

Inventories of forest host plants of the *Xanthomonas citri* bacterium in cashew orchards in western Burkina Faso

ABSTRACT

The presence of forest species in cashew orchards in Burkina Faso, linked to their lack of maintenance, serves as a hotspot for the emergence of pathogens such as bacteria from the *Xanthomonas citri* species. Identifying these forest species acting as reservoirs for this bacterium in various cashew orchards could contribute to their management. To this end, a forest inventory allowed us to identify the forest species present in cashew orchards, mango orchards, and mixed cashew/mango orchards. Molecular identification of 11 strains was carried out through the analysis of the *atpD* housekeeping gene sequences. The population sizes of strains inoculated on the CE420 cashew accession were measured 21 days post-inoculation. We have proven the existence of 18 forest species in the orchards. The analysis of *atpD* sequences demonstrated that all our strains derive from a common ancestor, *Xanthomonas citri*. The population sizes of strains 5DDCF, 6BFG, and 1BFG, isolated from *Ficus abutilifolia* (Miq.) Miq and *Combretum micranthum* G. Don, reached high densities of up to 8.10^8 CFU 21 days post-inoculation. *Xanthomonas* strains isolated from *Ficus abutilifolia* (Miq.) Miq and *Combretum micranthum* G. Don demonstrated their ability to colonize the leaf tissues of cashew. Mango, *Ficus abutilifolia* (Miq.) Miq, and *Combretum micranthum* G. Don were plant species that share the same ecological niche as cashew and serve as reservoirs for *Xanthomonas* bacteria pathogenic to cashew.

Keywords: cashew, forest species, *Xanthomonas*, hosts, housekeeping genes, Burkina Faso.

1. INTRODUCTION

Initially planted as a forest tree in Burkina Faso in 1970, the cashew was soon exploited for its fruit production [1]. Today, almost 45.076 households are involved in cashew nut production, covering an area of more than 255.000 hectares [2]. Cashew production in Burkina Faso has increased considerably over the past three years, from 72,899 tonnes in 2020 to 182,983 tonnes in 2022, an overall increase of 151.01%. The cashew sector in Burkina Faso has strong potential for export, job creation and added value for the national economy. Cashew nuts rank 2nd among agricultural export products after cotton, with 89.720 billion FCFA generated by exports, representing 3.16% of the country's total export value [2]. However this national production remains low compared to the average West African production. The financial profitability of all cashew nut production systems is assured by their low investment requirements [3]. Unfortunately, low yields in Burkina Faso, partly due to a lack of maintenance in the orchards, are still a cause for concern for producers, who are unable to meet their needs. Formerly produced in association with annual crops such as millet, sorghum, groundnuts and cowpeas, cashew orchards are now characterized by the presence of tree species from the same or different families, such as shea, dwarf, mango and citrus [4]. The cohabitation of cashew nuts with species from the same family, such as mango, coupled with the lack of orchard maintenance, which favours the development of forest species, could constitute an entry point for numerous biotic constraints in cashew nut orchards. In Burkina Faso, mango and cashew are two host species of the phytopathogenic bacterium *Xanthomonas citri* pv. *mangiferaeindicae*, the causal agent of mango and cashew black spot disease [5]. Fungal

pathogens such as *Colletotrichum gloeosporioides*, *Pestalotia heterocornis*, *Cephaleuros virescence* and two (02) bacterial genera, *Xanthomonas* and *Erwinia* have been associated with various symptoms observed on both mango and cashew trees in Burkina Faso [5, 6, 7]. The forest species present in the different orchards could constitute reservoirs of phytopathogenic agents that could favour their dissemination and compromise the health of cashew plants, and therefore their yield. Many crops, including cashew, are under constant threat from plant pathogens such as bacteria of the *Xanthomonas* genus, which are pathogens capable of infecting more than 400 plant species. The aim of this study is to identify the reservoir species of *Xanthomonas* bacteria that are pathogenic to cashew trees in western Burkina Faso.

2. MATERIAL AND METHODS

2.1 Inventory and sample collection

Plant species were inventoried and samples collected in nine (09) localities in western Burkina Faso (Figure 1). Forest species present in mango and cashew production orchards and mango-cashew association orchards were inventoried using the fixed-size plot or quadrat floristic inventory method [8]. The inventory was carried out in three (03) orchards of at least ½ ha per locality. The species were identified with the help of a forestry guide. However, species of doubt were placed in press for subsequent identification using the documents proposed by Arbonnier [9] and Bonnet [10]. The species classification followed the APG IV system and the scientific names were updated using the African Plant Database version 4.0.0 ([African Plant Database](#)). The symptoms collected concerned plant species showing common foliar symptoms due to *Xanthomonas* of mango and cashew. One symptomatic leaf and one apparently healthy leaf were collected per species.

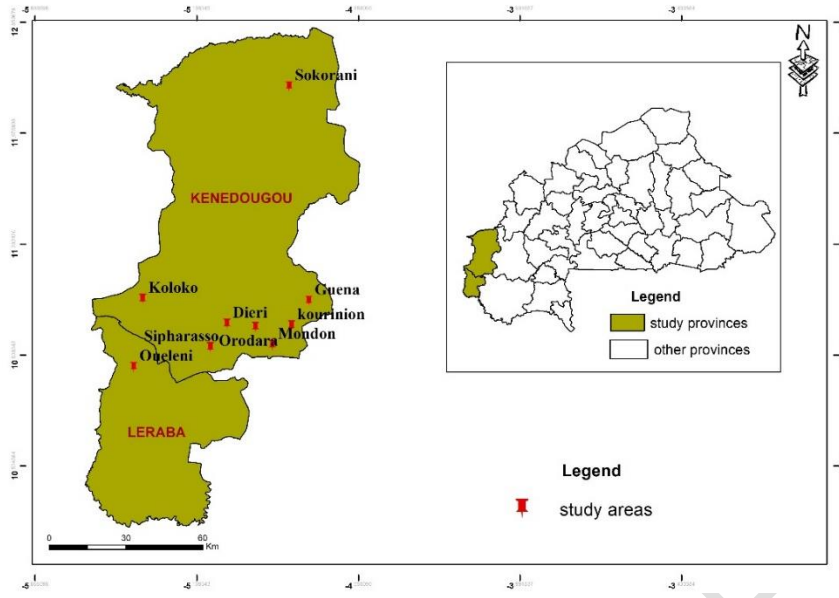


Figure 1: Mapping the prospecting and collection area

2.2 Isolation of bacteria

Bacterial isolation was carried out on collected leaves. Sections of leaf tissue were successively disinfected in 70% alcohol and sterile distilled water, then macerated in 1 ml Tris buffer (pH 7.2). The resulting macerate was left to stand for 30 minutes to allow the bacteria to diffuse. A volume of 50 μ l of the supernatant was then spread onto plates of YPGA medium (yeast extract, peptone, glucose, agar) enriched with cephalexin (40 mg/L), kasugamycin (20 mg/L) and propiconazole (50 mg/L). Cultures were incubated for 3 to 4 days at 28°C. One suspect bacterial colony was purified from each tissue sample and incubated for 48 hours. After incubation, 11 purified strains with morphological characteristics similar to the reference strain of *Xanthomonas* isolated by Zombré [5] were selected for molecular and pathogenic characterization.

2.3 Molecular identification of bacteria

2.3.1 DNA extraction and gene amplification

The strains were seeded on YPGA medium and incubated for 2 days in an oven at 28°C. Colonies grown on the medium were used for direct extraction of genomic DNA using the GeneJet DNA purification kit (Thermo Fisher Scientific, Inc., USA). The concentration and purity of the extracted DNA were assessed using Nanodrop.

Portions of the *gyrB* (GyrBU301: GCCGAGGTGATCCTCACCGT / GyrBL1307: GGCCGAGCCACCTGCCGAGT) genes encoding the DNA gyrase beta subunit and *atpD* (atpDU9: GGGCAAGATC GTTCAGAT / atpDL859: GCTCTTGGTC GAGGTGAT) encoding the F1-F0 ATPase subunit were amplified by PCR in 35 μ l reaction mixtures using a Biometra

thermal cycler. PCR was performed as follows: an initial denaturation phase at 95°C for 3 minutes, 30 reaction cycles comprising denaturation at 95°C for 30 seconds, hybridisation at varying temperatures depending on primer type, elongation at 72°C for 4 minutes and a final elongation phase for 10 minutes at 72°C and a stabilisation phase at 16°C. The presence of amplicons was revealed on a 1% agarose gel and visualised using an Ultraviolet lamp. PCR products were sequenced by Genewiz using the SANGER method.

2.3.2 Phylogeny of amplified genes

Consensus sequences of *atpD* gene were generated after correction of the raw sequences using Geneious version 9.1.8 created by Biomatters (<http://www.geneious.com>). Each consensus sequence was subjected to a Blastn query on NCBI to identify similar and distant sequences. *Xanthomonas citri* pv. *mangiferaeindicae* *atpD* sequences were obtained by reference genome mapping of strains LMG9322 and CFBP1717 isolated from mango and cashew in Burkina Faso [11]. All the fasta sequences (*gyrB* and *atpD*) were aligned using the Geneious pairwise muscle alignment tool. Based on these alignments, matrices of pairwise distances between strains were generated according to the gene amplified. The consensus sequence obtained by aligning *atpD* sequences was used to construct the Neighbor-Joining phylogenetic tree using the Tamura-Nei genetic distance model and for a Bootstrap analysis using 1000 replicates.

2.4 Pathogenicity evaluation

The sequenced strains were incubated for 24 h at 28°C on YPGA culture medium. For each 24h-old strain, an inoculum with an optical density (OD) of 0.2 at 600 nm corresponding to 10⁸ CFU/mL measured with a spectrophotometer was prepared. Inoculation was carried out on the youngest leaves of cashew accession CE420, which were already green and hardened, using a sterile 1 ml syringe without needle. The syringe orifice is inserted between the two secondary veins on the underside of the leaf, which has been disinfected on both sides with 70% alcohol. Using moderate mechanical pressure on the syringe plunger, an area of 1 cm² was infiltrated with the bacterial suspension on seven (07) plants per inoculum. Infiltration was carried out on three (03) leaves per plant, i.e. 06 inoculation points per leaf.

The bacterial population was quantified on the 21st day after inoculation. To do this, three fragments of leaves showing symptoms ($\approx 1 \text{ cm}^2$) were collected from the inoculation sites, disinfected and ground in a Bioreba extraction bag in 2 ml of sterile distilled water. The supernatants from the different grindings were used for six cascade dilutions. For each dilution, 50 μl was spread on YPGA medium and left to incubate for 3 days at 28°C. The number of colonies for each dilution was counted using a STUART SC6+ colony counter.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1. Inventory and samples collected

A total of 18 forest species showing foliar symptoms were observed in the various orchards surveyed (Figure 2). These included fruit species such as *Annona senegalensis* Pers., *Vitellaria paradoxa* Gaertn. f. and *Lannea velutina* A. Rich. In addition, symptomatic species of *Lannea velutina* A. Rich were observed in all localities except Sokoroni. In Koloko its

frequency of observation was the highest (30%) but remained low in Guena (9%). Of all the localities, Dieri-Deni, Oueleni and Sipharasso, characterised respectively by mixed orchards, had the highest number (09) of forest species showing bacterial leaf symptoms. Of these species, *Cochlospermum planchonii* Hook.F. and *Combretum micranthum* G. Don are still observed at frequencies of 10% in Dieri-Deni and 11% in Oueleni and Sipharasso respectively. The samples collected showed symptoms on the leaf blade, veins or petiole. Symptoms on the leaf blade and between the veins took the form of blackish (Figure 3. B & F), brick-red (Figure 3. A & I) or brownish (Figure 3. D) necroses most often marked by a yellow halo (Figure 3. H & C). Greyish cankers were much more common on the main vein or petiole of symptomatic species (Figure 2. B & G). Some of the secondary veins of cashew plants showed red burns that perforated the leaf blade (Figure 3. E).

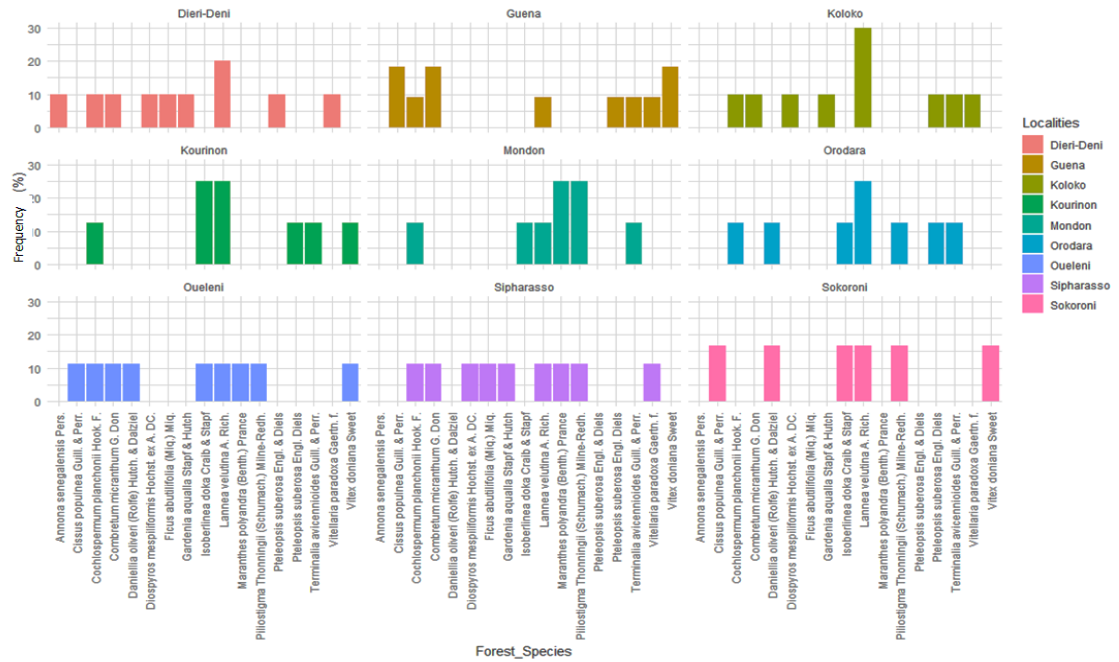


Figure 2: Frequency of observation of symptomatic forest species

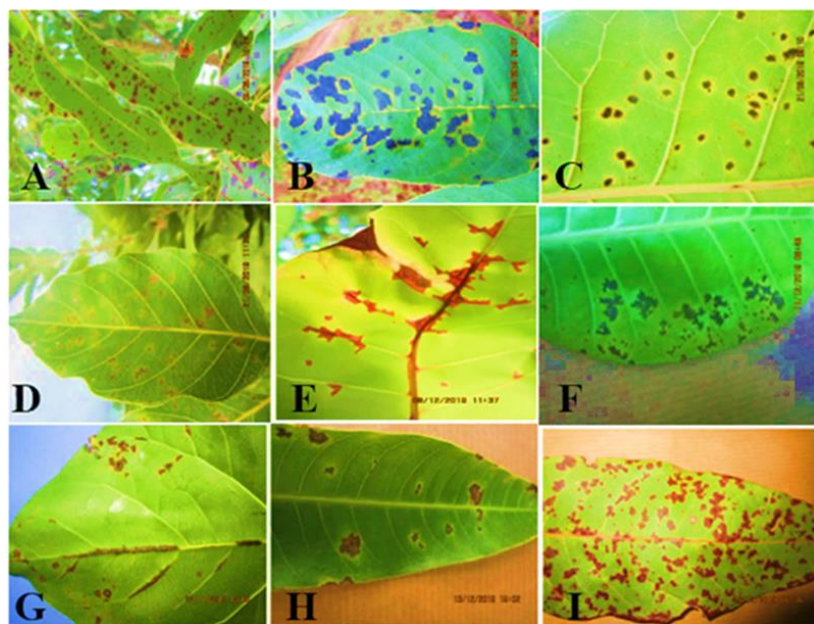


Figure 3: Leaf symptoms collected in (A & I) *Vitellaria paradoxa* Gaertn. f.; (B) *Annona senegalensis* Pers.; (D) *Combretum micranthum* G. Don; (G) *Ficus abutilifolia* (Miq.) Miq.; (E & F) *Anacardium occidentale* L. ; (H) *Mangifera indica* L.

3.1.2 Identity of the bacteria isolated

A total of 122 bacterial isolates were obtained from 136 leaf samples collected from the different orchards (Table 1). The sequenced strains were isolated from *Ficus abutilifolia* (Miq.) Miq (1BFG, 2BFG, 3BFG, 6BFG, 8BFG), mango (4BMI, 7BMI, 9BMI), cashew (11OAO) and *Combretum micranthum* G. Don (5DDCF).

Table 1: Number of bacterial strains isolated by localities.

Localities	Dieri-Deni	Guen-a	Koloko	Kouri-non	Mon-don	Oro-dara	Si-phar-asso	Soko-roni	Ouele-ni
Types of orchard	Mixed	Mango	Cashew	Cashew	Cashew	Cashew	Mixed	Cashew	
Number of symptomatic species	9	8	8	6	6	7	9	6	9
Number of samples collected	18	16	16	12	12	14	18	12	18
Number of bacterial strains	20	10	13	11	4	6	16	19	23

3.1.3. Molecular characteristics of strains

to $8,10^8$ per lesion were observed for strains 6BFG, 5DDCF and 1BFG isolated from *Ficus abutilifolia* (Miq.) Miq and *Combretum micranthum* G. Don. Additionally, strains isolated from mango (10BMI) and *Ficus abutilifolia* (Miq.) Miq (3BFG) exhibited log CFU/mL values close to the initial concentration, although not all reached 8.0. The population sizes of all other strains showed values of $1,10^5$ or less (Figure 5).

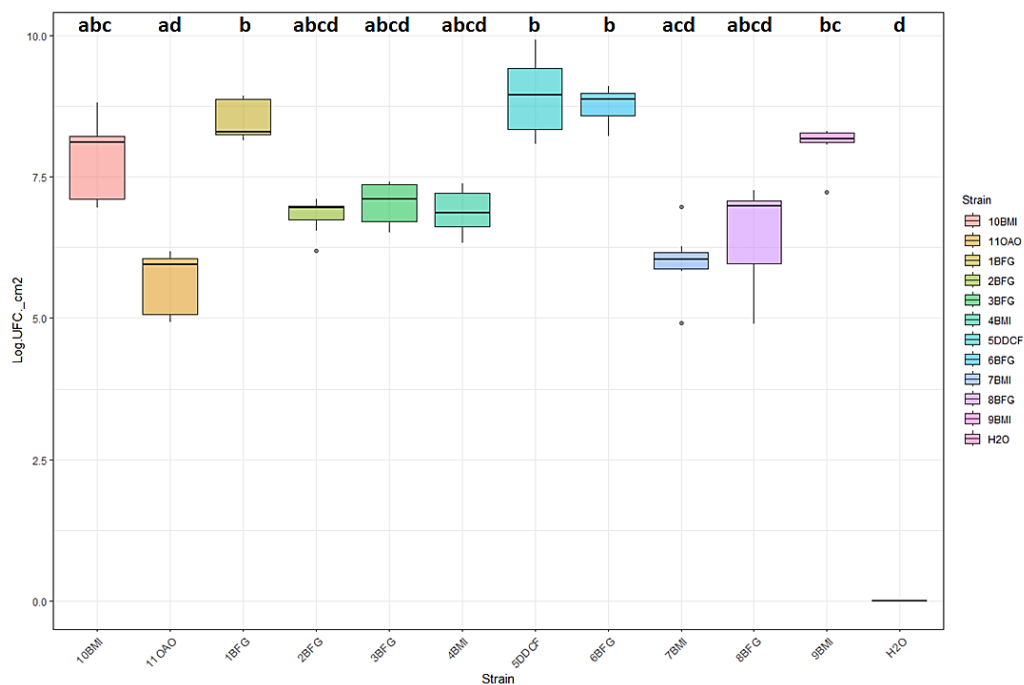


Figure 5: Bacterial population of strains 21 days after inoculation

3.2 Discussion

The presence of forest species in the various cashew orchards in western Burkina Faso would justify the intensification in the area of an agro-sylvo-pastoral system characterised by land use combining agriculture, tree and forest management and animal husbandry. According to Zampaligre et al [12], trees and shrubs constitute an important fodder reserve that provides additional nutrients for animals during critical periods. Some species are left in orchards voluntarily by growers because of their importance for food, medicine or socio-cultural reasons. Species such as *Annona senegalensis* Pers, *Vitellaria paradoxa* Gaertn. f. and *Lannea velutina* A. Rich are voluntarily preserved in orchards for the edibility of their fruit. These species are available at a time when granaries are empty and play a role in combating food insecurity [13].

The leaf symptoms are characterised by blackish necrotic areas most often marked by a yellowish halo. They are similar to the symptoms of bacteriosis due to *Xanthomonas citri* pv. *mangiferae* indica, a pathogen of mango and cashew trees in Burkina Faso, and related to those described by Ah-you et al. [14]. Bacteria of the genus *Xanthomonas* were isolated from similar symptoms originating from fruit species such as mango, pomegranate, peach and plum in India [15]. Cashew leaves showing brick-red burns along the veins are characteristic of desiccation in which fungi such as *Pestalotiopsis* sp., *Fusarium* sp., *Colletotrichum* sp., *Aspergillus* sp., and *Botrytis* sp. have been associated [7]. Pathogenicity tests have shown that *Colletotrichum gloeosporioides* and *Pestalotia heterocorni* cause anthracnose and pestalotiosis of cashew trees respectively [6].

Species such as *Ficus abutilifolia* (Miq.) Miq and *Combretum micranthum* G are reservoirs for *Xanthomonas* in the various cashew orchards in Burkina Faso. *Xanthomonads* producing non-pigmented colonies have been described as the agents responsible for bacterial dieback of ambarella (*Spondias dulcis* Forst., syn. *Spondias cytherea* Sonn.) in the French West Indies [16] and black spot affecting Brazilian pepper (*Schinus terebinthifolius* Raddi) in Réunion [17]. The *Xanthomonas* genus contains around 27 species [18] which can cause major diseases on more than 400 host plants [19].

Strains 7BMI, 4BMI, 2BFG, 8BFG, and 3BFG, characterized by identity percentages > 94%, reflect cases of minor variants originating from the same lineage. These strains may therefore be capable of adapting to slightly different local conditions. The high similarity of strains 11OAO, 9BMI, and 10BMI, but with a high genetic distance (~37%) from Group 1, could indicate significant evolutionary divergences between these strains and those of Group 1. This divergence in the *atpD* gene flow may be linked to the accumulation of mutations and a reduction in homologous recombination due to the presence of barriers [20]. Furthermore, the strains 6BFG, 5DDCF, and 1BFG, grouped within the same clade as *Xanthomonas citri* pv. *mangiferaeindicae*, reflect their shared evolutionary origin. They have inherited similar characteristics from their common ancestor *Xanthomonas citri*, and over the course of evolution, they have diverged while retaining genetic traits that link them in the same group. This common origin may explain the genetic similarities that place them in the same phylogenetic clade. The divergence of these strains can lead to their speciation to plant species such as *Ficus abutilifolia* (Miq.) Miq and *Combretum micranthum* G. The identification of bacterial species is based primarily on the genetic divergence between strains, rather than on reproductive isolation as is the case for the majority of eukaryotes [20].

The growth profile of the inoculated strains shows that they differ in terms of their adaptation to cashew trees and their potential pathogenicity. Strains 6BFG, 5DDCF, and 1BFG from the same clade as *Xanthomonas citri* pv. *mangiferaeindicae* with the highest bacterial populations are the most virulent or better adapted to cashew. A homology of pathogenicity or virulence genes acquired by horizontal transfer from the same *Xanthomonas citri* ancestor would exist between them. Its strains identified respectively as *Xanthomonas citri* pv. *citri* (11OAO, 7BMI) and *Xanthomonas citri* pv. *punicae* (4BMI) are therefore capable of developing adaptations to colonise the plant tissues of cashew nut plants or to protect themselves from their immune defences. Different pathovars of the same bacterial species can attack the same plant species, inducing different diseases with their own biological characteristics, which requires adapted control strategies [21]. The bacterial population sizes of these strains are characteristic of a compatible interaction between them and cashew. Similar population sizes ($3 \cdot 10^7$ to $9 \cdot 10^7$ cfu) characteristic of a compatible interaction were observed when cashew was inoculated with group II strains. Thus, the cashew tree is a potential host for its *Xanthomonas* species. Furthermore, while strains isolated from *Ficus abutilifolia* (Miq.) Miq can multiply efficiently, although with slightly reduced growth (3BFG), others (1BFG, 2BFG, 6BFG, and 8BFG) are able to colonise the cashew tree but less effectively than the previous strains. This may be explained by the selective pressure exerted by the cashew on its various strains. This is typical of interactions involving non-host species, resistant cultivars, or occasional pathogens. The same applies to the strain 5DDCF isolated from *Combretum micranthum* G, which shows minimal growth and poor adaptation in cashew tissues. Nevertheless, the cashew remains an alternative host for these bacterial strains. In addition to the mango tree, *Ficus abutilifolia* (Miq.) Miq and *Combretum micranthum* G represent potential reservoirs of *Xanthomonas* in various cashew orchards in Burkina Faso.

4. CONCLUSION

Xanthomonas bacteria cause significant diseases in economically important host plants such as the cashew tree. The analysis of *gyrB* and *atpD* gene sequences, coupled with the characterization of the pathogenic potential of strains isolated from mango, *Ficus* sp., and

Combretum sp., reveals that these species are reservoirs of *Xanthomonas* that share the same environment as the cashew tree in Burkina Faso. The presence of these species in mango and cashew orchards suggests that they should be considered in the implementation of integrated control methods for managing outbreaks, such as the bacterial blight of mango and cashew caused by *Xanthomonas citri* pv. *mangiferaeindicae* in Burkina Faso.

CONSENT

Not applicable

ETHICAL APPROVAL

Not applicable

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

pv: pathovars

NCBI: National Center for Biotechnology Information

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