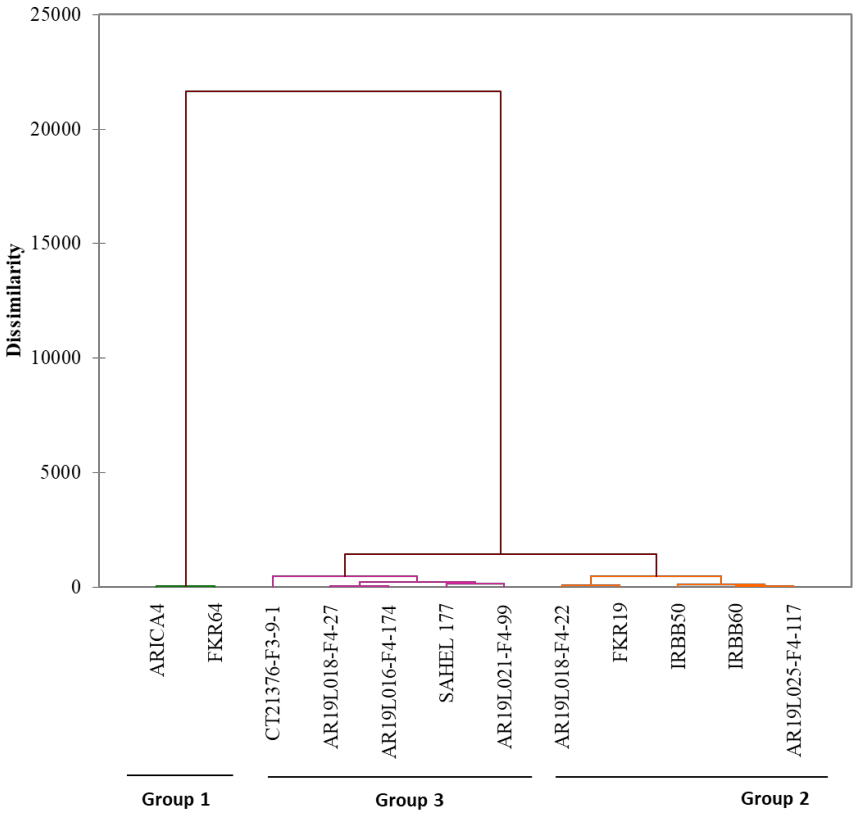


Figure 5 : Dendrogramme des lignées inoculées.

Discussion

The results of the analyses reveal a marked variability in the behavior of lines, their parents and local varieties with regard to bacterial disease. This diversity could be explained by the intrinsic genetic characteristics of each genotype. This finding is in line with the observations of several authors, including Ouédraogo SL [19], Tall H [20] and Wonni I [8], who have shown that disease progression depends on the presence or absence of specific resistance genes.

The AR19L018-F4-22 and AR19L025-F4-117 lines stood out for their high resistance, with lesions of less than 10 cm. This resistance is probably due to the presence of the *Xa4* and *xa5* genes, inherited from their respective parents, IRBB50 and IRBB60. In addition, variety CT21376-F3-9-1, a parent of line AR19L018-F4-22, also possesses these two genes.

Observations confirm that line IRBB60 shows stable resistance, unlike IRBB50, which shows a loss of resistance over time. However, lines produced by crossing these two parents, which carry pyramid genes, showed enhanced resistance to the Burkinabe strain BAI3. These results corroborate those of Diallo A [7], who reported the susceptibility of IRBB50 (*Xa4* + *xa5*) to the BAI3 strain, in contrast to the more stably resistant IRBB60 (*Xa4* + *xa5* + *xa13* + *Xa21*). Gautam RK [21] also highlighted the increased efficacy of Xa genes when combined (pyramided) in the same line.

The dominant *Xa4* gene confers resistance to races 1, 4, 5, 7, 8 and 10 of *Xoo* of Asian origin [22]. Several studies, including those by Gonzalez C [13], Wonni I [23] and Diallo A [14], have demonstrated its efficacy against African strains. In addition, Oña I [24] confirmed the efficacy of the *Xa4* gene under conditions of variable disease pressure in the field, noting a significant reduction bacterial blight outbreaks in IR varieties carrying this gene.

The *xa5* gene, recessive and located on chromosome 5, codes for the gamma subunit of the TFIIA transcription factor. It is effective against several races of *Xoo,* notably those from the Philippines [25], as well as certain African strains [13,14].

Interactions between *Xoo* and rice are regulated by the bacterium's TAL effectors, which target susceptibility genes

of the SWEET family. The *xa13* allele, for example, prevents induction of the *OsSWEET11* gene by the PthXo1 effector, conferring resistance to certain *Xoo* strains [26]. Conversely, lines possessing the *Xa13* allele, i.e. expressing *OsSWEET11*, remain susceptible.

Our results also confirm the susceptibility of the FKR64 variety and the resistance of FRK19 to Burkinabe strains of Xoo, as already observed by Wonni I [8] and Diallo A [14].

Impact of the Disease on Agronomic Performance

Analysis of variance revealed significant differences between the agronomic traits of inoculated and non-inoculated lines, indicating a negative impact of the disease on the performance of susceptible genotypes. With the exception of tillering, no agronomic traits could be assessed on highly susceptible genotypes such as ARICA3 and FKR64, due to total wilting at 45 days after inoculation, resulting in a complete loss of production. The absence of effective resistance genes to the BAI3 strain could explain this high susceptibility.

TAL effectors such as PthXo1, PthXo3 and avrXa7 target the OsSWEET11 and OsSWEET14 genes respectively, facilitating bacterial multiplication in rice tissues [27-29]. The BAI3 strain used in this study produces the TalC effector, which also activates OsSWEET14 via a separate binding site [30,31].

The drop in yield observed in inoculated lines is thus linked to a disruption in photosynthetic sugar accumulation, with a direct effect on grain filling. This hypothesis is supported by the negative and significant correlations between disease severity and yield components such as number of tillers per cluster, panicle length, panicle weight and grain weight per cluster. Similar findings were reported by Tall H [20]. The average sterility rate was 28.47% in inoculated lines, compared with only 18.57% in non-inoculated controls, an increase of over 10%. This sterility, strongly correlated with yield decline, is a critical indicator of the disease's impact. The number of panicles per poquet was also affected, dropping from seven (07) in the controls to five (05) in the inoculated lines.

Typology of Varieties According to Bacterial Blight Behavior
Factor analysis enabled us to group varieties into distinct classes according to their agronomic performance and level of resistance. Under non-inoculated conditions, ARICA3 and FKR64 performed well: thousand grain weights of 22.91 g and 20.46 g respectively, and grain weights per panicle of 14.69 g and 11.96 g. These results confirm those of Wopereis MCS [17] and Konate AK [32], who reported yields in excess of 5 t/ha for these varieties. However, under inoculation conditions, they fall into the group of highly susceptible varieties, characterized by a total loss of yield.

The use of susceptible varieties in areas with high vascular bacterial blight pressure exposes growers to drastic losses. On the other hand, the new lines AR19L018-F4-22 and AR19L025-F4-117, combining resistance and productivity, appear as to be promising candidates for enhancing food safety in a sustainable way.

Conclusion

The aim of the study was to assess the bacterial leaf blight disease behaviour of new varieties resulting from crosses between lines with the pyramided *Xa* gene.

Analysis of variance showed variability between the lines with regard to bacterial leaf blight disease. The new varieties AR19L025-F4-117 and AR19L018-F4-22 and their respective parents IRBB60 and CT21376-F3-9-1 proved to be more resistant at both 14 and 21 days after inoculation, with leaf lesion lengths of less than 10 cm. However, the agronomic performance of most lines was reduced when inoculated with Xoo strain BAI3. Varieties FKR64 and ARICA3 wilted completely after inoculation, with a yield loss of 100 %. The average sterility rate in the inoculated lines was 28.47 %, whereas it was only 18.57 % in the control lines. In addition, correlation analysis showed significant negative correlations between disease severity and the main yield components.

The new varieties AR19L018-F4-22 and AR19L025-F4-117 were selected as being productive and resistant to vascular bacterial disease. These lines could guarantee sufficient and sustainable production in Burkina Faso. However, multi-local trials should be carried out in areas endemic to vascular bacterial disease, particularly in the rice-growing plain of Bagré. In addition, molecular studies to determine the presence/absence of resistance genes should be considered in order to understand the resistance/susceptibility of the genotypes tested.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

IW, AKK and AB initiated and supervised the work. SK and AMNO conducted the semi-controlled evaluations. SK drafted the manuscript. All authors contributed to the correction of the manuscript.

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