



Plant Pathology Journal

ISSN 1812-5387

science
alert

ANSI*net*
an open access publisher
<http://ansinet.com>



Research Article

Pathogenic Diversity of *Xanthomonas* sp. Infecting Cashew (*Anacardium occidentale* L.) and Susceptibility of Genotypes Released in Côte d'Ivoire

¹Abenan Manou Natacha Kouman, ²Amoa Armist Tehua, ³Nakpalo Silué, ²Klinnanga Noel Koné, ⁴N'Dri Séverin N'Goran, ⁵Sibirina Soro, ⁶Eric-Olivier Tienebo, ⁶Kouabenan Abo and ^{1,4}Daouda Koné

¹Laboratory of Climate Change, Biodiversity and Sustainable Agriculture, Training and Research Unit, Biosciences, African Center of Excellence in Climate Change, Biodiversity and Sustainable Agriculture, University Félix Houphouët-Boigny 22 BP 582 Abidjan 22, Côte d'Ivoire

²Department of Plant Biology, Training and Research Unit, Biological Sciences, University Peleforo Gon Coulibaly, BP 1328 Korhogo, Côte d'Ivoire

³Department of Agriculture and New Technology, UFR Agriculture, Fisheries Resources and Agro-industry, University of San Pedro, B.P. V1800 San Pedro, Côte d'Ivoire

⁴Laboratory for Biotechnology, Agriculture and Biological Resource Development, Training and Research Unit, Biosciences, Pedagogical and Research Unit, Plant Physiology and Pathology, University Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire

⁵Laboratory of the Improvement of Agricultural Production, Training and Research Unit UFR Agroforestry, Pedagogical and Research Unit, Plant Physiology, University Jean Lorougnon Guédé, B.P. 150 Daloa, Côte d'Ivoire

⁶Laboratory of Phytopathology and Plant Biology, Félix Houphouët-Boigny National Polytechnic Institute, B.P. 1313, Yamoussoukro, Côte d'Ivoire

Abstract

Background and Objective: Bacterial blight caused by the genus *Xanthomonas* is one of the main biotic constraints of cashews in Côte d'Ivoire. The aim of this study was to evaluate the susceptibility of cashew genotypes to various isolates of *Xanthomonas* sp. in Côte d'Ivoire. **Materials and Methods:** Cashew tree nurseries, consisting on the one hand of "all-round" genotypes and the other hand of high-producer genotypes A24, A27 and A30, arranged in a greenhouse, were used as plant material for the various inoculations. A total of 44 *Xanthomonas* sp. isolates were used for pathogenicity testing. The inoculation was carried out using a 1 mL syringe topped with gum. A bacterial inoculum of 0.5 mL of each isolate, with a concentration of 10^8 CFU mL⁻¹, was infiltrated into young cashew leaves. At the same time, control was set up with plants whose leaves were infiltrated with sterile distilled water. Inoculation tests were repeated three times. **Results:** This experiment evaluated symptom onset time, incidence and severity index. The 44 *Xanthomonas* sp., isolates tested showed their pathogenicity in the marking zones where infiltrations were made on the different cashew genotypes tested after 30 days of incubation. Multivariate analysis generated four pathogenicity groups for the 44 isolates and two susceptibility groups for the cashew genotypes tested. **Conclusion:** These results suggested that these genotypes are susceptible to cashew bacterial blight in Côte d'Ivoire and that there is variability in the expression of pathogenicity among isolates of the causal agent.

Key words: Cashew, genotypes, *Xanthomonas* sp., pathogenicity, Côte d'Ivoire

Citation: Kouman, A.M.N., A.A.Tehua, N. Silué, K.N. Koné and N.S. N'Goran *et al.*, 2024. Pathogenic diversity of *Xanthomonas* sp. infecting cashew (*Anacardium occidentale* L.) and susceptibility of genotypes released in Côte d'Ivoire. Plant Pathol. J., 23: 1-11.

Corresponding Author: Kouman Abenan Manou Natacha, Laboratory of Climate Change, Biodiversity and Sustainable Agriculture, Training and Research Unit, Biosciences, African Center of Excellence in Climate Change, Biodiversity and Sustainable Agriculture, University Félix Houphouët-Boigny 22 BP 582 Abidjan 22, Côte d'Ivoire

Copyright: © 2024 Abenan Manou Natacha Kouman *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The cashew tree (*Anacardium occidentale* L.) is a tropical fruit tree whose cultivation contributes to the social and economic development of several cashew-producing countries worldwide^{1,2}. Native to South America, European explorers introduced the plant to Asia and Africa in the 16th century³. Cashew is a perennial plant cultivated mainly for producing cashew nuts and for cashew apples and certain liquids obtained from nut shells⁴.

The world's largest cashew-producing region is West Africa. In 2018, 1,795,000 tonnes of raw cashew nuts were produced, which accounts for over half (49%) of world production⁵.

Côte d'Ivoire, is the world's leading producer, with production estimated at 900,000 tonnes in 2020⁵.

The nut is Côte d'Ivoire's second-largest export after cocoa⁵. Cultivated mainly in the North, East and Central Regions, cashew provides producers with a substantial income and constitutes an income-generating activity enabling them to meet their vital needs⁶.

However, cashew cultivation is faced with numerous biotic constraints that affect cashew yields in terms of both quality and quantity⁷⁻¹⁰. Among these constraints, bacterial blight, caused by *Xanthomonas*, is responsible for considerable damage and is considered one of the major phytosanitary problems in tropical and subtropical zones^{11,12}. Symptoms can be observed mainly on leaves and fruit. They can also appear on young branches. On leaf blades, these symptoms appear as small, angular spots close to the veins¹³. According to Gama *et al.*¹⁴, who classified these symptoms as systemic, leaf lesions progress to the branches and stem, which can cause crown desiccation. On fruit, lesions can occur not only on developing nuts but also on mature ones. A dark, deformed, burn-like necrosis is usually observed in mature adults.

In Côte d'Ivoire, Kouman *et al.*¹⁵ revealed that cashew bacterial blight is present in all cashew-producing regions, with high infection rates of over 50%. Furthermore, given the allogamous nature of cashews and their use as planting material, Ivorian orchards are populations in which different trees with unknown characteristics coexist. As a result, large quantities of seeds can be sown without guarantee of obtaining a single plant exactly like the parent plant. Moreover, seeds lose their germinative power fairly quickly when stored in an ambient environment. It, therefore, seemed essential for research to move progressively from creating orchards by sowing to grafting seedlings. Thus, since 2014, the

CNRA has made three cashew genotypes identifiable in the collection available to growers by codes LAX3264, LAX4297 and LAZ330.

These different genotypes were selected on the basis of their high cashew nut yields per hectare¹⁶. However, no scientific information has been reported on the susceptibility of these three genotypes to bacterial blight in Côte d'Ivoire. As a result, it seemed appropriate to conduct further research into this disease, which has emerged in recent years. The present study aims to investigate the pathogenic diversity of the agent responsible for cashew bacterial blight in Côte d'Ivoire and the susceptibility of the genotypes disseminated.

MATERIALS AND METHODS

Study area: This study was carried out at the Scientific and Innovation Pole of the University Félix Houphouët-Boigny in Abidjan, Côte d'Ivoire, during the period from July, 2022 to May, 2023. Preparation of the inocula followed by inoculation under glass were carried out at the University's Industrial Research Unit.

Materials

Plant material: The plant material consisted of cashew nursery seedlings of the "tout-venant" (TV) genotype from the Yamoussoukro and Korhogo localities and the three cashew genotypes distributed as clones and supplied by an approved nurseryman in the Toumodi locality. These three genotypes, LAX3264, LAX4297 and LAZ330, distributed to growers under the names A24, A27 and A30, respectively, were used for pathogenicity testing and all-plant material.

Bacterial isolates of *Xanthomonas* sp.: A total of 44 bacterial isolates were tested in this study. They were isolated on YPGA culture medium from symptomatic organ samples (leaves, apples and nuts) of cashew trees from cashew-growing regions in Côte d'Ivoire. The isolates came from orchard samples from 44 localities in 19 administrative regions, belonging to six of the seven agroecological zones (AEZ) in Côte d'Ivoire (Table 1).

Methods

Preparation of plant material: Nurseries of the TV genotype cashew trees were established using seeds sown in sterilized soil contained in bags. These nurseries were placed in a greenhouse and watered regularly with 225 mL of water per plant every 2 days.

Table 1: Origins and characteristics of sample areas of cashew orchards affected by bacterial blight

Isolate	Locality	Region	Longitude (West)	Latitude (North)	Agroecological zone (AEZ)
XCI14	Abengourou	Indénié-Duablin	3°30'27,216"	6°48'4,98"	I
XCI23	Tanguélan	Indénié-Duablin	3°21'929"	7°12'241"	I
XCI07	Koudougou	Marahoué	5°40'55"	6°56'12"	II
XCI26	Bonoufla	Haut-Sassandra	6°28'37"	7°9'43"	II
XCI34	Zoukougbeu	Haut-sassandra	6°49'22"	6°46'49"	II
XCI42	Kouétinfla	Marahoué	6°1'0"	6°36'31"	II
XCI03	Minambo	Béliér	5°07'479"	7°07'947"	IV
XCI22	Bédiékro	Iffou	4°5'58,8"	6°55'42,9"	IV
XCI25	Bondoukou	Gontougo	2°45'483"	8°02'465"	IV
XCI28	Bondo	Gontougo	3°13'502"	8°12'836"	IV
XCI29	Dadiassé	Gontougo	2°56'960"	7°49'931"	IV
XCI36	Bongouanou	Moronou	4°25'36,102"	6°35'58,782"	IV
XCI43	Toumodi	Béliér	5°0'32"	6°35'54"	IV
XCI44	Dimbokro	N'Zi	4°49'2,244"	6°39'48,594"	IV
XCI02	Bouaké	Gbèkè	5°08'180"	7°39'268"	V
XCI05	Marahoué	Béré	6°14'27"	8°5'19"	V
XCI08	Lamadougou	Béré	5°55'54"	8°8'43"	V
XCI10	Adamasso	Béré	6°9'20"	7°58'54"	V
XCI18	Sakassou	Gbeke	5°16'57,893"	7°24'19,757"	V
XCI21	Forona	Worodougou	6°37'40"	8°6'56"	V
XCI24	Béoumi	Gbèkè	5°27'478"	7°38'456"	V
XCI31	Téguéla	Worodougou	6°37'32"	7°50'1"	V
XCI39	Benvé 2	Bafing	7°53'58,0"	8°14'48,8"	V
XCI40	Morifingso	Bafing	7°15'50,5"	8°32'34,2"	V
XCI41	Fadiadougou	Worodougou	6°38'2"	8°42'31"	V
XCI01	Niofouin	Poro	6°06'566"	9°36'406"	VI
XCI04	Madinani	Kabadougou	6°58'01,7"	9°35'20,8"	VI
XCI06	Dekokaha	Tchologo	5°11'963"	9°40'287"	VI
XCI09	Samatiguila	Kabadougou	7°34'14,2"	9°47'30,2"	VI
XCI11	Mahandiara	Bagoué	6°56'51"	9°26'12"	VI
XCI12	Tienny	Folon	7°58'06,9"	10°03'13,8"	VI
XCI13	Lamekaha	Tchologo	5°02'046"	9°21'220"	VI
XCI15	Pagala	Hambol	4°37'660"	8°24'455"	VI
XCI16	Torla	Tchologo	5°10'883"	9°58'970"	VI
XCI17	Tortiya	Hambol	5°40'115"	8°44'102"	VI
XCI19	Sinématiali	Poro	9°35'43,7"	5°21'10,8"	VI
XCI20	Kouto	Bagoué	6°25'704"	9°52'041"	VI
XCI30	Sirana	Kabadougou	7°49'44,8"	9°25'46,8"	VI
XCI32	Néguépié	Bagoué	6°32'02,8"	10°22'01,1"	VI
XCI33	Katogo	Poro	6°08'02,3"	10°09'54,7"	VI
XCI35	Arikokaha	Hambol	5°12'241"	8°47'089"	VI
XCI27	Niandégué	Bounkani	2°54'06"	9°16'12,228"	VII
XCI37	Carrefour	Bounkani	3°30'28,4"	9°35'57,4"	VII
XCI38	Vonkoro	Bounkani	2°44'39,0"	9°10'12,0"	VII

The 3 cashew genotypes (A24, A27 and A30) obtained by end-split grafting, aged four months, were also placed and watered under the same conditions.

Inoculum preparation: The inoculum was prepared from *Xanthomonas* isolates purified 48 hrs prior to inoculation. Bacterial colonies on YPGA culture medium were picked using a Pasteur pipette and transferred to test tubes containing sterilized distilled water in a fume hood. The tubes were then hermetically sealed with screws and incubated at 30°C for around 10 hrs. The resulting suspension served as inoculum.

Inoculation of plants with isolates of *Xanthomonas* sp.:

The 48 hrs before inoculation, the plants were placed in transparent plastic bags and then the leaves were sprayed to maintain high humidity. The actual inoculation was carried out using the modified infiltration technique described by Meyer *et al.*¹⁷. This modification involved using a syringe topped with an eraser to mark the leaves. Bacterial inoculum, previously prepared and contained in tubes, was calibrated to an optical density (OD) of 0.2 at a wavelength of 600 nm, corresponding to 10⁸ CFU mL⁻¹. Then, using a 1 mL syringe topped with an eraser, 0.5 mL of bacterial inoculum, at a

Table 2: Disease severity rating scale Moral and Trapero¹⁹

Note	Percentage of diseased leaf area (%)
1	No symptoms
2	1-4
3	5-9
4	10-19
5	20-29
6	30-44
7	45-59
8	60-75
9	>75

concentration of 10^8 CFU mL⁻¹, was infiltrated onto the underside of the leaf, on either side of the main vein, at a rate of three infiltration points per leaf. Three plants were required per isolate, with four leaves per plant. A negative control was made using sterile distilled water. The experiment was repeated three times. After inoculation, regular watering was carried out every 2 days to maintain the high humidity conditions conducive to disease expression. Cashew plants were regularly monitored until the appearance of the first symptoms. A total of 44 isolates from cashew-growing areas were evaluated. Experiments were conducted under average temperature conditions of 34°C and 80% relative humidity.

Parameters measured or observed: The incubation period is the time between the contamination of cashew plants by the bacterial isolate and the appearance of the first symptoms of bacteriosis.

The incidence of symptoms (I) is the rate of infection of the disease, taking into account the number of infected infiltration points where symptoms have appeared out of the total number of infiltration points, obtained with the formula of Cooke¹⁸:

$$I (\%) = \frac{N_m}{N_t} \times 100$$

where, N_m is number of infiltration points where symptoms appeared and N_t is total number of infiltration points.

The symptom severity index (Is) expresses the intensity of symptoms observed on the leaves of cashew plants. Symptoms were assessed every five days for one month, using the Moral and Trapero¹⁹ rating scale ranging from 1 to 9, as illustrated in Table 2.

From these scores, the symptom severity index was calculated using the formula of Song *et al.*²⁰:

$$I_s (\%) = \frac{\sum (X_i \cdot n_i)}{N \cdot Z} \times 100$$

where, X_i is grade (disease severity), n_i is number of times grade i , N is number of times plant was inoculated (number of infiltration points) and Z is highest grade on the scale.

Statistical analysis of data: The data collected were recorded in Excel 2019 and analyzed using Statistica version 7.1. First, they were subjected to a classification criterion Analysis of Variance (ANOVA). The Newman-Keuls test was used to classify means to determine homogeneous groups in case of a significant difference at the $\alpha = 0.05$ threshold. Then performed a multivariate analysis with R Studio. This was a hierarchical classification of principal components (HCPC). The HCPC (hierarchical clustering on principal components) approach thus made it possible to combine the three standard methods used in multivariate data analysis^{21,22}: (i) Principal component methods, (ii) Hierarchical clustering and (iii) Clustering by partition, in particular the k-means method.

RESULTS

Expression of bacterial blight symptoms after inoculation:

Disease expression was analyzed regarding the incubation period and the extent and types of symptoms appearing on the leaves.

The time between inoculating the leaves with the bacteria and the appearance of the first symptoms varied from 48 to 96 hrs from one genotype to another. Thus, the first symptoms of bacteriosis were observed two days after inoculation with *Xanthomonas* sp. on the different genotypes A24, A27, A30 and TV.

Inoculated cashew leaves developed various symptoms for all genotypes tested with *Xanthomonas* sp. isolates. Two types of necrosis were observed at the infiltration points, in contrast to the control (Fig. 1a-d). These were dark-brown, angular necrotic spots on the leaf blade around the veins of the TV genotype (Fig. 1e) but with a yellow halo on the disseminated clones A24 and A27 (Fig. 1f-g). Small and black, angular necrotic spots were also observed all over the inoculated leaf surface of genotype A30. These angular necrotic spots are also more numerous in the veins (Fig. 1h).

Average incidence of bacterial blight symptoms: The average incidence of brown spots ranged from 96.15 to 84.85%. The highest incidences were obtained on the TV genotypes, with a rate of 96.15%, followed by A24 (96.02%) and A30 (92.80 %). The lowest incidence was observed with genotype A27, with a rate of 84.85%. Statistical analysis showed a significant difference between the

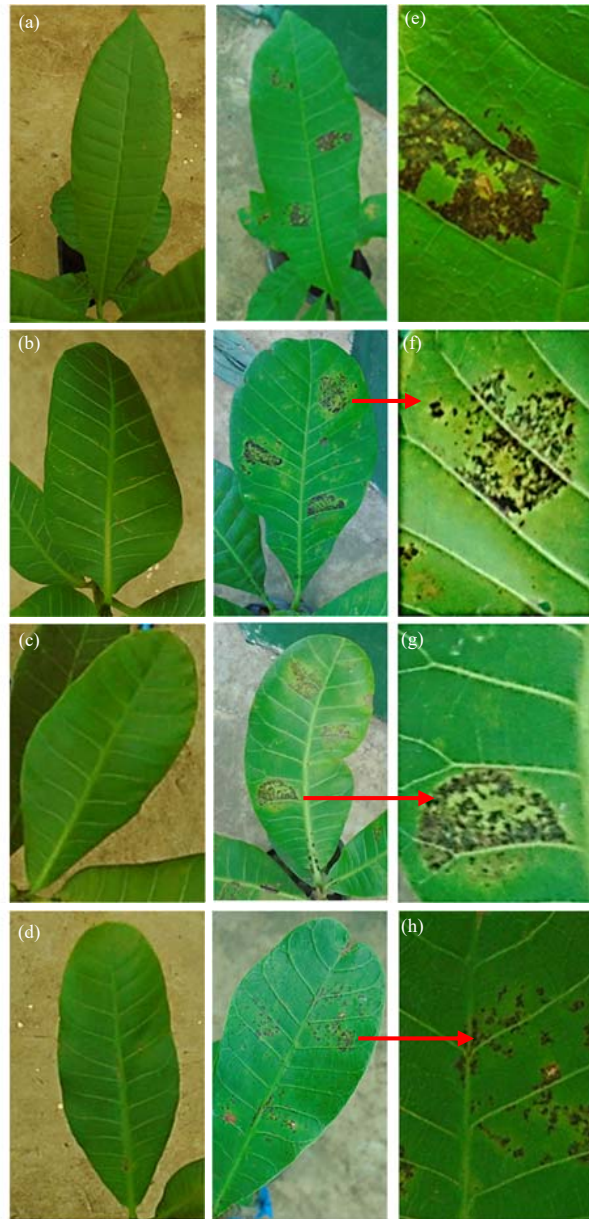


Fig. 1(a-h): Symptoms of bacterial blight caused by *Xanthomonas* sp. on cashew leaves 25 days after inoculation, (a) TV control, (b) A24 control, (c) A27 control, (d) A30 control, (e) TV inoculated, (f) A24 inoculated, (g) A27 inoculated and (h) A30 inoculated

observed symptoms ($F = 12.45$, $p = 0.000 < 0.05$). Thus, two homogeneous groups were determined: Group 1, with TV, A24 and A30 and group 2, with genotype A27 (Fig. 2).

Analysis of the results in Fig. 3 shows that for the TV and A24 genotypes, the incidence of symptoms increased with time, reaching a maximum value of around 96% 15 days for the TV genotype and 20 days for the A24 genotype after inoculation. For genotype A27, the incidence also increases up to day 20 after inoculation, reaching a maximum incidence of

85%. Finally, symptom incidence for genotype A30 reached its optimum value 10 days after inoculation, with a value of 92%.

Average severity of bacterial blight symptoms after inoculation: The severity index of bacterial blight symptoms varied according to genotypes A27, A24, A30 and TV. The highest severity indices were recorded for genotypes A24 (67.45%) and TV (74.03%), while the lowest was obtained with genotype A27 (41.87%). Statistical analysis revealed a

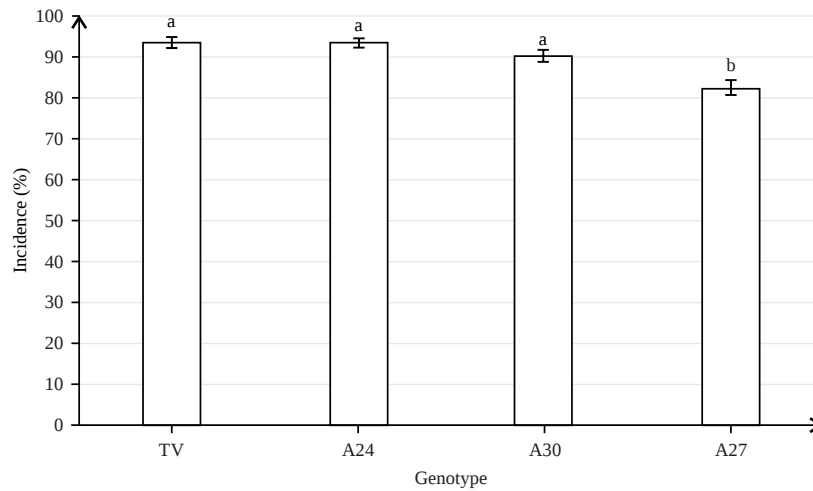


Fig. 2: Average Incidence of bacterial blight symptoms in the greenhouse on different cashew genotypes
Error bars surmounted by the same letter are statistically identical according to the Newman-Keuls test at threshold = 0.05

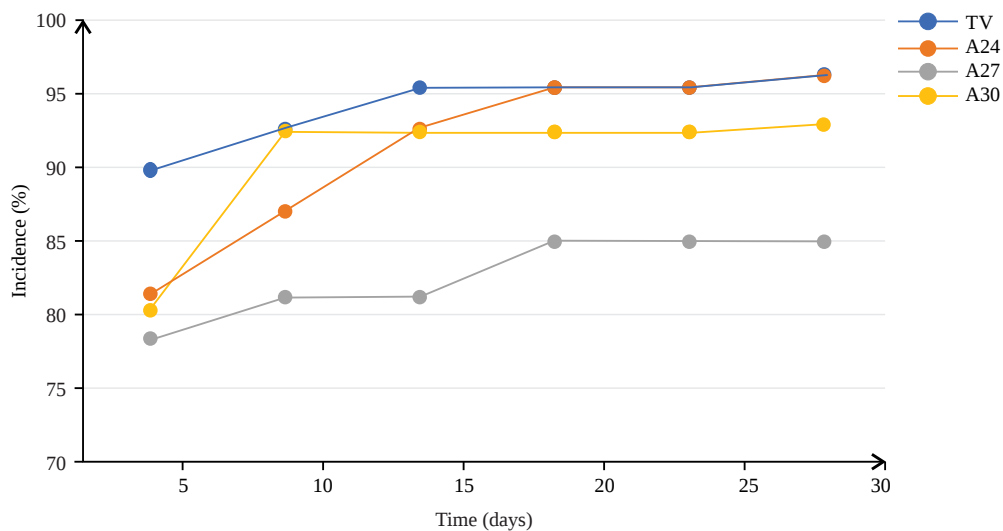


Fig. 3: Evolution of the average Incidence of bacterial blight symptoms in the greenhouse on different cashew genotypes

significant difference between genotype severity index means ($F = 31.98$, $p = 0.000 < 0.05$). Thus, three homogeneous groups were determined: Group 1, with genotypes A24 and TV, group 2, with genotype A30 and group 3, with genotype A27 (Fig. 4).

Figure 5 showed that the symptom severity index for genotypes A24 and TV increased until day 25 post-inoculation, then stabilized until day 30. This index corresponds to a maximum value of 74% for the TV genotype and 68% for the A24 genotype. For genotypes A27 and A30, the severity indices had a maximum value of 41% for genotype A27 on day 25 post-inoculation and 53% for genotype A30.

Pathogenicity groups for bacterial isolates of *Xanthomonas* sp.: The multivariate analysis generated 4 pathogenicity groups of bacterial isolates (Fig. 6) based on the incidence and severity index values expressed for each isolate and cashew genotype:

Class 1: The class 1 isolates, 17 in number, are statistically categorized by the parameter Incidence on A30 and TV genotypes, which have means (73.610 and 58.335%, respectively) significantly lower than the respective overall means (92.802 and 96.149%).

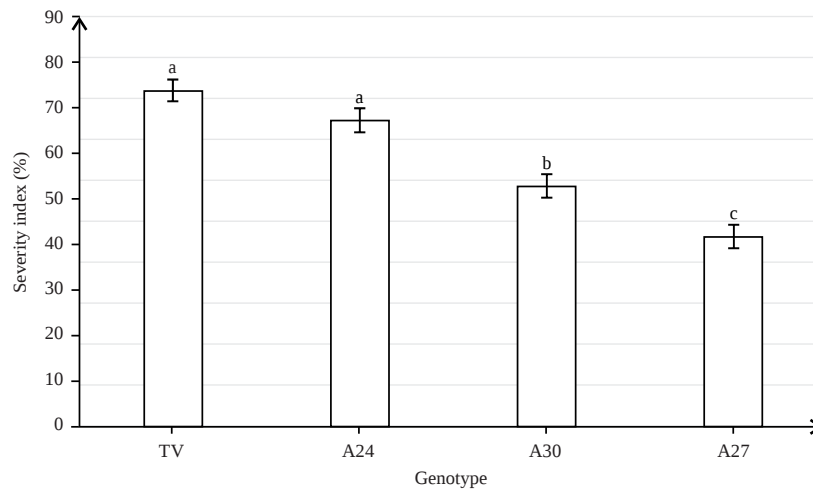


Fig. 4: Average severity of bacterial blight symptoms in the greenhouse on different cashew genotypes

Error bars surmounted by the same letter are statistically identical according to the Newman-Keuls test at threshold $p = 0.05$

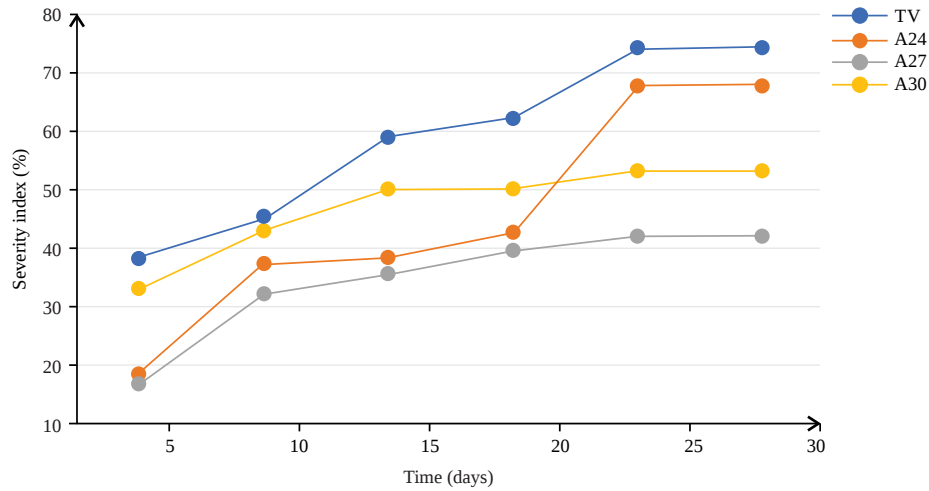


Fig. 5: Evolution of average bacterial blight symptom severity index in the greenhouse on different cashew genotypes

Class 2: Seventeen isolates formed class 2 and were categorized by two parameters: Incidence for genotype A24 and severity for genotypes A27, A30, A24 and TV. For incidence, the mean value for genotype A24 was 93.14%, significantly lower than the overall mean of 96.02%. As for severity, the values for genotypes A27, A30, A24 and TV were 33.44, 41.87, 51.71 and 59.42%, respectively, significantly lower than the overall averages of 41.87, 53.00, 67.45 and 74.03%.

Class 3: The class 3, comprising 2 isolates, was formed by the two parameters: Incidence for genotypes A30 and A27 and severity for genotype A27. For incidence, the respective mean values for genotypes A30 and A27 were 83.33 and 70.99%,

significantly lower than the respective overall means of 92.80 and 84.85%. As for severity, the average for genotype A27 is 30.21%, significantly lower than the overall average of 41.87%.

Class 4: The class 4, comprising eight isolates, was formed by the two parameters incidence for genotypes A27 and A30 and severity for genotypes A27, A30, TV and A24. For incidence, 95.83 and 98.26% averages are significantly higher than the overall averages of 84.85 and 92.80% for genotypes A27 and A30, respectively.

For severity, the four genotypes A27, A30, TV and A24 expressed mean values of 58.58, 68.89, 87.66 and 81.29%, all significantly higher than the overall averages of 41.87, 53.00, 74.03 and 67.45%, respectively.

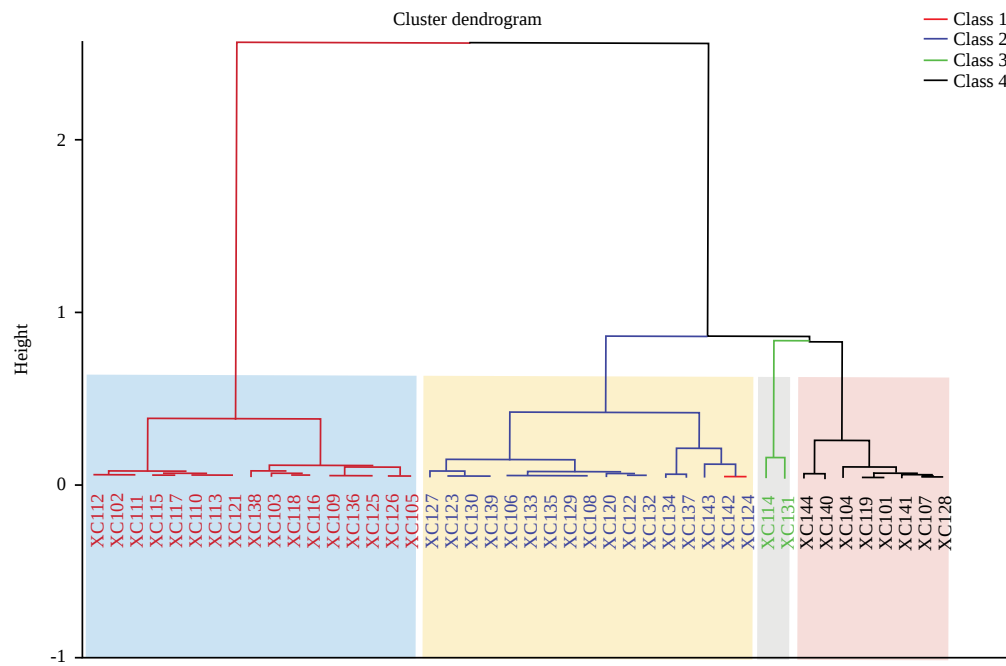


Fig. 6: Dendrogram of bacterial isolate pathogenicity classes

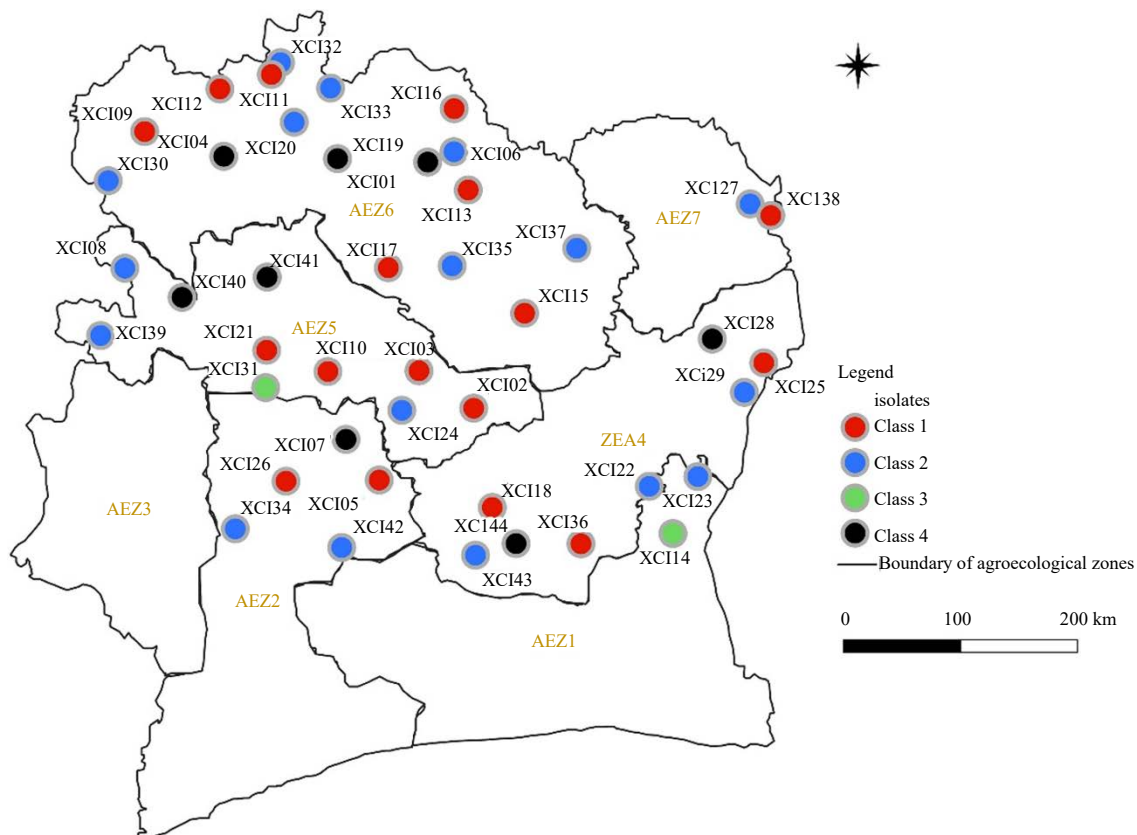


Fig. 7: Distribution of pathogenicity groups of bacterial isolates in cashew nut production zones according to agroecological zones in Côte d'Ivoire

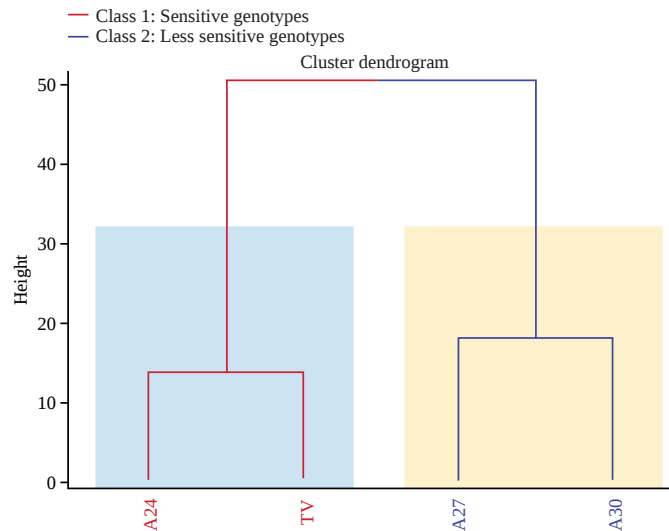


Fig. 8: Dendrogram of susceptibility classes of cashew genotypes distributed in Côte d'Ivoire

This classification shows that the pathogenicity classes are not affiliated with a specific ecological zone (Fig. 7). However, we note that classes 1 and 2, which have the highest number of isolates (17 each), are more present in agroecological zones V and VI, corresponding respectively to the transitional forest and humid tropical savannah zones, at 71% for class 1 and 53% for class 2.

Susceptibility groups of evaluated genotypes: The multivariate analysis generated two groups of genotypes: Class 1 for the more susceptible genotypes A24 and TV and class 2 comprised A27 and A30, less susceptible or more resistant genotypes (Fig. 8).

DISCUSSION

This study assessed the pathogenicity diversity of *Xanthomonas* sp. isolates. Following Koch's principle, the pathogenicity of isolates was tested under semi-controlled conditions on four cashew genotypes, including 3 high-producer genotypes (A24, A27 and A30) and TV genotype, using the infiltration technique. The tested *Xanthomonas* sp. isolates showed their ability to cause necrosis symptoms in the disseminated genotypes. These results corroborated those of previous studies. Studies in Brazil, Burkina Faso, Benin and Côte d'Ivoire have shown that isolates of the *Xanthomonas* genus are responsible for necrosis on cashew leaves observed on plantations. Thus, the most apparent necroses are characterized by angular, translucent, dark-brown spots on the leaf blade near the veins^{11,13,15,23}. The location of these

symptoms could be explained by the fact that the brown spots run from the main vein to the secondary veins. While the bacterium freely colonizes the leaf vessels, the veins themselves prevent the bacterium from progressing into the leaf blade, so spots in this area are small, angular and often very close to the veins²⁴.

The results obtained showed that the isolates tested were virulent. Indeed, between 48 and 96 hrs after inoculation, all isolates induced translucent spots on the leaves at the infiltration points, characteristic symptoms of bacteriosis on cashew leaves. These results were in line with those of Zombre *et al.*¹², who showed that mature cashew and mango leaves inoculated with bacterial suspensions of *Xanthomonas citri* pv. *anacardii* and *Xanthomonas citri* pv. *mangiferaeindicae* at 10^5 CFU mL⁻¹, reproduced typical cashew bacterial blight symptoms in Burkina Faso. These authors, therefore, concluded that cashew and mango are two host species affected by *Xanthomonas citri* pv. *mangiferaeindicae* in Burkina Faso. Furthermore, current study results indicate a high disease incidence in cashew nut production areas in Côte d'Ivoire. The pathogenicity of *Xanthomonas* isolates on the four genotypes showed overall high incidences and severities.

The classification of *Xanthomonas* sp. pathogenic isolates shows that the four pathogenicity classes are not correlated with a typical agroecological zone. This would indicate that the pathogenicity classes of *Xanthomonas* sp. isolates are present in all agroecological zones of Côte d'Ivoire.

The pathogenicity of *Xanthomonas* isolates on the four genotypes showed overall high incidences and severities

following inoculation tests. However, the two sets of genotypes generated after analysis showed that Class 1, made up of genotypes A24 and TV, is more susceptible than Class 2, made up of genotypes A27 and A30, which is less susceptible. The variability of these evaluation parameters between genotypes against *Xanthomonas* sp. is thought to be due to various factors, such as the pathogen virulence and its infection time. The resistance of particular genotypes to bacterial blight and their closed stomata could slow down the infiltration of inoculum into leaf tissue. Also, the diversity of pathogen expression could be due to environmental factors that vary in the cashew-growing area of Côte d'Ivoire. These results corroborated those of Garrett *et al.*²⁵, who showed that environmental conditions strongly influence plant pathogen attacks. They also showed that the onset of disease symptoms could be linked to bacterial virulence, genotype susceptibility and a favourable environment. As a result, these factors could explain the maximum severity recorded 25 days after inoculation, stabilizing up to day 30 in A24 and TV genotypes as a function of time, which is 80%. The optimum severity for genotypes A27 and A30 is around 60%. The same applies to the assessment of incidence. All four genotypes proved susceptible to cashew bacterial blight. However, genotypes A24 and TV were more susceptible than genotypes A27 and A30.

CONCLUSION

Pathogenicity tests with *Xanthomonas* sp. isolates confirmed the sensitivities of the disseminated and the TV genotypes. Evaluation of the susceptibility of four cashew genotypes to bacterial blight infection by *Xanthomonas* sp. showed that inoculated leaves of the genotypes showed angular necrosis typical of bacterial blight after artificial infiltration. The 44 *Xanthomonas* sp. isolates tested showed pathogenicity on the four cashew genotypes evaluated, with incidences and severity indices varying according to genotype. Overall, we can conclude that these genotypes are susceptible to high incidence and severity of bacterial blight. These results suggest that the high-productivity genotypes disseminated are susceptible to cashew bacterial blight in Côte d'Ivoire. Bacterial blight control strategies should, therefore, be considered.

SIGNIFICANCE STATEMENT

The aim of this study was to evaluate the susceptibility of cashew genotypes to various isolates of *Xanthomonas* sp. the agent responsible for cashew tree bacterial blight in

Côte d'Ivoire. This work is in line with on the distribution of cashew tree bacterial blight in Côte d'Ivoire. This study assessed the time to onset of symptoms, incidence and severity index. The 44 *Xanthomonas* sp. isolates tested showed their pathogenicity in the marking zones where infiltrations were made on the different cashew genotypes tested after 30 days of incubation. Multivariate analysis generated four pathogenicity groups for the 44 isolates and two susceptibility groups for the cashew genotypes tested.

ACKNOWLEDGMENTS

The authors would like to thank the Interprofessional Fund for Agricultural Research and Consulting (FIRCA), the Project to Promote the Competitiveness of the Cashew Value Chain (PPCA), the Cotton and Cashew Council (CCA), the National Program of Research on Cashew (PNRA). Our thanks also go to the West African Science Service Centre on Climate Change and Adapted Land Use (WASCAL)/African Center of Excellence in Climate Change, Biodiversity and Sustainable Agriculture (CEA-CCBAD) doctoral program at the University Félix HOUPOUËT-BOIGNY in Abidjan, Côte d'Ivoire, for the facilities made available to us to carry out this work.

REFERENCES

1. Martin, K.P., 2003. Plant regeneration through direct somatic embryogenesis on seed coat explants of cashew (*Anacardium occidentale* L.). Sci. Hortic., 98: 299-304.
2. Bezerra, M.A., C.F. de Lacerda, E.G. Filho, C.E.B. de Abreu and J.T. Prisco, 2007. Physiology of cashew plants grown under adverse conditions. Braz. J. Plant Physiol., 19: 449-461.
3. Bhat, M.G., K.V. Nagaraja and T.R. Rupa, 2010. Cashew research in India. J. Hortic. Sci., 5: 1-16.
4. Agboton, C., A. Onzo, F.I. Ouessou, G. Goergen, S. Vidal and M. Tamò, 2014. Insect fauna associated with *Anacardium occidentale* (Sapindales: Anacardiaceae) in Benin, West Africa. J. Insect Sci., Vol. 14. 10.1093/jisesa/ieu091.
5. Babatunde, O.P., O.O. Adeigbe, O.I. Sobowale, A.A. Muiyiwa and S.T. Balogun, 2023. Cashew production and breeding in 5 West African countries. J. Sci. Res. Rep., 29: 28-39.
6. Sinan, A. and N.K. Abou, 2016. Socio-economic impacts of cashew cultivation in the sub-prefecture of Odiénne (Ivory Coast) [In French]. Eur. Sci. J., 12: 369-383.
7. Moreira, R.C., J.S. Lima, L.G.C. Silva and J.E. Cardoso, 2013. Resistance to gummosis in wild cashew genotypes in Northern Brazil. Crop Prot., 52: 10-13.
8. Guy, B.K., O.Z. Franck, D. Dabé, L.K. Bertrand, K.K.I. Nazaire and D.D. Odette, 2021. Identification of agromorphological determinants favoring the propagation of bacterial disease in agroforestry farms of cashew trees in Côte d'Ivoire. Asian J. Adv. Agric. Res., 15: 29-37.

9. Ouali-N'Goran, S.W.M., E.N. Akessé, G.M. Ouattara and D. Koné, 2020. Process of attack on cashew tree branches by *Diastocera trifasciata* (Coleoptera: Cerambycidae) and the relationship between these attacks and the phenological stages in the Gbêkê Region (Central Côte d'Ivoire). Insects, Vol. 11. 10.3390/insects11080456.
10. Nakpalo, S., S. Sibirina, K. Tchoa, A. Kouabenan, K. Mongomake and K. Daouda, 2017. Parasitical fungi in cashew (*Anacardium occidentale* L.) orchard of Cote d'Ivoire. Plant Pathol. J., 16: 82-88.
11. Gama, M.A.S., K.C. da Silva Felix, A.M.F. da Silva, L.A. dos Santos, M.M.B. dos Santos and L.J. do Nascimento Souza, 2013. Cashew and bacterial diseases: Angular leaf spot and xanthomonas spot [In Portuguese]. Anais Acad. Pernambucana Ciênc. Agron., 10: 123-142.
12. Zombre, C., P. Sankara, S.L. Ouédraogo, I. Wonni and K. Boyer *et al.*, 2016. Natural infection of cashew (*Anacardium occidentale*) by *Xanthomonas citri* pv. *mangiferaeindicae* in Burkina Faso. Plant Dis., 100: 718-723.
13. Wonni, I., D. Sereme, I. Ouedraogo, A.I. Kassankagno, I. Dao, L. Ouedraogo and S. Nacro, 2017. Diseases of cashew nut plants (*Anacardium occidentale* L.) in Burkina Faso. Adv. Plants Agric. Res., 6: 78-83.
14. Gama, M.A.S., R.L.R. Mariano, F.M.P. Viana, M.A.S.V. Ferreira and E.B. Souza, 2011. Polyphasic characterization of pigmented strains of *Xanthomonas* pathogenic to cashew trees. Plant Dis., 95: 793-802.
15. Kouman, A.M.N., S. Soro, B. Camara, N. Silue and A.A. Tehua *et al.*, 2022. Distribution of cashew tree bacterial blight (*Anacardium occidentale* L.) in the cashew nut production areas of Cote d'Ivoire. Agron. Afr., 34: 21-33.
16. Kouakou, C.K., A. N'da Adopo, A.J.B. Djaha, D.P. N'da, H.A. N'da, I.A. Zoro Bi and K.K. Koffi *et al.*, 2022. Genetic characterization of promising high-yielding cashew (*Anacardium occidentale* L.) cultivars from Côte d'Ivoire. Biotechnol. Agron. Soc. Environ., 24: 46-58.
17. Meyer, D., E. Lauber, D. Roby, M. Arlat and T. Kroj, 2005. Optimization of pathogenicity assays to study the *Arabidopsis thaliana*-*Xanthomonas campestris* pv. *campestris* pathosystem. Mol. Plant Pathol., 6: 327-333.
18. Cooke, B.M., 2006. Disease Assessment and Yield Loss. In: The Epidemiology of Plant Diseases, Cooke, B.M., D.G. Jones and B. Kaye (Eds.), Springer, Dordrecht, Netherlands, ISBN: 978-1-4020-4581-3, pp: 43-80.
19. Moral, J. and A. Trapero, 2009. Assessing the susceptibility of olive cultivars to anthracnose caused by *Colletotrichum acutatum*. Plant Dis., 93: 1028-1036.
20. Song, W., L. Zhou, C. Yang, X. Cao, L. Zhang and X. Liu, 2004. Tomato Fusarium wilt and its chemical control strategies in a hydroponic system. Crop Prot., 23: 243-247.
21. Koh, K.Y., S. Ahmad, J.I. Lee, G.H. Suh and C.M. Lee, 2022. Hierarchical clustering on principal components analysis to detect clusters of highly pathogenic avian influenza subtype H5N6 epidemic across South Korean poultry farms. Symmetry, Vol. 14. 10.3390/sym14030598.
22. Maugeri, A., M. Barchitta, G. Basile and A. Agodi, 2021. Applying a hierarchical clustering on principal components approach to identify different patterns of the SARS-CoV-2 epidemic across Italian regions. Sci. Rep., Vol. 11. 10.1038/s41598-021-86703-3.
23. Houndahouan, D.E.T., R. Sikirou, A. Banito, A. Basso and V. Zinsou *et al.*, 2022. Assessment and diagnostic of cashew seedling diseases in nurseries in Benin. Net J. Agric. Sci., 10: 9-19.
24. Viana, F.M.P., J.E. Cardoso, H.A.O. Saraiva, M.A.S.V. Ferreira, R.L.R. Mariano and L.C. Trindade, 2007. First report of a bacterial leaf and fruit spot of cashew nut (*Anacardium occidentale*) caused by *Xanthomonas campestris* pv. *mangiferaeindicae* in Brazil. Plant Dis., 91: 1361-1361.
25. Garrett, M., A. Fullaondo, L. Troxler, G. Micklem and D. Gubb, 2009. Identification and analysis of serpin-family genes by homology and synteny across the 12 sequenced *Drosophilid* genomes. BMC Genomics, Vol. 10. 10.1186/1471-2164-10-489.