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Research Article

EVALUATION OF RESISTANCE IN ELITE CASHEW ACCESSIONS TO *XANTHOMONAS CITRI* PV. *MANGIFERAEINDICAE* IN BURKINA FASO

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ABSTRACT

Cashew (*Anacardium occidentale* L.) plays a vital socio-economic role in Burkina Faso, particularly benefiting rural communities and contributing to agricultural diversification. However, the sector faces significant challenges from pests and diseases, notably bacterial canker caused by *Xanthomonas citri* pv. *mangiferaeindicae*. This study evaluates the resistance of 15 elite cashew accessions to this pathogen under controlled greenhouse conditions. Two bacterial strains, LM6.1 and LH127.2, were used for inoculation. Disease progression was assessed using the area under the disease progression curve (AUDPC) and bacterial population quantification. Accessions CE200 and CE260 exhibited the highest tolerance, with significantly lower AUDPC values and bacterial populations, indicating strong potential for resistance breeding. In contrast, accessions CE10 and CE420 were highly susceptible. Positive correlations between AUDPC and bacterial populations underscore the utility of these metrics for resistance assessment. The findings highlight the genetic variability among cashew accessions, providing valuable insights for breeding programs aimed at developing resistant cultivars to enhance sustainable cashew production.

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INTRODUCTION

The cashew (*Anacardium occidentale* L.) plays a vital socio-economic role in Burkina Faso, contributing to job creation, income generation, and agricultural diversification. It is particularly important for rural communities, especially women, who are heavily involved in processing and marketing cashew nuts (Coulibaly, 2023).

In 2021, Burkina Faso produced 106,044 tons of raw cashew nuts (NBC). The country has 21 industrial units specializing in processing NBC into cashew kernels. These units processed 16.11% of the total production, equivalent to 17,087 tons, in 2021 (Tapsoba, 2022).

The cashew sector supports approximately 45,076 households and spans over 255,000 hectares. In 2019 and 2020, these processing units paid a total of 2.054

billion FCFA and 1.981 billion FCFA in wages, respectively, significantly contributing to poverty reduction and strengthening the national economy (Tapsoba, 2022).

The cashew, well adapted to drought, is an ideal crop for the Sahel region, where water scarcity is a major challenge (Coulibaly, 2023). However, the sector faces challenges such as low production quality and market volatility, necessitating the adoption of improved agricultural practices and commercial training for producers (Ouedraogo et al., 2023). This highlights the need for sustainable support to enhance the resilience and profitability of the sector.

Despite its high production potential, cashew cultivation is threatened by pests and pathogens. In Burkina Faso and West Africa, three major economically significant diseases have been identified viz. anthracnose (*Colletotrichum gloeosporioides*), dieback (*Lasiodiplodia theobromae*), and bacterial canker of mango and cashew (*Xanthomonas citri* pv. *mangiferaeindicae*) (Afouda et al., 2013; Zombré et al., 2016; Wonni, 2017; Dianda et al., 2023). *X. citri* pv. *mangiferaeindicae* emerged in several West African countries a decade ago, causing economically damaging outbreaks (Zombré et al., 2016). Although this bacterium primarily infects mango, the strains that emerged in Burkina Faso were the first to cause disease outbreaks on cashew, another member of the *Anacardiaceae* family (Zombré et al., 2016). Outbreaks have also been reported on cashew in Brazil; however, these were caused by a related pathogen, *X. citri* pv. *anacardii* (Ah-You et al., 2007; Gama et al., 2011).

This bacterial disease causes leaf lesions and fruit infections, resulting in significant economic losses. Its ability to spread through rain, wind, and agricultural tools (Sossah et al., 2024) complicates disease management. Strategies such as controlling the importation of plant material, eradicating infected plants, and using copper-based products (copper sulfate, copper hydroxide, and copper oxychloride) have been proposed (Pruvost, 1989; Zombré et al., 2017; Sossah et al., 2024). However, intensified treatments have led to the emergence of copper-resistant strains, necessitating more sustainable solutions.

The most effective and cost-efficient solution is the selection of cashew cultivars that are resistant or partially resistant to *X. citri* pv. *mangiferaeindicae*. To date, the only cultivars identified as resistant to *X. citri* pv. *mangiferaeindicae* are Heidi and Sensation, which were found in South Africa on mango (Du Plooy, 1991). However, no cashew cultivar resistant to *X. citri* pv. *mangiferaeindicae* has been identified worldwide.

The National Institute of Environmental and Agricultural Research of Burkina Faso maintains a collection of fifteen elite cashew accessions at the Farako-Bâ research station and in woodlots in the western part of the country. This study aims to evaluate the resistance of these elite cashew accessions under controlled greenhouse conditions in a hot and humid environment in Burkina Faso.

MATERIAL AND METHODS

Plant material

The plant material consisted of 15 elite cashew accessions selected from 820 candidate trees in the provinces of Kénédougou and Léraba (Table 1). These accessions were chosen based on surveys conducted in 2011, 2014, and 2015, which identified trees with exceptional production characteristics (Tarpaga et al., 2020). The nuts were sorted, soaked in a copper hydroxide-based fungicide-bactericide solution, and then planted in pots containing sterilized soil to prevent pathogen contamination. The resulting plants served as rootstocks.

Scions were collected from the INERA woodlots and grafted onto three-month-old rootstocks, which were disinfected to prevent contamination. The plants were regularly monitored, and any showing signs of disease were removed. They were maintained and watered until they achieved good vegetative growth after six months.

To ensure the long-term sustainability and conservation of these accessions, dedicated nurseries were established in the provinces of Kénédougou, Comoé, Léraba, and at the Farako-Bâ Research Station.

Biological material

Two strains of *X. citri* pv. *mangiferaeindicae*, designated LM6.1 and LH127.2, were used to evaluate the resistance of elite cashew accessions. Strain LM6.1 was isolated from cashew in 2015, while strain LH127.2 was obtained from mango in 2010. Both strains were characterized using the MLVA-12 scheme, which targets 12 specific microsatellites (Zombre et al., 2016) (Table 2).

Table 1. Origin and characteristics of elite cashew accessions screened for resistance and susceptibility to the bacterium *X. citri* pv. *mangiferaeindicae* (Tarpaga et al., 2020)

Elite accession	Province	Departement	Locality of origin	Average age	Year of identification
CE30	Kénédougou	Orodara	Sector 7	9 years	2015
CE40	Kénédougou	Orodara	Sector 7	12 years	2015
CE90	Léraba	Sindou	M'para	18 years	2015
CE110	Kénédougou	Kangala	Kangala	10 years	2015
CE200	Léraba	Sindou	M'para	17 years	2014
CE210	Léraba	Sindou	M'pogona	20 years	2014
CE260	Léraba	Sindou	M'Para	18 years	2014
CE301	Léraba	Sindou	Kawara	20 years	2014
CE400	Léraba	Sindou	M'para	20 years	2014
CE430	Kénédougou	Kangala	Kangala	10 years	2014
CE480	Léraba	Sindou	M'Pogona	14 years	2014
CE420	Kénédougou	Kangala	Kangala	10 years	2014
CE491	Léraba	Sindou	Kawara	20 years	2014
CE10	Léraba	Sindou	M'para	12 years	2015
CE511	Léraba	Sindou	M'Pogona	14 years	2014

Table 2. Characteristics of VNTRs (Variable Number of Tandem Repeats) in two strains of *X. citri* pv. *mangiferaeindicae* from Burkina Faso.

Strain	Province	Origin	Year	XL 6	XL 10	XL 11	XL 2	XL 8	XL 7	XL 5	XL 3	XL 9	XL 1	XL 4	XL 13
1	Comoé	Mango	2015	6	3	6	46	6	6	11	13	4	12	24	10
2	Kénédougo	Cashew	2014	6	3	6	44	6	6	10	14	4	12	24	10

Bacterial inoculum preparation and plant inoculation method

A 40 µl volume of each *X. citri* pv. *mangiferaeindicae* strain (LM6.1 and LH127.2) was plated on YPGA (Yeast extract, Peptone, Glucose, Agar) medium without antibiotics and incubated at 28°C for 24 h. After incubation, the bacterial suspension was adjusted to an optical density (OD) of 0.06 at 600 nm, corresponding to 10^8 CFU/ml. The suspension was applied to 10 designated zones on each leaf using a sterile 1 ml syringe. Before inoculation, leaves were disinfected with 70% ethanol. Three leaves were inoculated per plant.

Measured parameters

Area under the disease progression curve

Symptom progression was monitored daily from 3 to 25 days after inoculation (DAI). Each inoculation site was scored as either 0 or 1, based on the absence or presence of a visible black spot. This scoring system yielded a total score ranging from 0 to 10 for each strain on a given leaf, providing an accurate measure of disease progression.

Bacterial enumeration

The bacterial population of *X. citri* pv. *mangiferaeindicae* was quantified using the plate enumeration method. Inoculated leaves were collected on the 33rd DAI for each strain and accession. From each leaf, three symptomatic fragments (~1 cm^2) (Figure 1A) were excised from the inoculation sites, disinfected, and individually crushed in Bioreba extraction bags containing 2 ml of sterile distilled water.

Serial dilutions were prepared at concentrations ranging from 10^{-6} to 10^{-12}, with a final volume of 100 µl per dilution. Ten microliters of each dilution was plated on Petri dishes containing YPGA medium. After incubation at 28°C for 2 to 3 d, visible colonies from the appropriate dilutions were counted (Figure 1B), providing an accurate estimate of the bacterial population.

Data entry and analysis

Data entry and the construction of correlation curves between bacterial populations and area under the disease progression curve (AUDPC) values were performed using

Microsoft Excel 2013. Disease severity data were transformed into AUDPC values for each inoculated strain. AUDPC was calculated based on the cumulative appearance of visible lesions across ten inoculation sites per leaf. Two AUDPC values were obtained for each of the two strains per accession. The calculation followed the formula described by Jeger and Viljanen-Rollinson (2001):

$AUDPC = \sum_{i}^{n-1} [(Y_i + Y_{i+1})/2] (T_{i+1} - T_i)$

Where:

Y_i is the number of visible symptoms on day T_i,

n is the total number of observations.

The bacterial population was quantified using the formula given below:

$N = Nc \times D \times 10 \times 100 \times 2$

Where:

N is the number of bacteria/ml,

Nc is the number of colonies counted,

D is the reciprocal of the dilution concentration (10^{-6} to 10^{-12}) of the sample used for colony counting.

The bacterial population data were log-transformed to stabilize the means. Analysis of variance (ANOVA) for AUDPC values and bacterial populations was performed using GenStat software (version 11). Multiple comparisons of means for all pairs of AUDPC and bacterial population values were conducted using Tukey's test at a 5% significance level.

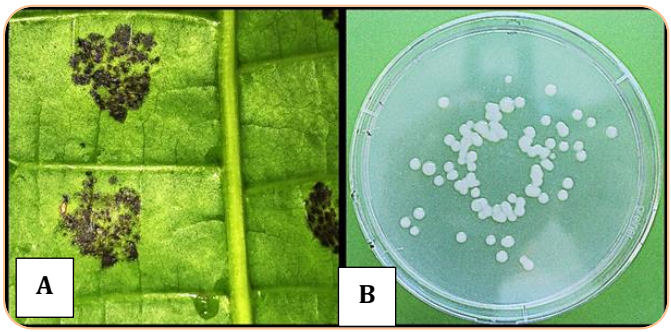

Figure 1. Counting of bacterial populations of *X. citri* pv. *mangiferaeindicae*. A: Lesions caused by *X. citri* pv. *mangiferaeindicae* at 33rd DAI. B: Development of colonies of *X. citri* pv. *mangiferaeindicae* after depositing a stock solution at a dilution of 10^{-6}.

RESULTS

Behavior of elite accessions based on AUDPC

The AUDPC values of the cashew accessions, assessed 11 days after inoculation with the two bacterial strains *X. citri* pv. *mangiferaeindicae* (LM6.1 and LH127.2), are presented in Table 3. These strains induced black lesions, with severity varying significantly among the accessions.

Table 3. Means of AUDPC at 11th day of inoculation on the leaves of cashew accessions with the strains LM6.1 and LH127.2 of *X. citri* pv. *mangiferaeindicae*.

Accessions	AUDPC	
	Strain	
	LM 6.1	LH 127.2
CE200	12.00 (1,000) a	12.33 (0,577) a
CE260	13.00 (1,000) a	13.00 (1,000) a
CE430	15.67 (0,577) b	15.00 (0,000) ab
CE400	16.67 (0,577) b	16.67 (1,154) b
CE110	37.67 (0,577) c	37.33 (0,577) c
CE210	37.67 (0,577) c	37.67 (0,577) c
CE40	37.67 (0,577) c	38.67 (0,577) c
CE480	37.67 (0,577) c	37.00 (1,000) c
CE30	38.33 (1,527) cd	38.33 (2,309) c
CE90	38.33 (1,527) cd	38.33 (2,309) c
CE301	38.67 (0,577) cd	38.67 (0,577) c
CE511	39.67 (0,577) d	39.67 (0,577) c
CE491	43.67 (0,577) de	43.33 (0,577) cd
CE10	46.67 (0,577) e	46.67 (1,154) d
CE420	48.00 (1,000) e	46.67 (0,000) d
Probability	< 0001	< 0001

AUDPC values were based on lesion development at the ten inoculation sites per leaf. A total of three AUDPC values per accession-strain combination were used to calculate the mean and standard error. Standard errors are presented in parentheses. AUDPC values with the same letter are not significantly different (P = 0.05).

Analysis of variance revealed a significant effect of accession on AUDPC values. Among the tested accessions, CE200 and CE260 exhibited the lowest AUDPC values (12 and 13, respectively), indicating greater tolerance to infection. These were followed by CE430 and CE400, which showed slightly higher values (15.67 and 16.67, respectively).

Conversely, some accessions displayed significantly higher AUDPC values, indicating increased susceptibility to the bacterial strains. In particular, accessions CE10 and CE420 recorded the highest AUDPC values of 46.67 and 48, respectively.

Variation in bacterial populations among elite accessions

Bacterial population counts recorded 33 days after inoculation are presented in Table 4. Significant differences in bacterial population sizes were observed among the tested accessions for both strains. Based on bacterial population levels, the accessions were classified into two distinct groups.

Table 4. Size of bacterial populations recorded from leaf lesions 33 days after inoculation of cashew accessions with strains LM6.1 and LH127.2 of *X. citri* pv. *mangiferaeindicae*.

Accessions	Log-transformed CFU lesion^{-n}	
	Strain	
	LM 6.1	LH 127.2
CE200	5.013 (0,015) a	5.027 (0,015) a
CE260	5.047 (0,005) a	5.033 (0,005) a
CE430	5.537 (0,055) b	5.527 (0,060) b
CE400	5.553 (0,049) b	5.557 (0,010) b
CE480	7.703 (0,005) c	7.690 (0,005) c
CE301	7.707 (0,005) c	7.690 (0,050) c
CE210	7.743 (0,037) cd	7.720 (0,005) cd
CE110	7.757 (0,055) cde	7.743 (0,085) cde
CE30	7.767 (0,057) cde	7.800 (0,005) e
CE40	7.767 (0,005) cde	7.743 (0,060) cde
CE90	7.790 (0,01) de	7.757 (0,005) de
CE511	7.823 (0,015) ef	7.803 (0,005) ef
CE491	7.877 (0,011) fg	7.853 (0,011) f
CE420	7.913 (0,011) g	7.940 (0,010) g
CE10	7.917 (0,066) g	7.920 (0,005) g
Probability	< 0001	< 0001

The mean size of bacterial populations, determined on a semi-selective KC medium, was obtained from three leaf lesions per accession and per strain. Standard errors are shown in parentheses. A comparison between cashew accessions was performed using Tukey's multiple comparison test on the entire data set for both strains. Bacterial population sizes with the same letter(s) do not differ significantly (P = 0.05).

The first group included accessions CE480, CE301, CE210, CE110, CE30, CE40, CE90, CE511, CE491, CE420, and CE10. These accessions exhibited symptoms characteristic of *X. citri* pv. *mangiferaeindicae*, such as rough black spots that expanded over time and developed an ash-gray coloration at the penetration sites by day 33. Under parasitic pressure, leaves often yellowed, with particularly high bacterial populations recorded in accessions CE420 and CE10, reaching 8.2 × 10^7 and 8.7 × 10^7 CFU per lesion, respectively. In this group, bacterial populations consistently exceeded 1 × 10^7 CFU per lesion, indicating a compatible interaction between the bacterial strains and these accessions.

The second group consisted of accessions with bacterial populations below 1 × 10^7 CFU per lesion, nearing the threshold of an incompatible interaction. Among them, accessions CE200 and CE260 had the lowest bacterial populations (1.03 × 10^5 to 1.1 × 10^5 CFU per lesion) and showed statistically similar values for both strains. They were followed by accessions CE430 and CE400, with bacterial populations ranging from 3.37 × 10^5 to 3.6 × 10^5 CFU per lesion.

Correlation between AUDPC and bacterial populations

Figures 2 and 3 illustrate the correlation between AUDPC values and bacterial populations for the *X. citri* pv. *mangiferaeindicae* strains LM6.1 and LH127.2. A significant positive correlation was observed in both cases, as indicated by the trend lines.

Lower bacterial population levels consistently corresponded to the lowest AUDPC values, regardless of the origin of the bacterial strain. This relationship is particularly evident in accessions CE200, CE260, CE430, and CE400, which belong to the second group. These accessions exhibit tolerance or resistance to infection, in sharp contrast to others that show significantly higher bacterial populations and AUDPC values. These findings underscore the correlation between AUDPC and bacterial populations as a key indicator of the susceptibility or resistance of elite accessions.

DISCUSSION

The results of this study provide important insights into the response of elite cashew accessions to *X. citri* pv. *mangiferaeindicae*, highlighting significant variability in tolerance and susceptibility to infection. This genetic diversity serves as a strong foundation for selecting desirable traits in breeding programs, thereby promoting sustainable pathogen management.

Correlation between AUDPC and bacterial populations

A significant positive correlation was observed between AUDPC values and bacterial populations, confirming their reliability as indicators of plant resistance or susceptibility. Accessions CE200 and CE260, which exhibited the lowest AUDPC values and bacterial populations, demonstrated an enhanced ability to limit bacterial multiplication. These findings align with those of Gagnevin et al. (1998), who reported that a reduction in bacterial populations is a key factor in plant resistance. Similarly, Ah-You (2007) described comparable mechanisms in interactions between *Xanthomonas* and *Anacardiaceae*, emphasizing their role in limiting infection.

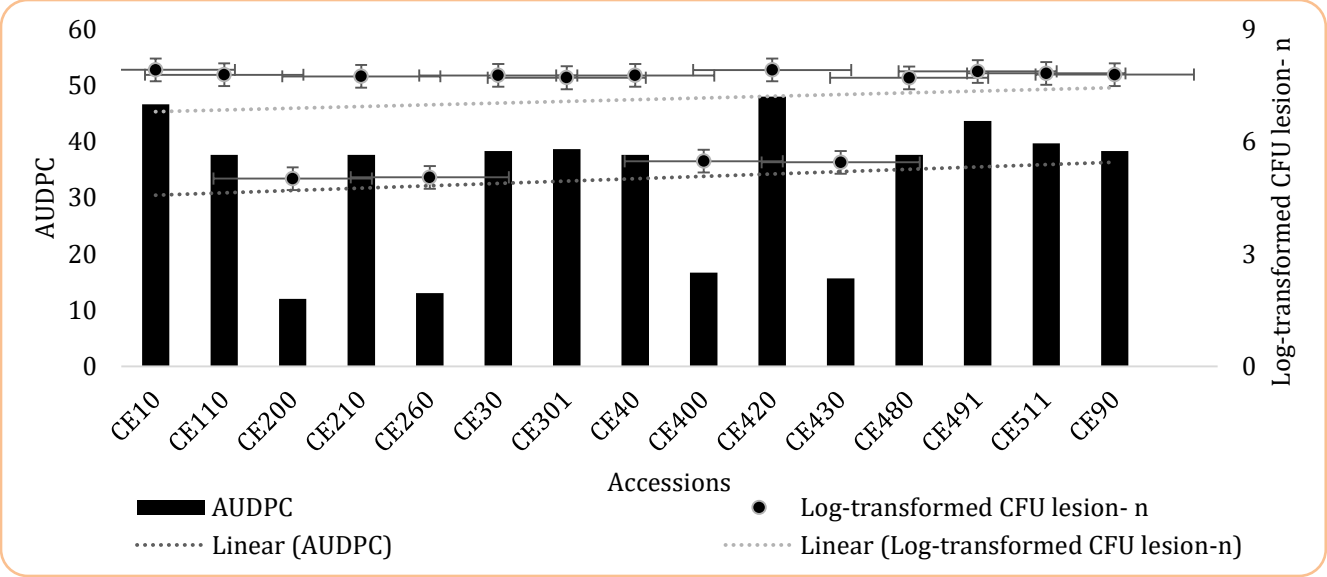

Figure 2. Correlation between AUDPC and the bacterial population of LM6.1 of *X. citri* pv. *mangiferaeindicae*.

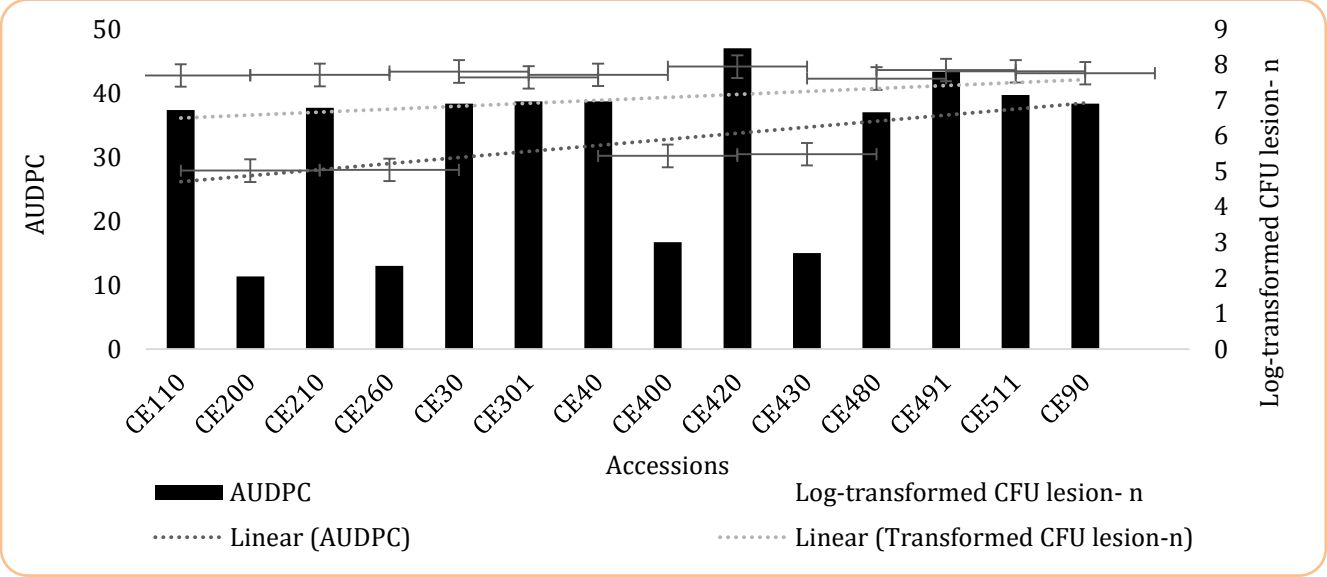

Figure 3. Correlation between AUDPC and the bacterial population of LH127.2 of *X. citri* pv. *mangiferaeindicae*.

Conversely, accessions CE10 and CE420, characterized by high AUDPC values and bacterial populations, exhibited increased susceptibility. Their inability to effectively restrict bacterial multiplication suggests their potential use as sensitive controls in future studies. These findings support the work of Pruvost (1989), who advocated for an integrated approach combining natural plant defenses with chemical or biological treatments to enhance pathogen management.

Role of genetic variability

Genetic variability among accessions plays a crucial role in resistance to infection. Kikutani (2015) demonstrated that the production of secondary metabolites, such as phenolic compounds, is a key defense mechanism against pathogens. Furthermore, Andersen et al. (2018) highlighted that activating specific immune responses following bacterial inoculation is essential for limiting infection. These findings explain the correlation between lower bacterial populations and reduced AUDPC levels in resistant accessions.

Implications for breeding and genetic improvement

Accessions CE200 and CE260 emerge as promising

candidates for developing bacterial blight-resistant cultivars. These accessions may aid in identifying genetic traits associated with resistance, as suggested by Gagnevin et al. (1998). Similarly, Ah-You (2007) emphasized the importance of selecting resistant genotypes in Anacardiaceae, a principle directly applicable to CE200 and CE260.

Moreover, accessions CE430 and CE400, which exhibit moderate tolerance, could serve as valuable genetic resources in environments with low pathogen pressure. Incorporating them into breeding programs would enhance parental line diversity and broaden the genetic base of cultivated varieties. Andersen et al. (2018) further noted that identifying resistance-related genes could guide more targeted and effective breeding strategies.

Potential resistance mechanisms

The results suggest that the resistance of elite cashew accessions is primarily based on basal defense mechanisms, which play a critical role in limiting infections. One key strategy is reducing bacterial multiplication in tissues, as highlighted by Gagnevin et al. (1998).

Another important defense mechanism involves the activation of physical and biochemical barriers, such as cell wall thickening and the production of toxic phenolic compounds. Kikutani (2015) emphasized the central role of secondary metabolites in plant defense, while Ah-You (2007) observed a significant reduction in bacterial proliferation due to these mechanisms. Additionally, Pruvost (1989) highlighted the importance of structural adaptations, such as cell wall thickening, in restricting bacterial disease progression.

Together, these mechanisms explain the increased tolerance observed in certain elite accessions. A deeper understanding of these processes could guide the selection and development of cashew cultivars resistant to *X. citri* pv. *mangiferaeindicae*, thereby contributing to sustainable pathogen management strategies.

This study provides valuable insights into the resistance of elite cashew accessions to *X. citri* pv. *mangiferaeindicae*. However, certain limitations must be considered. To enhance the reliability of these findings and support the development of cashew varieties that are both productive and resistant to bacterial blight, future research should include field trials, expanded genetic screening to encompass greater diversity, and in-depth molecular analyses to precisely identify the underlying resistance mechanisms.

CONCLUSION

This study evaluated the resistance of fifteen elite cashew (*Anacardium occidentale* L.) accessions to bacterial blight caused by *Xanthomonas citri* pv. *mangiferaeindicae* under controlled conditions in Burkina Faso. The results revealed significant variability in tolerance among the tested accessions, highlighting valuable genetic potential for breeding and variety improvement programs. Notably, accessions CE200 and CE260 exhibited remarkable tolerance, characterized by low AUDPC values and reduced bacterial populations, making them promising candidates for the development of resistant cultivars.

The strong correlation observed between AUDPC and bacterial populations confirms the relevance of these indicators for evaluating resistance and guiding disease management strategies. These findings also underscore the importance of basal defense mechanisms, such as limiting bacterial multiplication and synthesizing secondary metabolites, in infection tolerance.

Incorporating the identified accessions into genetic selection programs is a crucial step toward enhancing the resilience of the cashew sector to bacterial blight. This approach could not only improve the productivity and profitability of the industry but also contribute to sustainable agriculture capable of addressing climatic and epidemiological challenges. Furthermore, increased investment in research on the genetic and physiological mechanisms of resistance, as well as the conservation of local genetic resources, is essential to ensure the long-term sustainability of the cashew sector and to support the socio-economic development of rural communities reliant on cashew production.

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AUTHORS' CONTRIBUTION

CZ carried out the tests and collected data; VWT provided the elite accessions; CZ and BO analyzed the data; CZ, YT, BO, MS, ZOD, AO, and RSO wrote and revised the manuscript; IW and BS supervised the entire study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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