# OCCURRENCE, DISTRIBUTION AND CHARACTERISATION OF *Xanthomonas axonopodis* pv. *dieffenbachiae*, CAUSAL ORGANISM OF BACTERIAL LEAF BLIGHT OF COCOYAM IN NORTH WEST NIGERIA

BY

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## DECLARATION

I declare that the work in this Dissertation entitled "Occurrence, Distribution and Characterisation of *Xanthomonas axonopodis* pv. *dieffenbachiae,* Causal Organism of Bacterial Leaf Blight of Cocoyam in North West Nigeria" has been carried out by me in the Department of Crop Protection. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this Dissertation was previously presented for another degree or Diploma at this or any other Institution.

Abdulkareem ABDULLAHI _____ _____
(Student) Signature Date

## CERTIFICATION

This Dissertation entitled "OCCURRENCE, DISTRIBUTION AND CHARACTERISATION OF *Xanthomonas axonopodis* pv. *dieffenbachiae*, CAUSAL ORGANISM OF BACTERIAL LEAF BLIGHT OF COCOYAM IN NORTH WEST NIGERIA" by Abdulkareem ABDULLAHI meets the regulations governing the award of the degree of Master of Science in Crop Protection of the Ahmadu Bello University, and is approved for its contribution to scientific knowledge and literary presentation.

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## DEDICATION

This research is dedicated to the memories of my late father, Mallam Abdullahi Okumoh, brother, Mallam Isma'eel Abdullahi and sister, Mallama Bilqees Abdullahi. May you all dwell forever in *Jannatul-firdaus*, amen.

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**ABSTRACT**

Field surveys were conducted during 2014 wet season in three Local Government Areas each of Kaduna, Kano and Katsina States in North West Nigeria to determine the occurrence and distribution of bacterial leaf blight of cocoyam. A total of 81 cocoyam fields were surveyed in the three States. Sampling was done using a five, 2×2 $m^2$ quadrat in each of the nine fields assessed per Local Government Area. Disease incidence recorded in each field showed that the highest incidence of 93.7 % in Kaduna State was obtained in Kudan and the least 16.6 % in Giwa Local Government Areas. In Kano State, the highest 30.0 % and lowest 10.4 % incidence were recorded in Kiru and Bebeji Local Government Areas respectively. With respect to disease severity, Kudan and Giwa Local Government Areas in Kaduna State recorded the highest 45.3 % and the lowest 6.3 % respectively while Kiru and Bebeji Local Government Areas in Kano State recorded the highest 12.7 % and lowest 2.7 % respectively. Bacterial leaf blight was not found in any of the surveyed cocoyam fields in Katsina State. Bacterial isolates obtained from the diseased samples were used for pathogenicity and hypersensitive reaction (HR) tests on cocoyam and tobacco plants respectively and the results indicated that six isolates induced blight symptoms on cocoyam and elicited HR reactions on tobacco. Biochemical tests such as catalase tests, pectolytic tests, amylolytic tests, fluorescence on KB medium and production of acid from glycerol were carried out on the isolates and found that isolates were amylolytic and pectolytic. Furthermore, all the isolates were Gram negative, motile, oxidase negative, catalase positive and did not fluorescent on KB medium and produced no acids from glycerol. For molecular characterisation, a nearly full length of the 16S rDNA gene of selected isolates was PCR amplified using 16S rRNA primers (27F and 1492R) and referenced amplicons were sequenced at the Eurofins Genomics Company, Eurofins MWG

Operon LLC, Huntsville, Alaska, USA using Sanger sequencing. BLAST results of sequenced genes showed 98-100% homology to *Xanthomonas axonopodis* pv. *dieffenbachiae*. Maximum likelihood phylogenetic trees constructed for the 16S rDNA gene sequences revealed isolates were identical to the *X. axonopodis* pv. *dieffenbachiae* reference strains AC1 and XADK. Generally, all the isolates obtained were *X. axonopodis* pv. *dieffenbachiae.* This is the first report of *X. axonopodis* pv. *dieffenbachiae* causing bacterial leaf blight in cocoyam fields in North West Nigeria and the first report on molecular characterisation of the pathogen in Nigeria.

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# CHAPTER ONE

## 1.0 INTRODUCTION

Cocoyam (*Colocasia* and *Xanthosoma* spp.) belongs to the family Araceae. It is one of the oldest crops grown mostly in the tropics, for its edible corms, cormels and leaves and as an ornamental plant (Adelekan, 2012). Cocoyam is one of the basic food crops of major economic importance and ranks third in importance after cassava and yam among the root and tuber crops cultivated and consumed in Nigeria (Baruwa and Oke, 2012). It is superior to yam and cassava nutritionally, with higher protein, mineral nutrients and vitamin contents and its starch is more readily digested (Odebunmi *et al*., 2007). On a global scale, it ranks 14$^{th}$ as a vegetable crop with annual production figure of over 9 million tonnes (FAOSTAT, 2014). Africa accounts for at least 60% of world production and most of the remaining 40% is from Asia and Pacific regions (Mitra *et al*., 2007). Nigeria is the world's leading producer of cocoyam, with annual production of 3.27 million metric tonnes, accounting for about 36 percent of total world output of cocoyam (FAOSTAT, 2014).

Cocoyam is commonly grown for its starchy edible roots. *Colocasia* sp. is grown for its corm and cormels which are consumed after boiling, frying or roasting. The corms can be dried and used to make flour or sliced and fried to make chips. The leaves of the plant are also edible and are usually consumed as a vegetable after cooking in dishes such as stews. *Xanthosoma* sp. produces tubers much like potato and are boiled, baked, steamed or fried prior to consumption. Young leaves are eaten as a vegetable (Azeez and Madukwe, 2010).

The yield and quality of this crop are threatened by various abiotic and biotic factors. Diseases caused by fungi and bacteria are among biotic factors militating against the production of cocoyam in Nigeria. The diseases include phytophthora leaf blight caused by *Phytophthora colocasiae* (Bandyopadhyay *et al.*, 2011; Zarafi *et al*., 2012), corm rot induced by some species of fungi such as *Aspergillus niger, Fusarium solani*, *Fusarium oxysporum* and *Sclerotium rolfsii* (Ugwuoke *et al*., 2008), corm soft rot induced by *Pectobacterium* spp. (Amodu and Akpa, 2012) and the bacterial leaf blight (BLB) of cocoyam induced by *Xanthomonas axonopodis* pv. *dieffenbachiae* (Opara *et al*., 2013).

### 1.1 Justification of the Study

*Xanthomonas axonopodis* pv. *dieffenbachiae* (*Xad*) is considered the most serious bacterial pathogen causing extensive crop losses in commercial foliage plant production worldwide because of its virulence and broad host range within the Araceae family (Donahoo *et al*., 2013). Bacteria leaf blight of cocoyam was first reported in Nigeria at Umudike, South-Eastern Nigeria where the crop was found to be highly susceptible to the pathogen (Opara *et al*., 2013). Reports of cocoyam diseases from farmers' fields in Kaduna State prompted a preliminary survey of some cocoyam fields in Kaduna State where bacterial leaf blight was observed among symptoms of diseases threatening cocoyam production in the State. The existence of *Xad* in North West Nigeria became highly probable due to transfer of planting materials among cocoyam growers and climate change. There was the need to assess the status of this disease in North West Nigeria where the crop occupies a very important position in the farming system. The crop is grown to fill hunger period and to provide instant cash to the farmer, with an average yield of 7000 kg/ha (NAERLS and NAPFS, 2010).

Efficient management of this pathogen requires a sensitive and reliable diagnostic tool (Robene-Soustrade *et al*., 2006). A review of available literature has shown that limited reports on the identity of the pathogen in Nigeria have been focused on biochemical method of identification (Opara *et al*., 2013). This method, apart from being time consuming, is highly limited in species differentiation. Molecular characterisation of *Xad* using Polymerase chain reaction (PCR) based technique has proven to be a fast, sensitive and reliable method for determining genetic relationships among pathogenic organisms (Khoodoo and Jaufeerally-Fakim, 2004; Robene-Soustrade *et al.,* 2006). This technique has become indispensable for quarantine purposes and effective breeding for resistance to bacterial diseases in order to achieve desirable disease management.

This research will provide valuable information that will form the basis for cocoyam bacterial leaf blight disease management decisions and breeding cocoyam cultivars with resistance to the pathogen. Molecular characterisation of the pathogen in the region will also provide information that could be used to map the pathogen in the region, a tool that could serve quarantine purposes.

### 1.2 Objectives of the Study

The objectives of this study are to:

1) determine the incidence and distribution of bacterial leaf blight of cocoyam in Kaduna, Kano and Katsina States
2) characterise isolates of bacterial leaf blight pathogen.

# CHAPTER TWO

# 2.0 LITERATURE REVIEW

## 2.1 Origin and Distribution of Cocoyam

Cocoyam is an ancient crop grown throughout the tropics and subtropics (Moore and Lawrence, 2003). The name 'cocoyam' is used for two members of the Araceae family, *Colocasia esculentum* (L.) Schott and *Xanthosoma sagittifolium* (L.) Schott (Talwana *et al.,* 2009). *C. esculentum* is considered to have originated in the Indo-Malaysian region, particularly eastern India and Bangladesh from where it spread eastwards into southern Asia and the Pacific Islands and westwards to Egypt. It spread southwards and westwards into East Africa and West Africa (Talwana *et al.,* 2009). *Xanthosoma sagittifolium* on the other hand, is native to tropical Central and South America and the Caribbean, where it has been cultivated and consumed since the pre-Columbian times (Valerio, 1988; Ki-zerbo, 1990; Onwueme, 1994). According to Brown (2000), *X. sagittifolium* reached West Africa between the 16th and 17th centuries and was spread further by traders, missionaries and other explorers.

*C. esculentum* is widely grown in South E0astern region of Nigeria while *X. sagittifolium* is more common in South West region of Nigeria (Opara *et al.,* 2013). However, both species are widely cultivated in northern Nigeria (Alinnor and Akalezi, 2010; Amodu and Akpa, 2012).

## 2.2 World Production Trend of Cocoyam

Cocoyam is currently grown in nearly every tropical region of the world (Tipton *et al.,* 1990). World production was estimated at over 12 billion British pounds in 2012 (FAOSTAT, 2012). Approximately 1.2 million hectares were devoted to cocoyam worldwide in 2014, producing about 9 million metric tonnes. Leading producers are

Nigeria, China mainland, Cameroun and Ghana who account for about 90 percent of total world production (FAOSTAT, 2014) (Table 1).

In Nigeria and on annual basis, area under cocoyam cultivation ranges between 400,000 and 500,000 hectares, largely in the South East region (NAERLS and NPAFS, 2010). In 2014, over 600,000 hectares were cultivated to produce about 3,300,000 metric tons of cocoyam (FAOSTAT, 2014). In North West Nigeria, cocoyam production is mostly carried out in three States, namely Katsina, Kaduna and Kano States with 49,840, 31,040 and 350 MT respectively (NAERLS and NPAFS, 2010). Production figures for other States in this region are not available.

### 2.3 Importance of Cocoyam

Cocoyam is grown as a root crop for its edible corms and cormels and the leaves are used as vegetable throughout the humid tropics. The crop can guarantee food security, because it is relatively affordable and can feed many low income earners (Azeez and Madukwe, 2010). In South Pacific, it is sometimes referred to as the mother of life because of cultural and religious importance attached to its cultivation. It holds great value in the customs and social structure of the Palauan people. In American Samoa, there are social-cultural purposes for which cocoyam is grown. It is grown to share with friends and relatives, and as gifts to local chiefs and pastors, and is an important food for Sunday feasts when family and friends come together. It is used in important occasions such as traditional ceremonies, celebrations, holidays, and funerals by the people of American Samoa (Tipton *et al.*, 1990).

It is one of the most valuable tuber crops in Nigeria. Cocoyam cormels are of great economic, social and nutritional importance to the people of Nigeria (Ugulu, 1996).

Table 1: Ten major cocoyam producing countries in the world, 2014

| Rank* | Country | Production Area (ha) | Production Quantity (Metric tonnes, MT) |
|---|---|---|---|
| 1 | Nigeria | 639,980 | 3,273,000 |
| 2 | China, mainland | 94,010 | 1,822,090 |
| 3 | Cameroun | 170,540 | 1,637,900 |
| 4 | Ghana | 200,000 | 1,299,000 |
| 5 | Papua New Guinea | 35,090 | 272,770 |
| 6 | Madagascar | 37,800 | 234,820 |
| 7 | Japan | 13,300 | 166,000 |
| 8 | Central African Republic | 40,030 | 132,740 |
| 9 | Egypt | 3,300 | 115,950 |
| 10 | Philippines | 15,850 | 114,800 |

Source: FAOSTAT (2014).

*Ranking based on production quantity

Cocoyam can be processed into flour and chips. The flour is highly digestible and therefore suitable for feeding infants, making confectionaries and baby food (Iwuoha and Kalu, 2000). Cocoyam leaves are also used as vegetable for preparing soup in various parts of the world (Onwubuya and Ajani, 2012). It contains 0.6-1.18% crude fiber, 0.6-1.3% ash, 14-30% proteins, 13-29% carbohydrate, 0.16-0.36% fat, 63-85% moisture and a good amount of vitamin C (Okonmah and Eruotor, 2012).

The potential of cocoyam as a good source of renewable energy for producing bio-fuel was demonstrated by Adelekan (2012), who reported that 10 million tonnes of cocoyam is potentially able to produce $1.2 \times 10^9$ litres of ethanol, equivalent to $7.6 \times 10^8$ litres gasoline or $39.5 \times 10^9$ litres of methane which when combusted would produce $179.3 \times 10^7$ MJ energy.

## 2.4 Constraints to Cocoyam Production in Nigeria

In spite of the numerous social, cultural and economic benefits derived from cocoyam, its production is faced with many challenges which are caused by abiotic and biotic factors.

### 2.4.1 Abiotic factors

Increase in the rate of land degradation which has led to decreased soil nutrients availability is one of the abiotic factors militating against production of cocoyam (Onwubuya and Ajani, 2012). The ignorance of the nutritive value and diversities of food forms from cocoyam by a large percentage of the populace is a major limiting factor to general acceptability and large scale production of the crop in Nigeria. The notion that cocoyam is a poor man's crop is still prevalent among the populace in Nigeria (Onyenweaku and Okoye, 2006).

### 2.4.2 Biotic factors

Cocoyam is subjected to losses due to attack by pests and diseases. These include insect pests such as taro beetle (*Papuana* spp.), cocoyam root aphid (*Patchiella reaumuri*), mealy bug (*Maconellicoccus hirsutus*), armyworm (*Spodoptera litura*), spiraling whitefly (*Aleurodicus dispersus*), tobacco whitefly (*Bemisia tabaci*), Aphids (*Aphis gossypii*); mites such as spider mites (*Tetranychus* spp.) and molluscs such as the giant African snail (*Lissachatina fulica*) (Carmichael *et al*., 2008).

Diseases caused by nematodes, viruses, fungi and bacteria are among biotic factors hampering production of cocoyam worldwide. The nematodes include root-knot nematodes (*Meloidogyne* spp.), which damage cocoyam roots when the crop is planted in infested soils. Galls on the root and swelling and malformations on the corm are characteristic of attack by this nematode. Severe attacks will stunt the plants and render it chlorotic (Carmichael *et al*., 2008). Other nematodes inducing diseases in cocoyam include *Pratylenchus* spp., *Helicotylenchus* spp., *Rotylenchulus* spp. and *Longidorus* spp. (Carmichael *et al*., 2008).

Fungal diseases include root and corm rots induced by *Pythium* spp. and *Phytophthora* spp. (Ooka, 1980), corm and/or tuber storage rot caused by *Aspergillus niger, Fusarium solani*, *Botryodiplodia theobromae, Fusarium oxysporum, Corticium rolfsii, Geotrichum candida* and *Sclerotium rolfsii* (Ugwuoke *et al*., 2008) and taro leaf blight (TLB) induced by *Phytophthora colocasiae* (Bandyopadhyay *et al*., 2011). TLB is a devastating disease of cocoyam in Nigeria that has continued to threaten cocoyam production since it was first reported in 2009 (Chiejina and Ugwuja, 2013).

There is little information on virus diseases of cocoyam in Nigeria, but reports from Florida, U. S. A and Solomon Islands show an aphid transmitted Dasheen mosaic virus and the Alomae and Bobone virus diseases respectively on cocoyam (Ooka, 1980).

The major bacterial diseases of cocoyam are bacterial soft corm rot induced by *Pectobacterium* spp. (Ooka, 1980; Amodu and Akpa, 2012) and bacterial leaf blight of cocoyam induced by *Xanthomonas axonopodis* pv. *dieffenbachiae* (Pohronezny *et al*., 1985; Opara *et al.*, 2013).

## 2.5 Bacterial Leaf Blight of Cocoyam

Bacterial leaf blight (BLB) of cocoyam was first reported in Florida in 1979, but the causal organism was not identified (Pohronezny *et al.*, 1985). A bacterium very closely resembling *Xanthomonas campestris* pv. *dieffenbachiae* (McCulloch and Pirone) Dye in physiological and morphological features was earlier reported by Berniac (1974) in Guadeloupe, Caribbean Islands as the causal organism of bacterial leaf blight of cocoyam. He suggested *X. campestris* pv. *aracearum* (Berniac) Dye as the name for the cocoyam strain and all leaf-spotting Xanthomonads. However, Pohronezny *et al.* (1985) reported that the causal organism of bacterial blight of cocoyam in Florida was *Xanthomonas campestris* pv. *dieffenbachiae* and the bacteria isolated from the leaf blights of cocoyam fitted physiological and biochemical descriptions of *Xanthomonas campestris* and that disease reactions were identical with those for *X. campestris* pv. *dieffenbachiae*.

In 1995 the *X. campestris* pv. *dieffenbachiae.* was reclassified as *X. axonopodis* pv. *dieffenbachiae* based on DNA-DNA homology studies (Vauterin *et al*. 1995). In that study, five *X. campestris* pv. *dieffenbachiae* strains were compared with 178 strains

from 33 other pathovars of *X. campestris*. The five *X. campestris* pv. *dieffenbachiae* strains used, included three Anthurium strains from Brazil and two Dieffenbachia strains from the United States. The strains used in the study seem limited with regards to hosts of origin and excluded the incorporation of any strains from Aglaonema, Philodendron, Syngonium, or Xanthosoma.

### 2.5.1 Symptomatology

Early symptoms of bacterial leaf blight of cocoyam in the field are small, water-soaked spots, more evident on the abaxial leaf surface. The sports enlarged and produce necrotic spot of about 2 cm in diameter surrounded by prominent chlorotic halos. Pronounced water-soaking continue on the abaxial leaf surface. Leaf spots were often delimited by veins, but infections progressed into veins and proceeded basipetally, resulting in streaks of infected tissues (Pohronezny *et al.*, 1985). Some lesions coalesced, resulting in large dead areas. A cream to light yellow bacterial exudate was often seen on the undersides of young lesions, especially in the morning. A water-soaked and chlorotic leaf margin result when the infection occurs through the hydathodes (Pohronezny *et al.*, 1985).

### 2.5.2 Disease cycle and epidemiology

Pathogen inoculum may be present in the field on infected crop residues from a previous season or be introduced at planting within infected cormels (Pohronezny *et al.*, 1985). The pathogen usually enters through the wounds or stomata on the lower leaf surface, where they survive and multiply. Some bacteria are deposited near hydathodes (Pohronezny *et al.*, 1990). Symptoms appear 7 to 18 days after infection. Temperature above 25 ºC and relative humidity exceeding 85% favour disease development. The organisms are easily spread from leaf to leaf and plant to plant by

splashing rain water, contaminated tools, insects and poor handling of infected plants (EPPO, 2013). Seed borne inoculum allows long range dispersal and introduction of the pathogen into new areas or fields. Since the pathogen is systemic, it is capable of symptomless epiphytic transfer that enables undetected passage through quarantine (Pohronezny *et al.*, 1985; EPPO, 2013).

### 2.5.3 Host-pathogen interaction

#### *2.5.3.1 Host range*

Most of the hosts of this pathogen are in the Araceae family. Some of the known hosts include *Aglaonema* spp. (Aglaonema), *Anthurium* spp. (Anthurium), *Caladium* spp. (Caladium), *Colocasia esculenta* (old cocoyam), *Dieffenbachia maculate* (Dieffenbachia), *Epipremnum aureum*, *Philodendron* spp. (Philodendron), *Rhaphidophora* spp., *Scindapsus pictus*, *Spathiphyllum* spp. (Spathiphyllum), *Syngonium podophyllum* and *Xanthosoma* spp. (new cocoyam) (Pohronezny *et al.*, 1985; Chase *et al.*, 1992; Robene-Soustrade *et al.*, 2006).

#### *2.5.3.2 Infection of the host by bacterial blight pathogen*

During infection, bacteria enter the plant through natural openings such as stomata or hydathodes or wounds to reach intercellular spaces, where they establish an intimate relationship with the plant cell (Donahoo, 2003). The bacteria multiply at the point of entry and spread through the xylem vessels (Donahoo, 2003). Genes in both the bacteria and the host determine the outcome of interactions between the two. In a susceptible plant, infection with a virulent strain of *Xanthomonas* leads to bacterial multiplication, giving rise to small water soaked lesions that later become necrotic in a compatible reaction, leading to a diseased condition (Donahoo, 2003).

When resistance genes are present in the plant and matching avirulence genes in the bacterium, a hypersensitive reaction (HR) is induced in the host (incompatible reaction) (Bonas and van den Ackerveken, 1999). Recognition of the pathogen by a resistant host requires functional hypersensitive resistance and pathogenicity (hrp) genes, and is determined by a pair of corresponding genes, one for avirulence in the bacterium and the other for resistance (R) in the plant (Bonas and van den Ackerveken, 1999). The pathogenicity of the bacteria depends on a specialized secretion system which secretes accessory proteins into the extracellular spaces (Ellis and Jones, 1998). The most important secretion system implicated in this is the Type Three Secretion System (TTSS or T3SS), through which effector proteins are delivered directly into the host cell (Ellis and Jones, 1998).

### 2.5.4 Economic importance of bacterial leaf blight of cocoyam

The economic importance of bacterial leaf blight in the field in Nigeria is yet to be determined, as disease assessment and yield loss studies due to the disease have not been reported. Opara *et al*. (2013) reported over 70% incidence of *Xad* on cocoyam in screen house experiment at Umudike, South East Nigeria. From Florida in U. S. A., about 74-100% incidence of *Xad* on cocoyam was reported from a survey of commercial cocoyam fields in 1983 (Pohronezny *et al*., 1985). The disease was reported in anthurium as a limiting factor, with a loss of 2.74 million USD recorded in anthurium industry in 1989 in Hawaii, USA (Shehata and Nishijima, 1989). Production losses exceeding $5 million have been reported in Hawaii (Chase *et al*, 1992). In spite of the use of strict sanitation to control the disease, there has been more than a 50% loss of plants in Hawaii (Fukui *et al.*, 1998).

### 2.5.6 Characterisation of bacterial leaf blight pathogen

Diagnosis of *X. axonopodis* pv. *dieffenbachiae* infection has over the years been based on biochemical identification, pathogenicity tests, or serological tests (Pohronezny *et al*., 1985; Lipp *et al*., 1992). Such tests are time consuming and do not give the true identity of the pathogen. Molecular approaches based on deoxyribonucleic acids (DNA) polymorphisms have been developed for the taxonomic study of plant pathogenic bacteria (Verdier *et al*., 1994). Rapid methods based on specific polymerase chain reaction (PCR) amplification, have been used for the detection and identification of *Xanthomonas* spp. (Khoodoo and Jaufeerally-Fakim, 2004; Trindade *et al.*, 2005; Robene-Soustrade *et al*., 2006).

Robene-Soustrade *et al*. (2006) developed a reliable, highly specific and sensitive PCR based diagnostic protocols for *X. axonopodis* pv. *dieffenbachiae* in which a nested PCR assay was designed using the sequence from an *X. axonopodis* pv. *dieffenbachiae* specific fragment identified from randomly amplified polymorphic DNA (RAPD ) based PCR. The fragments were characterised further as sequence-characterised amplified region (SCAR) markers that were used to design specific primers for the nested PCR technique, which was used to characterise the bacterial strains in the study. Using nested PCR markers developed for the detection of the anthurium blight pathogen, Robene-Soustrade *et al.* (2006) found that pathogenic strains could be detected in planta.

Accurate identification and typing of bacteria currently relies on a number of approaches, including 16S rDNA sequence analysis, DNA genotyping methods restriction fragment length polymorphism (RFLP), RAPD, repetitive extragenic palindromic sequence (Rep)-PCR, and amplified fragment length polymorphism

(AFLP), and phenotypic data fatty acid methyl ester (FAME) analysis, carbon source utilization (BIOLOG, Hayward, CA); however, species are currently defined based on DNA-DNA homology (Stackebrandt *et al*., 2002). Using genus Xanthomonas as a model, AFLP and Rep-PCR were compared with DNA-DNA homology studies (Rademaker *et al*., 2000) and a high correlation was observed between the two genotyping techniques, suggesting that these fingerprinting methods may function as core techniques in polyphasic taxonomy. One exception was Xanthomonas DNA homology group 9, which exhibited low DNA-DNA homology values (Vauterin, *et al.*, 1995; Rademaker *et al*., 2000). In another study, DNA from 339 Xanthomonas strains composing 80 pathovars across all 20 DNA homology groups were subjected to Rep-PCR. DNA homology group 9 was divided further into six subgroups, with strains originating from anthurium placed in subgroup 9.4 and strains from dieffenbachia in subgroup 9.6 (Rademaker *et al*., 2000).

#### *2.5.6.1 16S rRNA gene sequence based identification*

As huge varieties of bacteria are present in the environment, the range and complexity of the techniques to be utilized for their identification purpose is highly bewildering (Spratt, 2004). However, the use of nucleotide sequence data from 16S rRNA gene has been regarded to be the most suitable practice not only to identify but also to draw the phylogenetic relationship for all microorganisms on earth. Some of the reasons why the use of 16S rRNA gene for identification has become popular include (i) occurrence of the gene in all organisms performing the same function, (ii) the gene sequence is conserved sufficiently containing conserved, variable and hyper-variable regions, and (iii) around 1500 bp of sequence size which is relatively easy to sequence and large enough to contain sufficient information for identification and analysis of phylogeny (Clarridge III, 2004). This technique became popular only after sufficient deposition of

16S rRNA gene sequences in the database as well as the availability of suitable primers for gene amplification.

The major advantage of the comparative sequencing and analysis of the 16S rRNA gene is the rapid and accurate identification. In conventional techniques certain groups of bacteria are difficult to identify and require special equipment and expertise for their identification such as gas chromatography and mass spectroscopy for anaerobes whereas sequence based identification does not rely on them (Clarridge III, 2004). Certain slow growing bacteria are present which take considerable time for their growth and hence their identification by conventional techniques becomes tedious. In this regard, 16S rRNA gene sequencing represents a unique technology yielding the result in considerably less time. Another important advantage is that some isolates with atypical morphological characteristics can also be detected using this technique (Gee *et al.*, 2004). Novel bacterial genera as well as the discovery of new species can be accomplished by combining the phenotypic characteristics, 16S rDNA sequences, sequences of other genes as well as the results of DNA–DNA hybridization tests (Staley, 2006).

Some of the disadvantages of 16S rDNA gene sequencing analysis include cost and technical considerations as the primers are very expensive. Also it is not possible to differentiate between species that share the same sequence of the gene. Identification of bacterial species based on sequence analysis of the 16S rRNA gene relies on matching the obtained sequence with the existing sequence. Matching with a sequence that was incorrectly identified leads to incorrect identification. Other problems associated with the use of 16S rDNA gene are sequencing artefacts and problems with the purity of bacterial isolates which may lead to incorrect identification. Problems associated with

sequence analysis of the 16S rRNA gene when identifying bacterial species argue for the use of alternative methods to confirm findings. Among these alternative methods is matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) which relies on exploiting differences in bacterial protein profiles (Tshikhudo *et al.*, 2013).

### 2.5.7 Management of bacterial leaf blight

Resistant varieties have been advocated for the management of plant diseases generally but no cocoyam variety has been reported to be resistant to this disease. The management strategies for *Xad* in other hosts, particularly *Anthurium* spp. consist principally of prevention, sanitation, and the use of axenically propagated plants (Robene-Soustrade *et al.*, 2006). Antibiotics such as streptomycin and oxytetracycline have been reported as a management option for bacterial leaf blight on *Anthurium* spp. in Hawaii, USA (Sato, 1983). The only documented management strategy for this disease in Nigeria is the use of plant extracts (Opara *et al.*, 2013). In their work, 50 g powdered extracts each from *Cymbopogon citrates* (lemon grass), *Piper guineense* (black pepper) seed, *Citrus sinensis* (orange) peel dissolved in 100 ml of sterile water and later diluted to 20%, were tested for their efficacy against BLB and were found to be as effective as standard synthetic pesticides (1% Streptomycin sulphate and 10% Copper oxychloride) in reducing disease incidence and severity on cocoyam.

Different chemical products such as streptomycin, Cupric hydroxide, mancozeb and oxytetracycline have been used for management of this disease in Hawaii, USA (Sato, 1983).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Incidence and Distribution of Bacterial Leaf Blight of Cocoyam

Incidence and distribution of bacterial leaf blight of cocoyam were determined between August and September 2014 in Kaduna, Kano and Katsina States of North West Nigeria (Table 2). In each State, three Local Government Areas (LGAs) were visited and in each LGA, three farming communities were selected. In each farming community, three cocoyam fields were surveyed. In each field, cocoyam leaves were examined for symptoms of bacterial leaf blight, thereafter, a 2 by 2 $m^2$ quadrat was set each at four corners and at the centre of the field. The total number of plants in the quadrat was recorded. Disease incidence was assessed by counting the number of plants showing bacterial leaf blight symptoms in each quadrat and the total number of plants in that quadrat. The disease incidence was calculated using the formula:

$$\text{Disease incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plants examined}} \times 100$$

Disease Severity was recorded per plot using a disease scale of 0-5 developed by Opara *et al*. (2013):

0 = no disease symptom visible on the plant;

1 = less than 10% of plant affected;

2 =10-30 % of plant affected;

3 = 31-50% of plant affected;

4 = 51 - 70% of plant affected;

5 = above (>) 70% of plant affected.

Table 2: Surveyed Locations in North West Nigeria

| State | Local Government Area | Town/Village | Coordinates and Altitudes |
|---|---|---|---|
| Kaduna | Sabongari | Tudun Sarki | N $11^0$07.236', E $007^0$44.307'; 625 m |
| | | Bomo | N $11^0$09.012', E $007^0$41.503'; 630 m |
| | | Yan Sarki | N $11^0$07.263'; E $007^0$35.459'; 702m |
| | Kudan | Jaja | N $11^0$14.065', E $007^0$38.663'; 660 m |
| | | Hunkuyi | N $11^0$16.786', E $007^0$39.430'; 630 m |
| | | Musawa | N $11^0$13.477'; E $007^0$40.409'; 658m |
| | Giwa | Shika | N $11^0$11.725'; E $007^0$34.532'; 670 m |
| | | Giwa | N $11^0$15.489'; E $007^0$27.704'; 660m |
| | | Yakawada | N $11^0$15.997'; E $007^0$23.788'; 638m |
| Kano | Kiru | Yelwan Paki | N $11^0$30.016', E $008^0$09.627'; 586 m |
| | | Dankargo | N $11^0$30.570'; E $008^0$10.410'; 586 m |
| | | Dangora | N $11^0$32.473'; E $008^0$09.822'; 575 m |
| | Bebeji | Gwarmai | N $11^0$32.039'; E $008^0$15.382'; 575 m |
| | | Bebeji | N $11^0$37.991'; E $008^0$19.390'; 495 m |
| | | Unguwandankali | N $11^0$30.217'; E $008^0$15.983'; 485 m |
| | Rogo | Zarewa | N $11^0$24.602'; E $007^0$49.632'; 696 m |
| | | Fulatan | N $11^0$23.220'; E $007^0$51.953'; 676 m |
| | | Babbarika | N $11^0$25.052'; E $007^0$50.015'; 695 m |
| Katsina | Sabuwa | Sabuwa | N $11^0$10.729'; E $007^0$07.261'; 666 m |
| | | Tashan Nadaya | N $11^0$12.874'; E $007^0$10.066'; 712 m |
| | | Ungwar Maiwasa | N $11^0$10.724'; E $007^0$ 05.332'; 665 m |
| | Dandume | Dandume | N $11^0$27.427'; E $007^0$13.420'; 707 m |
| | | Kufar Maitsamiya | N $11^0$27.376'; E $007^0$13.470'; 708 m |
| | | Mahuta | N $11^0$27.068'; E $007^0$16.482'; 709 m |
| | Funtua | Dutsen Reme | N $11^0$31.708'; E $007^0$20.417'; 706 m |
| | | Mairuwa | N $11^0$31.324'; E $007^0$18.008'; 702 m |
| | | Dankawo | N $11^0$23.856'; E $007^0$19.066'; 658 m |

Disease Severity Index (DSI) was evaluated using the formula as follows:

$$\text{DSI} = \frac{\text{Sum of individual disease scores}}{\text{Total number of plants scored x maximum score}} \text{ x } 100$$

Leaf samples were collected from each field, wrapped in labeled paper bags and taken to the Bacteriology Laboratory of the Department of Crop Protection, Ahmadu Bello University, Zaria for isolation.

Coordinates and altitudes for each location were recorded using a handheld Global Position System (GPS) navigator manufactured by Garmin, USA. Unstructured questionnaire was used to obtain some cropping information from the surveyed areas.

### 3.2 Characterisation of *Xanthomonas* spp.

#### 3.2.1 Isolation of the organism, hypersensitive reaction and pathogenicity tests

Sampled leaves were surface-sterilized with 3.5 % w/v sodium hypochlorite and washed thoroughly with sterile water for 3 minutes before isolation. Small pieces (3 mm) of advancing lesions on leaves were excised from the boundary between diseased and the healthy tissues using sterile scalpel. Excised tissue was rinsed with sterilized tap water (STW) for 1 minute before it was placed in a Petri dish containing 3.0 ml of STW, shredded with sterile needles and allowed to stand for 10 minutes. The suspension was then streaked on nutrient agar (Difco) plates, placed upside down in an incubator set at 27 $^0$C for 48-72 hours. Pure cultures were obtained after sub-culturing twice. The pure cultures were then stored in sterilized tap water (STW) in McCartney bottles at 4 $^o$C.

Twenty-four hour old cultures of the bacterial colony obtained were adjusted with spectrophotometer to an optical density of 0.3 at 600 nm of light, corresponding to a bacterial concentration of $10^8$ cfu/ml. Isolates corresponding to different locations were

designated numerically with the prefix 'CE' to indicate cocoyam genus (*Colocasia esculenta*) origin. A total of six isolates namely CE2, CE3, CE4, CE6, CE7 and CE11 designating isolates from Tudun Sarki, Jaja, Yelwan Paki, Hunkuyi, Shika and Gwarmai were tested for hypersensitive reaction (HR) on tobacco and pathogenicity on cocoyam.

Inoculum concentration of $10^8$ cfu/ml was used for hypersensitive reaction test on tobacco leaves according to Wick (2010). The mesophyll layer of the lower surface of a healthy mature tobacco leaf was infiltrated by injecting 1 ml suspension of the inoculum into the leaf using a handheld hypodermal syringe. Another leaf injected with 1 ml sterile distilled water (SDW) served as control. The tobacco leaves were left for 24 hours and observed for HR development.

Cocoyam corms were planted into 30 cm diameter pots filled with heat sterilized soil at the rate of one corm per pot. The bacteria isolate was tested for pathogenicity on 15 day-old cocoyam plants (*Colocasia esculenta*). Two methods of inoculation were used; hypodermal syringe method (Pohronezny *et al*., 1985) and sand paper technique (Salaheddin *et al*., 2005) (Plate 1). In the hypodermal syringe method, 0.3 ml bacteria suspension was injected into the lower surface of cocoyam leaf using a hypodermal syringe. In the sand paper technique, cocoyam leaf was held between two folds of a sand paper and gentle pressure applied, and then the sand paper removed. Thereafter, 1 ml bacteria suspension was sprayed on the lower leaf surface using a handheld sprayer.

Inoculated seedlings were covered with transparent polyethylene bags for 24 hours to ensure post inoculation period of high humidity for bacteria incubation (Pohronezny *et al*., 1990). Plant inoculated with 1 ml SDW served as control. Leaves were observed




Plate 1: Hypodermic syringe (A) and sand paper (B) methods of inoculation of *Xanthomonas axonopodis* pv. *dieffenbachiae* on cocoyam

for symptoms of bacterial leaf blight for 21 days after inoculation and records on days to symptoms appearance were taken. Number of lesions developed was recorded every two days. Twenty-five days after inoculation, the disease severity was assessed on 10 leaves selected at random from each pot by using disease scale of 0-5 developed by Opara *et al*. (2013) used in item 3.1.

Diseased leaves were collected, bacteria isolated from them using the method described above and compared with the initial organisms used for inoculation to confirm Koch's postulate. The treatments consisted of six bacterial isolates and two methods of inoculation which were laid in a completely randomized design (CRD) with three repetitions.

### 3.2.2 Biochemical tests

*3.2.2.1 Test for Gram reaction*

For Gram reaction, the alternative potassium hydroxide (KOH) test was done for all samples. Twenty-four hour old culture was added to 3% KOH solution on clean glass slide using flame-sterilized needle. The bacterial suspension was stirred against the KOH solution while occasionally lifting the loop for slimy and thickened slurry.

*3.2.2.2 Test for fluorescence on King's Medium B (KB)*

Actively growing *Xanthomonas* cells were transferred from a 24 hour old culture onto King's Medium B using quadrant streaking procedure. Inoculated plates were incubated at 30 $^{0}$C for 48-72 hours and observed under ultraviolet light for fluorescence.

*3.2.2.3 Test for amylolytic reaction*

Twenty-four hour old cultures of *Xanthomonas* spp. isolates were transferred to nutrient agar plates amended with 0.2% (w/v) soluble starch. Cultures that emerged

were incubated at 30 $^0$C for 72 hours. Plates were flooded with Lugol's iodine solution and observed for any change of colour which signifies starch hydrolysis.

*3.2.2.4 Test for Pectolytic reaction*

Bacterial isolates were grown on sodium pectate gel medium which contained pectate substrate and incubated at 28 $^o$C for three days. Production of degrading enzymes was detected by observing depressions in the gel around the colony where the substrate has been degraded.

*3.2.2.5 Test for catalase*

A drop of 3% hydrogen peroxide ($H_2O_2$) was placed on glass slide, onto which a loopful of bacteria from a 24 hour old culture was suspended and observed for bubbling and production of gases.

*3.2.2.6 Oxidase reaction*

One ml 1 % aq. w/v solution of N'-tetramethyl-p-phenylene-diaminedihydrochloride solution was prepared and 100 µl of the solution were placed on a new piece of Whatman No. 1 filter paper with a clean Pasteur pipette. Part of a colony was removed with a sterile toothpick and smeared onto the moistened paper. This was observed for colour changes which should occur within 30 seconds for positive results.

*3.2.2.7 Gelatine test*

Tubes containing 12 % w/v gelatine solution were inoculated with the 24 hour bacterial isolates and incubated for 15 days at 20 $^o$C. This was kept at 5 $^o$C for 15 minutes before determining gelatine liquefaction.

*3.2.2.8 Acid production*

Solutions of Dye's medium C and carbohydrate sources were prepared in tubes and inoculated with the isolates. This was incubated at 28 $^o$C for 2-10 days and observed for

colour changes. Yellow colour formation indicates acid production from carbohydrate. Substrates that were tested included glucose, fructose, mannose, lactose, galactose, sucrose, glycerol and arabinose.

### 3.2.3 Molecular characterisation

Molecular characterisation was carried out at the Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University, U.S.A. Only four out of the six isolates confirmed to be pathogenic on cocoyam were characterised using PCR as the other two isolates could not grow on nutrient broth yeast (NBY) medium prior to DNA extraction.

*3.2.3.1 DNA extraction, genomic finger printing and agarose gel electrophoresis*

Bacteria cultures grown at 28 °C on NBY medium (8 g liter$^{-1}$ nutrient broth, 2 g liter$^{-1}$ yeast extract, 2 g liter$^{-1}$ $K_2HPO_4$, 0.5 g liter$^{-1}$ $KH_2PO_4$, 15 g agar, 974 ml distilled water, 25 ml 20 % glucose, 1 ml 1 M $MgSO_4$; pH 7.2) for 24 hours were cultivated in NBY liquid medium (NBY without agar) for 16 hours at 28 °C with agitation.

16S rDNA was extracted using Wizard Genomic DNA Purification Kit (Promega, 2005). One millilitre bacteria culture was added to a 1.5 ml micro-centrifuge tube and centrifuged at 13,000 × g for 2 minutes to pellet bacteria cells. Supernatant was removed and transferred to a 1.5 ml collection tube. Six hundred microlitre of Nuclei Lysis solution was added by gentle pipetting until the cells were resuspended. This was incubated at 80 °C for 5 minutes to lyse the cells and cooled to 25 $^{o}$C. Three microlitre of RNase solution was added to the cell lysate, mixed thoroughly, incubated at 37 °C for 30 minutes and cooled to 25 $^{o}$C. Two hundred microlitre protein precipitation solution was added to the RNase-treated cell lysate and vortexed vigorously at high

speed for 20 seconds to mix the protein precipitation solution with the cell lysate. Solution was incubated on ice for 5 minutes and centrifuged at 13,000 × g for 3 minutes. Supernatant containing the DNA was transferred to a clean 1.5 ml microcentrifuge tube containing 600 µl of isopropanol room temperature and gently mixed by inversion. It was then centrifuged at 13,000 × g for 2 minutes. Supernatant was poured off gently and the tube drained on clean absorbent paper. Six hundred microlitre of 70% ethanol at room temperature was added and the tube inverted gently several times to wash the DNA pellet. It was centrifuged at 13,000 × g for 2 minutes and the ethanol carefully aspirated. Tube was drained on clean absorbent paper and the pellet allowed air-drying for 10–15 minutes. One hundred microlitre of DNA rehydration solution was added to the tube and incubated at 65 °C for 1 hour to rehydrate DNA. Genomic DNA thus extracted was stored at -20 $^{o}$C until further use.

PCR protocol was used to amplify the purified bacterial genomic DNA. The I6S rRNA primers used for the amplification were forward: 27F (5'AGAGTTTGATCMTGGCTCAG-3') and reverse: 1492R (5'-GGYTACCTTGTTACGACTT-3'). The component of PCR master mix included buffer (10 µl), $dNTP_s$ (1.5 µl), $MgCl_2$ (5 µl), forward primer (1.5 µl), reverse primer (1.5 µl), Taq polymerase (0.5 µl) and sterile water (30 µl). Two microlitre of the purified DNA of each sample was pippeted into a separate PCR tube. Fifty microlitre of the master mix was added to each of the DNA samples, vortexed and centrifuged before placing in the thermocycler. The amplification program included initial denaturation at 94 °C for 3 minutes, 32 cycles consisting of denaturation at 94 °C for 20 seconds, annealing at 56 °C for 30 seconds, and extension at 72 °C for 1 minute 10 seconds, and an extra extension step at 72 °C for 5 minutes.

Amplification of the DNA was confirmed by gel electrophoresis. Agarose was prepared at the desired concentration (w/v) in 1xTris-Acetate-EDTA (TAE) buffer (1.0 % gel), by dissolving 0.4 g Agarose in 40 ml buffer and boiled in microwave oven with intermittent shaking until all the agarose was completely dissolved. The edges of the gel tray was sealed with a tape and comb placed at one end of the tray surface. The Agarose solution was allowed to cool to about 50 ºC and then poured into the gel tray to a thickness of 4-5 mm and the gel allowed settling. The tape was removed and the gel tray then placed in the electrophoresis unit and TAE buffer used to fill the unit so that there was 2-3 mm of buffer over the gel surface. The comb was carefully removed and 1.6 µl of loading buffer (15% Ficoll$^{R}$ 400, 0.03 % bromophenol blue, 0.03 % Xylene cyanol FF, 0.4 % orange G, 10 mM Tris-HCl (pH 705) ) was mixed with 8 µl of PCR product and loaded slowly into the wells. A known *Xanthomonas* strain, MSCTI and sterile distilled water were loaded to serve as positive and negative control respectively. DNA molecular weight marker (1 kb) was also loaded and the order of sample loading in the gel was recorded. Ethidium bromide solution (0.5 µg/ml) was added to the buffer before running the gel.

Electrophoresis unit was then connected to the power pack and power supply turned on until the bromophenol blue dye reached the bottom of the gel (approximately 30 min at 100 V, for DNA to migrate 7 cm from the wells in a 1.0 % gel). The gel was removed and observed under UV Transilluminator. Photograph of the gel was taken using an orange filter fitted camera. The resulting amplicons were sequenced at the Eurofins Genomics Company, Eurofins MWG Operon LLC, Huntsville, Alaska, USA using Sanger sequencing. 16S rDNA gene sequences from the *Xanthomonas* spp. isolates were blasted against sequences of related Xanthomonads from the National Centre for

Biotechnology Information (NCBI) online database (www.ncbi.nlm.nih.gov) to determine genomic relatedness.

### 3.3 Statistical Analysis

The data collected on pathogenicity were analyzed using Analysis of Variance (ANOVA) procedure of Statistical Analysis Software (SAS, 2002) and the means separated using Duncan Multiple Range Test (DMRT) at 5 % level of probability. The *Xanthomonas* spp. isolates were analysed for their evolutionary history using the maximum likelihood method. The analysis was done following the Tamura-Nei model (Tamura and Nei, 1993). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach, and then selecting the topology with the superior log likelihood value. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.*, 2013). Tajima's neutrality statistics (*D*) (Tajima, 1989) was also calculated.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Incidence and Distribution of Bacterial Leaf Blight

Mean bacterial blight incidence and severity observed in the Local Government Areas (LGAs) of the States are shown in Figures 1 and 2. In Kaduna State, the incidence in Kudan (93.7 %) was highest, followed by Sabongari (40.4 %) least in Giwa (16.6 %). For Kano State, Kiru LGA (30.0 %) had the highest disease incidence, followed by Bebeji (10.4 %) and there was no incidence of the disease in Rogo LGA. There were no symptoms of bacterial blight on cocoyam plants in fields surveyed in Katsina State. The disease severity in Kaduna State was highest in Kudan LGA (45.3 %), followed by Sabongari LGA (14.4 %), while Giwa LGA (6.3 %) had the least. In Kano State, Kiru LGA (12.7 %) had the highest disease severity, followed by Bebeji LGA (2.7 %). The mean bacterial blight incidence and severity for Kaduna and Kano States are shown in Figure 3, with Kaduna State having higher disease incidence and severity index than Kano State.

The different symptoms observed in the locations were mostly spots and blight, consisting of small, water-soaked lesions, usually surrounded by prominent chlorotic halos (Plate 2).

Figures 4 and 5 show the distribution of bacterial leaf blight in the surveyed areas in North West Nigeria. In Kaduna State, bacterial leaf blight was observed in four out of nine locations surveyed. These locations included Jaja and Hunkuyi in Kudan LGA and Tudun Sarki in Sabongari LGA. In Kano State, only Yelwan Paki in Kiru LGA and Gwarmai in Bebeji LGA showed the presence of bacterial leaf blight out of the nine locations surveyed.

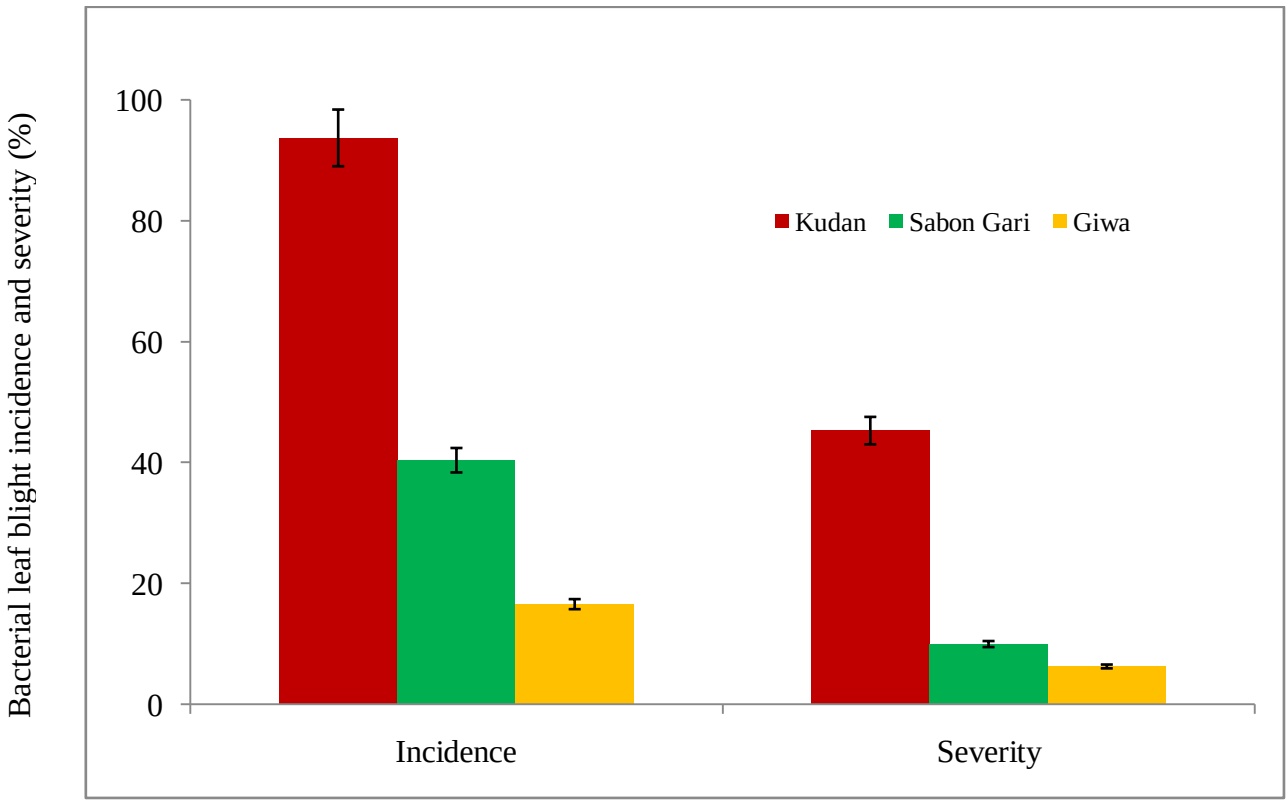



Figure 1: Incidence and severity of bacterial leaf blight on cocoyam leaves in three Local Government Areas of Kaduna State, Nigeria during 2014 wet season.

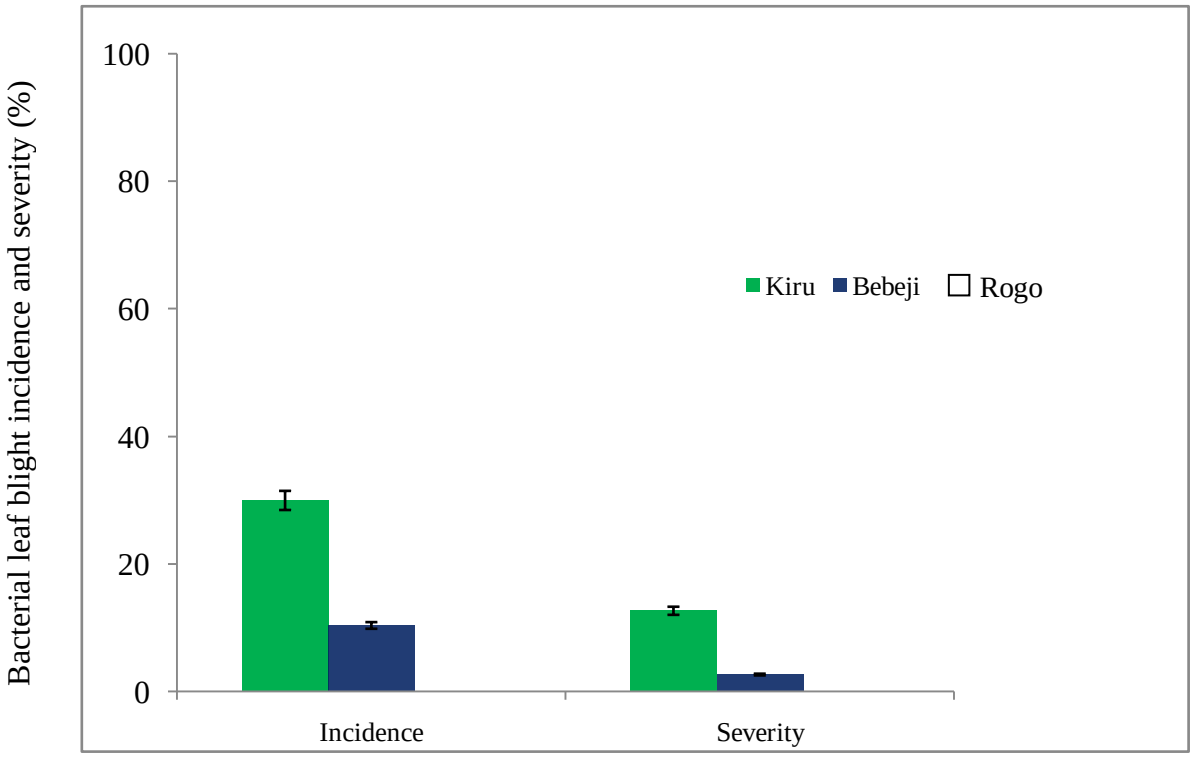



Figure 2: Incidence and severity of bacterial leaf blight on cocoyam leaves in three Local Government Areas of Kano State, Nigeria during 2014 wet season.

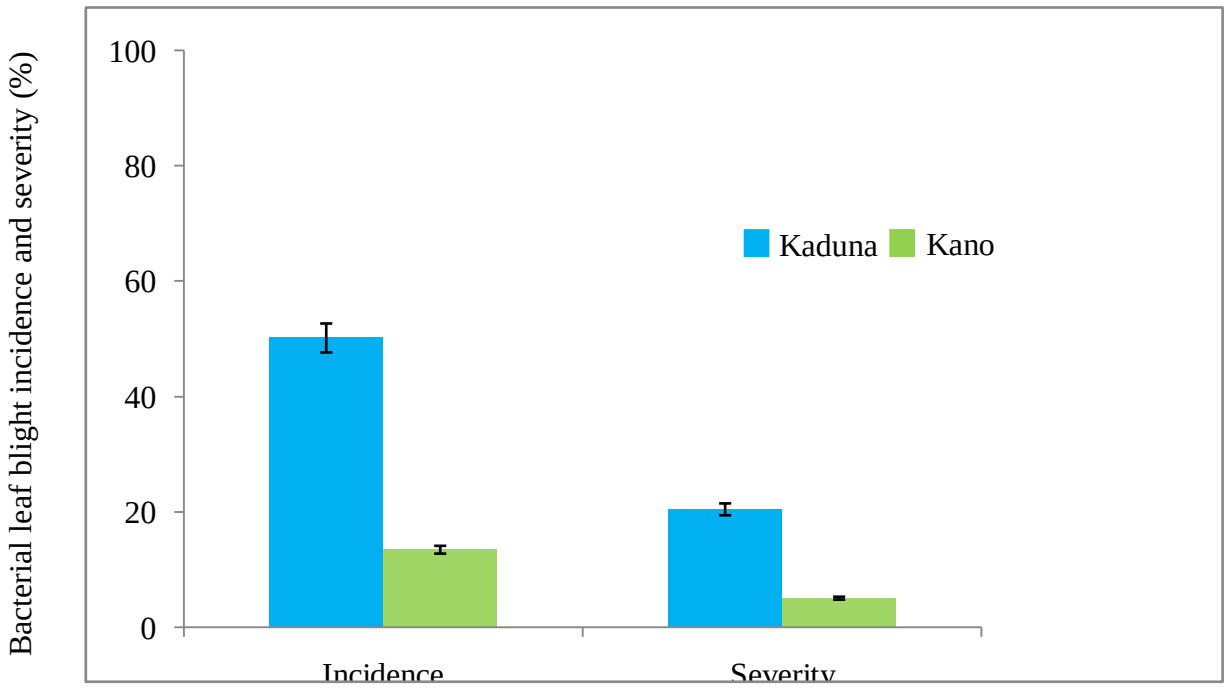



Figure 3: Incidence and severity of bacterial leaf blight on cocoyam leaves in Kaduna and Kano States, Nigeria during 2014 wet season




Plate 2: Typical symptoms (blight and spot) of bacterial leaf blight on cocoyam leaf in a field in Jaja, Kaduna State.

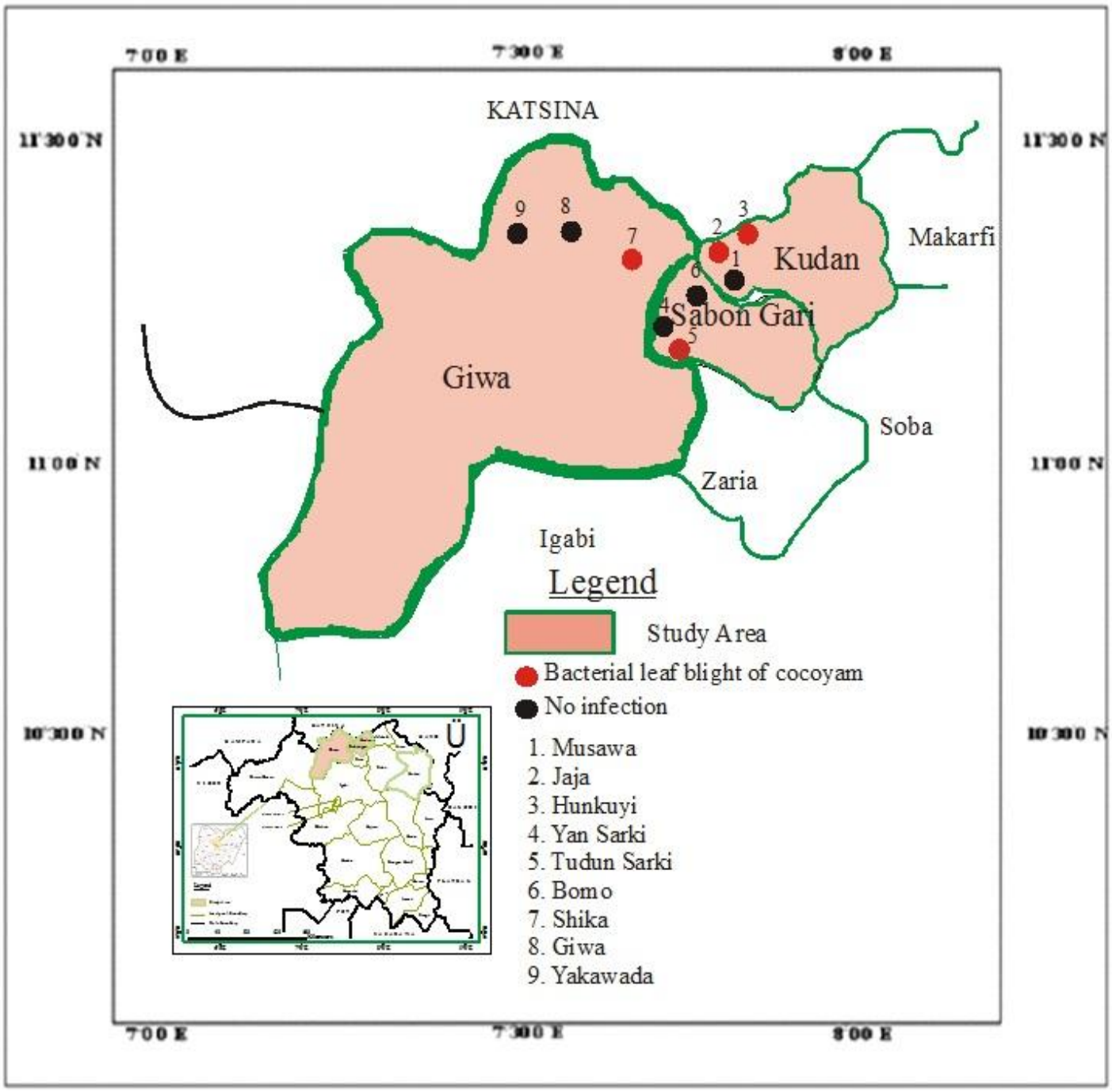



Figure 4: Map showing distribution of bacterial leaf blight of cocoyam in the surveyed locations in Kaduna State during 2014 wet season

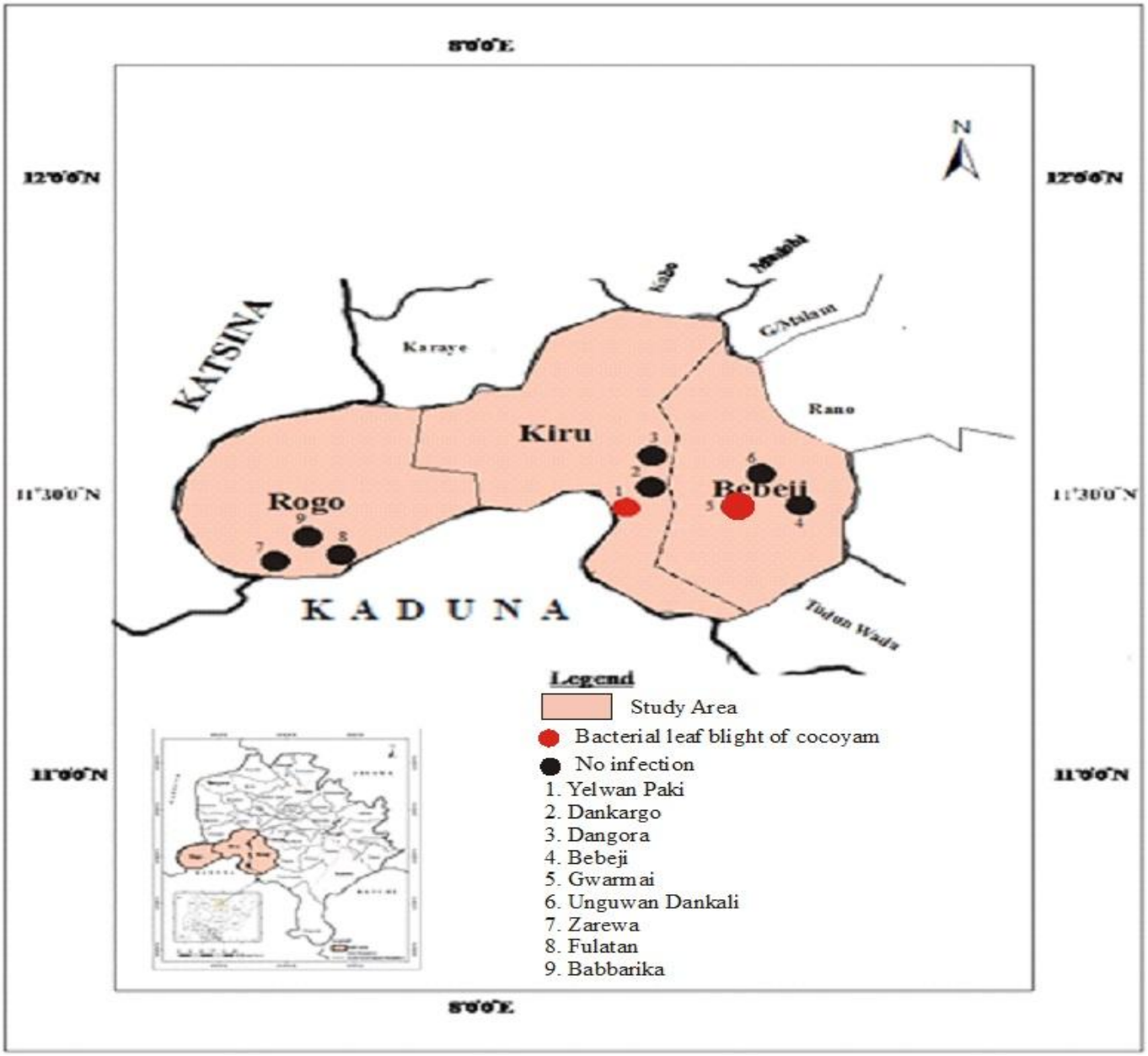



Figure 5: Map showing distribution of bacterial leaf blight of cocoyam in the surveyed locations in Kano State during 2014 wet season

## 4.2 Characterisation of *Xanthomonas* spp.

### 4.2.1 Tobacco hypersensitive and pathogenicity tests

The isolated bacteria formed yellow, convex, mucoid colonies on nutrient agar medium. The six isolates (CE2, CE3, CE4, CE6, CE7 and CE11) elicited hypersensitive reaction (HR) on tobacco and were found to be pathogenic on cocoyam. On tobacco, a clear HR was observed 48 hours after suspension of the inoculum was infiltrated into leaf tissues (Plate 3). Control leaves did not show similar reaction. On cocoyam, symptoms appeared 2-5 days after inoculation on the leaves. Prominent chlorotic halos developed around the lesions which were similar to those observed in the field (Plate 4).

The data on Table 3 shows the effect of the isolate and method of inoculation of *Xanthomonas axonopodis* pv. *dieffenbachiae* on disease development. There was significant difference at 5 % level on number of lesions produced by the various isolates. Isolate CE2 had the highest number of lesions 29.7 that was significantly higher than those of the other isolates, followed by CE4 which was statistically at par with CE3, CE6, CE7 and CE11. Isolate CE2 also recorded significantly higher disease severity index than the other isolates, followed by isolate CE6 which was statistically similar to isolates CE4 and CE11 and higher than isolates CE3 and CE7, while disease severity recorded on isolates CE3, CE4, CE7 and CE11 were statistically the same. Incubation periods among Isolates CE6, CE7 and CE11 were statistically similar ($P>0.05$) and statistically higher than those of isolates CE2, CE3 and CE4, except with isolate CE6 ($P<0.01$). The effect of inoculation method on lesion number, severity index and incubation period was significantly different ($P=0.05$). Sand paper method had higher lesions of 30.7 and severity index of 37.6 % than hypodermic syringe

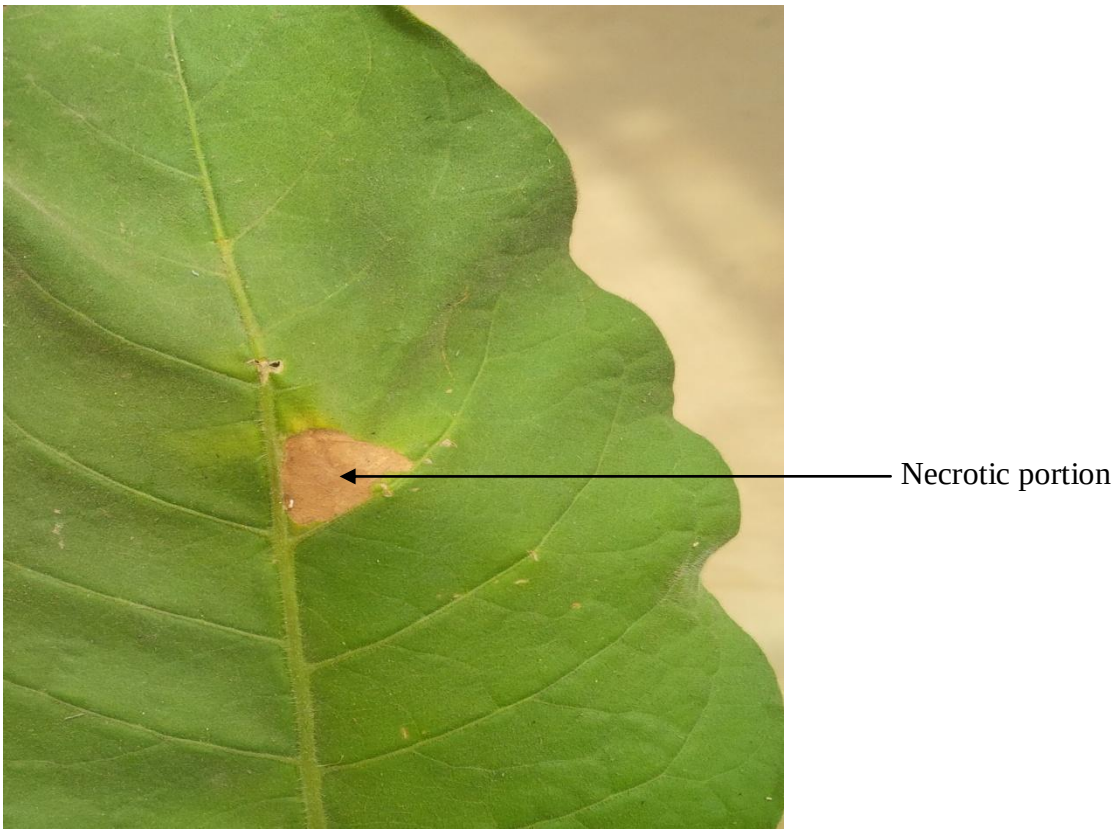



Plate 3**:** Necrotic portion on tobacco leaf inoculated with *Xanthomonas axonopodis* pv. *dieffenbachiae* showing hypersensitive reaction

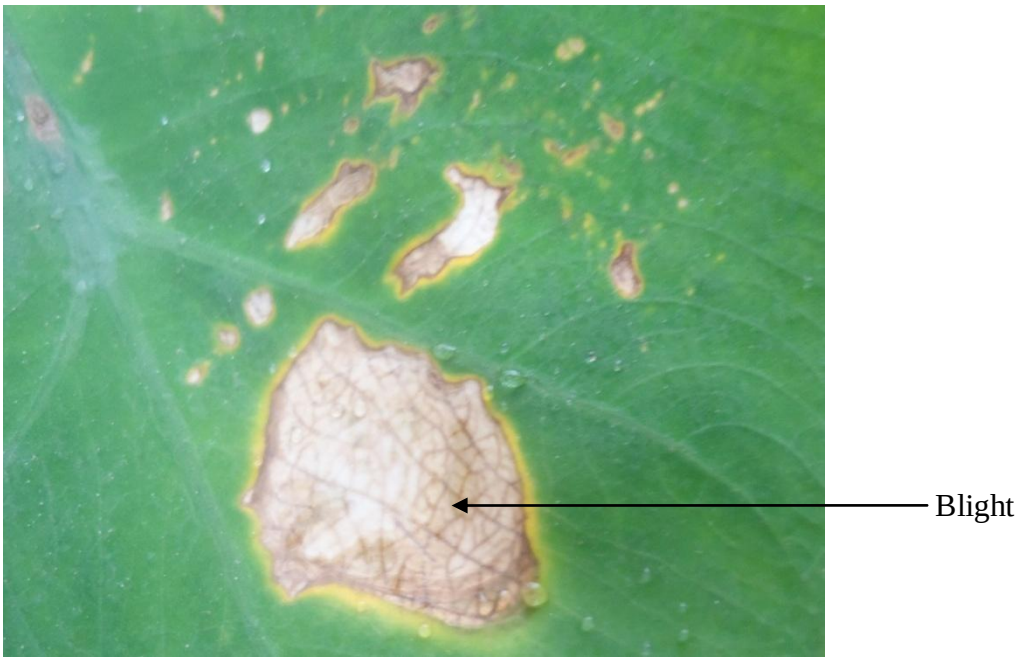



Plate 4: Leaf blight symptoms on cocoyam inoculated with *Xanthomonas axonopodis* pv. *dieffenbachiae* in the screen house

Table 3: Number of lesions, disease severity index and incubation period of *Xanthomonas* isolates on cocoyam with different inoculation methods in the screen house

| | Number of lesions* | Disease Severity index (%)* | Incubation period (days)* |
|---|---|---|---|
| **Isolate** | | | |
| CE2 | 29.7[a] | 48.0[a] | 2.8[b] |
| CE3 | 26.0[b] | 28.0[c] | 3.3[b] |
| CE4 | 26.8[b] | 32.7[bc] | 3.2[b] |
| CE6 | 25.3[b] | 35.3[b] | 3.5[ab] |
| CE7 | 26.2[b] | 28.7[c] | 4.5[a] |
| CE11 | 25.5[b] | 34.0[bc] | 4.5[a] |
| S.E.± | 0.93 | 1.92 | 0.33 |
| **Inoculation method** | | | |
| Sandpaper | 30.7[a] | 37.6[a] | 3.1[b] |
| Hypodermic syringe | 22.5[b] | 31.3[b] | 4.2[a] |
| S.E.± | 0.53 | 1.11 | 0.19 |
| **Interaction** | | | |
| Isolate*inoculation | NS | NS | NS |
| S.E.± | 1.31 | 2.72 | 0.46 |

*Means followed by the same letter along a column are not significantly different by DMRT at P = 0.05

CE2 = Tudun Sarki isolate

CE3 = Jaja isolate

CE4 = Yelwan Paki isolate

CE6 = Hunkuyi isolate

CE7 = Shika isolate

CE11 = Gwarmai isolate

S.E. = Standard error

NS = not significant

with 22.5 and 31.3 %, respectively. However, incubation period by hypodermic syringe (4.2 days) was higher than 31.3 days obtained when sand paper method was used. Interaction of isolates and inoculation methods was not significant on the parameters taken.

### 4.2.2 Biochemical tests

All the isolates were Gram negative, did not produce fluorescent pigment on King's medium B and were oxidase negative, catalase, amylolytic, pectolytic and liquefaction of gelatin positive, producing acid from glucose, fructose, mannose, lactose, galactose, sucrose, arabinose and not from glycerol (Table 4).

### 4.2.3 Molecular characterisation

*4.2.3.1 Gel electrophoresis of amplified 16S rDNA*

The 16S rDNA of each isolate amplified using 16S rRNA primers had the expected band size of 1465 bp (Plate 5).

*4.2.3.2 Phylogenetic analysis*

Basic Local Alignment Search Tool (BLAST) results for the isolates showed 98-100 % genetic relatedness to three *Xad* strains in the Genbank. Phylogenetic tree of sequenced isolates is shown in Figure 6. Isolates CE2 and CE6 are more closely related to each other than to other isolates, though isolate CE2 has accumulated eight times as many genetic changes or mutations as isolate CE6. However, isolates CE2 and CE3 have undergone more genetic change than either isolate CE6 or CE4. The isolates obtained from North West Nigeria form the same clade with three *Xanthomonas axonopodis* pv. *dieffenbachiae* strains on the dendogram (Figure 7). The calculated Tajima Neutrality test (Tajima, 1989) shown in Table 5 indicates that there are 18 segregating sites for the four isolates tested from Nigeria with a negative Tajima statistic, *D*.

Table 4: Biochemical reaction of the isolates

| Tests | Isolates | | | | | |
|---|---|---|---|---|---|---|
| | CE2 | CE3 | CE4 | CE6 | CE7 | CE11 |
| Gram's reaction | - | - | - | - | - | - |
| Fluorescence on KB | - | - | - | - | - | - |
| Oxidase activity | - | - | - | - | - | - |
| Catalase Activity | + | + | + | + | + | + |
| Amylolytic Activity | + | + | + | + | + | + |
| Pectolytic Activity | + | + | + | + | + | + |
| Gelatin test | + | + | + | + | + | + |
| Acid production from: | | | | | | |
| Glucose | + | + | + | + | + | + |
| Fructose | + | + | + | + | + | + |
| Mannose | + | + | + | + | + | + |
| Lactose | + | + | + | + | + | + |
| Galactose | + | + | + | + | + | + |
| Sucrose | + | + | + | + | + | + |
| Arabinose | + | + | + | + | + | + |
| Glycerol | - | - | - | - | - | - |

+ and - indicate positive and negative reactions, respectively to the test indicated.

CE2 = Tudun Sarki isolate

CE3 = Jaja isolate

CE4 = Yelwan Paki isolate

CE6 = Hunkuyi isolate

CE7 = Shika isolate

CE11 = Gwarmai isolate

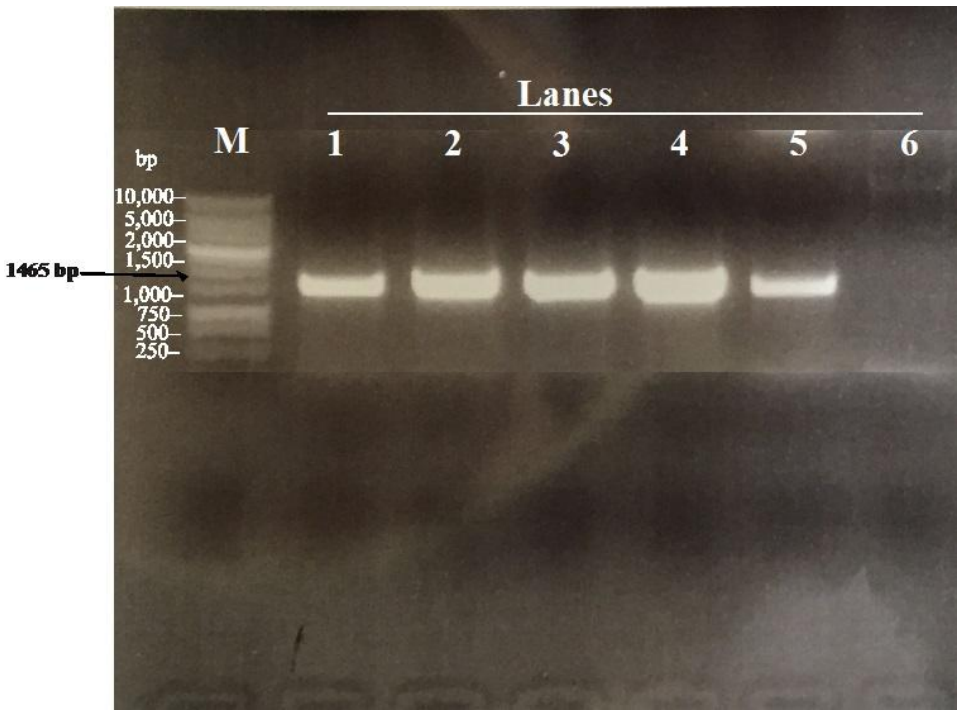



Plate 5**:** Electrograph of amplified 16S rDNA genes of *X. axonopodis* pv. *dieffenbachiae* isolates from North West Nigeria.

M = molecular marker

Lanes: 1 = CE2 (Tudun Sarki isolate)

2 = CE3 (Jaja isolate)

3 = CE4 (Yelwan Paki isolate)

4 = CE6 (Hunkuyi isolate)

5 = MSCTI (positive control)

6 = sterile distilled water (negative control)

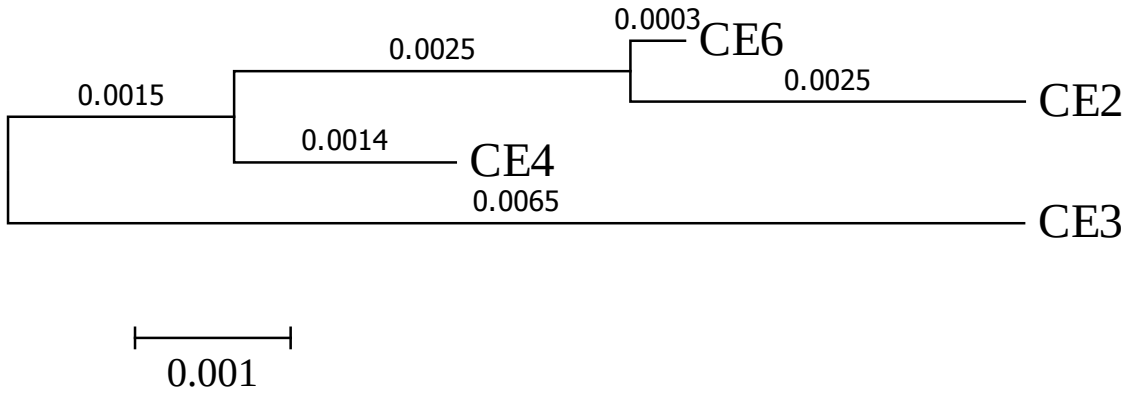



Figure 6: A phylogram showing phylogenetic relatedness of all four isolates from 16S rDNA amplified sequence.

CE2 = Tudun Sarki isolate

CE3 = Jaja isolate

CE4 = Yelwan Paki isolate

CE6 = Hunkuyi isolate

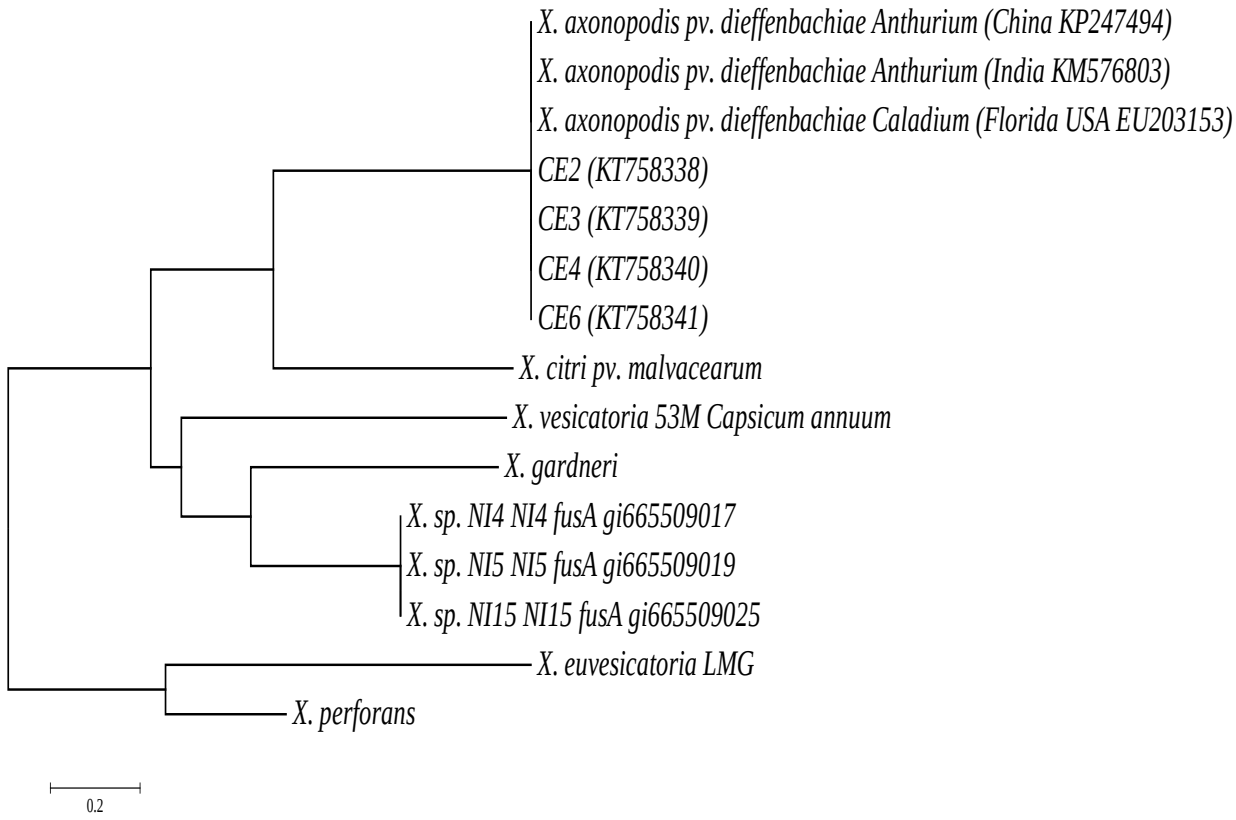



Figure 7: Phylogenetic tree showing relatedness of gene sequences of *Xanthomonas* isolates from North West Nigeria to other *Xanthomonas* spp.

CE2 = Tudun Sarki isolate

CE3 = Jaja isolate

CE4 = Yelwan Paki isolate

CE6 = Hunkuyi isolate

Table 5: Results from Tajima's Neutrality Test

| ***m*** | ***S*** | $p_s$ | $\Theta$ | $\Pi$ | ***D*** |
|---|---|---|---|---|---|
| 4 | 18 | 0.012748 | 0.006953 | 0.006492 | -0.678399 |

*Abbreviations*: *m* = number of sequences, *S* = Number of segregating sites, $p_s = S/n$, $\Theta = p_s/a_1$, $\pi$ = nucleotide diversity, and *D* is the Tajima test statistic.

# CHAPTER FIVE

## 5.0 DISCUSSION

The bacterial leaf blight of cocoyam was recorded in Kaduna and Kano States out of the three States surveyed, with Kaduna State recording a significantly higher incidence and severity of the disease than Kano State. The high incidence and severity in Kaduna State may be attributed to higher rainfall in Kaduna State during the period of the survey than in Kano State (Appendix 6). Most of the fields surveyed in Kaduna State, such as those in Kudan and Giwa LGAs were wetlands (Appendix 7), a condition which favours the pathogen. Anonymous (2001) states that *Xanthomonas axonopodis* pv. *dieffenbachiae* is favoured by warm and moist conditions. Hot, humid, and rainy weather conditions are ideal for disease development and the spread of *Xad* (Deng *et al.*, 2010). The disease incidence recorded in some Local Government Areas in Kaduna and Kano States was in line with the earlier report of Opara *et al*. (2013)**,** who reported over 65-68% incidence of BLB at Umudike, Abia State in South East Nigeria. A 74-100% incidence of BLB was also reported from a survey of commercial cocoyam fields in Florida, U. S. A. (Pohronezny *et al*., 1985). However, the disease severity index was generally low, averaging 2 or less, indicating that less than 30 % of the foliar area was damaged by *Xad*. For example, Pohronezny *et al*. (1985) reported a less than 10 % disease severity of bacterial leaf blight in their survey of commercial cocoyam fields infected by *Xad* in Florida.

Reports of this disease on other Araceae hosts such as *Anthurium*, *Syngonium* and *Dieffenbachiae* show that bacterial leaf blight is the most serious bacterial disease causing extensive crop losses in commercial foliage plant production (Khoodoo and Jaufeerally-Fakim, 2004; Robene-Soustrade *et al.*, 2006; Donahoo *et al*., 2013).

The result showed that bacterial leaf blight did not occur in the locations surveyed in Katsina State despite the State's cocoyam production status in North West Nigeria. Original source of inoculum of this pathogen is unknown. It is possible the pathogen was introduced into North West Nigeria in cocoyam planting stock from South East Nigeria, as some farmers (about 42%) in the areas where bacterial leaf blight occurred in Kaduna State, confirmed South East Nigeria to be one of the sources of their planting materials (Appendix 7). Systemic nature of the pathogen in cocoyam and other aroids may allow its transfer from crop to crop in contaminated corms and cormels used for plantings (Pohronezny *et al*., 1985). This could be the reason why the disease did not occur in Katsina State as most farmers in Katsina State used locally sourced planting materials in every season (Appendix 9).

*Xanthomonas axonopodis* pv. *dieffenbachiae* is distributed worldwide. The pathogen has been reported in Africa, Asia, Europe, Americas and Oceania (EPPO, 2015). Most of the reports of this pathogen from these regions are on *Anthurium* sp. while reports on cocoyam came only from America and Africa (Pohronezny *et al*., 1985; Opara *et al*., 2013). Anonymous (2009) distinguishes at least two groups of strains affecting Araceae, 'strains that are highly virulent to *Anthurium*, and with a broad host range and are the causal agent of bacterial blight of *Anthurium*' and 'strains originating from other Araceae genera that are primarily pathogenic to their host or origin and weakly or not pathogenic to Anthurium'.

Sand paper method of inoculation produced higher symptoms expression as indicated by disease severity index and number of lesions than hypodermic syringe method. Pressing with sandpaper produces micro wounds which provided routes for entry of inoculum leading to faster development of the disease compared to infiltration by

hypodermic syringe (Salaheddin *et al,* 2005). Wounding with sand paper produced more infection sites and weaker plant tissues for disease development. In addition, *Xanthomonas axonopodis* pv. *dieffenbachiae* is a vascular pathogen. At the point of entry into the host, either through the stomata, hydathodes or wounds, the bacteria multiply and spread through the xylem vessels (Donahoo, 2003). Plant wounding (such as the use of sand paper) exposes the xylem vessels and the bacteria can infect the xylem tissues (Munoz-Bodnar *et al.*, 2015). The flow of water and other substances through the vascular vessels could increase bacterial mobility and rate of infection (Munoz-Bodnar *et al.*, 2015).

Syringe-infiltration introduces bacterial inoculum into the leaf mesophyll such that symptoms quickly appear in the infiltrated leaf area; however, bacteria colonize the neighboring tissues slowly if they do not reach the mid-vein (Ruz *et al*., 2008). This method is best suited for bacterial pathogens such as tomato bacterial spot pathogens which colonize the apoplast of the leaf mesophyll (Kim *et al*., 2008). Sand paper method may be highly useful in screening large numbers of cocoyam cultivars for resistance to bacterial leaf blight. There was significant difference ($p<0.05$) among the isolates with regards to disease severity index and number of lesions produced, with the isolate from Tudun Sarki (CE2) having the highest disease severity index and number of lesions. It may be that Tudun Sarki isolate is most virulent among the *Xad* isolates from North West Nigeria.

Bacterial organisms isolated from diseased leaf of cocoyam collected from North West Nigeria fit biochemical descriptions of *Xanthomonas axonopodis*. Biochemical tests showed that the isolates are pectolytic, confirming Chase *et al*. (1992) report which stated that strains from *Colocasia*, *Dieffenbachia* and *Philodendron* were highly

pectolytic, while those obtained from *Xanthosoma* and *Syngonium* are non-pectolytic. Acids were not produced from glycerol by isolates obtained from various locations in North West Nigeria which confirms the findings of Pohronezny *et al*. (1985) and Opara *et al*. (2013) who reported that strains from cocoyam did not produce acid from glycerol. However, the variability in acid production from glycerol may preclude the usefulness of this biochemical test in the differentiation of *X. axonopodis* pathovars. This is in line with the findings of Pohronezny *et al*. (1985) who reported lack of acid production in a known *Xad* strain from other aroids in Florida.

BLAST results of 98-100 % genetic relatedness in the Genbank and formation of same clade by the isolates on the phylogenetic trees with three *Xad* strains, re-affirmed that isolates from North West Nigeria are *Xanthomonas axonopodis* pv. *dieffenbachiae.* 16S rDNA profiling has been widely used for the genetic differentiation of plant pathogens including pathovars of *Xanthomonas axonopodis* (Vauterin *et al*., 1995; Deng *et al.*, 2010). This technique though useful for such identification, *X. axonopodis* strains do not always produce a consistent and unique profile with 16S rDNA (Vauterin *et al*., 1995). Host range has been used to define the pathovar *Xad.* This pathogen has been shown to be serologically diverse and heterogeneous in carbon source use, fatty acid analysis, rep-PCR and random amplified polymorphic DNA analysis (Chase *et al.* 1992; Khoodoo and Jaufeerally-Fakim, 2004). Strains of *Xad* from different species of aroids are differentiated primarily by host range with strains being generally more pathogenic on the host of origin. Strains from Anthurium and Syngonium could be differentiated using restriction analysis PCR amplification products, whereas strains from other hosts were generally difficult to distinguish from each other by physiological, serological, or molecular techniques (Chase *et al*., 1992; Lipp *et al*.,

1992; Khoodoo and Jaufeerally-Fakim, 2004; Robene-Soustrade *et al*., 2006). Little is known about the host range of strains from cocoyam but one strain from cocoyam was pathogenic to Dieffenbachiae in Florida, USA (Pohronezny *et al*. 1985).

It is observed from the Tajima's neutrality test (Tajima, 1989) that there are 18 segregating sites for the sequences of the four isolates. This is equivalent to the number of nucleotide positions at which polymorphism is found (Fu, 1995). This result is also supported by the codon based test for neutrality, estimates of evolutionary divergence, and the Fisher's exact test for neutrality of sequences. The number of segregating sites is a valuable descriptor of genetic variation, which, together with theoretical predictions, can be used to make inferences about population history (Wakeley, 1998). The Tajima's *D* test is a widely used test of neutrality in population genetics and it measures the allele frequency distribution of nucleotide sequence data (Simonsen, *et al.*, 1995). The statistics can be influenced by both population history and natural selection. The negative Tajima's *D* signifies an excess of low frequency polymorphisms relative to expectation. This could be important information when considering breeding cocoyam for resistance to *Xad*.

This is the first report of *Xanthomonas axonopodis* pv. *dieffenbachiae* in North West Nigeria and first report on molecular characterisation of the pathogen in Nigeria. Appendices 2-5 show the sequences of the isolates deposited in the Genbank and their accession numbers (http://www.ncbi.nlm.nih.gov/nuccore).

# CHAPTER SIX

## 6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

### 6.1 Summary

Field surveys were conducted during the 2014 wet season in three Local Government areas each of Kaduna Katsina and Kano States of Nigeria to determine the presence of bacterial leaf blight of cocoyam caused by *Xanthomonas axonopodis* pv. *dieffenbachiae*. A total of eighty one cocoyam fields were surveyed, that was twenty seven in each of the States and diseased leaf samples were collected. Cocoyam leaves were examined for symptoms of bacterial leaf blight; disease incidence and severity were recorded per field, per location, then per Local Government Area in two States. Isolates of *Xanthomonas* spp. collected from cocoyam fields were characterised using biochemical tests and polymerase chain reactions (PCR) of 16S rRNA genes. The results showed presence of bacterial leaf blight of cocoyam in Kaduna and Kano States, with Kaduna State having higher incidence and severity of the disease than Kano State. The results showed that the disease was not found in Katsina State. Pathogenicity test on cocoyam showed positive results with isolates from Kudan, Sabongari and Giwa LGAs in Kaduna State, Kiru and Bebeji LGAs in Kano State. Sandpaper method of inoculation proved to be more efficient in test for pathogenicity than hypodermal syringe method. Biochemical analysis revealed that isolates were amylolytic and pectolytic and did not produce acids from glycerol. Further phylogenetic analysis confirmed cocoyam isolates from North West Nigeria to be *Xanthomonas axonopodis* pv. *dieffenbachiae*.

## 6.2 Conclusions

Bacterial leaf blight of cocoyam occurred in Kaduna and Kano States and the disease is more prevalent in Kaduna than in Kano State. There was no incidence of the disease in Katsina State. This is the first report of bacterial leaf blight of cocoyam in North West Nigeria and first report on molecular characterisation of the pathogen in Nigeria. The nucleotide sequences of the isolates (CE2, CE3, CE4 and CE6) have been deposited in the Genbank and can be accessed on http://www.ncbi.nlm.nih.gov/nuccore. The sequences represent the first submission of *Xanthomonas axonopodis* pv. *dieffenbachiae* (causal organism of bacterial leaf blight of cocoyam) on any plant in West Africa and on cocoyam in Africa.

## 6.3 Recommendations

Based on the findings of this study, the following recommendations are suggested:

1) An effective strategy which could help in the integrated management of the disease in the surveyed areas is recommended. This is because bacterial leaf blight is one of the numerous diseases currently threatening cocoyam production in North West Nigeria.
2) Studies on the occurrence of bacterial leaf blight should be extended to other cocoyam producing areas in Nigeria with hitherto little or no information on the disease. Consequently, a full profile of the *Xanthomonas* causing bacterial blight in cocoyam can be identified.
3) Farmers in the surveyed areas in Katsina State should make sure that cocoyam corms are not obtained for planting from areas where bacterial leaf blight has been reported.

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## APPENDICES

Appendix 1: Analysis of variance procedure for number of lesions, disease severity index (%) and incubation period (days)

Analysis of variance Table for number of lesions

| Source | Degree of freedom | Sum of squares | Mean square | F-value | Probability |
|---|---|---|---|---|---|
| Isolate | 5 | 76.92 | 15.38 | 2.99 | 0.0307 |
| Method of inoculation | 1 | 600.25 | 600.25 | 116.78 | <0.0001 |
| Isolate*method of inoculation | 5 | 40.25 | 8.05 | 1.57 | 0.2074 |
| error | 24 | 123.33 | 5.14 | | |
| Total | 35 | 840.75 | | | |

Analysis of variance Table for disease severity index (%)

| Source | Degree of freedom | Sum of squares | Mean square | F-value | Probability |
|---|---|---|---|---|---|
| Isolate | 5 | 1576.89 | 315.38 | 14.19 | <0.0001 |
| Method of inoculation | 1 | 348.44 | 348.44 | 15.68 | 0.0006 |
| Isolate*method of inoculation | 5 | 78.22 | 15.64 | 0.70 | 0.6260 |
| error | 24 | 533.33 | 22.22 | | |
| Total | 35 | 2536.88 | | | |

Analysis of variance Table for incubation period (days)

| Source | Degree of freedom | Sum of squares | Mean square | F-value | Probability |
|---|---|---|---|---|---|
| Isolate | 5 | 14.81 | 2.96 | 4.63 | 0.0042 |
| Method of inoculation | 1 | 10.03 | 10.03 | 15.67 | 0.0006 |
| Isolate*method of inoculation | 5 | 0.14 | 0.03 | 0.05 | 0.9988 |
| error | 24 | 15.33 | 0.64 | | |
| Total | 35 | 40.31 | | | |

Appendix 2: *Xanthomonas axonopodis* pv. *dieffenbachiae* strain Tudun Sarki isolate (CE2) 16S ribosomal RNA gene, partial sequence

GenBank: KT758338.1 FASTA Graphics PopSet

LOCUS KT758338 1453 bp DNA linear BCT 06-OCT-2015
DEFINITION *Xanthomonas axonopodis* pv. *dieffenbachiae* strain CE2 16S ribosomal RNA gene, partial sequence.
ACCESSION KT758338
VERSION KT758338.1 GI:932249343
KEYWORDS .
SOURCE *Xanthomonas axonopodis pv. dieffenbachiae*
ORGANISM *Xanthomonas axonopodis pv. dieffenbachiae*
Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Xanthomonas.
REFERENCE 1 (bases 1 to 1453)
AUTHORS Abdullahi, A., Lu, S., Bandyopadhyay, R., Alao, SEL, Banwo, O. O. and Alabi, O.
TITLE Molecular Characterisation of *Xanthomonas axonopodis* pv. *dieffenbachiae* Strains Infecting Cocoyam in North West Nigeria
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1453)
AUTHORS Abdullahi, A., Lu, S., Bandyopadhyay, R., Alao, SEL., Banwo, O. and Alabi, O.
TITLE Direct Submission
JOURNAL Submitted (05-SEP-2015) Crop Protection, Ahmadu Bello University, Zaria
COMMENT Sequences were screened for chimeras by the submitter using MEGA 6.06.

ORIGIN

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   1 ctaacacttg ccagtcgaac ggcagcacag taagagcttg ctcttatggg tggcgagtgg
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 121 acgctaatac cgcatacgac ctacgggtga aagcggagga ccttcgggct tcgcgcggtt
 181 gaatgagccg atgtcggatt agctagttgg cggggtaaag gcccaccaag gcgacgatcc
 241 gtagctggtc tgagaggatg atcagccaca ctggaactga gacacggtcc agactcctac
 301 gggaggcagc agtggggaat attggacaat gggcgcaagc ctgatccagc catgccgcgt
 361 gggtgaagaa ggccttcggg ttgtaaagcc cttttgttgg gaaagaaaag cagtcggtta
 421 atacccgatt gtgtctgacg gttaccccaa aagaataagc acccccgggc tcaacttcgg
 481 tgccagcagc ccggcggtaa atacgagaaa gggtgcaagc gttactctgg aattactggg
 541 gcgtaaagcg tgcgtaggtg gtggtttaag tctgttgtga aagcccctgg gctcaacctg
 601 ggaattgcca gtggatactg ggatcactag agtgtggtag agggtagcgg gaatttcccg
 661 ggtgtagcag tgaaatgcgt agagatcggg aggaacatcc cgtggcgaaa ggcggctacc
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 841 tcgaagctaa cgcgttaagt tcgccgcctg gggagtacgg tcgcaagact gaaactcaaa
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 961 gaaccttacc tggtcttgac atccacggaa ctttccagag atggattggt gccttcggga
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1081 cccgcaacga gcgcaaccct tgtccttagt tgccagcacg taatggtggg aactctaagg
1141 agaccgccgg tgacaaaccg gaggaaggtg gggatgacgt caagtcatca tggcccttac
1201 gaccagggct acacacgtac tacaatggta gggacagagg gctgcaaacc cgcgagggca
1261 agccaatccc agaaacccta tctcagtccg gattggagtc tgcaactcga ctccatgaag
1321 tcggaatcgc tagtaatcgc agatcagcat tgctgcggtg aatacgttcc cgggccttgt
1381 acacaccgcc cgtcacacca tgggagtttg ttgcaccaga agcaggtagc ttaaccttcg
1441 gaggcgctgc cac
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Appendix 3: *Xanthomonas axonopodis* pv. *dieffenbachiae* strain Jaja isolate (CE3) 16S ribosomal RNA gene, partial sequence

GenBank: KT758339.1 FASTA Graphics PopSet

LOCUS KT758339 1399 bp DNA linear BCT 06-OCT-2015
DEFINITION *Xanthomonas axonopodis* pv. *dieffenbachiae* strain CE3 16S ribosomal RNA gene, partial sequence.
ACCESSION KT758339
VERSION KT758339.1 GI:932249344
KEYWORDS .
SOURCE *Xanthomonas axonopodis pv. dieffenbachiae*
ORGANISM *Xanthomonas axonopodis* pv. *dieffenbachiae*
Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Xanthomonas.
REFERENCE 1 (bases 1 to 1399)
AUTHORS Abdullahi, A., Lu, S., Bandyopadhyay, R., Alao, SEL., Banwo, O. and Alabi, O.
TITLE Molecular Characterisation of *Xanthomonas axonopodis* pv. *dieffenbachiae* Strains Infecting Cocoyam in North West Nigeria
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1399)
AUTHORS Abdullahi, A., Lu, S., Bandyopadhyay, R., Alao, S., Banwo, O. and Alabi, O.
TITLE Direct Submission
JOURNAL Submitted (05-SEP-2015) Crop Protection, Ahmadu Bello University,
COMMENT Sequences were screened for chimeras by the submitter using MEGA 6.06.

ORIGIN

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 121 gctaataccg catacgacct acgggtgaaa gcggaggacc ttcgggcttc gcgcggttga
 181 atgagccgat gtcggattag ctagttggcg gggtaaaggc ccaccaaggc gacgatccgt
 241 agctggtctg agaggatgat cagccacact ggaactgaga cacggtccag actcctacgg
 301 gaggcagcag tggggaatat tggacaatgg gcgcaagcct gatccagcca tgccgcgtgg
 361 gtgaagaagg ccttcgggtt gtaaagccct tttgttggga aagaaaagca gtcggttaat
 421 acccgattgt tctgacggta cccaaagaat aagcaccggc taacttcgtg ccagcagccg
 481 cggtaatacg aagggtgcaa gcgttactcg gaattactgg gcgtaaagcg tgcgtaggtg
 541 gtggtttaag tctgttgtga aagccctggg ctcaacctgg gaattgcagt ggatactgga
 601 tcactagagt gtggtagagg gtagcggaat tcccggtgta gcagtgaaat gcgtagagat
 661 cgggaggaac atccgtggcg aaggcggcta cctggaccaa cactgacact gaggcacgaa
 721 agcgtgggga gcaaacagga ttagataccc tggtagtcca cgccctaaac gatgcgaact
 781 ggatgttggg tgcaatttgg cacgcagtat cgaagctaac gcgttaagtt cgccgcctgg
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 961 tttccagaga tggattggtg ccttcgggaa ccgtgaaaca ggtgctgcat ggctgtcttc
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1141 ggatgacgtc aagtcatcat ggcccttacg accagggcta cacacgtact acaatggtag
1201 ggacagaggg ctgcaaaccc gcgagggcaa gccaatccca gaaaccctat ctcagtccgg
1261 attggagtct gcaactcgac tccatgaagt cggaatcgct agtaatcgca gatcagcatt
1321 gctgcggtga atacgttccc gggccttgta cacaccgccc gtcacaccat gggagtttgt
1381 tgcaccagaa gcaggtagc
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Appendix 4: *Xanthomonas axonopodis* pv. *dieffenbachiae* strain Yelwan Paki (CE4) 16S ribosomal RNA gene, partial sequence

GenBank: KT758340.1 FASTA Graphics PopSet

LOCUS KT758340 1422 bp DNA linear BCT 06-OCT-2015
DEFINITION Xanthomonas axonopodis pv. dieffenbachiae strain CE4 16S ribosomal RNA gene, partial sequence.
ACCESSION KT758340
VERSION KT758340.1 GI:932249345
KEYWORDS .
SOURCE *Xanthomonas axonopodis* pv. *dieffenbachiae*
ORGANISM *Xanthomonas axonopodis* pv. *dieffenbachiae*
Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Xanthomonas.
REFERENCE 1 (bases 1 to 1422)
AUTHORS Abdullahi, A., Lu, S., Bandyopadhyay, R., Alao, SEL., Banwo, O. O. and Alabi, O.
TITLE Molecular Characterisation of *Xanthomonas axonopodis* pv. *dieffenbachiae* Strains Infecting Cocoyam in North West Nigeria
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1422)
AUTHORS Abdullahi, A., Lu, S., Bandyopadhyay, R., Alao, SEL., Banwo, O. and Alabi, O.
TITLE Direct Submission
JOURNAL Submitted (05-SEP-2015) Crop Protection, Ahmadu Bello University, Zaria

COMMENT Sequences were screened for chimeras by the submitter using MEGA 6.06.

ORIGIN

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   1 gctaccatga agtcgaacgg cagcacagta agagcttgct cttatgggtg gcgagtggcg
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 181 atgagccgat gtcggattag ctagttggcg gggtaaaggc ccaccaaggc gacgatccgt
 241 agctggtctg agaggatgat cagccacccc ggaactgaga cacggtccag actcctacgg
 301 gaggcagcag tggggaatat tggacaatgg gcgcaagcct gatccagcca tgccgcgtgg
 361 gtgaagaagg ccttcgggtt gtaaagccct tttgttggga aagaaaagca gtcggttaat
 421 acccgattgt tctgacggta cccaaagaat aagcaccggc taacttcgtg ccagcagccg
 481 cggtaatacg aagggtgcaa gcgttactcg gaattactgg gcgtaaagcg tgcgtaggtg
 541 gtggtttaag tctgttgtga aagccctggg ctcaacctgg gaattgcagt ggatactgga
 601 tcactagagt gtggtagagg gtagcggaat tcccggtgta gcagtgaaat gcgtagagat
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1321 ctgcggtgaa tacgttcccg ggccttgtac acaccgcccg tcacaccatg ggagtttgtt
1381 gcaccagaag caggtagctt aacctcggga aggcgctgcc ac
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Appendix 5: *Xanthomonas axonopodis* pv. *dieffenbachiae* strain Hunkuyi isolate (CE6) 16S ribosomal RNA gene, partial sequence

GenBank: KT758341.1FASTA Graphics PopSet
LOCUSKT 758341 1416 bp DNA linear BCT 06-OCT-2015
DEFINITION Xanthomonas axonopodis pv. dieffenbachiae strain CE6 16S ribosomal RNA gene, partial sequence.
ACCESSION KT758341
VERSION KT758341.1 GI:932249346
KEYWORDS .
SOURCE *Xanthomonas axonopodis* pv. *dieffenbachiae*
ORGANISM *Xanthomonas axonopodis* pv. *dieffenbachiae*
Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Xanthomonas.
REFERENCE 1 (bases 1 to 1416)
AUTHORS Abdullahi, A., Lu, S., Bandyopadhyay, R., Alao, SEL., Banwo, O. and Alabi, O.
TITLE Molecular Characterisation of *Xanthomonas axonopodis* pv. *dieffenbachiae* Strains Infecting Cocoyam in North West Nigeria
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1416)
AUTHORS Abdullahi, A., Lu, S., Bandyopadhyay, R., Alao, SEL., Banwo, O. and Alabi, O.
TITLE Direct Submission
JOURNAL Submitted (05-SEP-2015) Crop Protection, Ahmadu Bello University,
COMMENT Sequences were screened for chimeras by the submitter using MEGA 6.06.

ORIGIN

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   1 atgcagtcga acggcagcac agtaagagct tgctcttatg ggtggcgagt ggcggacggg
  61 tgaggaatac atcggaatct actctttcgt gggggataac gtagggaaac ttacgctaat
 121 accgcatacg acctacgggt gaaagcggag gaccttcggg cttcgcgcgg ttgaatgagc
 181 cgatgtcgga ttagctagtt ggcggggtaa aggcccacca aggcgacgat ccgtagctgg
 241 tctgagagga tgatcagcca cactggaact gagacacggt ccagactcct acgggaggca
 301 gcagtgggga atattggaca atgggcgcaa gcctgatcca gccatgccgc gtgggtgaag
 361 aaggccttcg ggttgtaaag ccctttgtt gggaaagaaa agcagtcggt taatacccga
 421 ttgttctgac ggtacccaaa gaataagcac cggctaactt cgtgccagca gccgcggtaa
 481 tacgaagggt gcaagcgtta ctcggaatta ctgggcgtaa agcgtgcgta ggtggtggtt
 541 taagtctgtt gtgaaagccc tgggctcaac ctgggaattg cagtggatac tggatcacta
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 661 gaacatccgt ggcgaaggcg gctacctgga ccaacactga cactgaggca cgaaagcgtg
 721 gggagcaaac aggattagat accctggtag tccacgccct aaacgatgcg aactggatgt
 781 tgggtgcaat ttggcacgca gtatcgaagc taacgcgtta agttcgccgc ctggggagta
 841 cggtcgcaag actgaaactc aaaggaattg acgggggccc gcacaagcgg tggagtatgt
 901 ggtttaattc gatgcaacgc gaagaacctt acctggtctt gacatccacg gaactttcca
 961 gagatggatt ggtgccttcg ggaaccgtga gacaggtgct gcatggctgt cgtcagctcg
1021 tgtcgtgaga tgttgggtta agtcccgcaa cgagcgcaac ccttgtcctt agttgccagc
1081 acgtaatggt gggaactcta aggagaccgc cggtgacaaa ccggaggaag gtggggatga
1141 cgtcaagtca tcatggccct tacgaccagg gctacacacg tactacaatg gtagggacag
1201 agggctgcaa acccgcgagg gcaagccaat cccagaaacc ctatctcagt ccggattgga
1261 gtctgcaact cgactccatg aagtcggaat cgctagtaat cgcagatcag cattgctgcg
1321 gtgaatacgt tcccgggcct tgtacacacc gcccgtcaca ccatgggagt ttgttgcacc
1381 agaagcaggt agcttaacct tccggagggc gctgcc
```

Appendix 6: Mean Monthly Rainfall and Temperature of Kaduna, Katsina and Kano States, March to October, 2014

| Months | Rainfall (mm) | | | Temperature ($^{o}$C) | | |
|---|---|---|---|---|---|---|
| | Kaduna | Kano | Katsina | Kaduna | Kano | Katsina |
| March | 3.6 | 0.0 | 0.0 | 36.2 | 36.1 | 37.6 |
| April | 144.2 | 16.9 | 0.0 | 36.1 | 38.8 | 39.7 |
| May | 259 | 75.7 | 38.1 | 33.5 | 36.3 | 37.6 |
| June | 401.6 | 88.9 | 39.5 | 31.6 | 35.6 | 36.6 |
| July | 328.2 | 481.5 | 178.9 | 30.0 | 32.3 | 33.3 |
| August | 986.2 | 509.6 | 178.7 | 28.7 | 30.0 | 30.7 |
| September | 580.4 | 186.2 | 46.3 | 30.3 | 31.4 | 32.1 |
| October | 131.2 | 17.2 | 0.0 | 32.3 | 33.6 | 35.9 |

Source: NAERLS and FDAE (2014)

Appendix 7: Cropping information obtained from cocoyam farms surveyed in Kaduna State

| State | LGA | Location | Field | Farm size (ha) | Cropping pattern | Associated crop | Age of crop (months) | Source of corm | Topography | GPS Coordinates and Altitude |
|---|---|---|---|---|---|---|---|---|---|---|
| Kaduna | Kudan | Jaja | 1 | 0.40 | Mixed cropping | Okra | 4 | SEN | lowland | N $11^0$14.065$'$;E $007^0$38.663$'$; 660m |
| | | | 2 | 0.35 | Sole cropping | | 4 | SEN | lowland | N $11^0$14.030$'$;E $007^0$37.203$'$; 680m |
| | | | 3 | 0.40 | Sole cropping | | 3 | LS | lowland | N $11^0$14.075$'$;E $007^0$36.257$'$; 630m |
| | | Hunkuyi | 1 | 0.20 | Mixed cropping | Maize | 4 | SEN | lowland | N $11^0$16.786$'$;E $007^0$39.430$'$; 630m |
| | | | 2 | 0.10 | Mixed cropping | Maize | 5 | SEN | lowland | N $11^0$16.715$'$;E $007^0$40.222$'$; 595m |
| | | | 3 | 0.32 | Sole cropping | | 4 | SEN | upland | N $11^0$16.516$'$;E $007^0$39.325$'$; 650m |
| | | Musawa | 1 | 0.14 | Mixed cropping | Sugarcane | 5 | LS | lowland | N $11^0$13.477$'$;E $007^0$40.409$'$; 658m |
| | | | 2 | 0.30 | Sole cropping | | 5 | LS | lowland | N $11^0$13.426$'$;E $007^0$41.209$'$; 662m |
| | | | 3 | 0.12 | Sole cropping | | 4 | LS | upland | N $11^0$13.417$'$;E $007^0$40.312$'$; 640m |
| | Sabongari | Tudun Sarki | 1 | 0.4 | Mixed cropping | Maize | 4 | LS | lowland | N $11^0$07.236$'$;E $007^0$54.307$'$; 625m |
| | | | 2 | 0.35 | Mixed cropping | Maize | 3 | LS | upland | N $11^0$07.255$'$;E $007^0$54.106$'$; 630m |
| | | | 3 | 0.12 | Sole cropping | | 4 | MM | upland | N $11^0$07.245$'$;E $007^0$54.113$'$; 659m |
| | | Bomo | 1 | 0.09 | Sole cropping | | 3 | LS | upland | N $11^0$09.012$'$,E $007^0$41.503$'$; 630m |
| | | | 2 | 0.10 | Mixed cropping | Maize | 3 | LS | upland | N $11^0$09.032$'$ E $007^0$41.303$'$; 630m |
| | | | 3 | 0.20 | Mixed cropping | Maize | 3 | LS | upland | N $11^0$09.119$'$, E $007^0$41.256$'$; 630m |
| | | Yan Sarki | 1 | 0.12 | Sole cropping | | 4 | LS | upland | N $11^0$07.263$'$; E $007^0$35.459$'$; 702m |
| | | | 2 | 0.08 | Mixed cropping | Okra | 5 | LS | upland | N $11^0$07.269$'$; E $007^0$35.692$'$; 702m |
| | | | 3 | 0.13 | Sole cropping | | 4 | LS | upland | N $11^0$07.450$'$; E $007^0$35.205$'$; 695m |
| | Giwa | Shika | 1 | 0.12 | Mixed cropping | Okra | 5 | LS | lowland | N $11^0$11.725$'$; E $007^0$34.532$'$; 670m |
| | | | 2 | 0.03 | Sole cropping | | 5 | LS | Lowland | N $11^0$11.458$'$; E $007^0$33.139$'$; 680m |
| | | | 3 | 0.10 | Sole cropping | | 4 | LS | upland | N $11^0$11.525$'$; E $007^0$34.502$'$; 640m |
| | | Giwa | 1 | 0.15 | Mixed cropping | sugarcane | 3 | LS | lowland | N $11^0$15.239$'$; E $007^0$27.514$'$; 630m |
| | | | 2 | 0.15 | Sole cropping | | 2 | LS | lowland | N $11^0$15.489$'$; E $007^0$27.704$'$; 660m |
| | | | 3 | 0.08 | Sole cropping | | 2 | MM | lowland | N $11^0$15.200$'$; E $007^0$27.586$'$; 670m |
| | | Yakawada | 1 | 0.35 | Sole cropping | | 4 | LS | upland | N $11^0$15.997$'$; E $007^0$23.788$'$; 638m |
| | | | 2 | 0.2 | Mixed cropping | Maize | 4 | LS | upland | N $11^0$16.005$'$; E $007^0$23.715$'$; 589m |
| | | | 3 | 0.28 | Sole cropping | | 3 | LS | upland | N $11^0$16.035$'$; E $007^0$23.658$'$; 625m |

LGA = Local Government Area; LS = Locally sourced; SEN = South East Nigeria; MM = Makarfi Market

Appendix 8: Cropping information obtained from cocoyam farms surveyed in Kano State

| State | LGA | Location | Field | Farm size (ha) | Cropping pattern | Associated crop | Age of crop (months) | Source of corm | Topography | GPS Coordinates and Altitude |
|---|---|---|---|---|---|---|---|---|---|---|
| Kano | Kiru | Yelwan-paki | 1 | 0.25 | Sole cropping | | 5 | LS | upland | N 11$^0$30.016$^{'}$, E 008$^0$09.627$^{'}$; 586 m |
| | | | 2 | 0.10 | Sole cropping | | 4 | LS | upland | N 11$^0$30.008$^{'}$, E 008$^0$09.661$^{'}$; 582 m |
| | | | 3 | 0.07 | Sole cropping | | 2 | LS | upland | N 11$^0$30.001$^{'}$, E 008$^0$09.667$^{'}$; 581 m |
| | | Dankargo | 1 | 0.36 | Sole cropping | | 3 | LS | upland | N 11$^0$30.587$^{'}$; E 008$^0$10.418$^{'}$; 584 m |
| | | | 2 | 0.25 | Sole cropping | | 3 | LS | upland | N 11$^0$30.570$^{'}$; E 008$^0$10.410$^{'}$; 586 m |
| | | | 3 | 0.32 | Sole cropping | | 4 | LS | upland | N 11$^0$30.400$^{'}$; E 008$^0$10.545$^{'}$; 586 m |
| | | Dangora | 1 | 0.18 | Mixed cropping | maize | 4 | LS | upland | N 11$^0$32.473$^{'}$; E 008$^0$09.822$^{'}$; 575 m |
| | | | 2 | 0.20 | Sole cropping | | 3 | LS | upland | N 11$^0$32.203$^{'}$; E 008$^0$09.830$^{'}$; 572 m |
| | | | 3 | 0.30 | Sole cropping | | 4 | LS | upland | N 11$^0$32.403$^{'}$; E 008$^0$09.836$^{'}$; 573 m |
| | Bebeji | Bebeji | 1 | 0.08 | Sole cropping | | 5 | LS | upland | N 11$^0$37.991$^{'}$; E 008$^0$19.390$^{'}$; 495 m |
| | | | 2 | 0.11 | Sole cropping | | 3 | LS | upland | N 11$^0$37.982$^{'}$; E 008$^0$19.415$^{'}$; 405 m |
| | | | 3 | 0.10 | Sole cropping | | 5 | LS | upland | N 11$^0$38.001$^{'}$; E 008$^0$19.340$^{'}$; 500 m |
| | | Gwarmai | 1 | 0.28 | Sole cropping | | 3 | LS | upland | N 11$^0$32.054$^{'}$; E 008$^0$15.365$^{'}$; 576 m |
| | | | 2 | 0.16 | Sole cropping | | 3 | LS | upland | N 11$^0$32.039$^{'}$; E 008$^0$15.382$^{'}$; 575 m |
| | | | 3 | 0.25 | Sole cropping | | 4 | LS | upland | N 11$^0$32.045$^{'}$; E 008$^0$15.376$^{'}$; 575 m |
| | | Ungwan-dankali | 1 | 0.18 | Sole cropping | | 4 | LS | upland | N 11$^0$30.217$^{'}$; E 008$^0$15.983$^{'}$; 485 m |
| | | | 2 | 0.14 | Sole cropping | | 4 | LS | upland | N 11$^0$30.220$^{'}$; E 008$^0$15.996$^{'}$; 476 m |
| | | | 3 | 0.26 | Sole cropping | | 3 | LS | upland | N 11$^0$30.317$^{'}$; E 008$^0$15.973$^{'}$; 483 m |
| | Rogo | Zarewa | 1 | 0.24 | Sole cropping | | 4 | LS | upland | N 11$^0$23.895$^{'}$; E 007$^0$48.895$^{'}$; 704m |
| | | | 2 | 0.02 | Sole cropping | | 4 | LS | upland | N 11$^0$24.156$^{'}$; E 007$^0$48.943$^{'}$; 704 m |
| | | | 3 | 0.29 | Sole cropping | | 3 | LS | upland | N 11$^0$24.602$^{'}$; E 007$^0$49.632$^{'}$; 696 m |
| | | Fulatan | 1 | 0.30 | Mixed cropping | sugarcane | 4 | LS | lowland | N 11$^0$23.220$^{'}$; E 007$^0$51.973$^{'}$; 676 m |
| | | | 2 | 0.26 | Sole cropping | | 3 | LS | lowland | N 11$^0$23.242$^{'}$; E 007$^0$51.953$^{'}$; 675 m |
| | | | 3 | 0.12 | Sole cropping | | 4 | LS | upland | N 11$^0$23.515$^{'}$; E 007$^0$51.490$^{'}$; 689 m |
| | | Babbarika | 1 | 0.32 | Sole cropping | | 3 | LS | upland | N 11$^0$24.941$^{'}$; E 007$^0$50.211$^{'}$; 693 m |
| | | | 2 | 0.10 | Sole cropping | | 3 | LS | upland | N 11$^0$24.906$^{'}$; E 007$^0$50.227$^{'}$; 695 m |
| | | | 3 | 0.18 | Sole cropping | | 3 | LS | upland | N 11$^0$25.052$^{'}$; E 007$^0$50.015$^{'}$; 695 m |

LGA = Local Government Area; LS = Locally sourced; SEN = South East Nigeria; MM = Makarfi Market

Appendix 9: Cropping information obtained from cocoyam farms surveyed in Katsina State

| State | LGA | Location | Field | Farm size (ha) | Cropping pattern | Associated crop | Age of crop (months) | Source of corm | Topography | GPS Coordinates and Altitude |
|---|---|---|---|---|---|---|---|---|---|---|
| Katsina | Sabuwa | Tashan-nadaya | 1 | 0.24 | Sole cropping | | 4 | LS | upland | N $11^0$12.917'; E $007^0$10.090'; 716 m |
| | | | 2 | 0.08 | Sole cropping | | 3 | LS | upland | N $11^0$12.874'; E $007^0$10.066'; 712 m |
| | | | 3 | 0.12 | Sole cropping | | 3 | LS | upland | N $11^0$12.815'; E $007^0$10.396'; 712 m |
| | | Ungwar-maiwasa | 1 | 0.35 | Sole cropping | | 5 | LS | upland | N $11^0$10.683'; E $007^0$ 05.465'; 668 m |
| | | | 2 | 0.05 | Sole cropping | | 4 | LS | upland | N $11^0$10.724'; E $007^0$ 05.332'; 665 m |
| | | | 3 | 0.08 | Sole cropping | | 2 | LS | upland | N $11^0$10.668'; E $007^0$ 05.594'; 666 m |
| | | Sabuwa | 1 | 0.26 | Sole cropping | | 3 | LS | upland | N $11^0$10.623'; E $007^0$07.968'; 662 m |
| | | | 2 | 0.40 | Sole cropping | | 3 | LS | upland | N $11^0$10.712'; E $007^0$07.146'; 665 m |
| | | | 3 | 0.33 | Sole cropping | | 4 | LS | upland | N $11^0$10.729'; E $007^0$07.261'; 666 m |
| | Dandume | Dandume | 1 | 0.17 | Sole cropping | | 3 | LS | upland | N $11^0$27.732'; E $007^0$13.065'; 713 m |
| | | | 2 | 0.42 | Sole cropping | | 3 | LS | upland | N $11^0$27.671'; E $007^0$13.027'; 709 m |
| | | | 3 | 0.35 | Sole cropping | | 4 | LS | upland | N $11^0$27.429'; E $007^0$13.420'; 707 m |
| | | Kufar-maitamiya | 1 | 0.06 | Sole cropping | | 4 | LS | upland | N $11^0$27.370'; E $007^0$13.371'; 708 m |
| | | | 2 | 0.12 | Sole cropping | | 4 | LS | upland | N $11^0$27.376'; E $007^0$13.470'; 708 m |
| | | | 3 | 0.11 | Sole cropping | | 3 | LS | upland | N $11^0$27.380'; E $007^0$13.471'; 708 m |
| | | Mahuta | 1 | 0.70 | Sole cropping | | 5 | LS | upland | N $11^0$27.068'; E $007^0$16.482'; 709 m |
| | | | 2 | 0.65 | Sole cropping | | 4 | LS | upland | N $11^0$27.450'; E $007^0$16.751'; 707 m |
| | | | 3 | 0.50 | Sole cropping | | 4 | LS | upland | N $11^0$27.451'; E $007^0$16.481'; 709 m |
| | Funtua | Dutsen-reme | 1 | 0.01 | Sole cropping | | 4 | LS | upland | N $11^0$31.708'; E $007^0$20.417'; 706 m |
| | | | 2 | 0.02 | Sole cropping | | 3 | LS | upland | N $11^0$31.743'; E $007^0$20.427'; 706 m |
| | | | 3 | 0.06 | Sole cropping | | 3 | LS | upland | N $11^0$31.708'; E $007^0$20.417'; 706 m |
| | | Mairuwa | 1 | 0.15 | Mixed cropping | sugarcane | 4 | LS | lowland | N $11^0$32.406'; E $007^0$17.924'; 696 m |
| | | | 2 | 0.23 | Sole cropping | | 4 | LS | lowland | N $11^0$31.324'; E $007^0$18.008'; 702 m |
| | | | 3 | 0.26 | Sole cropping | | 5 | LS | upland | N $11^0$30.594'; E $007^0$18.430'; 709 m |
| | | Dankawo | 1 | 0.31 | Sole cropping | | 3 | SEN | upland | N $11^0$23.958'; E $007^0$19.932'; 687 m |
| | | | 2 | 0.25 | Sole cropping | | 3 | SEN | upland | N $11^0$23.797'; E $007^0$20.008'; 687 m |
| | | | 3 | 0.08 | Mixed cropping | maize | 3 | SEN | upland | N $11^0$23.856'; E $007^0$19.945'; 688 m |

LGA = Local Government Area; LS = Locally sourced; SEN = South East Nigeria