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Prevalence and polymerase chain reaction detection of *Xanthomonas axonopodis* pv. *manihotis* causal agent of cassava bacterial blight disease in Osun state, Southwestern Nigeria

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Abstract

Bacterial blight is one of the major diseases of cassava, caused by *Xanthomonas axonopodis* pv. *manihotis (Xam)* Bondar (Vauterin et al.) in Nigeria. It induces extensive necrosis on the leaves, twigs and stems systemically, which accounts for significant yield loss. Field survey for symptoms of cassava bacterial blight (CBB) disease was conducted across ten Local Government Areas (LGAs) notable for cassava production in Osun State. Disease incidence and severity were determined by visual examination and scoring of infected plants. Leaf samples obtained from 60 farmers' fields spread over the ten LGAs during the rainy season of 2016 were bioassayed using polymerase chain reaction (PCR) to detect the presence of the pathogen. Two Xam specific primers, 1525R (5'-AGGAGGTGATCCAGCC-3'). and 27F (5'-AGAGTTTGATC (A/C)TGGCTCA-3') were used to amplify 16s gene in total nucleic acid extracted from diseased leaf samples. Leaf samples from Ife North LGA had the highest incidence and severity of 76.10% and 4.5 respectively of CBB disease. PCR results showed high DNA concentration and presence of *Xam* in eight LGAs with infectivity varying from mild to highly infected. However, further studies will be required to determine the diversity among Xam isolates in the State in order to elicit appropriate control measures.

Keywords: Bioassay, Disease severity, Germplasm, Polymerase chain reaction, *Xanthomonas axonopodis* pv. *manihotis*

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Introduction

Cassava (*Manihot esculenta* Crantz) is a root tuber which provides a rich source of carbohydrate for over 700 million people worldwide, mainly in developing countries of tropical regions (FAOSTAT, 2015). The crop serves as a source of income for peasant farmers in the tropics where hunger, starvation and unemployment prevails thereby contributing positively to poverty alleviation. Cassava is a staple food in Nigerian and majority of the tubers produced is consumed locally as traditional meals (Simonyan and Joshua, 2014). The resilience of cassava enables it to grow successfully under a wide range of agroecological zones where cereals and other crops cannot thrive, making it a suitable crop for poor farmers to cultivate under marginal environments in Africa (Samura et al. 2014). Its production requires very low inputs and gives significant harvests where other crops would fail (Lebot et al., 2015).

Although cassava displays high productivity under drought and poor soil conditions, it is susceptible to field diseases and postharvest deterioration (Patil et al., 2015). The major cassava disease limiting productivity in different parts of Africa is cassava bacterial blight (CBB). It causes significant yield loss ranging between 12 and 100% in infected fields, especially when a virulent species or more

aggressive strains of the pathogen occur in a region where highly susceptible cultivars are grown and under favourable environmental conditions (Ogunjobi, 2006; López and Bernal, 2012; Chenge et al., 2017).

The pathogen, *Xanthomonas axonopodis* pv. *manihotis*, causes cassava bacterial blight (Martin et al., 2017) and has been ranked as one of the top ten most important bacterial phytopathogens (Trujillo et al., 2014). The bacterium enters the leaves through wounds or natural openings mainly hydathodes and stomata (Mansfield et al., 2012). Once inside, it multiplies rapidly in the intercellular spaces of the mesophyll and subsequently in the vascular tissue of the plant, where it acquires the ability to move systemically (Verdier et al., 2004). Severe CBB outbreak causes shortages in the supply of healthy planting materials during the growing and next planting season (Emmanuel, 2007). Symptoms of CBB include blighting of leaves, shoots and twigs necrotic appearance on the vascular tissues, angular leaf spot and secretion of exudates from infected stems (Trujilo et al., 2014). Cassava is propagated mainly by stem cuttings and the pathogen is thus frequently spread through infected planting material.

The disease can be controlled through cultural methods including the use of chemical sprays uninfected planting material and breeding for resistance.

Although the production of healthy planting material can reduce the incidence of the disease, this method is limited by ability of *X. axonopodis* pv. *manihotis* to survive in tissue without causing symptoms (Ogunjobi, 2006; Onyeka et al., 2008). Effective breeding for resistance in cassava requires information on the diversity and geographical distribution of the pathogen and this can be facilitated by using sensitive detection techniques. As a consequence of quarantine procedures, international exchange of cassava germplasm is limited and occurs through true seeds or material propagated in-vitro (Dixon et al., 2002; Mamba-Mbayi et al., 2014). The success of a cassava seed certification programme will depend on the availability of reliable tests for detecting the pathogen in seeds and vegetative materials. Conventional methods for identifying and detecting *X. axonopodis* pv. *manihotis* include isolating the bacterium in culture media and conducting immunoassays of tissue extracts (Mc Donald and Linde, 2002). Enzyme-linked immunosorbent assays for *X. axonopodis* pv. *rnanihotis* may improve detection and are rapid, but immunological reactions are not entirely specific because of cross-reactions with some strains of other pathovars of *X. axonopodis* (Chenge et al., 2017). Therefore, this study evaluated a more sensitive and precise method using polymerase chain reaction (PCR) for the distribution and detection of *X. axonopodis* pv. *manihotis*.

Materials and Methods

Field survey for cassava bacterial blight (CBB) diseased leaf samples

Field sampling for symptoms of CBB was carried out across ten Local Governments Areas (LGAs) that were notable for cassava production in Osun state in the rainy season between May and June 2016. Surveyed locations were selected to ensure that adequate cassava fields were available for sampling. Relevant data on cassava production status in the state was obtained from extension officers at the Agricultural Development Project (ADP) of the state and this was helpful in the choice of major cassava producing LGAs that were surveyed. Six farms were visited in each Local Government Area (LGA) and ten diseased cassava leaf samples were collected per farm using random sampling technique and a total of 60 farms were surveyed. Cassava farms were surveyed at intervals of 5 km and the coordinates for each sampling site were recorded using a global positioning system (GPS) device. The locations where the samples of diseased cassava leaves were collected are shown in Figure 1

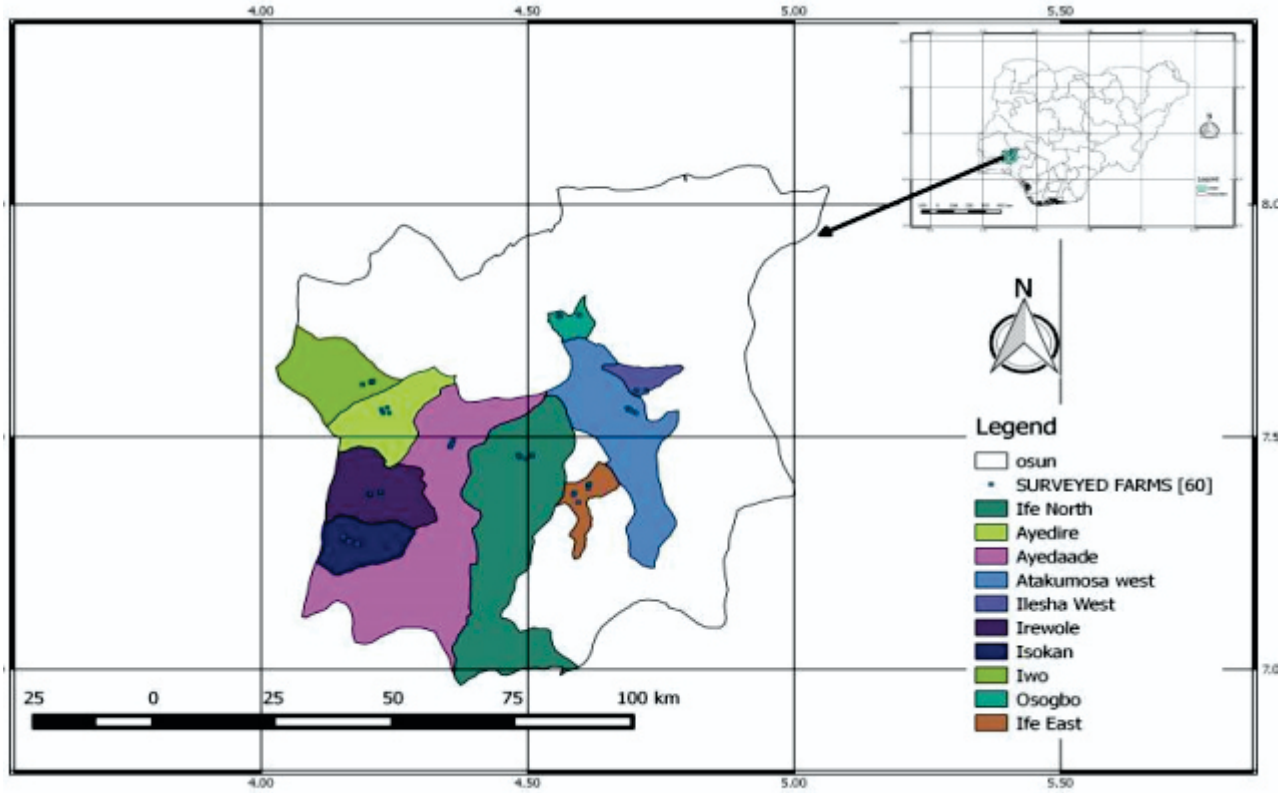

Figure 1: Map of Osun state showing locations where samples of cassava leaves infected with cassava bacterial blight were collected

Evaluation of incidence and severity of cassava bacterial blight

Fifty cassava plants were randomly sampled for CBB disease symptoms in each of the six farms with a total of 300 plants in each LGA. Incidence of CBB was recorded as the number of cassava plants showing leaf symptoms of the disease expressed as percentage of the total number of plants assessed (IITA, 1990).

$$Incidence = \frac{Number\ of\ infected\ plants \times 100}{Total\ number\ of\ plants\ per\ treatment}$$

The severity of CBB was scored on a rating scale of 1 – 5 using the scale by Wydra and Msikita (1998): 1 = no symptom; 2 = only angular leaf spots; 3 = angular leaf spots, wilting, blighting, defoliation; and some exudates on stem/petioles; 4 = blighting of leaves, wilting, defoliation, exudates, and tip dieback; 5 = blighting of leaves, wilting, defoliation, exudates, tip dieback, and plant stunting.

Total nucleic acid extraction of Xam from infected cassava leaves

DNA of the pathogen was extracted from symptomatic leaf samples using the Cetyl trimethylammonium bromide (CTAB) protocol (Doyle and Doyle, 1987) One hundred milligram of homogenized leaf sample was ground in 1000µl of extraction buffer in a sterile mortar and pestle and the macerated sap was poured into sterile ependorf tubes, vortexed briefly and incubated in a water bath at 60°C for 10 minutes, cooled to room temperature and Phenol, Chloroform and Iso-amyl alcohol were then added at ratio 25:24:1 respectively. The solution was vortexed and centrifuged (12000 rpm for 10 min). Four hundrerd and fifty microlitre of the supernatant was dispensed into new sterile tube and 300µl of cold isopropanol was added which helped to precipitate the DNA. The sample was mixed, incubated for 1hour at -20°C and centrifuged (at 12000 rpm for 10 min) to sediment the DNA. Thereafter, the supernatant was decanted; five hundred microlitre (500µl) of 70 % ethanol was added to the DNA pellets and centrifuged at 12,000 rpm for 5 min. Ethanol was decanted and the DNA air-dried at 37°C for 30 min. The DNA pellet was later suspended and then suspended in 50µl TE buffer and stored at -20°Cfor further.

Amplification of Polymerase Chain Reaction (PCR)

An aliquot of 0.25 µl of forward (27F 0.2µM) and 0.25 µl of reverse primers (1525R 0.25µM) purchased from the Bioscience unit of the International Institute of Tropical Agriculture (IITA), Ibadan and sequence of 5'-AGGAGGTGATCCAGCC-3' and 5'-AGAGTTTGATC(A/C)TGGCTCA-3' respectively were used in PCR. Each contained 12.5 µl in 200 µl thin-walled tubes mixed with 2 µl of DNA template and 0.06µl Taq DNA polymerase (Ochman et al. 1988). An aliquot of 0.75 µl master mix consisting of $MgCl_2$, 0.25 µl of dNTPs (0.2mM), 2.5 µl of buffer, 6.44 µl of sterile distilled water was then added to the solution. The mix was vortexed, centrifuged and 12.5 µl aliquots master mix were loaded into each PCR tube (200 µl size). The start cycle was for 35 times, one initial denaturizing cycle at 94 °C for 30 seconds during annealing step at 50°C for 30 seconds extension and elongation step at 72 °C for 30 seconds.. This was followed by final extension step at 72°C for 5 min (Arnheim and Erlich, 1992). Amplified PCR products were analyzed using gel electrophoresis in (1.5%), agarose stained with ethidium bromide, visualized and photographed under UVB 310 nm trans illuminator. PCR reagents were briefly vortexed and centrifuged an then12.5µl PCR master mix was loaded into each 200µl PCR tube. The DNA template used as positive control was *X. axonopodis* pv. *phaseoli* LMG 7455.

Agarose Gel Electrophoresis

One and half grammes of agarose powder was mixed with electrophoresis buffer by dissolving Tris-acetate-EDTA (TAE) in 40 ml distilled water to make 1.5% gel and heated to melting point in a microwave oven Model JEP2261WPSL, USA. About 0.5 ug/ml of ethidium bromide was added to the mixture and allowed to cool to 60ºC. The mix was then poured into a casting tray suspension to solidify. The gel was allowed to polymerize and run with Tris acetate buffer containing 100 nM 0.1M Tris-HCl pH 8.0, 1.4 M NaCl, and 20 mM EDTA pH 7.5 for 1h. Samples containing DNA mixed with loading buffer were then loaded into the sample wells, and voltage of 150V was applied and ran for 1 h or until dye migration. The distant DNA which migrated in the gel was assessed visually under ultraviolet light migration of the tracking dyes like bromophenol blue and xylene cyanol dyes. The result of the nucleic acid concentration was read by using nanodrop spectrophotometer MN 900 AA wavelength 900-200ng/uL, Nanodrop Technologies, USA.

Data analysis

Data on incidence and severity of CBB disease were analysed using Generalised Linear Model Statistical Analysis System Ver. 9.1, Institute 2002, USA. Means was separated using Least Significant Difference (LSD) test at $p \leq 0.05$.

Results

Incidence and severity of cassava bacterial blight in the surveyed areas

Leaf samples that were collected from surveyed farms in Ife North and Isokan Local Government Areas (LGAs) had the highest mean incidences of 76.10 and 74.40% of CBB respectively, while the lowest incidence of 33.90% occurred at Ife East LGA (Table 1). Ayedaade LGA had the lowest mean severity score of 1.5 among the LGAs visited while cassava leaf samples that were obtained in Ife North had the highest mean severity of 4.5.

Table 1: Mean incidence and disease severity rating of cassava bacterial blight in surveyed Local Governments Areas (LGAs) in Osun state

LGA	Incidence	Severity
Atakumosa West	43.71	2.87
Ayedaade	50.74	1.53
Ayedire	65.47	3.52
Ife East	33.91	3.28
Ife North	76. 12	4.50
Ilesa West	55.23	2.32
Irewole	41.81	4.31
Isokan	74.47	3.84
Iwo East	61.10	3.73
Osogbo	54.76	3.04
SE±	0.54	0.03
LSD	12.16	0.65

The disease infection rate was mainly characterised by moderate to severe infection with seven of the ten LGAs belonging to this category. Only three of the LGAs had severity rating of < 3.0, which implies that cassava samples obtained from these locations exhibited mild symptoms of CBB

Amplification of Polymerase Chain Reaction (PCR)

Five of the test leaf samples evaluated from six farms in Ife North LGA (OK1, OK2, OK4, OK5 and OK6) had high DNA concentration that ranged between 1252.6 and 6847.9 ng/µl and were also of high protein content (1.46 – 2.19 nm) (Table 2). Although sample OK3 had very low DNA concentration of 23.8 ng/µl, the PCR result, however, showed that the leaves were highly infected (+++). Conversely, sample OK1 with high DNA concentration of 3128.1 ng/µl and protein of 2.1nm showed mild infection in the sample during PCR process. Diseased leaf samples collected from cassava farms in Osogbo LGA (OS1-0S6) also had a very high DNA concentration of *X. axonopodis* pv. *manihotis* that occurred in the range of 827.4-2444.4 ng/µl and high protein (1.94- 2.13 nm). However, three of the samples (OS1, OS2 and OS5) proved to be disease-free (-) in the PCR result even though they showed symptoms of cassava bacterial blight disease when they were collected from farmers' fields during survey.

Although infected leaf samples that were collected in Ayedaade and Ilesa west LGAs all had high DNA concentration of the pathogen, the PCR result however, showed that all the samples were free from disease (-) even though they were symptomatic during physical examination of the leaves in the field. All the test leaf samples evaluated from Isokan LGA had high DNA concentration of *X. axonopodis* pv. *manihotis* that ranged between 940.7 and 2000.8 ng/µl but the PCR result, however, showed that one of the samples was disease-free (-). Five of the test leaf samples obtained from Iwo LGA had high DNA concentration ranging between 1477.5 and 2135.9 ng/µl and high protein that ranged from 1.91-2.11 nm. The PCR showed that all samples from this LGA were infected with *X. axonopodis* pv. *manihotis* with infectivity ranging from moderate to high. Similarly, the PCR result showed that all samples from Ife East LGA were infected with *X. axonopodis* pv. *manihotis* the blight causing pathogen with infectivity ranging from moderate to high. Although all the test leaf samples evaluated from cassava farms in Ayedire LGA had high DNA concentration of *X. axonopodis* pv. *manihotis,* the PCR result however, indicated that sample OL6 was disease-free (-) even though it was symptomatic on the field while the remaining five samples were infected with *X. axonopodis* pv. *manihotis* with infectivity ranging from mild to high.

Five of the test leaf samples evaluated from Irewole LGA had high DNA concentration that ranged between 707.80-1781.00 ng/µl, however, sample IK3 had low DNA concentration value of 57.70 ng/µl. All the samples had high protein purity that ranged from 2.10-2.25 nm. The PCR result however, indicated that sample IK5 was disease-free (-) even though it was symptomatic on the field while the remaining five samples were highly infected with *X. axonopodis* pv. *manihotis*. All the test leaf samples collected from cassava farms in Irewole and Atakumosa west LGAs had high DNA concentration and protein of *X. axonopodis* pv. *manihotis*, but the PCR result showed that samples IK5 and AT6 were disease-free respectively, regardless of being symptomatic on visual examination in the field.

Table 2: DNA concentration and PCR indexing of *X. axonopodis* pv. *manihotis* in diseased leaf samples

SN	Sample ID	Source (LGA)	Nucleic acid (ng/µl)	Protein 260/280 (nm)	Purity 260/230 (nm)	PCR result
1	0K1	Ife North	3128.1	2.1	2.23	+
2	OK2	Ife North	6847.9	2.11	2.18	+++
3	OK3	Ife North	23.8	1.87	0.99	+++
4	OK4	Ife North	1252.6	2.19	2.35	+++
5	OK5	Ife North	1268.9	1.46	1.47	+++
6	OK6	Ife North	3828.1	2.12	2.19	+++
7	OS1	Osogbo	1531.3	1.98	1.66	-
8	OS2	Osogbo	827.4	1.94	1.42	-
9	OS3	Osogbo	1475	2.09	1.89	++
10	OS4	Osogbo	1196.1	2.13	2.18	+++
11	OS5	Osogbo	2444.4	2.12	2.2.3	-
12	OS6	Osogbo	957.7	2.10	2.41	++
13	GB1	Ayedaade	3621.4	2.08	2.11	-
14	GB2	Ayedaade	1243.2	2.09	1.97	-
15	GB3	Ayedaade	1540	2.08	2.13	-
16	GB4	Ayedaade	1236.1	2.06	1.97	-
17	GB5	Ayedaade	1704.2	2.09	2.04	-
18	GB6	Ayedaade	767.5	2.06	1.77	-
19	SE1	Ilesa West	909.9	2.04	1.81	-
20	SE2	Ilesa West	68.6	1.99	1.22	-
21	SE3	Ilesa West	1068.4	2.09	1.85	-
22	SE4	Ilesa West	83.5	1.80	0.94	-
23	SE5	Ilesa West	93.1	1.65	0.71	-
24	SE6	Ilesa West	1431.6	2.16	2.13	-
25	IS1	Isokan	1313.1	2.13	2.13	+++
26	IS2	Isokan	2000.8	2.08	2.10	+++
27	IS3	Isokan	1745.4	2.05	1.86	++
28	IS4	Isokan	1986.9	2.16	2.13	+
29	IS5	Isokan	940.7	2.10	2.01	-
30	IS6	Isokan	1505.4	2.06	1.86	++
31	IW1	Iwo East	248	1.91	1.33	++
32	IW2	Iwo East	1477.5	2.08	1.94	+++
33	IW3	Iwo East	1726.6	2.10	2.12	++
34	IW4	Iwo East	2135.9	2.18	2.15	+++
35	IW5	Iwo East	1568.9	2.11	2.12	+++
36	IW6	Iwo East	1740.1	2.14	2.07	+++
37	AK1	Ife East	809.5	2.11	2.09	++
38	AK2	Ife East	1789.3	2.08	1.93	+++
39	AK3	Ife East	36.9	1.83	0.98	++
40	AK4	Ife East	2252.4	2.08	2.98	++
41	AK5	Ife East	1367.6	2.03	1.77	++
42	AK6	Ife East	1077.1	2.06	1.80	++
43	OL1	Ayedire	1132.3	2.11	2.31	++
44	OL2	Ayedire	1203.2	1.78	1.11	+++

45	OL3	Ayedire	882.2	1.75	0.82	+++
46	OL4	Ayedire	785.6	1.96	1.35	+
47	OL5	Ayedire	671.7	2.01	1.64	+
48	OL6	Ayedire	1845.1	2.01	1.75	-
49	IK1	Irewole	1110.9	2.17	1.97	+++
50	IK2	Irewole	914.7	2.12	2.04	+++
51	IK3	Irewole	57.7	2.25	1.32	+++
52	IK4	Irewole	1642.9	2.11	2.18	+++
53	IK5	Irewole	1781	2.13	2.25	-
54	IK6	Irewole	707.8	2.13	2.14	+++
55	AT1	Atamakusa West	965.1	1.99	1.59	+
56	AT2	Atamakusa West	914.6	2.13	2.06	++
57	AT3	Atamakusa West	2420.2	2.11	2.14	++
58	AT4	Atamakusa West	1720.2	2.03	1.78	+
59	AT5	Atamakusa West	1581.7	2.09	1.99	+
60	AT6	Atamakusa West	2146.2	2.12	2.14	-

Legend
- Negative = disease free
+++ Positive = Highly infected
++ Positive = Moderately infected
+ Positive = Mildly infected

Agarose Gel Electrophoresis

Leaf samples from five of the six farms evaluated for the presence of the blight pathogen, *X. axonopodis* pv. *manihotis* in Ife north LGA showed thick bands in the Gel electrophoresis, which was an indication that the pathogen was present in those locations (Plate 1a) However, there was presence of faint band in sample 1 and this implies that the pathogen was absent in that farm location or mild inoculum concentration. The blight pathogen was less predominant in Osogbo LGA, with the presence of band in only three of the six farm locations evaluated, while it was absent in the remaining three sampled locations. There was, however, absence of bands in all symptomatic leaf samples collected from the six farms each in Ayedaade and Ilesa west LGAs (samples 13-24), which is an indication that the pathogen was not present in the samples even though they showed symptoms of bacterial blight diseases on the field. (Plates 1a and b).

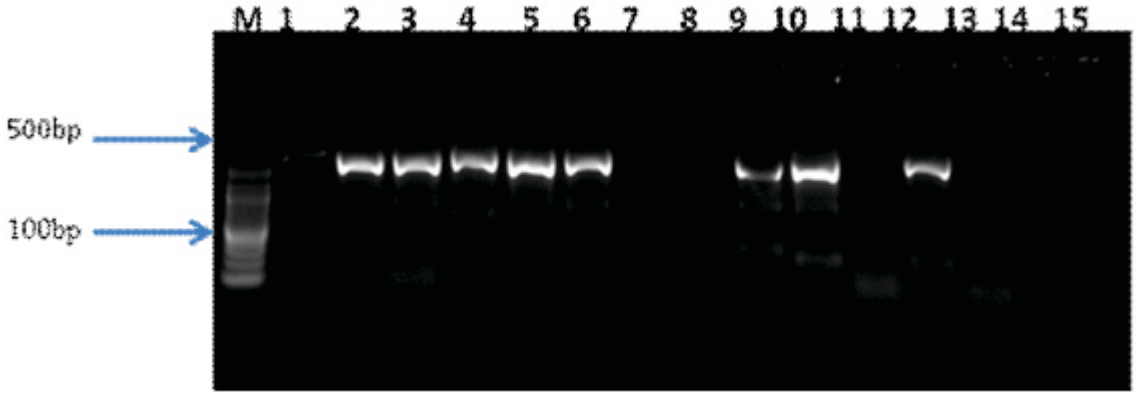

Plate1a: Agarose Gel electrophoresis showing the result of Polymerase chain reaction using 1525R and 27F to amplify 16s gene in total nucleic acid extracted from diseased samples (1-15). Samples 1-6 = Leaf specimens collected from Ife North LGA, Samples 7-12 = Leaf specimens collected from Osogbo LGA, Samples 13-15 = Leaf specimens collected from Ayedaade LGA. M= Standard reference ladder

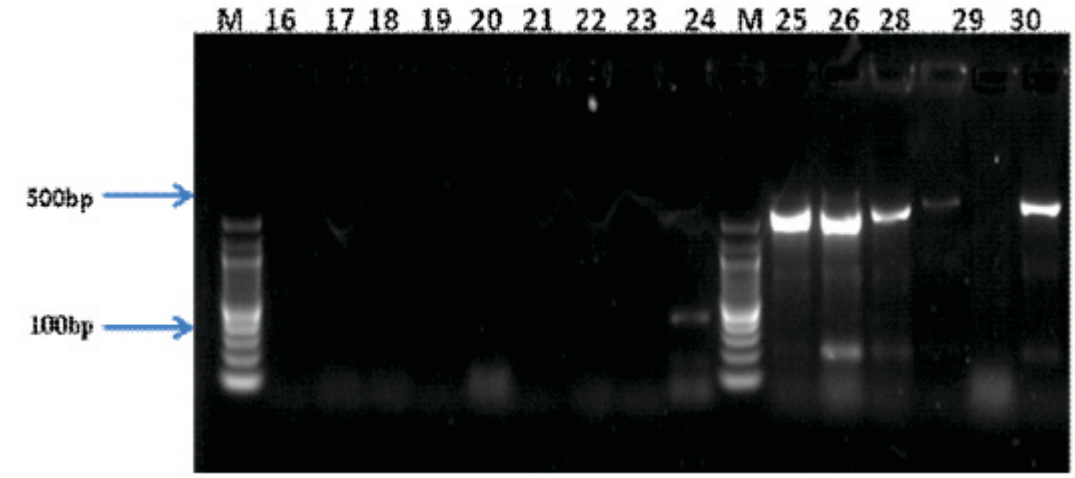

Plate 1b: Agarose Gel electrophoresis showing the result of Polymerase chain reaction using 1525R and 27F to amplify 16s gene in total nucleic acid extracted from diseased samples (16-30). Samples 16-18 = Leaf specimens collected from Ayedaade LGA, Samples 19-24 = Leaf specimen collected from Ilesa West LGA, Samples 25-30 = Leaf specimen collected from Isokan LGA. M= Standard reference ladder

The blight pathogen was present in all the samples evaluated from six cassava farms each from Iwo East and Ife East LGAs (samples 31-42) as indicated by the presence of bands in the gel electrophoresis result while only three of the six sampled farms in Ayedire LGA showed the presence of *X. axonopodis* pv. *manihotis* even though all the test leaf samples collected from the farmers' fields showed leaf blight symptoms (Plate 1c). The Gel electrophoresis result showed that the bacterial blight pathogen was present in five of the leaf samples obtained from six farmers' fields (49, 50, 51, 52 and 54) in Irewole LGA as indicated by the presence of bands. The pathogen was, however, absent in only one sampled location as indicated by the absence of band (Plate 1d). Similarly, the pathogen was also present in five of the six surveyed farm samples (samples 55-59) collected from Atakumosa LGA. However, two of the sampled farms showed presence of faint bands which was an indication of lower inoculum concentration

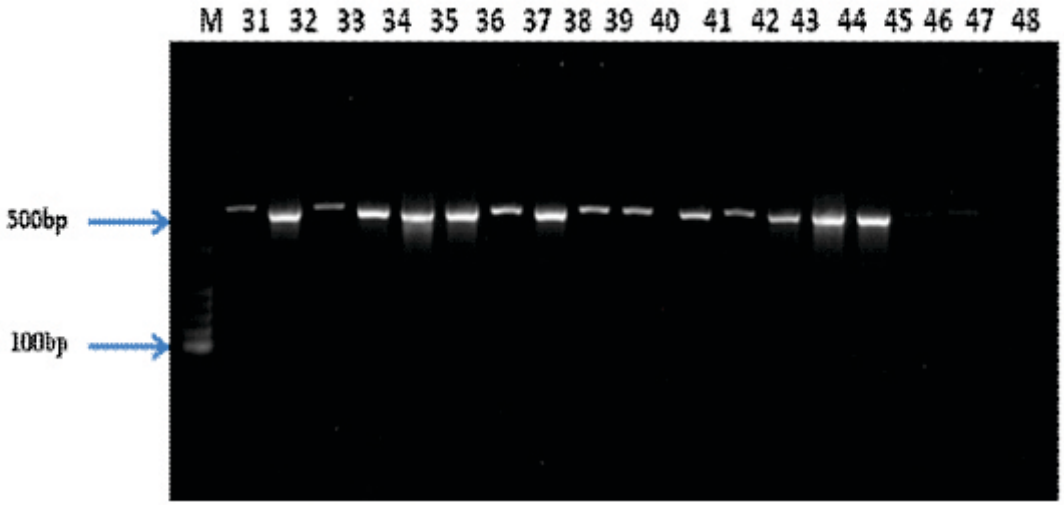

Plate 1c. Gel electrophoresis showing the result of Polymerase chain reaction using 1525R and 27F to amplify 16s gene in total nucleic acid extracted from diseased samples (31-48). Samples 31-36= Leaf specimens collected from Iwo East LGA, Samples 37-42 = Leaf specimens collected from Ife East LGA. Samples 43-48 = Leaf specimens collected from Ayedire LGA.

M= Standard reference ladder

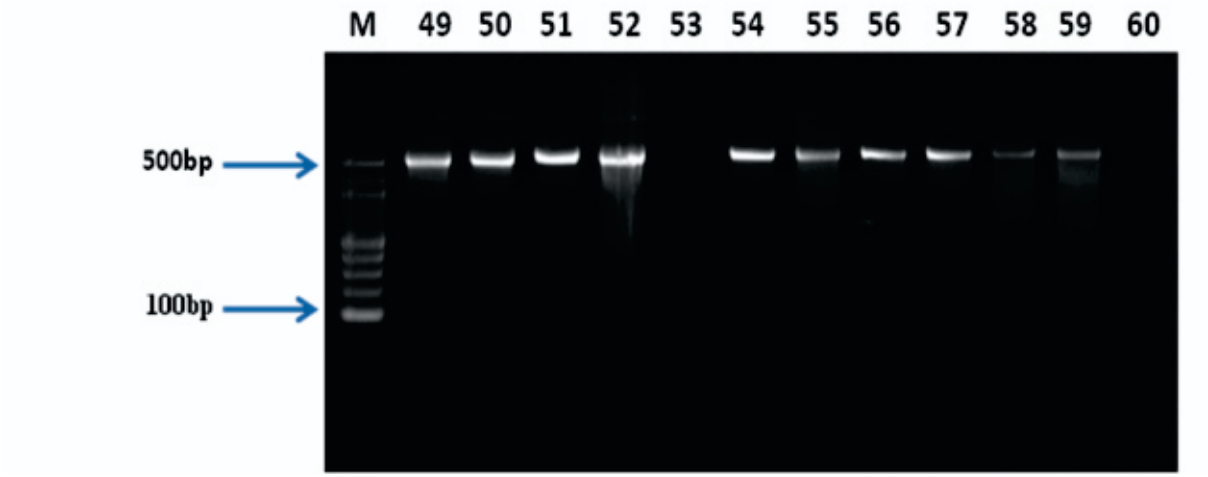

Plate 1d: Gel electrophoresis showing the result of Polymerase chain reaction using 1525R and 27F to amplify 16s gene in total nucleic acid extracted from diseased samples (49-60). Samples 49-54 = Leaf specimens collected from Irewole LGA.Samples 55-60 = Leaf specimens collected from Atakumosa West LGA. M= Standard reference ladder

Discussion

Field survey showed significant variation in incidence and severity of CBB among the different LGAs in Osun state; the variability in the degree of infection of the disease is most likely to make accurate forecast of an impending epidemic of the disease difficult. Previous authors have reported variations in the dynamics of CBB across various ecozones (Onyeka et al., 2008). There was high incidence and severity of CBB in seven of the ten LGAs evaluated by visual examination of symptoms in the field. The remarkable presence of the disease may be due to the location of the state in the Derived savanna agroecological zone, which is characterised by moderate annual rainfall ranging between 1300 mm and 1500 mm (Atehnkeng et al., 2008). This result is consistent with the findings of Onyeka et al. (2008) and Martin et al. (2017) who reported higher prevalence of CBB in the Savanna ecozone relative to the occurrence of the disease in the humid rainforest. The high incidence of the disease in the Savanna agroecology could be attributed to the inherent ability of the causal organism, *X. axonopodis* pv. *manihotis* to survive in plant debris during periods of unfavourable climatic conditions, especially the dry season (Herrera-Campo, 2011; Fanou and Wydra, 2014). Therefore, the occurrence of the disease is enhanced by dry climatic conditions in the savanna than the humid climatic conditions in the rain forest zone. The cropping system adopted by the peasant farmers could also influence the incidence and severity of CBB in the state (Zinou et al., 2005). It was observed during the survey that farmers across the various surveyed farms practised monoculture which enhanced the incidence and rapid spread of the disease. The cultivation of susceptible cultivars of cassava by the farmers is another factor that could encourage the development and outbreak of the disease as most of the farms visited used CBB-infected cuttings obtained from other local farmers or from previous season's harvest.

The cassava plants showed varying severity in symptom expression; some plants did not show symptoms of cassava bacterial blight while others had symptoms on only few leaves in the whole plants. However, some plants had symptoms expressed on all parts of the plant. This is consistent with the findings of Wydra and Msikita (1998) who rated cassava bacterial blight disease into varying categories ranging from no symptom, mild, moderate, severe to very severe symptoms based on disease expression on diseased plants. The symptoms observed on the leaves and stems were similar to those described by Zandjanakou-Tachin et al. (2007) and Martin et al. (2017), which were expressed in the form of translucent and angular spots characteristic of *X. axonopodis* pv. *manihotis* infection. The symptoms observed on the field were not only limited to the leaf but the whole plant (candlestick symptom) which appeared as extensive leaf wilt, defoliation, stunting and dieback. The endemic nature of cassava bacterial blight as vascular disease observed on the field survey correlates previous report by Zinou et al. (2004) and Zinga et al. (2008). It was observed

from the survey that infection of cassava with *Xanthomonas axonopodis* pv. *manihotis* was partially dependent on the age of the plant among other factors, as young plants were in most cases free from the disease. Conversely, matured plants showed obvious symptoms of CBB disease. This agrees with the findings of Zinou et al. (2004) who reported higher severity of cassava bacterial blight disease in old cassava leaves relative to the young plants.

Polymerase chain reaction (PCR) techniques have rapidly become the standard for detection and identification of plant pathogens, including bacteria (Alexander et al., 2004). This is due to the fact that these techniques overcome many of the shortcomings due to their sensitivity, greater accuracy, specificity and more rapid results than conventional techniques (Schaad et al., 2001). The PCR and Gel electrophoresis results showed the presence of *Xanthomonas axonopodis* pv. *manihotis* in eight of the ten surveyed LGAs with infectivity ranging from mild to highly infected. The high occurrence of the disease in the surveyed LGAs may be because the survey was conducted in the rainy season when environmental factors such as relative humidity and temperature were very favourable for the penetration and subsequent proliferation of the pathogen in tissues of the host, unlike the dry season, which is a difficult phase of survival for *Xanthomonas axonopodis* pv. *manihotis.* This tallies with earlier submission of Banito et al. (2008) who reported that the severity and incidence of cassava bacterial blight are highly correlated to the amount of rainfall. Conversely, the pathogen was found to be absent in two LGAs even though leaf samples collected from these areas showed cassava bacterial blight disease symptoms during the field survey. This implies that visual examination of cassava leaf samples for occurrence of CBB may be misleading and does not suffice in the diagnosis of the disease. This corroborates previous report of Bock et al. (2010) that if assessments of disease severity are inaccurate or done without precision, incorrect conclusions might be drawn and wrong inferences made.

However, non-detection of the pathogen by PCR in samples that showed symptoms of the disease may be attributed to the possibility of the diseased samples showing symptoms of other diseases such as leaf spot and anthracnose on visual field examination which are similar to blight symptoms at early stages of disease development. For most of the analysed samples, the DNA concentration as well as their purity measured using a spectrophotometer fell between the standard range of 1.7 and 2.1nm (Banito et al., 2008). This showed that the DNA was free from contamination which makes the PCR results from this study reliable. Due to its specificity and sensitivity, the PCR assay in this study has shown considerable potential as a reliable procedure for detecting and identifying the cassava bacterial blight pathogen in infected plant tissue.

This study showed that cassava fields surveyed were significantly infected with *X. axonopodis* pv. *manihotis* and this constitutes a serious threat to cassava production in Osun state. Since all major diseases of cassava can be transmitted by planting of infected propagules, the availability of disease-free stem cuttings and resistant cultivars is crucial to reducing epidemics of the disease in cassava production. Therefore, there is need for a multiplication programme for the supply of clean improved planting materials to the farmers as this remains the only viable option for the management of the disease. The presence of this pathogen in the different cassava growing regions of Osun state should be considered as significant threat to food security particularly among the peasant cassava farmers. Constant surveillance, phytosanitation and improvement on existing diagnostics as well as quarantine measures must be enforced in the state to prevent an epiphytotic of CBB disease. Information on prevalence of CBB obtained in this study will provide a baseline for the development of the disease management programme in the fast growing cassava enterprise in Osun state. However, further study is required to evaluate the genetic diversity of Xam in the state in order to understand the existing strains of the pathogen with a view to enhancing adequate selection of sources of resistance for cassava breeding programme.

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