

*Full Length Research Paper*

# Identification of resistance to *Xanthomonas phaseoli* pv. *manihotis*, agent of cassava (*Manihot esculenta* Crantz) bacterial blight in Burkina Faso

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Received 25 October, 2022; Accepted 10 March, 2023

**Cassava bacterial blight (CBB) is rife in all production areas in Burkina Faso. The use of resistant varieties is known as the best control against this disease. Our objective is to identify resistant varieties within a collection of local and introduced cassava varieties in Burkina Faso. Eleven varieties of cassava were screened in the field for two successive years using a randomized block of Fisher design with three repetitions. Disease severity was collected monthly during the wet season. The results indicate that all cassava varieties exhibited symptoms with varying incidence levels and an average severity rate of 8.48%. The V8 (Chair jaune) and V10 (4(2)1425) varieties were resistant regardless of growing season. However, varieties V5 (91/02312), V11 (Locale Santidougou), V1 (94/0270) and V7 (92/0325) were the most susceptible. Cassava varieties, evaluated under irrigated growing conditions, will be tested in a rainfed cropping system that ensures national cassava production. In addition to their susceptibility to cassava bacterial blight, other biotic constraints including viral and fungal diseases will be evaluated. However, the high-performance varieties will be disseminated according to their adaptability to the different agro-ecological areas and can be used in varietal breeding program.**

**Key words:** Cassava, Resistance, *Xanthomonas phaseoli* pv. *manihotis*.

## INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important source of calories in the world with tubers production estimated at 302 662 494 tons in 2020. African cassava occupied 64% of this production, distributed among 39 countries. Nigeria is the first producing country in the world with 60 001 531 tons of tubers produced in 2020 (Faostat, 2022). Cassava is an important staple crop as a source of food and income for hundreds of millions of people in tropical countries (Zárate-Chaves et al., 2021). Also, it is used as animal food and serves as a raw material in industries for the production of ethanol,

vinegar, adhesives, textiles and printing (Diallo et al., 2013). In Burkina Faso, the development of cassava cultivation resumed in 1995 thanks to the adoption of Strategic Plan 1 of the policy of diversification of growth sectors supported by the FAO and IITA (Diancoumba, 2008). The main production areas are Cascades, Hauts Bassins and South-western regions. Annual production in 2020 was estimated at 4 244 tons of tubers (Faostat, 2022). Cassava is adapted to poor and marginal lands, and its cultivation requires few agricultural inputs, which favors the expansion of its production in all

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agroecological zones of Burkina Faso (Ouédraogo et al., 2010). Despite all these interesting characteristics, cassava production remains limited by biotic and/or abiotic constraints that lead to low yields. Biotic constraints include common leaf and stem pests such as *Phenacoccus manihoti*, *Mononychellus tanajoa*, *Zonocerus Variegatus*, *Aleurodicus dispersus*, and *Bemesia tabaci* (James et al., 2000). African cassava mosaic and cassava brown streak disease are also very important, as they can reduce tuber production by 20-90 and 60-70% respectively (Hillocks and Thresh, 2000; Zacarias and Labuschagne, 2010; Earnnet, 2015). In addition, Msikita et al. (2005) reported tuber rots caused by fungi with losses of over 80%. Bacteria such as *Erwinia herbicola*, *Agrobacterium tumefaciens* Biovar 1, *Xanthomonas campestris* pv. *cassavae*, and *Xanthomonas axonopodis* pv. *manihotis* also cause cassava diseases (Abessolo, 2013). Among these bacterial diseases, Cassava Bacterial Blight (CBB), caused by *Xanthomonas phaseoli* pv. *manihotis* (Xpm), causes significant tuber production losses ranging from 20 to 100% (Earnnet, 2015). Symptoms appear initially as brown to dark-brown water-soaked translucent angular spots on the leaf tissues browning at later stages, occasionally surrounded by a chlorotic halo (Zarate-Chaves et al., 2021). The vascular infection of the plant results in the presence of gummy exudate on veins, petioles and stems, canker on stems and vascular necrosis. According to Lozano (1986), roots of infected plants remain asymptomatic. In Burkina Faso, confirmation of the pathogen was made from samples collected in August of 2011 and October of 2012, on ten months-old field cassava grown in two localities in Cascades region (Wonni et al., 2015). Several control methods exist for cassava bacterial blight such as cultural, chemical, and heat therapy methods. According to FAO (2013), the disinfecting cuttings by soaking them in hot water at 50°C for about 50 minutes will rid them of the bacteria. Indeed, the best strategy for effective management of major crop diseases is the selection of resistant varieties (Ferguson et al., 2019) through inoculations of leaves or stems in the greenhouse (Mbaringong et al., 2017); and observation of symptom evolution in the field under high disease pressure over several crop cycles (Boher and Verdier, 1995). This strategy is used to control CBB worldwide (Jorges et al., 2001; Wydra et al., 2004; Banito et al., 2008; FAO, 2013; Mamba-Mbayi et al., 2014; Mbaringong et al., 2017; Affery et al., 2018; Kante, 2020; Toure et al., 2020). Several authors including Sanchez et al. (1999), Wydra et al. (2004), Mbaringong et al. (2017), and Fanou et al. (2018) reported that several genes were involved in plant resistance to bacterial blight. Cassava resistance is described as polygenic and derived from interspecific crosses between *Manihot esculenta* and the wild parent *Manihot glaziovii* (Hahn et al., 1979). Jorge et al. (2000) identified 12 QTLs (Quantitative Trait Loci) in cassava involved in resistance. In West Africa, recent studies in

Ivory Coast carried by Affery et al. (2018) and Toure et al. (2020) have identified cassava varieties resistant to Xpm. However, cassava genotypes resistant to Xpm, have not yet been identified in Burkina Faso. Therefore, this study aims to evaluate the phenotype of 11 cassava genotypes against CBB under field conditions.

## MATERIALS AND METHODS

### Study site

The trials were conducted for two successive years, in 2020 and 2021, in the irrigated perimeter of the Kou Valley located in western Burkina Faso, 20 km northwest of Bobo-Dioulasso in the rural commune of Bama (11°23'10"N ; 4°23'17"W). The perimeter covers an area of 1260 ha of developed land. The climate is South Sudanese and is characterized by an alternating rainy season from May to October and a dry season from November to April (Guinko, 1984). Climatic data for the years 2020 and 2021 were provided by the meteorological station of Bama and are presented in Figure 1. The monthly mean values for rainfall, number of rainy days, temperature and relative humidity range from 0 to 368.8 mm, 0 to 19 days, 24 to 38.1°C and 31 to 82.8% respectively.

### Plant material

The plant material used consists of 11 cassava varieties, eight of which originate from Burkina Faso and three (03) from Ivory Coast. The characteristics of these varieties are given in Table 1.

### Experimental design

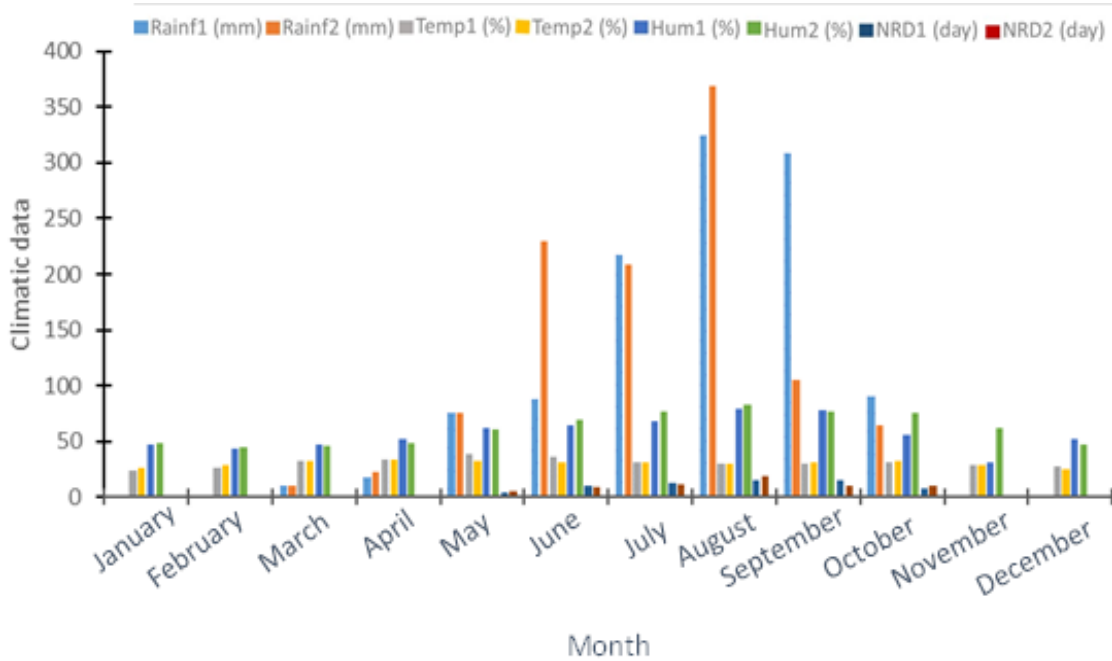
Cassava varieties were planted in February 2020 and 2021, at the same site in the Kou Valley irrigated perimeter. The experimental design was a randomized block of Fisher with three replications (Figure 2). The total area of the trial is 672 m<sup>2</sup> (42 m x 16 m). Each block contains 11 elementary plots of 8m<sup>2</sup> each (2 m x 4 m). The space between the three blocks and between elementary plots were 2 m.

### Plant culture

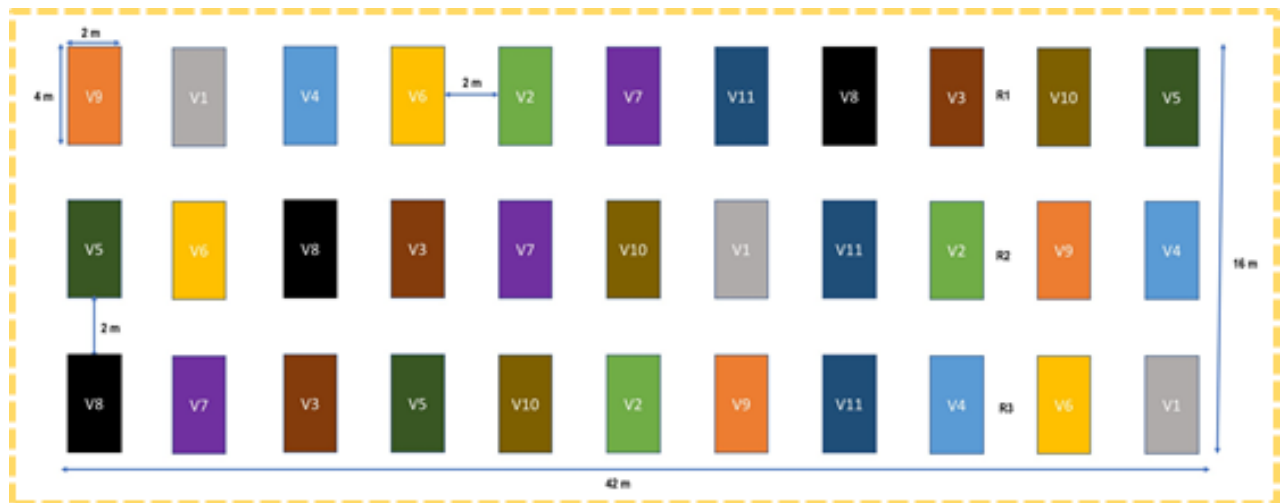
The soil were weeded before the plowing and then staked to delimit the elementary plots. The cuttings were soaked in an ash solution at a rate of five handfuls per 5l of water, for one minute, in order to eliminate mealybug and mite populations. They were then planted so that the eyes of the nodes were positioned outward. Each elementary plot had 15 plants with five plants per line, with a spacing of one meter between lines and plants. Irrigation was carried out every other day until the winter season when water was supplied during pockets of drought. NPK 15-15-15 were applied at 45 days after planting at a rate of 50g/plant as recommended by FAO (2013).

### Data collection

The plants were monitored regularly and the assessment of severity began when the first symptoms appeared. For this purpose, the Banito (2003) scale was used to score the five plants in the center line of each elementary plot (Table 2). The disease severity rate Mbayi et al. (2014) as follows:



**Figure 1.** Mean values of climatic data during the two years of experimentation in the Kou Valley. Rainf1: Rainfall 2020; Rainf2: Rainfall 2021; Temp1: Temperature 2020; Temp2: Temperature 2021; Hum1: Relative Humidity 2020; Hum2: Relative Humidity 2021; NRD1: Number of rainy days 2020; NRD2: Number of rainy days 2021. Source: INERA (2020 and 2021)



**Figure 2.** Schematic representation of experimental design of the trial in two years. V1: 94/0270; V2: 92/0427; V3: Yavo; V4: 92/0067; V5: 91/02312; V6: Bocou 5; V7 : 92/0325; V8: Chair jaune; V9: Bonoua; V10: 4(2)1425; V11: Locale Santidougou; R1: Repeat 1; R2: Repeat 2; R3: Repeat 3. Source: Authors

$SD (\%) = (N1 \times 1 + N2 \times 2 + N3 \times 3 + N4 \times 4 + N5 \times 5) / (N \times I) \times 100$ ; with N1 to N5 the number of plants having the score 1 to 5, and I corresponding to the maximum score. Climatic data such as temperature, rainfall, number of rainy days and relative humidity were collected at the Bama weather station located within the irrigated area.

**Data analysis**

The data were analyzed with R software version 4.1.2. The nonparametric Kruskal Wallis test were used to compare varieties and the correlation between CBB severity and climatic data were determined by Pearson's method.

**Table 1.** Characteristics of cassava varieties used for screening.

Code	Name	Genetic nature	Breeder	Maintainer	Cycle (Month)	Yield (T/Ha)	Other characteristics	Source
V10	4(2)1425	Line	IITA	INERA	6-12	30-40	Susceptible to virus	CNS (2014)
V5	91/02312	Line	IITA	INERA	6-12	30-40	Susceptible to virus	CNS (2014)
V4	92/0067	Line	IITA	INERA	6-12	30-40	Susceptible to virus	CNS (2014)
V7	92/0325	Line	IITA	INERA	6-12	30-40	Susceptible to virus	CNS (2014)
V2	92/0427	Line	IITA	INERA	6-12	30-40	Susceptible to virus	CNS (2014)
V1	94/0270	Line	IITA	INERA	6-12	30-40	Susceptible to virus	CNS (2014)
V3	Yavo	ND	ND	ND	ND	ND	Resistant to CBB	Affery et al. (2018)
V6	Bocou 5	ND	IITA	ND	ND	40	ND	Vernier et al. (2018)
V9	Bonoua	ND	ND	CNRA	12-20	15	Susceptible to virus	N'Zué et al. (2013)
V8	Chair jaune	ND	ND	ND	ND	ND	ND	
V11	Locale Snt	ND	ND	ND	ND	ND	ND	

**ND:** Not Determined; **Snt:** Santidougou; **CNS:** « Comité National des Semences » (National Seed Committee); **IITA:** « Institut International d'Agriculture Tropicale » (International Institute of Tropical Agriculture); **INERA:** « Institut de l'Environnement et de la recherche Agricoles » (Institute of the Environment and Agricultural Research), CNRA « Centre National de Recherche Agronomique » (National Center for Agronomic Research). Source: Affery et al. (2018); Vernier et al. (2018); CNS (2014); and N'Zué et al. (2013).

**Table 2.** Banito's severity scale (2003).

Class	Phenotype
1	No symptoms
2	Presence of angular leaf spot
3	Presence of angular leaf spot, burning, wilting, defoliation and sometimes presence of exudates on stem, petioles or leaves
4	Presence of burning, wilting, defoliation, exudation and dieback
5	Presence of burning, wilting, defoliation, exudation, formation of lateral abortive shoots, stunted growth, complete decline

Source: Author

## RESULTS

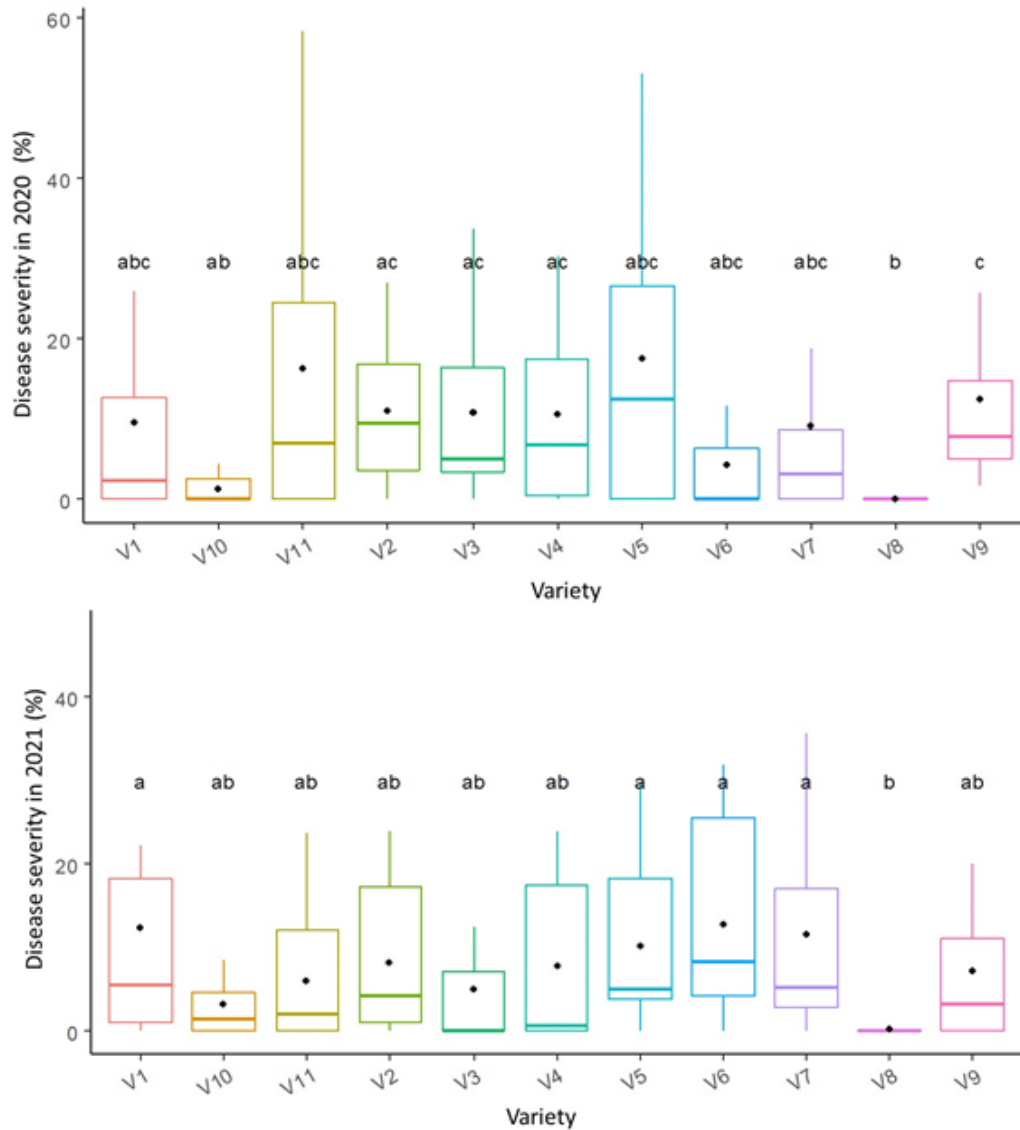
### Phenotype of the varieties for each year of experimentation

The first CBB symptoms appeared in July, five months after the establishment of the crop, and evolved progressively throughout the observation period (July to October). Figure 3 shows comparative analysis of CBB severity on cassava varieties. It showed in 2020, that all cassava genotypes, except the Chair jaune variety, showed CBB symptoms with varying levels of severity. Overall, an average severity rate of 9.30% was observed. On the other hand, the highest severity values were obtained on varieties V5 and V11 with respective rates (represented by point in the boxplot) of 17.58 and 16.16%; unlike V10 and V6 varieties, which expressed low severity with respective values of 1.2 and 4.31%. However, analysis of variances, represented by the letters on the boxplot, reveals a very highly significant difference ( $p$ -value = 0.0002) between the varieties. Thus, the V8 variety is different from varieties V2, V3, V4 and V9. Similarly, V9 variety is statistically different from

V10 variety. Unlike the first year, all 11 cassava varieties showed CBB symptoms in year 2021. The V8 variety, which was not affected by CBB in 2020, showed symptoms eight months after planting, specifically in October. The comparative analysis (Figure 3) showed that all 11 varieties showed CBB in variable proportions with an average rate of 7.67%. Thus, the Chair V8, V10 and V3 varieties recorded the lowest severity rates (represented by point in the boxplot) with values of 0.28, 3.23 and 4.89% respectively. However, the highest rates were observed on varieties V6, V1, V7 with respective values of 12.76, 12.30 and 11.53%. Furthermore, the analysis of variances, represented by the letters on boxplots showed significant differences between the varieties ( $p$ -value = 0.005). Except for variety V8, which is different from varieties V1, V5, V6 and V7, the other varieties are not statistically different.

### Phenotype of cassava varieties over the two years of experimentation

At the end of the assessment over the two years, the



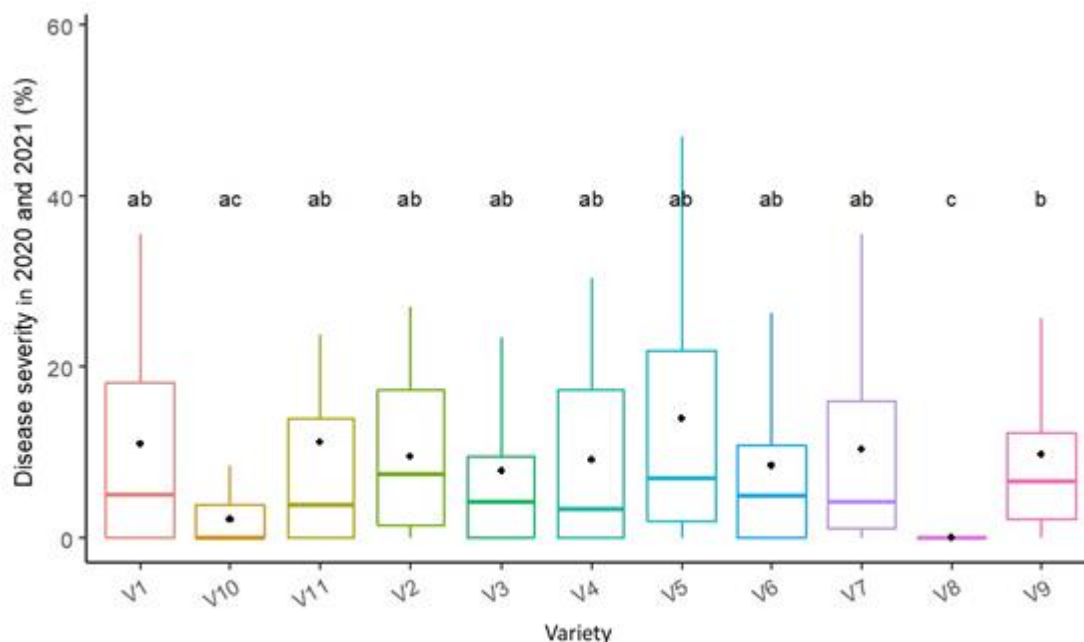
**Figure 3.** Comparison of cassava varieties phenotype against CBB for each year of experiment. The boxplot summarizes the severity of CBB on each of the 11 varieties, allowing to visualize the extreme values and to understand the CCB distribution. The central value of the boxplot (horizontal line) is the median. The black points represent the mean value of severity recorded by each variety. The mean values of the severity rate followed by the same letter do not differ significantly at the 5% threshold according to the Kruskal-Wallis test ( $p < 0.05$ ). V1: 94/0270; V2: 92/0427, V3: Yavo, V4: 92/0067, V5: 91/02312, V6: Bocou 5, V7: 92/0325, V8: Chair jaune, V9: Bonoua, V10: 4(2)1425, V11: Locale Santidougou. Source: Author

severity of CBB was variable. The main symptoms observed were angular spots (Figure 4A), burns (Figure 4B), wilting of leaves (Figure 4C). No symptoms of exudation on the stem, formation of lateral abortive shoots, stunting, and complete decline of the plant were observed. Figure 5 presents a comparison of the average CBB severity rate on the varieties for the two years of experiment. Over the two years, an average severity rate of 8.48% was recorded. Varieties V5, V11, V1 and (V7)

were the most severely infected with values of 13.83, 11.09, 10.92 and 10.29% respectively; while V8 and V10 showed the lowest average severity rates with values of 0.14 and 2.21% respectively. The varieties V3, V6, V4, (V2) and V9 recorded average severity rates between 7.78 and 9.80%. However, the analysis of variance, presented the letters on the boxplot, revealed a very highly significant difference between the varieties ( $p$ -value = 0.0001). Indeed, the variety V8 is different from



**Figure 4.** Evolution of CBB symptoms on the variety V5. A: Angular leaf spot; B: Leaf burning; C: Leaf wilt.  
Source: Author



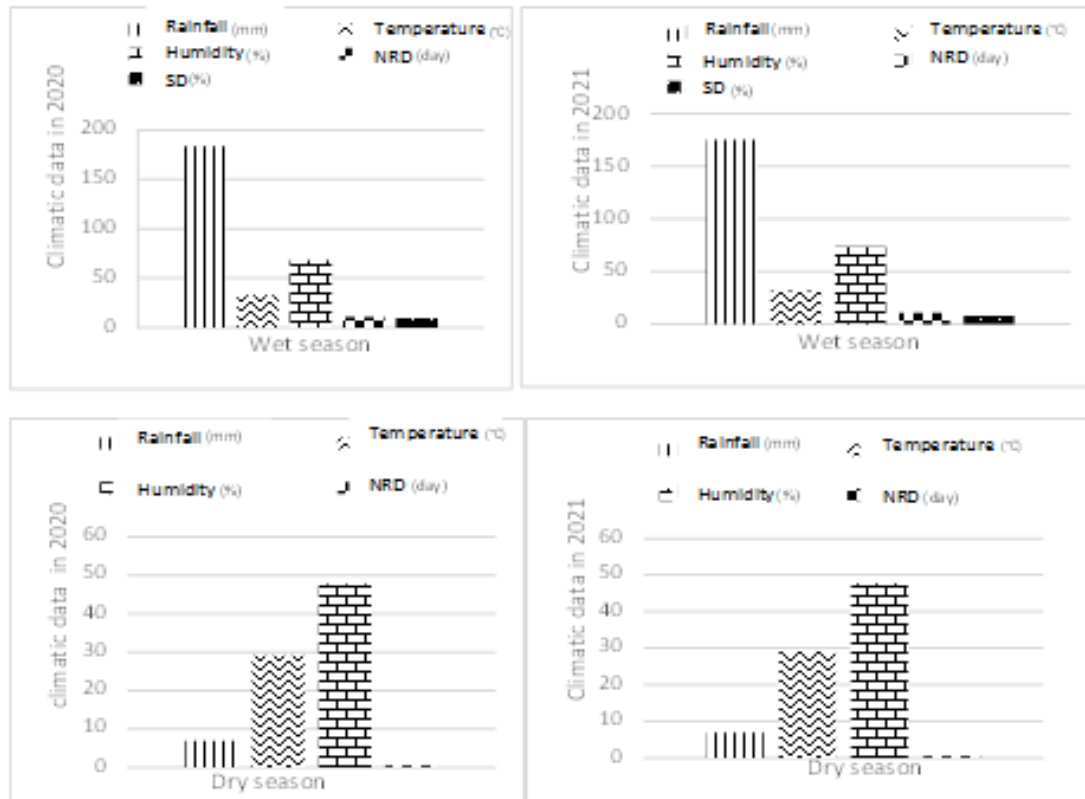
**Figure 5.** Comparison of cassava varieties phenotype against CBB for cumulate data of the two years of experiment. The boxplot summarizes the severity of CBB on each of the 11 varieties, allowing to visualize the extreme values and to understand the distribution of observations. The central value of the boxplot (horizontal line) is the median. The points represent the mean value of severity recorded by each variety for the cumulate data of two years of experiment. The mean values of the severity rate followed by the same letter do not differ significantly at the 5% threshold according to the Kruskal-Wallis test ( $p < 0.05$ ). V1: 94/0270; V2: 92/0427, V3: Yavo, V4: 92/0067, V5: 91/02312, V6: Bocou 5, V7: 92/0325, V8: Chair jaune, V9: Bonoua, V10: 4(2)1425, V11: Locale Santidougou.  
Source: Author

all the other varieties, except V10 variety. Also, variety V10 is statistically different only from variety V9.

**Disease severity under the environmental conditions**

The wet season in 2020, was characterized by a mean rainfall of 183.75 mm, a mean temperature of 32.75°C, a

mean relative humidity of 67.97% and a mean rainy day of 10.83 days. During the dry season, the averages of parameters such as rainfall, temperature, relative humidity, and number of rainy days were respectively 6.95 mm, 29.07°C, 47.65% and 0.5 for the same year. In year 2021, these parameters were respectively 175.35 mm; 31.34°C, 73.69%, 10.67 days for wet season and 8.3 mm, 30.52 °C, 46.70% and 0.75 days for dry season



**Figure 6.** Average of climatic data and CBB severity during the two years of experimentation. The mean value of each variable is presented for wet season and dry season. NRD : Number of rainy day.  
Source: Author

(Figure 6). However, the expressions of CBB in wet season (Severity Rate) were 9.30 and 7.67% respectively in 2020 and 2021 (Figure 6). The highest disease severity ( $13.83 \pm 12.32\%$ ) was recorded on variety V5; while the lowest rates (SD = 0.14 and 2.21%) were respectively recorded on varieties V8 and V10.

### Correlation between climatic factors and disease severity

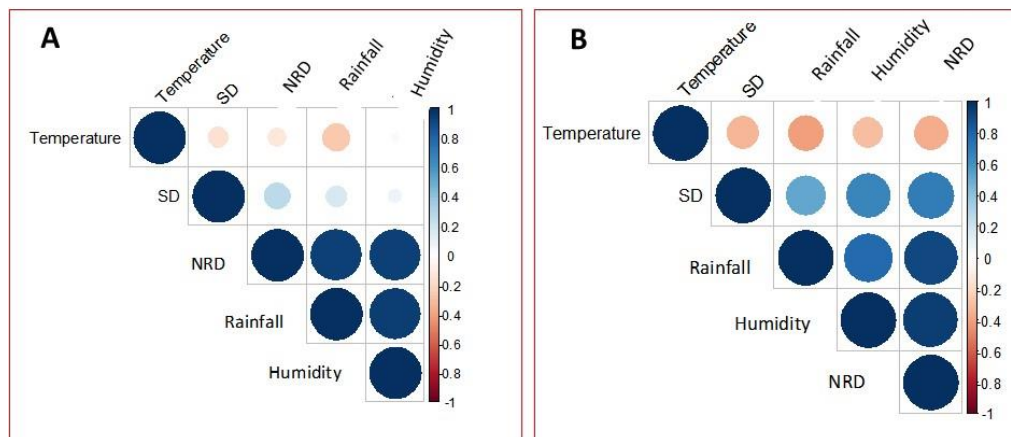
The correlogram with values between -1 and 1 gives the relationship between the different studied parameters in first year of experiment (Figure 7A). The graph showed the existence of a weak correlation between climatic factors and CBB severity rate. This correlation was positive for rainfall, number of rainy days (NRD) and relative humidity with disease severity and respective coefficients of 0.16, 0.27 and 0.08. On the other hand, it was negative for temperature with a coefficient of -0.16. In the second year of experiment (2021), the analysis showed a correlation between climatic data and CBB severity (Figure 7B). This correlation was positive for rainfall, number of rainy days and relative humidity with coefficients of 0.51, 0.69 and 0.65 respectively. In

contrast, it was negative for temperature with a coefficient of -0.33. The interpretation of the significance test of these coefficients (Table 3) shows that in first year, an increase in rainfall, the number of rainy days (NRD) and relative humidity leads to a non-significant increase of disease severity ( $p = 0.67, 0.49$  and  $0.83$  respectively for rainfall, number of rainy days and relative humidity) and vice versa. Also, an increase in temperature leads to a non-significant decrease of the expression of CBB ( $p = 0.68$ ) and vice versa. For the second year of experiment (2021), this test revealed that an increase of rainfall resulted in a non-significant increase of CBB severity ( $p = 0.16$ ) and vice versa. An increase in the number of rainy days and relative humidity resulted in a significant increase of CBB severity ( $p = 0.03$  for NRD and  $p = 0.05$  for humidity) and vice versa. The analysis also showed that an increase in temperature led to a non-significant decrease in the CBB severity ( $p = 0.37$ ).

## DISCUSSION

### Phenotype of cassava varieties against CBB

Screening of varieties in the field, is recognized as



**Figure 7.** Correlogram of climatic data and the CBB. Positive correlations are displayed in blue and negative correlations in red. The intensity of the color and the size of the circles are proportional to the correlation coefficients. On the right of the correlogram, the color legend shows the correlation coefficients and the corresponding colors. SD: disease severity (%); NRD: Number of rainy day (day); Humidity: relative humidity (%), Rainfall (mm). Source: Author

**Table 3.** P-value of Pearson's coefficients of correlation.

Variables	2020	2021
	Disease severity (SD)	
Disease severity (SD)		
Rainfall	0.67	0.16
Number of rainy days (NRD)	0.49	0.03 *
Temperature	0.68	0.37
Humidity	0.83	0.05*

\* Significant at the level of 5%.

Source: Author

powerful for the identification of resistant varieties, especially since the plant is confronted with several biotic and abiotic factors that can influence its phenotype (Maraite et al., 1982; Restrepo et al., 2000; Verdier et al., 2001; Jorge and Verdier, 2002; Wydra et al., 2004, Bondnar et al., 2015, Mbaringong et al., 2017). The variable phenotype of cassava varieties to CBB, observed in this study could be explained by the intrinsic characteristics of each variety, more or less favoring infection and colonization of cassava cells by the pathogen. Several authors including Sanchez et al. (1999), Wydra et al. (2004), Mbaringong et al. (2017), Fanou et al. (2018) reported several genes involved in cassava resistance to CBB. Furthermore, Jorge et al. (2000) identified six cassava genomic regions involved in resistance. Indeed, cassava resistance to bacterial vascular disease is described as polygenic and additively inherited, deriving from interspecific crosses between *Manihot esculenta* and the wild parent *Manihot glaziovii* (Hahn et al., 1979). Moreover, these differential

responses could be related to the pathogenic variability of *Xpm* strains present at the experimental site. Indeed, an analysis of the genetic structure of *Xpm* strains at the Kou Valley site (Wonni et al. unpublished), indicated a genetic diversity of 0.57. Moreover, on the Kou valley site, this genetic diversity is variable from one year to the next, which would certainly explain the seasonal variability of the CBB. Despite the high severity of CBB, the presence of exudation, formation of abortive shoots and complete plant decline were not observed on any variety. These results suggest that all varieties possess more than one CBB resistance gene, but their efficacy varies according to the strains present at the site. In Benin, Djinadou et al. (2018) observed a low level of CBB attack on both beta-carotene biofortified (yellow pulp) cassava varieties, and white pulp varieties. Results obtained by Affery et al. (2018) in Ivory Coast, revealed that traditional cassava varieties show more susceptibility to CBB than improved varieties.

### Interactions between climatic factors and vascular bacterial disease

The seasonal variability of the phenotype of cassava varieties could be explained by the levels of interactions between cassava genotypes, environment and inoculum pressure (Hahn et al., 1979; Boher and Agboli, 1992; Jorge et al., 2001; Banito et al., 2007; Toure et al., 2020). Indeed, the first symptoms were observed from July, precisely at five months after planting. During the dry season, environmental conditions were unfavorable for pathogen multiplication, so no symptoms were observed on the plants. At the beginning of the rainy season, the pathogen starts an epiphytic life, multiplies and reaches a



sufficient level to cause CBB symptoms. This would express the correlation between the rate of disease severity and climatic parameters during the two crop years. Thus, the results reveal that an increase in rainfall, number of rainy days and relative humidity during the rainy season leads to an increase in CBB expression regardless of the year of experimentation. In contrast, a decrease in temperature leads to an increase in CBB expression. Indeed, Daniel and Boher (1985) demonstrated the importance of the dry season-rainy season transition period, where the bacterium multiplies and constitutes the primary inoculum of *Xpm* responsible for disease establishment. The significance of the correlation between the number of rainy days and the rate of disease severity in 2021 could explain the appearance of the disease on the Chair jaune variety at the end of the observation. Also, the significance of the correlation between climatic factors indicates that the interaction between temperature, number of rainy days and relative humidity could lead to favorable conditions for disease expression on varieties. For Affery et al. (2018), susceptibility to bacterial blight is closely related to the varieties grown, their vegetative stages and climatic conditions in Côte d'Ivoire. Elad and Pertot (2014) also showed that abiotic stress such as increased temperatures, changes in rainfall amount and pattern, increased CO<sub>2</sub> and ozone levels, drought, etc., could increase plant susceptibility to diseases. Also, many aspects of a pathogen's biology such as spore production and germination and growth rate are highly dependent on temperature, relative humidity and, in the case of foliar pathogens, often on leaf moisture (Colhoun, 1973; Kaiser and Huber 2001). Similarly, Banito et al. (2007) demonstrated that disease expression is dependent on agroecological factors such as ecozone, plant age, planting density, cropping system, soil type and moisture. In contrast, plants respond to environmental changes by regulating their gene expression patterns such as their phenology (including senescence), sugar and starch content, nitrogen and phenolic content, root and shoot biomass, ability to grow, and ability to adapt to climate change (Elad and Pertot, 2014).

## Conclusions

The objective of the present study was to identify resistant varieties against cassava bacterial blight by field screening at a site under high natural pressure of the pathogen. Results showed that all varieties were infected by CBB, but with varying severity levels. Of the 11 tested varieties, V5 (91/02312), V11 (local Santidoukou), V9 (Bonoua) were the most infected by CBB. However, V8 (Chair jaune), V10 (4(2)1425), V4 (92/0067) and V3 (Yavo) recorded the fewest leaf symptoms. In addition, environmental factors such as the number of rainy days,

and relative humidity significantly influenced the CBB expression, contrastly to rainfall and temperature. In order to determine the adaptability of the varieties according to the agroclimatic zones, it is necessary on the one hand to screen the 11 varieties under greenhouse conditions with the strains representative of the diversity in Burkina Faso. On the other hand, these varieties must be tested under rainfed conditions on several production sites. Eventually, the resistant varieties could be genotyped in order to better understand and exploit the resistance genes in a varietal improvement program.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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