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Elite local rice varieties resistant to bacterial leaf streak caused by *Xanthomonas oryzae* pv. *oryzicola* under field conditions in Burkina Faso

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This study aims to evaluate the phenotype of nine genotypes of rice, during two consecutive seasons, in plots of rice farmers in irrigated plains Kou valley and Di. A Fisher block was implanted with three replicates at both sites on plots that had previously been shown to have a high incidence of bacterial leaf streak (BLS). The incidence, severity, growth rate of the disease, and the average yield of the different genotypes tested were assessed. In addition, climatic data including temperature and hygrometry were recorded in order to establish correlations between the various parameters measured. It was noted that the first symptoms appeared on susceptible varieties as of 30 DAT and progressed over time to reach higher levels (100%) by 72 DAT. The results show that FKR19, WAB181-18, FKR45N, and FKR49N genotypes were shown to be resistant despite the high pressure of BLS. However, high temperature and hygrometry significantly influenced the BLS severity (r = 0.8), which had a significant effect on the potential yield of the tested varieties (P = 0.00014). Therefore, adhesion to the cropping calendar and use of resistant varieties are some of the best strategies to reduce the incidence of BLS in rice-growing conditions in Burkina Faso.

Key words: Rice, bacterial leaf blight, *Xanthomonas oryzae* pv. *oryzicola*, resistant.

INTRODUCTION

Rice consumption is increasing due to population growth, increasing urban areas, and changes in eating habits. Meanwhile, the global supply of rice is declining due to reduction in the area available for rice cultivation in favour of other crops (biofuels, wood, etc.) and climate change leading to droughts and floods (SNDR, 2011). Although there is ample potential to increase rice production in Africa, rice imports represent a third of the total amount of

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rice traded on the world market. A number of measures are being implemented, however, by African countries to intensify rice cultivation.

In Burkina Faso, rice ranks fourth after sorghum, millet, and maize in terms of the area of cultivation, the amount produced, and the level of consumption. Indeed, national rice production only covers less than half of the population's consumption needs, which are estimated to be 475,000 tonnes of milled rice annually. Thus, efforts are being made to increase the national production of rice through irrigation schemes, the use of improved varieties, and promotion of the rice sector (Presao, 2011).

Despite these considerable efforts, the increase in rice production is limited by biotic, abiotic, and socio-economic constraints. In terms of the biotic constraints, rice is subject to serious diseases that can reduce the yield, including rice blast, rice yellow mottle virus, bacterial leaf blight disease, and bacterial leaf streak (BLS) (Seré and Nacro, 1992; Seré et al., 1994; Seremé et al., 2014; Wonni et al., 2011, 2014).

BLS caused by *Xanthomonas oryzae* pv. *oryzicola* (*Xoc)* has been reported in many African countries including Mali, Nigeria, Senegal, Niger, Ivory Coast, Madagascar, Uganda, Burundi, and Burkina Faso (Wonni et al., 2011; Poulin et al., 2014; Afolabi et al., 2014a, b; Diallo et al., 2021).

In Burkina Faso, BLS is present in the main rice-growing areas of Bagre and Itenga in the Central-Eastern region, Bama and Banzon in the Hauts-Bassins region, Niassan and Di in the Boucle Mouhoun region, and Karfiguela and Douna in the Cascades region (Wonni et al., 2011, 2014; Barro, 2015; Barro et al., 2021). BLS symptoms consist of water-soaked lesions that develop into translucent yellow streaks with visible exudates at the leaf surface. BLS develops in the field at any growth stage of rice. *Xoc* is an intercellular pathogen that enters plants through wounds or by invading open stomata (Ou, 1985). It then multiplies in the substomatal chamber and colonizes the apoplast of the mesophyll cells (Mew, 1987; Niño-Liu et al., 2006). *Xoc* oozes from natural openings in strands or strings on the leaf surface, and exudates can spread the disease from plant to plant by direct contact or indirectly via irrigation water and by windblown rain (Mew et al., 1993). *Xoc* is a seed-borne and a seed-transmitted pathogen (Xie and Mew, 1998). Yield losses due to this disease depend on the rice variety being cultivated and the climatic conditions, but typically range from 10 to 20% (Ou, 1985). Although significant yield losses have not yet been observed in Burkina Faso, BLS has a high leaf incidence of up to 100% in certain rice plots of the most irrigated sites (Wonni, 2013; Zougrana, 2017).

In light of the BLS distribution and its prevalence in the main rice-growing sites, there is a need to develop and/or identify resistant rice genotypes that are adapted to different cultivation areas.

Indeed, Wonni et al. (2016), under greenhouse inoculation conditions, identified local varieties of rice with a broad spectrum of resistance to the various African *Xoc* strains. However, these varieties remain to be evaluated in a field environment in order to assess their resistance and stability to BLS. The aim of this study was to identify rice genotypes that are resistant to BLS.

MATERIALS AND METHODS

Study sites

The tests were carried out at rice-growing sites known for their previous infestation with BLS disease reported by Wonni et al. (2011, 2014) and Zougrana (2017), which are the irrigated plains of the Kou valley and the Di plains.

The Kou valley plain is located at 30 km from Bobo-Dioulasso in the rural municipality of Bama at an altitude of 300 m above sea level between longitude 04°22'W and latitude 11°22'N. It extends over 1,200 ha with a total water control (Sontie, 2006). The climate is typical of southern Sudan, with annual rainfall ranging from 1100 to 12,000 mm (Yameogo et al., 2013).

The Di irrigated plain is located in the northwest of Burkina Faso at 326 km from Bobo-Dioulasso. It covers an area of 2,240 ha with total water control. The area lies at an altitude of 277 m above sea level between longitude 3°20'W and latitude 13°18'N. The climate is typical of northern Sudan, with annual rainfall ranging from 600 to 900 mm.

Rice genotypes tested

Nine varieties of rice whose phenotypes against African *Xoc* strains were evaluated by Wonni et al. (2016) under artificial inoculation conditions were tested under field conditions. Two rainfed and three irrigated/lowland varieties used by producers were included. The cultivars CG14, WAB56-50, and WAB181-18, which are the parents of NERICA varieties FKR45N and FKR49N, were also tested. The choice of these varieties is justified by their adoption by producers and consumers in Burkina Faso (Table 1).

Field tests for resistance to BLS

Experimental design

The tests were set up in the Kou valley and the Di plains from July 15, 2019 to 2020 in one farmer's field per site where BLS infection was observed during the wet seasons in 2017 and 2018. The experimental design was a Fisher block randomized to three replicates separated from each other by a distance of 1 m. The main factor that was assessed was the varieties and the second factor was the disease incidence. Each elementary plot had an area of 4 m^2, separated from each other by a distance of 0.5 m. The total area of the experimental design was 176 m^2. The good rice cultivation practices recommended by the national research agency were scrupulously applied.

Data collection

Several parameters were collected at each site to assess the degree of resistance or susceptibility of the various genotypes tested.

(i) The disease incidence was determined for each plant from the 30th DAT, and then every 14 days until maturity. This consisted of

Table 1. Genotypes tested in this study.

Accession	NERICA ref	Ecosystem	Subspecies	Backcross/comment	Source	Phenotype
IR64		Irrigated			IRRI	S
Wab56-50		Upland	*O. sativa* ssp. *japonica*	Recurrent parent for upland NERICA	Africarice	S
Wab181-18		Upland	*O. sativa* ssp. *japonica*	Recurrent parent for upland NERICA	Africarice	R
FKR19		Upland, irrigated	*O. sativa* ssp. *japonica*	Mashuri × IET1444	INERA	R
FKR45N	NERICA12	Upland	*Japonica*/*O. glaberrima*	WAB56-50/CG14/WAB56-50	Africarice	R
FKR49N	NERICA13	Upland	*Japonica*/*O. glaberrima*	CG14/WAB181-18/WAB181-18	Africarice	R
FKR62N	NERICA-L-19	Irrigated, lowland	*Indica*/*O. glaberrima*	TOG5681/3*IR64	Africarice	S
TS2		Irrigated, lowland			INERA	S
CG14		Upland, lowland	O.glaberrima	Donor parent for upland NERICAs	Africarice	S

S: Susceptible; R: resistant.
Source: Wonni et al. (2016).

counting the number of diseased plants per genotype in each elementary plot and determination of the incidence according to the following formula:

$$I= \sum_{i=1}^{n}(\frac{xi}{X} \times 100);$$

where **n** = the number of repetitions, *xi* = the number of diseased plants per elementary plot, and **X** = the total number of rice plants per elementary plot.

(ii) The foliar incidence was evaluated for 10 plants chosen at random on the two diagonals in each elementary plot. It was calculated by counting the number of infected leaves out of the total number of leaves according to the following formula:

$$IF= \sum_{i=1}^{n}(\frac{xi+\cdots+xi+1}{X}) \times 100;$$

where n = the number of repetitions, xi = the number of diseased leaves/plant, and X = the total number of rice leaves.

To determine the resistance level of each genotype, IRRI scale (2002) was used.

(iii) The epidemic growth rate (r) was expressed in units per day and assessed using the formula described by Rapilly (1991):

r = (log (1 / (1-x2)) - log (1 / (1-x1)) / (t2-t1);

where x1 and x2 denote the disease severity expressed as a percentage and t2 - t1 = the days between two observations.

(iv) The disease severity (S) was evaluated for the 10 plants chosen to estimate the disease incidence. The severity (S), expressed as a percentage of the total tissue area, was calculated by using the scale of Kauffman et al. (1973) as follows:

S = [(n1x1) + (n3x3) + n5x5) + n7x7) + n9x9)] x100 / (n1 + n3 + n5 + n7 + n9) x9;

where n1 to n9 are the numbers of leaves denoted from 1 to 9.

(v) Paddy yield: The three central lines of each elementary plot were harvested at maturity. The panicles were dried in the sun and were then seeded and the seeds weighed. The average yield per genotype was determined by calculating the average paddy yield of the three elementary plots of each genotype tested.
(vi) Climate data were collected at the meteorological station of the Kou valley and the Di plains. The temperature, hygrometry, and rainfall were recorded from June to November of each year.

Data analysis

Microsoft Excel 2010 software was used for data entry and to calculate the incidence, severity, and growth rate of BLS. Statistica 7.1 software was used for ANOVA tests and to establish the correlation between severity and yield. The comparison of averages was done by the Newman Keuls test at the 5% level.

RESULTS

Incidence per plant

Irrespective of the site and the year of cultivation, the first symptoms appeared as of the 30th DAT with low incidence (5.2%) and progressed over time to reach higher levels (94 to 100%) by the 72nd DAT on all of the susceptible genotypes. Thus, two genotypes groups could be distinguished according to their behavior against BLS. Group 1, which included the WAB181-18, FKR19, FKR45N, and FKR49N genotypes, comprised those that were resistant to BLS. Group 2 comprised the susceptible genotypes, such as the TS2, FKR62N, CG14, IR64, and WAB56-50 genotypes. However, their susceptibility varied according to the site, ranging from 58.33 to 100% (Figure 1).

Leaf incidence

The leaf incidence was significant for all of the susceptible varieties, irrespective of the site and the season at both sites. These comprised the IR64, FKR62N, TS2, and CG14 genotypes. In the Kou valley plot, the highest leaf incidence was recorded with CG14 (91%). In the Di plains plot, the CG14, FKR62N, and WAB56-50 genotypes were the most infected, with 99.63, 95.3, and 100% foliar incidence, respectively. Despite heavy

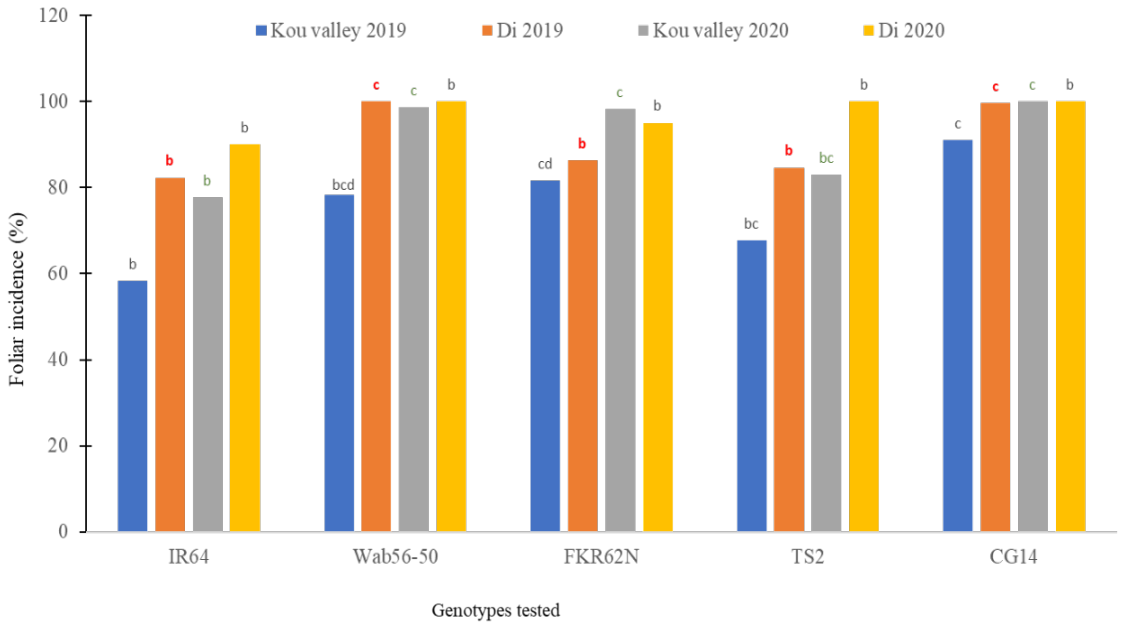

Figure 1. Foliar incidence of BLS on susceptible varieties in the both sites. Histograms with the same colour, followed by the same letter are not significantly different at the 5% threshold according to the Newman Keuls test.

Table 2. The averages (%) of incidence, severity of BLS and yields (t/ha) obtained per genotype.

Genotype	2019						2020					
	Kou Valley			Di			Kou Valley			Di		
	IF	Sev	Yield	IF	Sev	Yield	IF	Sev	Yield	IF	Sev	Yield
IR64	58.33^{b}	14.30^{b}	6.4^{b}	82.3^{b}	31.37^{b}	5.97^{b}	77.66^{b}	30^{b}	3.75^{ab}	90.06^{b}	44.9^{bc}	3.75^{ab}
Wab56-50	78.33^{bcd}	47.43^{e}	4.6^{a}	100^{c}	57.89^{c}	6.8^{b}	98.66^{c}	60^{cd}	2.5^{a}	100^{b}	68.66^{d}	3.48^{ab}
Wab181-18	0^{a}	0^{a}	4^{a}	0^{a}	0^{a}	4.2^{a}	0^{a}	0^{a}	2.92^{a}	0^{a}	0^{a}	3.98^{ab}
FKR19	0^{a}	0^{a}	6.5^{b}	0.01^{a}	0^{a}	6.8^{b}	0.01^{a}	0^{a}	6.82^{bcd}	0.03^{a}	0^{a}	5.75^{c}
FKR45N	0^{a}	0^{a}	4.4^{a}	0^{a}	0^{a}	3.6^{a}	0^{a}	0^{a}	2.02^{a}	0^{a}	0^{a}	3.28^{ab}
FKR49N	0^{a}	0^{a}	4.5^{a}	0^{a}	0^{a}	3.3^{a}	0^{a}	0^{a}	2.95^{a}	0^{a}	0^{a}	2.9^{a}
FKR62N	81.66^{cd}	38.03^{d}	6^{b}	86.33^{b}	47.02^{bc}	6.95^{b}	98.33^{c}	46.46^{c}	6.37^{cd}	95.03^{b}	53.5^{bcd}	3.5^{ab}
TS2	67.66^{bc}	28.33^{c}	6.5^{b}	84.66^{b}	32.76^{b}	6.62^{b}	83^{bc}	30^{b}	6.05^{cd}	100^{b}	40.1^{b}	3.4^{ab}
CG14	91^{c}	42.46^{de}	4.75^{a}	99.63^{c}	62.96^{c}	4.15^{a}	100^{c}	63.33^{d}	4.32^{abc}	100^{b}	65.1^{cd}	2.45^{a}

IF: Foliar incidence; Sev: disease severity. The values followed by the same letter in a column are not significantly different at the 5% level according to the Newman Keuls test.

pressure from BLS, WAB181-18, FKR45N, and FKR49N exhibited no symptoms at either site during the two experimental seasons (Table 2).

Average yield

The average yield of the tested genotypes varied from season to season and between the two sites. The lowest yields were recorded for FKR45N, FKR49N, WAB181-18, and CG14, between 2.02 and 4.75 t/ha. However, FKR62N, FKR19, and TS2 had the best yields, varying from 6 to 6.82 t/ha in the Kou valley versus 3.4 to 6.95 t/ha in the Di plains plot. Table 2 shows the average yields obtained by genotype at each site and by study year.

The correlation analysis between the disease severity and the yields showed a strong overall negative correlation that was very highly significant ($r = -0.74$; $p = 0.00014$) (data not shown). As the severity level of BLS influences the yield, we observed that the yield was low when the severity was high.

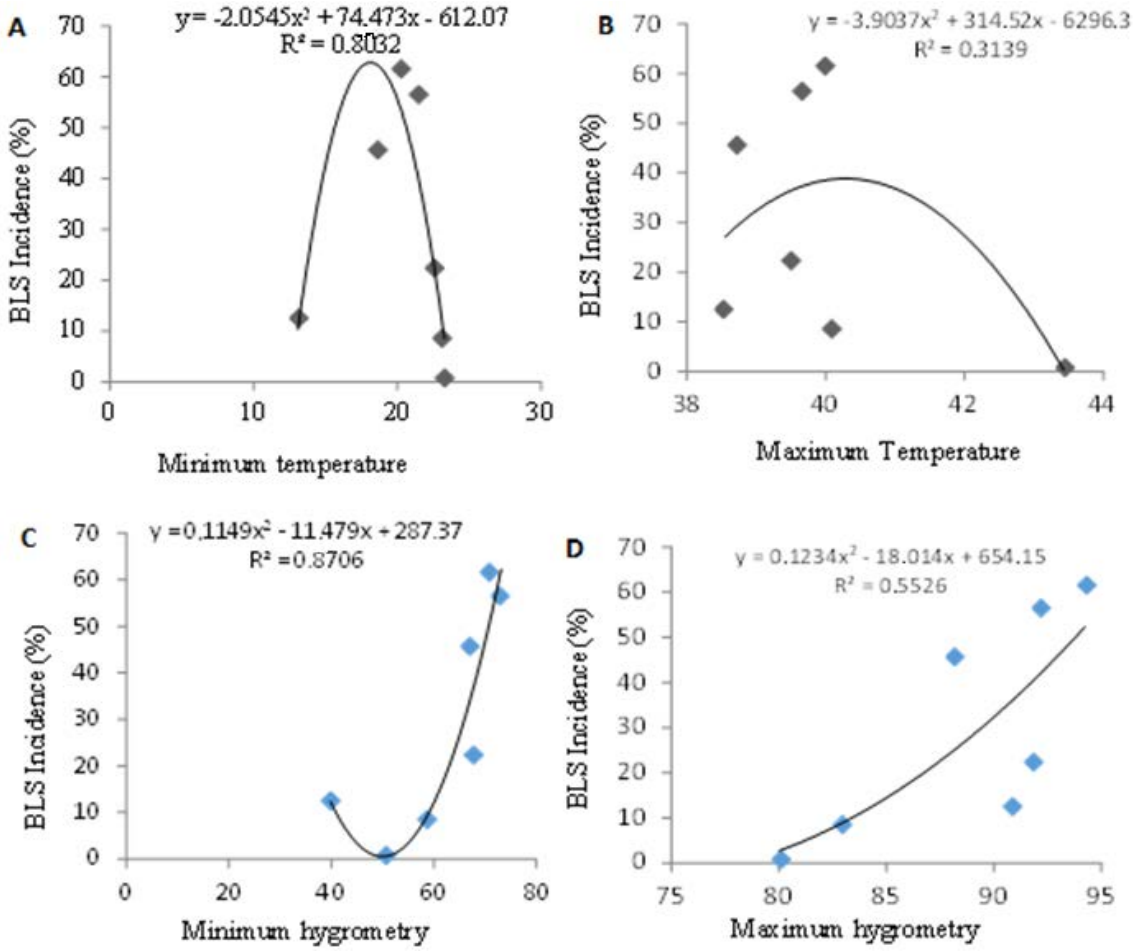

Figure 2. The linear regression curve of BLS incidence according temperature and hygrometry. A: Minimum temperature; B: Maximum temperature; C: Mimimum hygrometry; D: Maximum hygrometry.

Disease severity

The results show that the disease severity correlated with the disease incidence. In general, for all of the susceptible genotypes, the severity was greater in the Di plains plot (31.3 to 68.66%) than in the Kou valley which varied from 14.30 to 63.33%. Of these, WAB56-50 and CG14 were the most severely infected at both sites (Table 2).

BLS growth rate on the susceptible varieties

Interestingly, we noticed that the growth rate of BLS varied according to the vegetative stage of the susceptible genotypes, which were IR64, FKR62N, TS2, CG14, and WAB56-50. At the tillering stage, the growth rate of BLS was low in the Kou valley plot ($0.007 \leq r1 \leq 0.014$) and at Di ($0.35 \leq r1 \leq 0.6$). From maximum tillering to flowering, the growth rate of BLS (r2) increased significantly at both sites ($0.18 \leq r2 \leq 0.503$ in the Kou valley and $0.42 \leq r2 \leq 1.49$ at Di). At panicle initiation, the growth rate of BLS was greatly decreased for varieties IR64, WAB56-50, TS2, and FKR62N in the Kou valley, except CG14 C, for which the BLS symptoms increased. However, at this phase in the Di plot, CG14 and TS2 exhibited the highest growth rate (r3) (data not shown).

Relationship between temperature, humidity, and BLS incidence

Linear regression analysis between the BLS incidence and the climatic factors revealed a very significant regression. Figure 2A shows a polynomial curve whereby the disease incidence increased from 12.5 to 61.56% as the temperature varied from 13.07 to 20.32°C. Figure 2B shows that the BLS incidence increased when the minimum and the maximum temperatures were close to 20.32 and 40°C, respectively. Figure 2C and D indicates that the BLS incidence increased with humidity, amounting to between 40 and 95%.

DISCUSSION

During the two years of the study, the IR64, FKR62N, TS2, WAB56-50, and CG14 genotypes displayed differential reactions to BLS according to the site and the year, unlike the FKR19, WAB181-18, FKR45N, and FKR49N genotypes, which exhibited resistance. Several factors could be explained the variations of varieties susceptibility observed on the both site. The Di site was developed in 2015, and is full of several weed hosts, including *Oryza longistaminata*, both within and along the edges of some plots. In addition, this site borders the Sourou River whose banks are mainly populated by *O. longistaminata*. However, the plain of the Kou Valley was developed in the 1960s and is less invaded by *O. longistaminata*. Also, producers grow fewer varieties there; in contrast in Di site, where several varieties are produced, sometimes with introductions from neighboring countries such as Mali.

In addition, the variations observed in the behavior of the susceptible varieties relate to one of their intrinsic qualities, which is the absence of an effective resistance gene. These results are consistent with those of Wonni et al. (2015, 2016) who showed that these rice genotypes were highly susceptible to BLS under artificial inoculation conditions. Cultivars WAB56-50 and CG14, which belong to the glaberrima species, were found to be susceptible like FKR62N, which is an interspecific derived from the cross between cultivars TOG5681 and IR64, which are both susceptible to BLS.

Therefore, the cultivation of FKR62N and TS2 varieties requires the application of good agricultural practices aimed at mitigating the effect of BLS on their potential yield. These will include the use of healthy seeds, the rational use of nitrogen, and the control of weeds in general and in particular those which are potential reservoirs of *Xoc*. Indeed, Bradbury (1986) and Wonni et al. (2014) found that several Poaceae and Cyperaceae are natural host plants for *Xoc*. These comprise *Echinochloa colona*, *Eleusine indica*, *Digitaria horizontalis*, *Rottboellia cochinchinensis*, *O. longistaminata*, *Sacciolepis africana*, *Paspalum vaginatum*, and *Paspalum polystachyum*. These weed species are very abundant in rice plots wherever rice is grown in Burkina Faso.

Moreover, national research should consider an improvement program to develop resistance of these varieties to BLS while preserving their potential productivity.

Interestingly, the FKR19, WAB181-18, FKR45N, and FKR49N genotypes were confirmed to be resistant to BLS, as reported by Wonni et al. (2015, 2016). Despite the diversity of *Xoc* strains identified at the Di plains and the Kou valley sites (Wonni et al., 2014), these rice genotypes harbored one or more resistance genes. Indeed, these varieties, screened under artificial inoculation conditions, exhibited hypersensitive reactions.

While the WAB181-18, FKR45N, and FKR49N genotypes remained immune to BLS infection, the FKR19 genotype nevertheless exhibited symptoms with a very low incidence (≤ 0.03%). These results may indicate the presence of more than one gene responsible for the FKR19 phenotype in regard to BLS. In contrast, the immunity of the resistance genotype could be due to a specific resistance gene. These varieties have a japonica genetic background and are suitable for rainfed rice cultivation, except FKR19. This adaptability to upland ecology may explain the low yields recorded for these genotypes in our study. Therefore, these results are more interesting as they reveal, for the first time, resistant rice varieties in greenhouse and field conditions in Burkina Faso.

Zhao et al. (2004) reported that a resistance gene against *Xoc* had yet to be characterized in cultivated rice. However, to control BLS in Asia, a dominant maize gene, *Rxo1*, has been isolated and characterized. It confers resistance in maize to *Xoc* and it also prevents the development of *Xoc* when it is expressed as a transgene in rice (Zhao et al., 2005). Recently, a recessive resistance gene called bls1 was localized on chromosome 6 of *Oryza rufipogon* (He et al., 2012). In addition, Triplett et al. (2016) were able to determine that the resistance of the Carolina Gold rice variety is conferred by a single dominant locus, *Xo1*, located on a fragment of DNA of 1.09 Mbp on chromosome 4.

Conclusion

This study aimed to assess the behavior of different rice genotypes against BLS in natural infection conditions. The results show that four varieties, namely FKR19, WAB181-18, FKR45N, and FKR49N were resistant against BLS. However, these varieties are more suitable for rainfed rice cultivation and are not highly productive, except for FRK19, which is compatible with lowland and irrigated rice-growing systems. On the other hand, the TS2 and FKR62N varieties, which constitute the two most cultivated and consumed varieties in Burkina Faso, were shown to be highly susceptible to BLS. Therefore, identification of effective resistance genes against *Xoc* strain diversity, and improvement of elite susceptible varieties against BLS, remain essential in light of the spread and incidence of this disease in irrigated rice cultivation in Burkina Faso and in West Africa in general.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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