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# Pathological Variation in Cassava Bacterial Blight (CBB) Isolates in Nigeria

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**Abstract:** Cassava (*Manihot esculenta* Crantz), a major food crop in tropical lowlands, is susceptible to many pathogens that decrease yields. The most common biotic constraint to its production worldwide is Cassava Bacterial Blight (CBB) caused by *Xanthomonas axonopodis* pv *manihotis* (*Xam*). We determined the virulence characteristics of *Xam* strains isolated on cassava plants, in a nationwide survey of disease in Nigeria, on six different cassava cultivars in the greenhouse to know the possible pathogenic variation in their population structure. The cassava varieties were Isu, 96/0037 and 60142, known to be susceptible to CBB from the field records and 30572, 4(2)1425 and 92/0430 with moderate resistance. Out of 72 strains examined, 12.9% were classified as highly virulent (mean score>4) and 71.9% were grouped as virulent, with symptom development in classes 3 to 4. Also, 12.5% were considered as less virulent strains (>2.5<3) and 2.8% were non-pathogenic. It was observed that 4(2)1425 was most resistant cultivar compared to other varieties examined. Statistically, two clones were most susceptible, 96/0037 was able to resist 8.2% of the *Xam* populations and was susceptible to 58.5%; 60142 resisted 8.8% and was susceptible to 61.9%. We suggested possible strain specificity among the cassava cultivars, since the response to CBB differed from one cultivar to another. More resistant cassava accessions need to be developed to guarantee food security in Nigeria.

**Key words:** Virulence · pathological variation · *Xanthomonas axonopodis* pv. *manihotis* · cassava

## INTRODUCTION

Cassava (*Manihot esculenta* Crantz), native to South America, is a major food crop in tropical lowlands where it grows successfully in poor soils and under drought conditions [1, 2]. Cassava, however, is susceptible to many pathogens that decrease yields. More than thirty bacterial, fungal, viral, virus-like mycoplasma and nematode agents have been reported to attack cassava [3]. The most common biotic constraint to its production worldwide is cassava bacterial blight (CBB), caused by a vascular pathogen, *Xanthomonas axonopodis* pv. *manihotis* (*Xam*). Yield losses in different areas of Africa and South America may be anywhere between 12% and 90% [4, 5]. During the epiphytotic years of 1970-1975 when losses in central Africa were as high as 80%, CBB contributed immensely to starvation in Zaire [6].

Host-plant infection occurs through stomata and epidermal wounds, causing symptoms of angular leaf spot [7]. The pathogen then penetrates vascular tissues in stems as the disease progresses, to the whole plant [6]. Pathogen multiplication and the attendant production of bacterial slime block vascular tissues, leading to leaf wilt, production of exudates and dieback [7]. Bacterial exudates are spread to other plants through splashing during heavy rain and, to a much less extent, by insects. Because cassava is vegetatively propagated, planting materials form another significant vehicle for disease dissemination [8].

The use of disease-free planting materials and adoption of appropriate cultural practices have been suggested as ways of reducing disease incidence and crop loss [6]. However, these disease control measures have not been fully adopted in traditional cassava producing regions, making host-plant resistance the preferred method of control [8]. The wild relative *Manihot glaziovii* and *Manihot. esculenta* have been identified as sources of resistance to CBB [9, 10] from which Polygenic additively inherited resistance has been developed. Resistance can also be found in a genetically broad range of cassava accessions and is not limited to one or a few 'lineages' of the host [11]. Whether the two types of resistance to CBB act in the same way or are controlled by the same alleles is still unknown.

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Differences in virulence among *Xam* isolates were first described by Robbs *et al.* [12] in South American countries. The variability of *Xam*, based on pathogenicity and on physiological, biochemical and (recently) molecular characterization, reveals greater genetic diversity in Latin America than in Africa or Asia [2, 13-16].

Virulence variation was observed among isolates from Brazil and Africa [17, 18]. Fifty-two isolates collected in Colombia from different locations were grouped into four groups of virulence after inoculation onto three cassava varieties. Isolates also showed differences in the rate of symptom development, suggesting variation in aggressiveness [15, 19]. More recently, 10 pathotypes were defined among 91 *Xam* isolates in Venezuela using five cassava cultivars as differentials [2]. Restrepo *et al.* [20] confirmed the variation in the Colombian population of *Xam* when pathotype groups were defined and differences in aggressiveness were shown using 17 cassava cultivars. A high level of pathogenic diversity was reported to exist within each edapho-climatic zone of Colombia in their study.

In recent times the populations of these important pathogenic bacteria have not been monitored in Nigeria to know the pathogenic behavior and diversity within the population structure of the bacteria as a means to develop effective control measures against the pathogens. Hence, we reported the pathogenic variation in Nigerian populations of the bacteria.

## MATERIALS AND METHODS

**Cassava cultivars:** Six cassava varieties were usedthat have been observed to have contrasting levels of field resistance to CBB in the cassava fields of International Institute of Tropical Agriculture (IITA), Ibadan, over the years. The varieties were Isu, 96/0037 and 60142, known to be susceptible to CBB from field records and 30572, 4(2)1425 and 92/0430 with moderate resistance. Woody stems of the six cassava varieties free from CBB were selected from IITA's cassava plots. Cuttings 15 cm in length were surface sterilized for 20 min with 1% solution of Chlorox (5.25% sodium hypochlorite) containing 8 to 10 drops of Tween 20 to facilitate adhesion of the sodium hypochlorite to the stem cuttings.

**Bacterial isolates and Inoculation techniques:** All 72 field isolates collected in 2000 from all the agro-ecological zones of Nigeria and identified as 68 strains of *Xanthomonas axonopodis* pv *manihotis* and 4 strains of *Xanthomonas axonopodis* pv. *cassavae* [21] were evaluated for their virulence characteristics. The bacteria were cultured on Glucose Yeast Calcium Agar (GYCA) [22]. A 2-day-old culture of each bacterial isolate was washed off GYCA plates with 20 ml of sterile distilled water. A few drops of the bacterial cell suspension containing approximately $10^6$ to $10^8$ cells/ml was injected into the stem and leaf lamina of disease-free plants using a hypodermic syringe and needle. Stems and leaves of the plants were inoculated with the bacterial suspension. Inoculated plants were incubated for 48 hours in transparent polyethylene bags to ensure a relative humidity of 80-82% and day temperature of 28-30°C, conditions that would support the establishment of bacteria in the plants.

**Methods of disease assessment:** The assessment of the severity of CBB was based on observation of disease symptoms on the inoculated plants at 10 day intervals for 30 days, starting from the first 10 days after inoculation. On the leaves, observations were concentrated on the three inoculated leaves per plant and on new leaves developing after inoculation. For plants that were inoculated by the stem injection method, leaves above the inoculation points were considered.

**Statistical analyses:** Duncan's new multiple range tests (DNMRT) of SAS; version 8 [23] were used to compare treatment means.

## RESULTS

The pathogenicity of the bacterial isolates was assessed on six cassava varieties. Isu, 96/0037 and 60142 (susceptible to CBB) and 30572, 4(2)1425 and 92/0430 (moderate resistant). The pathogenicity test carried out in the greenhouse indicated that these bacteria varied in their virulence. Of the 72 strains tested, 12.9% were classified as highly virulent (mean score>4); 71.9% were grouped as virulent with symptom development in classes 3 and 4. Also 12.5% were considered as less virulent strains (>2.5<3) and 2.8% were non-pathogenic (Fig. 1). The yellow variants of the bacteria had moderate virulence on the cassava (with symptom development in the following range: (93, 3.8; 90B, 3.7; 92Y, 3.6 and 90A, 3.3) and were grouped with the virulent class (Table 1). The statistical analysis revealed that there were significant differences in the virulence level of the bacterial strains (Table 1). Thus, pathologically, four strains were identified to be present in the Nigerian

Table 1: Pathological-typing and variation in virulencw levels of cassava bacterial blight population on Nigeria

| Treatment | 10 days after inoculation | 20 days after inoculation | 30 days after inoculation | Treatment | 10 days after inoculation | 20 days after inoculation | 30 days after inoculation |
|---|---|---|---|---|---|---|---|
| 131B | 1.75 | 3.00 | 4.17a | 104B | 1.33 | 2.75 | 3.58 |
| 41B | 1.25 | 2.92 | 4.08b | 98 | 1.25 | 2.42 | 3.50g |
| 52A | 1.42 | 3.00 | 4.00c | 88 | 1.33 | 2.50 | 3.50 |
| 18 | 1.33 | 1.92 | 4.00 | 45B | 1.17 | 2.58 | 3.50 |
| 119A | 1.67 | 3.00 | 4.00 | 117 | 1.17 | 2.17 | 3.50 |
| 115A | 1.50 | 3.00 | 4.00 | 114A | 1.08 | 2.83 | 3.50 |
| 109B | 1.55 | 2.91 | 4.00 | 113B | 1.98 | 2.92 | 3.50 |
| 106B | 1.50 | 2.92 | 4.00 | 1.2B | 1.25 | 1.92 | 3.50 |
| 55 | 1.17 | 2.08 | 3.92d | 92M | 1.50 | 2.58 | 3.42h |
| 128 | 1.67 | 3.00 | 3.92 | 73 | 1.33 | 2.50 | 3.42 |
| 1.24B | 1.75 | 2.92 | 3.92 | 69 | 1.17 | 2.33 | 3.42 |
| 113A | 1.27 | 3.00 | 3.91 | 40 | 1.42 | 2.17 | 3.42 |
| 89A | 1.42 | 2.83 | 3.83e | 1A | 1.67 | 2.58 | 3.42 |
| 48A | 1.25 | 2.17 | 3.83 | 130B | 1.50 | 2.50 | 3.42 |
| 32A | 1.08 | 2.83 | 3.83 | 103A | 1.25 | 2.33 | 3.42 |
| 118B | 1.17 | 2.92 | 3.83 | 90A | 1.42 | 2.75 | 3.33I |
| 93 | 1.42 | 2.92 | 3.75 | 83 | 1.42 | 2.50 | 3.33 |
| 89B | 1.25 | 3.00 | 3.75 | 76A | 1.50 | 2.75 | 3.33 |
| 22B | 1.42 | 2.67 | 3.75 | 32B | 1.42 | 2.17 | 3.33 |
| 127 | 1.67 | 3.08 | 3.75 | 23B | 1.08 | 2.17 | 3.33 |
| 124A | 1.75 | 2.75 | 3.75 | 9 | 1.33 | 2.50 | 3.25j |
| 118A | 1.75 | 3.00 | 3.75 | 38B | 1.33 | 2.45 | 3.00k |
| 110B | 1.25 | 3.00 | 3.75 | 121B | 1.25 | 2.42 | 3.00 |
| 96A | 1.25 | 2.92 | 3.67 | 120A | 1.42 | 2.08 | 3.00 |
| 90B | 1.25 | 2.75 | 3.67 | 27A | 1.08 | 2.75 | 2.921 |
| 62 | 1.25 | 2.67 | 3.67 | 121A | 1.33 | 2.33 | 2.92 |
| 39 | 1.25 | 2.33 | 3.67 | 119B | 1.33 | 2.33 | 2.92 |
| 114B | 1.58 | 2.92 | 3.67 | 38A | 1.64 | 2.36 | 2.91 |
| 112A | 1.42 | 3.00 | 3.67 | 80 | 1.50 | 2.58 | 2.83m |
| 103B | 1.33 | 2.92 | 3.67 | 11B | 1.25 | 2.33 | 2.83 |
| 92Y | 1.17 | 2.25 | 3.58f | 23A | 1.17 | 2.00 | 2.50n |
| 59 | 1.17 | 2.42 | 3.58 | 28A | 1.09 | 1.64 | 2.36o |
| 131A | 1.58 | 2.58 | 3.58 | 74A | 1.08 | 1.67 | 2.08p |
| 130A | 1.25 | 2.75 | 3.58 | 13B | 1.25 | 1.50 | 2.08 |
| 129 | 1.50 | 2.50 | 3.58 | 5A | 1.17 | 1.17 | 1.58q |
| 125B | 1.58 | 2.58 | 3.58 | 5B | 1.08 | 1.17 | 1.17 |
| 112B | 1.33 | 3.00 | 3.58 | Control | 1.0 | 1.17 | 1.17 |

Mean values for 12 observation

Means with diffrent letters are signification diffrent

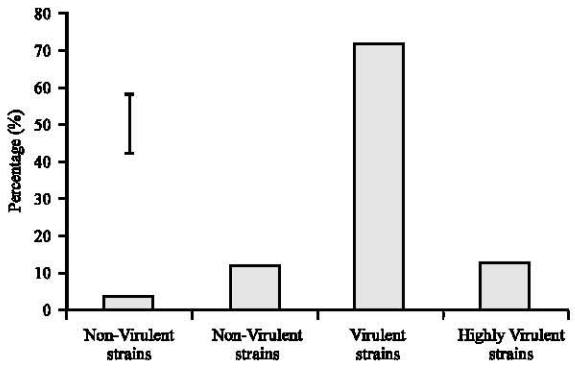


Fig. 1: Virulence characteristics of 72 *Xanthomonas axonopodis* pv. *Manihotis* strains isolated in Nigeria on 6 cassava cultivars

population of *Xathomonas axonopodis* pv. *Manihoti:* non-virulent, the less virulence, the virulent and highly or extremely virulent. Four isolates of the bacteria were considered non-virulent. These were 28A, 23A, 13B and 5B, all from the humid forest agro-ecological area. Only very few of the bacteria were able to maintain consistent virulence across the six cassava varieties. These were 131B, 41B, 52A, 18, 119A, 115A, 109B and 106B, all from different agro-ecological zones, from the humid forest to the arid and semiarid zones.

The six cassava varieties used in the pathogenicity test were also compared on the basis of disease symptom manifestation. It was observed that 4(2)1425 was most resistant to the infection compared to other varieties

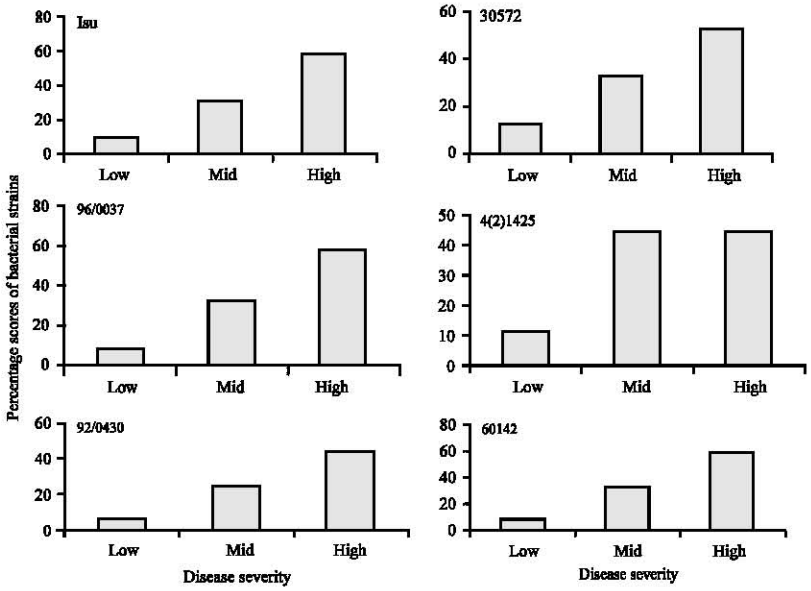


Fig. 2: Susceptibility patterns of six different cassava genotypes to *Xanthomonas axonopodis* pv. *Manihotis* population in Nigeria under a controlled environmental in the screen-house

Table 2: Statistical evaluation of the response of six cassava genotypes to CBB inoculation in the screenhouse

| Clones | 10 days after inoculation | 20 days after inoculation | 30 days after inoculation |
|---|---|---|---|
| Isu | 1.44371[a] | 2.41060[c] | 3.3775[b] |
| 30572 | 1.44079[a] | 2.52318[b] | 3.29801[c] |
| 96/0037 | 1.30263[c] | 2.76160[a] | 3.38411[b] |
| 4(2) 1425 | 1.26316[d] | 2.42105[c] | 3.24342[d] |
| 92/0430 | 1.31126[c] | 2.43709[c] | 3.32450[c] |
| 60142 | 1.37333[b] | 2.52000[b] | 3.43333[a] |

Mean values for 152 observations

Means with the same letter in a column are not significantly different

examined, (Table 2). Statistically, Isu and 30572 were the most susceptible clones in the first 10 days of inoculation, considering the average of 152 observations. There was no significant difference in the response of 96/0037 and 92/0430 with Duncan new multiple range tests for means separation in the first 10 days of observation. On the 20 days, 96/0037 was the most susceptible and Isu was the most resistant clone. At the close of the experiment 60142 was the least resistant clone; 30572 and 92/0430 were not significantly different and were next to 4(2)1425 in resistance to symptom manifestation. The response of each of these cassava genotypes to bacterial infection is shown in Fig. 2. Ten percent of the total bacterial isolates were not aggressive to Isu, while 58.5% were highly aggressive; 34% of the bacteria were mildly aggressive against 30572 and 32.7% against 92/0430. Both clones were able to resist 12.9% of the bacteria strains. Only 44.2% of the bacterial isolates were highly aggressive against 4(2) 1425; this clone was able to resist most of the aggressive *Xam* populations, hence, 45% of the bacterial strains were mildly aggressive on the genotype. This is the most resistant of the six cassava accessions examined in the greenhouse environment; two clones were most susceptible, 96/0037 was able to resist 8.2% of the *Xam* populations and was susceptible to 58.5 %; 60142 resisted 8.8% and was susceptible to 61.9%. A few of the bacteria that were aggressive against 60142 seemed to be mild on 92/0430 and low in virulence on 4(2)1425. This suggests the possibility of strain specificity to cassava clones.

## DISCUSSION

Inoculation of six cassava varieties with the *Xam* strains collected from different agro-ecological zones of Nigeria revealed four levels of virulence, indicating a high level of diversity within the pathogen population. The highly virulent group was present in all the zones. The statistical analysis revealed that there were significant differences in the virulence level of the bacterial strains. The virulence strains were not ecologically influenced in distributions, that is, they were not restricted to a particular agro-ecological area of the country. In this study, virulence variation was examined in terms of stem and leaf reactions to all the bacterial

strains on susceptible and resistant cassava cultivars. Very few strains were non-virulent. The majority were classified as virulent and highly virulent. Variation in the aggressiveness of *Xanthomonas axonopodis* pv. *manihotis* in Colombia has been reported [16] but the pathogenic structure has no correlation with the geographical origin of the strains. Differences in *Xam* virulence have been reported previously [17, 24, 25]. Five levels of virulence were distinguished among 32 strains from different areas of Brazil and from CIAT when Alves and Takatsu, [25] inoculated two cassava cultivars differing in resistance. They reported that distribution of virulent and weakly virulent isolates of *Xam* was not restricted to distinct geographical regions [25]. Pathogenic variability was also studied by Maraite *et al.* [26] using five strains of *Xam* and 13 cassava cultivars from Africa, Asia and South America with varying degrees of field resistance. Their data revealed significant differences in virulence among the strains besides the existence of some pathogenic specialization, because some strains were particularly virulent on specific cultivars and less on others. The authors proposed the need of further studies on a large number of plants per cultivar and a wider range of strains to confirm the results. Wydra and Msikita [27] stated that the disease occurred frequently in all ecozones of Ghana, Bénin and Nigeria and no ecozonal differentiation in the occurrence of highly virulent strains was also observed. All these reports emphasized that the virulence of the strains studied was not correlated with biochemical and physiological properties or geographical distribution. There have been repeated regional epidemic outbreaks of CBB, 1996 to 2000, in West and Central Africa. This may be due to the development and emergence of highly virulent strains of *Xam*.

From the reactions of the cassava cultivars in the greenhouse to bacteria inoculation, no variation was observed in initial colonization of leaves by the bacteria on either the susceptible or the resistant cultivars. It seems likely that the infection on the leaves by the bacteria occurred at the same rate in both resistant and susceptible cultivars. However, the spread of the disease symptoms was restricted to the leaves only in resistant cultivars. This suggests that resistance to the bacterial infection and the defense gene mechanism in the plant express their action in the vascular tissue of the stem: leaf infection is not enough to determine resistant cultivars. Kpémoua *et al.* [28] stated that resistance is expressed as a reduced rate of disease development in stems with the number of infected xylem vessels lower in resistant than in susceptible cultivars and that the defense mechanism had been characterized and included phenol production and xylem vessel occlusion by lignin-like compounds. Boher and Daniel [29] also reported in a similar observation that, during the epiphytic phase and intercellular multiplication in the mesophyll, there were no limiting factors or actions on parasite development in resistant varieties. However, the healing rate of lamina wounds, the high inoculum threshold required for ensuring infection or else rapid shedding of infected leaves, thus preventing stem contamination, are factors which may account for the resistant behavior of some varieties.

The six cassava cultivars used in accessing the virulence variation among the bacterial isolates also manifested variations in their responses to the disease infestation. Clone 4(2)1425 showed the highest level of resistance to the majority of the bacteria and 30572 was next in being able to resist bacterial infection; Statistically, two clones were most susceptible, 96/0037 was able to resist 8.2% of the *Xam* populations and was susceptible to 58.5%; 60142 resisted 8.8% and was susceptible to 61.9%. This suggested that there could be strain specificity among the cassava cultivars, since the response to bacterial disease differed from one cultivar to another. Maraite *et al.* [26] reported the existence of some pathogenic specialization, because some strains were particularly virulent on specific cultivars and less on others. However, no clear interactions between cassava accessions and strains of the bacteria have yet been established. In such studies, there is a lack of suitable ways of selecting the cassava varieties that could be used for establishing an appropriate set of host differentials since there are more than 6000 cassava accessions available in the world cassava collections held in CIAT [11]. Lozano [6] reported that a clear distinction of races appeared to be difficult among the bacterial populations. Races of the pathogen have not been described in the literature.

This may possibly explain the occurrence of CBB in several cassava growing regions of the world.

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