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First Report of *Phytophthora sansomeana* (E.M. Hansen) Causing Leaf Blight of taro (*Colocasia esculenta* (L.) Schott) in South Eastern Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Leaf blight and corm rot of taro (*Colocasia esculenta* (L.) Schott) caused by *Phytophthora colocasiae* Raciborski is the most disparaging disease of taro inducing severe losses in yield and quality of taro corms. Investigations were carried out in Umudike in 2016 and 2017 to determine the pathotypes present in that locality with a view to screening them on available taro genotypes for resistant cultivars. Symptomatic taro plant samples were collected from Taro Project Farm of the National Root Crops Research Institute, Umudike. Leaf tissue sections were excised from the periphery of lesions on symptomatic leaf, surface sterilized in 10% Sodium Hypochlorite solution for 2 minutes, rinsed in three changes of sterile distilled water and plated on Water Agar (WA). Mycelia emerging from diseased tissues after incubation were aseptically transferred to Corn Meal Agar (CMA). A total of 15 fungal isolates under 6 groups of species were obtained and these isolates were transferred to Carrot Agar (CA) from time to time until pure cultures were obtained. Based on varied cultural patterns, morphological characteristics and microscopic examination, the isolates were identified as *Phytophthora* sp, *Fusarium* sp FSSC, *Fusarium solani, F. oxysporum* and *F.*

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equiseti. Pathogenicity test confirmed that only *Phytophthora* sp induced leaf blight on detached leaf of taro plant. Molecular characterization of the isolates via Internal Transcribed Spacer (ITS) sequence analyses confirmed that the already implicated *Phytophthora* species was *Phytophthora sansomeana*. This is apparently the first report of *P. sansomeana* as a taro leaf blight pathogen in addition to the already known *P. colocasisae.*

Keywords: Colocasia esculenta; phytophthora sansomeana; leaf blight; pathogenicity; molecular identity.

1. INTRODUCTION

Taro (*Colocasia esculenta*), commonly referred to as cocoyam is an herbaceous perennial plant. It is an invaluable dietary crop whose starchy underground corms, highly nutritive leaves, petioles and flowers constitute important food in Nigeria and other developing countries of the world. Several studies have established the high nutritional quality of taro in terms of its digestibility, crude protein contents, essential minerals and vitamins [1-4]. There has been increasing interest in the use of taro as a specialty food in the management of gluten allergy in infants, celiac alimentary disorders and diabetes. It is used by convalescing adults due to its exceptionally small starch granule size, 1-4µ [5]. Taro has a high economic potential [6], not only as food but also as an agro-industrial raw material for brewery [7], confectionery [8], pharmaceutical [9,10] and livestock industries [11].

Phytophthora colocasiae Raciborski, the causal agent of taro leaf blight (TLB) is acclaimed globally, the most disparaging pathogen of taro plant. TLB and post-harvest rot of corms incited by this pathogen are the most destructive diseases of taro and the major factors limiting its production and storage worldwide [12-15]. *Phytophthora,* an age long formidable genus attacking a wide range of plants, is a cosmopolitan obligate plant pathogen of the Class Oomycetes [16-18]. The swift development of infections has had deleterious consequences both for peasant and commercial production of taro and these ranges from mild, heavy to total loss of taro crops in the field [19], postharvest deterioration of corms [14,20], resulting to nutrient depletions in the infected corm [1] scarcity and exorbitant prices of corms, among others. Symptoms of infection are seen in the form of small water-soaked, dark brown, and round to irregular necrotic lesions on the adaxial leaf lamina [21], which rapidly become enlarged to 2.5-5.0 cm within few days. Additional infections occur even on the petiole as the disease progresses and adjacent lesions coalesce to cover extensive leaf area, quickly destroying it within 7-14 days. Yield losses of up to 50 % [22,23] in severe cases have been reported and more than 70% in extreme severe cases [24].

The outbreak in 2009 of TLB in Nigeria decimated taro production and has continued to cause scarcity, exorbitant prices, hunger and lack of planting materials and there is not yet a sustainable bio-friendly control measure. These have led to scores of scientific investigations in order to come up with measures that can abate this disease and boost taro production [25, 23, 24, 26,27,28, 29]. Such investigations like isolation, identification and pathogenicity of the causal pathogen have been done in different areas where taro is grown. Yet there is the need to identify the strains that are prevalent in specific agro-ecological zones in order to select from the available taro genetic resources cultivars that are resistant to the strains which could be subjected to massive production. A comprehensive knowledge of the various forms/pathotypes of this organism is paramount to determining the level of damage each strain can cause, the predominance of each strain in a particular location and the best control approaches to be adopted. *Phytophthora colocasiae* associated with leaf blight of taro has been shown to vary in pathogenicity [30]. Our observation of different degrees of blights on infected taro leaves in the experimental plots led to the hypothesis that there may be different forms. Based on this observation, isolation, identification and pathogenicity of the isolates associated with taro leaf blight in the study site was carried out.

2. MATERIALS AND METHODS

2.1 Preparation of Culture Media

Dehydrated Corn-Meal Agar (2.1g) Water Agar (2.0g) was separately dissolved in 100ml of distilled water. The mixture was homogenized on

a hot plate at 50^{o}C for 4 minutes, and then sterilized by autoclaving for 15 minutes at 121^{o}C and 103 KN M^{-2}. The autoclaved medium was allowed to cool to 40^{o}C and later amended with Ampicillin 250 mgl^{-1} and Nystatin 10 mgl^{-1} to exclude bacteria contaminants according to Misra *et al*. [30]. The medium was poured into 90 mm Petri dishes and allowed to solidify.

2.2 Isolation and Identification of Fungal Pathogen

Aseptic procedures were followed in the sterilization of materials during the experiment. Isolation technique of Ugwuja and Chiejina [12] was adopted. Infected taro leaf samples were collected from infected plants (Plate 1) in the Research Farm of National Root Crop Research Institute Umudike (NRCRI) and transported to Phytopathology Laboratory of NRCRI for assay. By means of sterile scalpel, thin leaf tissue fragments (5 x 5 mm) were excised from the periphery of lesions, surface sterilized in 10% potassium hypochlorite for 2 minutes, rinsed in three changes of sterile distilled water and plated in Water Agar (WA) plates. Following incubation in Petri dishes at 25±2 ^{o}C) for 3 days, mycelia emerging from each tissue fragments were aseptically transferred to Corn-Meal Agar (CMA) amended with Ampicillin 250 mgl^{-1} ;PCNB 10 mgl^{-1} and Nystatin 10 mgl^{-1}. Three transfers of colony growth were aseptically done from CMA cultures to clean CMA plates until pure cultures were obtained. Identification of isolates was based on observed culture growth patterns, mycelia colour and microscopic examinations of vegetative and reproductive structures [18,31]. Isolates were preserved in screw capped McCartney agar bottle slants for subsequent use. Identification of these isolates was based on macroscopic examination of cultural characteristics of colonies in PDA plates, microscopic examination of their fruiting bodies and comparison with those in mycological manual [31] and molecular characterization.

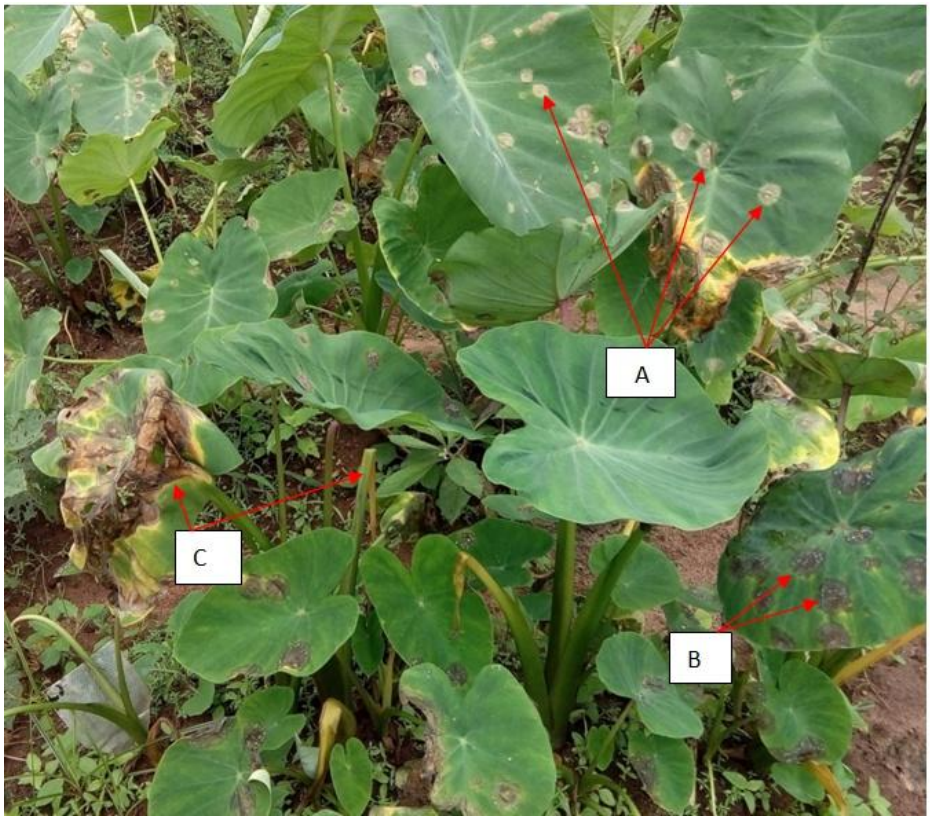

Plate 1. Field view of leaf blight infected taro plants at different stages of infection. (A): leaves with light brown circular lesions at primary infection stage (B): leaf with large necrotic areas at advanced infection stage and (C): totally damaged leaf with remant of petiole. Source: National Root Crops Research Institute Farm, Umudike

2.3 Pathogenicity Test

Pathogenicity test for all the isolates was carried out on detached leaves of taro using a modified technique of Brooks [32]. To confirm if the isolates obtained from the diseased taro leaf tissues were responsible for the blight symptoms, previously isolated cultures maintained in McCartney agar slants were used. Fresh subcultures were made on CMA and used on the seventh day. Inoculation was done on detached healthy leaves of a highly susceptible variety NCe001. Each detached leaf was washed under a running tap, rinsed in two changes of sterile distilled water and placed in a sterile plastic tray lined with moist sterile cotton wool. Agar discs (2 mm diameter) cut from cultures of a given isolate were each introduced unto the sterile leaf surface at 3 different position using a sterile cork-borer and moistened with a drop of sterile water. Due to the slippery nature of the cocoyam leaf surface the discs were held in place with a masking tape. The set-up was placed in an incubator maintained at 25±2 ^{o}C for three days after which the inoculated leaves were checked for development of leaf blight symptoms. Re-isolation of the pathogen from inoculated leaves that developed symptoms was carried out to confirm that they were the original organisms which produced the symptom on the diseased leaves. Sterile agar discs were inoculated unto controls.

2.4 Extraction of DNA

DNA Extraction was completed utilizing a ZR DNA mini prep extraction pack and convention provided by Inqaba Biotechnological, Pretoria, South Africa. By means of a sterilized spatula, thick growth (200 mg) of uncontaminated culture of each isolate was scratched off and moved to basing dab lysis tube. Lysis liquid (750 ul) was added to the cylinder and the cylinder was held in particle mixer (homogenizer) fitted with a 2 ml tube holder assembly and processed at most highest speed for 5 minutes. The ZR slamming dab lysis tube was centrifuged at 10,000 x g for 1 minute. Four hundred (400) micro litre of suprnatant was moved to a Zymo-Spin IV turn Filter (orange top) in an assortment tube and centrufuged at 7000 x g (Relative Centrifugal Force) for one minute.

DNA selective absorber referred to as buffer (1200 ul) was added to the filtrate in the collection tubes bringing the final volume to 1600 ul and 800 ul was then moved to a Zymo-Spin IIC segment in the collection tube and centrifuged at 10,000 x *g* for 1 minute. The filtrate was disposed of from the assortment tube and the rest of the volume was moved to the equivalent Zymo-spin and spun. Two hundred micro liters' (200 ul) DNA Pre-Wash buffer was added to the Zymo-turn IIC in another assortment tube and spun at 10,000 x g for one minute followed by the addition of 500 µl of fungal/bacterial DNA Wash Buffer and centrifugation at 10,000xg for oneminute. This step was rehashed for the rest of the volume in the assortment tube. The Zymo-spin IIC column was moved to a clean 1.5 µl centrifuge and 100 µl of DNA elution buffer was added to the segment framework and centrifuged at 10,000 x g for 30 seconds to elute the DNA. The ultra-unadulterated DNA was then kept in the refrigerator at – 20^{o} C degree for other downstream assays.

2.5 Internal Transcribed Spacer (ITS) Amplification

The Internal Transcribed Spacer (ITS) region of the isolate Ribosomal DNA (rDNA) genes was amplified using ITS4:5'-5'-TCCCTCCGCTTA TTGATGC-3'-3' and ITS6:5'GAGGTGAA GTCGT AACAG G-3'Primers on an ABI 9700 Applied Bio-Systems Thermal Cycler at a final volume of 30 micro liters for thirty-five cycles. The PCR mix included: Inqaba's X2 Dream Taq Master Mix, Taq polymerase, DNTPs, Mgcl, 0.4 M concentration primers, and the extracted DNA sample. The PCR conditions were as follows: basic denaturation, 95^{o}C for 5 minutes; denaturation, 95 ^{o}C for 30 seconds; annealing, 53 ^{o}C for 30 seconds; extension, 72 ^{o}C for 30 seconds and thirty-five cycles, and final extension, 72 ^{o}C for 5 minutes.The product was resolved on a 1% Agarose gel at 120V for 15 minutes and visualized on a blue light transilluminator [30].

2.6 Sequencing

Sequencing was performed by Inqaba Biotechnological, Pretoria, South Africa, using the Big Dye Terminator kit on a 3510 ABI sequencer. The sequencing was conducted at a final volume of 10 ul, and the components included 0.25 ul Big Dye ® terminator v1.1/v3.1, 2.25 ul 5x Big Dye sequencing buffer, 10 uM Primer PCR primer, and 2-10 ng PCR template per 100bp. The conditions for sequencing were the following: 32 cycles of 96 °C for 10 seconds, 55 °C for 5 seconds and 60 °C for 4 min.

2.7 Phylogenetic Analysis

Using the bioinformatics algorithm, the sequences obtained were modified. Trace editing for similar sequences was downloaded using BLASTN from the Pretoria, South Africa database of the National Center for Biotechnology Information (NCBI). Such sequences were linked using Cluster X. In MEGA 6.0 (Saitou and Nei, 1987) the evolutionary history was inferred using the Neighbor-Joining process**.** The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor, 1969).

3. RESULTS

3.1 Isolates

A total of fifteen isolates (01-015) were identified from the diseased taro leaves (Table 1). These include 1 *Phytophthora sansomeana* E.M. Hansen and Reeser of strain JHN243,3 *Fusarium solani* of strain HHNCJ01, 4 *Fusarium solani* of strain DE25, 1 *Fusarium solani* of strain MF996559, 1 *Fusarium oxysporium*f. cucumerinum wpII30-1, 2 *Fusarium equiseti* of strain Indo 186, 1 *Fusarium equiseti* of strain 176, and 2 unknown *Fusaruim* species designated as Km979504 and Ku950734.

3.2 Pathogenicity

Among the 15 identified isolates, only *P. sansomeana* (isolate 02) developed typical symptoms of taro leaf blight on detached taro leaves 4 days after inoculation. Water soaked dark-brown circular lesions were observed on the adaxial surfaces of the inoculated leaves after 4 days. Clear orange-yellow liquids oozed from the surface of the lesions and later dried into pallets. Whitish haze of spores surrounded the lesion at the periphery and advanced into the green portions of the leaves as shown in Plates 2a and 2b. Re-isolation from the inoculated detached leaves also gave rise to the same isolate that produced typical symptoms, which closely resembled those arising from natural infections on the leaves of cocoyam varieties. The other isolates showed no effect on the cocoyam leaves.

Table 1. Isolates with their related strains and frequency of occurrence

S/N	Isolates	Related strain	Frequency
1	*Phytophthora sansomeana*	JHN243	1
2	*Fusarium solani*	HHNC101	3
3	*Fusarium solani*	DE25	4
4	*Fusarium solani*	MF996559	1
5	*Fusarium oxysporum*	WPII30-1	1
6	*Fusarium equiseti*	Indo 186	2
7	*Fusarium equiseti*	173	1
8	*Fusarium sp.*	KM979504	1

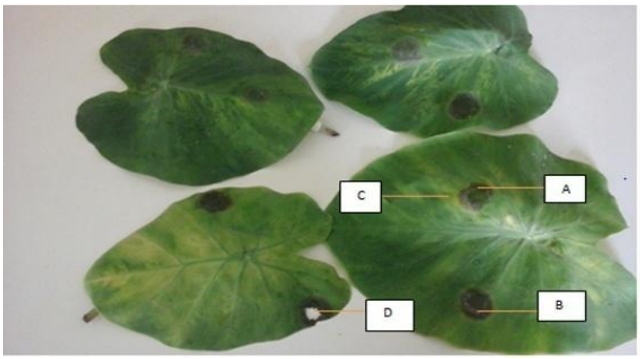

Plate 2. Photograph of inoculated detached leaves of taro with various symptoms of taro leaf blight 4 days after inoculation (Dai) including A: Dark brown necrotic lesion, B: Whitish band of mycelium, C: Yellowing zone of leaf and D: fallout of leaf tissue known as halo four days after inoculation

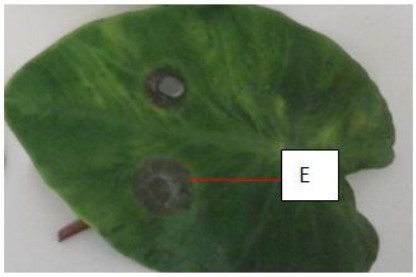

Plate 3. Photo plate of inoculated detached taro leaf with whitish haze of spores (E) surrounding lesions

Plate 4. Photograph of inoculated detached taro leaves without symptoms of TLB 5 Dai

3.3 Description of Fungal Isolates

Table 2. Macroscopic and microscopic characteristics of the fungal isolates

Fungi	Macro features	Micro features
Phytophthora Sansomeana	Colonies growth on Carrot Agar plates had no definite pattern. Young mycelia were fluffy; white at the top and creamy at the reverse side of the plate.	Sporangia were ovoid to ellipsoidal sporangia borne on irregularly branched sporangiophores with hyphal swellings at branch points. Sporangia were semi-papillate and deciduous with very short pedicel. Both hyphae and sporangiophores were non-septate
***Fusarium* sp. FSSC**	Colonies grew fast on media and produced white cottony mycelium which appeared slightly gray at the back of Petri dish.	slender conidiophores bearing clusters of short slender micro conidia and thick macro conidia
Fusarium equiseti	Colonies grew rapidly on Carrot Agar with white fluffy mycelium which measured 5-7 mm within 5 days. Concentric growth pattern with definite band of mycelia were features observed on the plates	Highly coiled intertwined mycelia with sparsely distributed macro and micro conidia were seen
Fusarium oxysporum	White cottony mycelia were seen on Agar plates, became purple with orange sporodochia and dark purple base. Colony grew rapidly, covering entire plate within 5 days.	Conidiophores were short, single, lateral and later arranged in densely branched clusters. Macroconidia were fusiform, slightly curved, pointed at the tips, mostly with three septa and scattered. Microconidia were abundant, mostly non-septate or mono-septate and ellipsoidal to cylindrical, straight or curved
***Fusarium* sp**	Colonies were white and fluffy, became whitish brown at the older part and creamy at the base. The growth was rapid reaching a diameter of 5cm within 4 days.	Macroconidia were 3-5 septate, cylindrical, moderately curved with blunt end cells, arranged in clusters and formed after 7 days from short multi branched conidiophores. Microconidia were short, cylindrical to oval, one to two celled and also arranged in clusters
Fusarium solani	Colonies grew rapidly reaching 4-5 cm in 4 days. Aerial mycelia were white to creamy becoming yellowish brown when sporodochia were present	Macroconidia were fusiform with 3-5 septa, cylindrical, often moderately curved and formed after 4-7 days on short multi-branched conidiophores. These conidia often had short blunt apical cell. Microconidia wereabundant, cylindrical to oval, 1-2 cell.

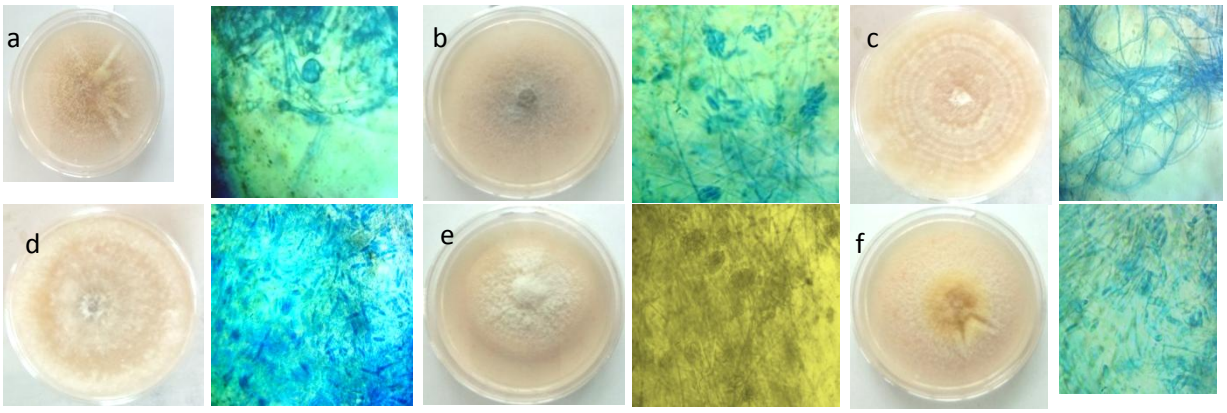

Plate 5. Morphology and photomicrograph of fungal isolates (a-f X40). a. *Phytophthora sansomeana,* b. *Fusarium* sp. c. *Fusarium equisetti* ,d. *Fusarium oxysporum,* e. *Fusarium* sp. f. *Fusarium solani*

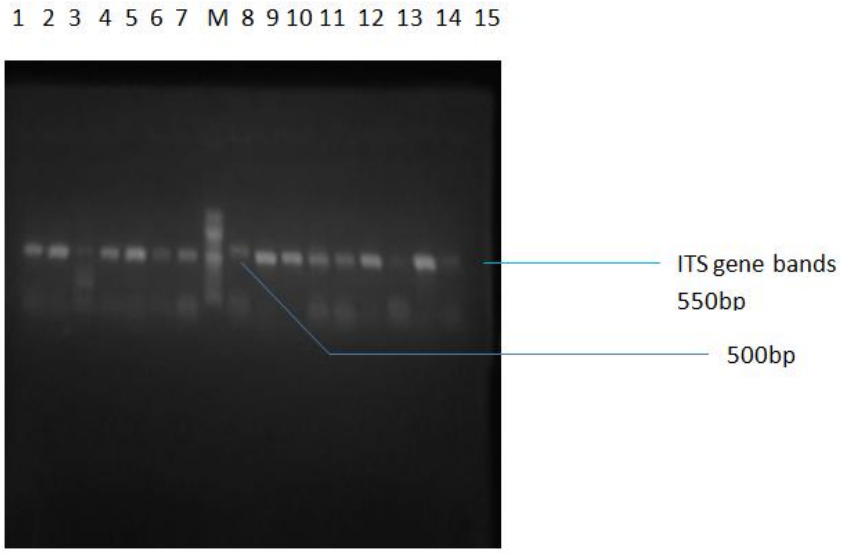

Plate 6. Agarose gel electrophoresis of the amplified ITS gene (550 bp) of the isolates. M represents the 100 bp molecular ladder

3.4 Internal Transcribed Spacer (ITS) Sequence

The Internal Transcribed Spacer (ITS) sequence obtained from the isolates produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The ITS sequence analysis of the isolate 02 showed 100 % similarity to *Phytophthora sansomeana.* The result obtained showed revealed a total of 15 clusters amongst the isolates with varying percentage similarities. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the ITS of the isolate 02 within the *Phytophthora* sp. and revealed a closely relatedness to *Phytophtmeana* strain JNH243 and then other *Phytophthora* sp. respectively (Fig. 1). The dendrogram revealed a close relatedness between 07, 08, 09, 010, 011, 13, 14 and 015 (*Fusarium solani*) at 99.4 % similarity. These isolates showed close relatedness to *Fusarium* HHNCJ01 and DE25 strains. Isolate 03 and 04 (*Fusarium equisetum*) had 99.2 % similarity and showed relatedness whereas isolate 012 (*Fusarium equisetum*) showed more relatedness to isolate 03 and 04 respectively.

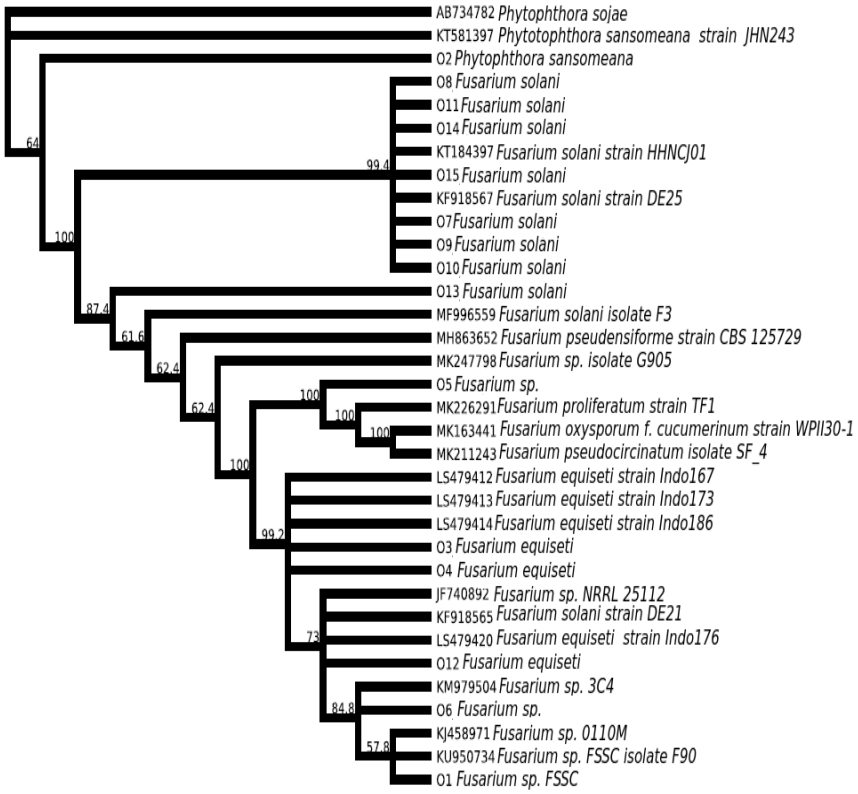

Fig. 1. Phylogenetic tree of the evolutionary distance between the isolates

Table 3. Isolates and their accession numbers

S/N	Isolates	Accession Number
1	02 - *Phytophthora sansomeana*	MN453372
2	01 - *Fusarium* - sp. FSSC	MN473246
3	03 - *Fusarium equiseti*	MN473249
4	05- *Fusarium oxysporum*	MN473248
5	06 - *Fusarium* sp.	MN473247

4. DISCUSSION

The results from this study demonstrated that rapidly developing molecular techniques such as the Polymerase Chain Reaction (PCR) and Internal Transcribed Sequence (ITS) analyses have revolutionized identification and classification of organisms [33], McCartney *et al*. Application of such techniques for identification of fungi is important for rapid and accurate identification. In the current study, a different pathogen, *Phytophthora sansomeana* E.M Hansen & Reeser instead of the usual *P. colocasiae* was isolated and established as a causal pathogen of taro leaf blight through its pathogenicity and ITS sequence analyses. This suggests that a spontaneous mutant of *P. colocasiae* might have evolved given its similarity in morphology, growth characteristics with the later and its ability to induce similar blight symptoms on taro leaf tissue. The above example suggests that there may be several mistakes regarding the identification of pathogenic agents based only on morphological observation [33]. From available records, *P. sansomeana* does not seem to show host specificity having been found causing blight, wilt and rot in different host plants including soybean, pea, corn, white clover and white carrot [34, 35, 36, 37, 38]. Infections from this pathogen is therefore expedient, given the presence of the pathogen, a susceptible host and optimum environmental conditions.

The result of the present study disagrees with earlier reports [30, 12], which associated only *P.*

colocasiae to TLB. Adomako *et al*. isolated and identified 80 isolates of *P. colocasiae* from diseased taro leaves in Ghana based on morphology and sequence homology. Padmaja *et al*. also obtained 4 isolates of *P. colocasiae* based on morphology and sequence homology as well as Misra *et al* [30] who obtained 14 isolates. Brooks [30,32] Lin *et al*. [39] and Misra *et al*. [23] isolated and confirmed *P. colocasiae* as the causal pathogen of TLB from different regions where leaf blight of taro have occurred.

*Phytophthora sansomeana*is a new species of *Phytophthora* segregated from *Phytophthora megasperma* [37]. It was first characterized based on isolates from Douglas-fir seedlings, white clover, and white carrot in nurseries in Oregon New York, United States. It was implicated the first time as causal pathogen of soybean root rot in China [38], wilting and stunting of corn in Ohio [36] and root rot of field pea in Canada. From the above reports it is therefore evident that *P. sansomeana* does not exhibit host specificity and is capable of infecting a wide range of crops given the required environmental conditions. The disparity in results obtained by earlier workers who studied taro leaf blight disease and the present results suggests that a new pathogen that can cause leaf blight in taro has evolved and this is a new and remarkable discovery in the field of plant pathology.

The inability of the other isolates obtained from the diseased leaf tissues to cause infection suggests that they are mere opportunistic organisms that took advantage of the nutrient sink in the depleted tissues for their own growth and metabolism [40]. Other roles played by these opportunistic organisms in the disease process are yet to be determined [42-43].

5. CONCLUSION

The present research has established that a new pathogen *Phytophthora sansomeana* caused taro leaf blight disease in South Eastern Nigeria. The results were obtained by the combination of the conventional and molecular biotechniques methods of pathogen identification. Hence the need to complement the conventional method with molecular techniques cannot be overemphasized.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ugwuja FN, Onyeka TJ, Chiejina NV, Ugwuoke KI. Effect of Phytophthora leaf blight disease of taro (*Colocasia esculenta (L*.) Schott) on proximate and phytochemical constituents of infected corms; 2020.
2. Adane T, Shimelis A, Negussie R, Tilahun B, Haki GD. Effect of processing method on the proximate composition, mineral content and antinutritional factors of taro (*Colocasia esculenta, L*.) grown in Ethiopia. African Journal of Food, Agriculture, Nutrition and Development. 2013;13:7383–7398.
3. Alcantara RM, Hurdata WA, Dizon EI. The nutritional value and phytochemical components of taro (*Colocasia esculenta (L.) Schott.)* powder and its selected foods. Journal of Nutrition and Food Science. 2013;3(2):207- 212.
4. Semsek S, El SN. In vitro starch digestibility, estimated glycemic index and antioxidant potential of taro (*Colocasia esculenta L. Scott*) corm. Food Chemistry. 2015;168:257-261.
5. Temesgen M, Retta N. Nutritional potential, health and food security benefits of taro *colocasia esculenta (L*): a review. Food Science and Quality Management. 2015;36:23-30.
6. Babayemi OJ, Bamikole MA. Nutrient value and in vitro gas production of African wild cocoyam (*Colocasiaesculentum*). African Journal of Food, Agriculture and Nutrition Development. 2009;9 (1): 593-607.
7. Eneh CO. Towards food security and improved nutrition in Nigeria: Taro (*Colocasiaantiquorum*) grit as carbohydrate supplement in energy food drink. African Journal of food Science. 2013;7(10): 355-360.
8. Ukpabi UJ, Chijioke U, Mbanaso ENA. Feasibility of producing acceptable carotene and energy rich taro crisps with deep palm-oil frying in Nigeria. Pakistan Journal of Nutrition. 2013;12(9):811-814.
9. Kundu N, Campell P, Hampton B, Lin C, Ma X, Ambulos N, Zhao XF, Goloubewa O, Holt D, Fulton AM. Antimetastic activity of

compound isolated from *Colocasia esculenta* (taro). Anti-Cancer Drugs. 2012;2:200-211.

10. Brown AC, Shovic A, Ibrahim SA, Holck P, Huang A. A non-dairy probiotic's (poi) influence on changing the gastrointestinal tract's microflora environment. Alternative Therapies in Health Medicine. 2005;11(1):58-64.
11. Buntha P, Borin K, Preston TR, Ogle B. Digestibility and nitrogen balance studies in pigs fed diets with ensiled taro (*Colocasia esculenta*) leaves as replacement for fish meal. Livestock Resources and Rural Development; 2008.
12. Ugwuja FN, Chiejina NV. Preliminary investigation of the causes of leaf rots of cocoyam in Nsukka zone of Nigeria. Nigerian Journal of Plant Protection. 2011;25:203-213.
13. Omane E, Oduro KA, Cornelius EW, Opoku IY, Akrofi AY, Sharma K, Kumar PL, et al. First report of leaf blight of taro (*Colocasia esculenta*) caused by Phytophthora colocasiae in Ghana. Plant Disease. 2012;96(2):292-293.
14. Mbong GA, Fokunang CN, Lum AF, Bambot MB, Tembe EA. An overview of Phytophthora colocasiaeof cocoyams: A potential economic disease of food security in Cameroon. Discourse Journal of Agriculture and Food. 2013;1(9):140-145.
15. Omeje TE, Ugwoke KI, Adinde JO, Ogwulumba SI, Unigwe LO. Effect of cropping season on the control of taro leaf blight (*Phytophthora colocasiae*) of cocoyam (*Colocasia esculenta L. Schott*.) in south eastern Nigeria. International Journal of Advanced Biological Research. 2016;6(1):30-39.
16. Wawra S, Belmonto R, Lobach L, Saraiva M, Willems A, Van West P. Secretion, delivery and function of oomycete effector proteins. Current opinion in microbiology. 2012;15:685-691.
17. Stassen JH, Ackerveken GV. How do Oomycetes effectors interfere with plant life? Current opinion in Biology. 2011;14:407- 414.
18. Drenth A, Sendall B. Practical Guide to Detection and Identification of Phytophthora. Version 1.0. CRC for Tropical Plant Protection Indooroopily, Australia. 2001;42.
19. Mishra AK, Sharma K, Misra RS. Effect of benzyl amino purine on the pathogen growth and disease development of taro leaf blight caused by *Phytophthora colocasiae*. Journal of Plant Pathology. 2008;90:191-196.
20. Brooks FE. Taro leaf blight. The plant health instructor. American Phyto-pathological Society; 2005. Available:http://www.apsnet.org/edcenter/intropp/lessons/fungi/Oomycetes/Pages/TaroLeafBlight.aspx (Retrieved January, 2019).
21. Chiejina NV, Ugwuja FN. Incidence of Phytophthora leaf-blight disease of cocoyam in Nsukka area of South-Eastern Nigeria. Journal of Botanical Research. 2013;4(1):21-.24.
22. Brunt J, Hunter D, Delp C. A bibliography of taro leaf blight. Secretariat of the Pacific Community (SPC)/AusAID taro genetic resources: conservation and utilization. Noumea, New Caledonia. 2001;93.
23. Misra RS, Sharma K, Mishra AK. Phytophthora leaf blight of taro (*Colocasia esculenta*)- a review. The Asian and Australian Journal of Plant Science and Biotechnology. 2008;2(2):55-63.
24. Nelson S, Brooks F, Teves G. Taro leaf blight in Hawaaii. Plant Diseases Bulletin No. PD -71; University of Hawaii: Manoa, HI, USA. New Caledonia; 2011.
25. Semisi ST, Mauga T, Chan E. Control of leaf blight disease. *Phytophthora colocasiae Racib*. in taro, *Colocasia esculenta (L.*) Schott with phosphorous acid. Journal of South Pacific Agriculture. 1998;5:77–83.
26. Shakywar RC, Pathak SP, Sah D. Management of leaf blight of taro through integrated approaches. Technofame. 2012;1:43-49.
27. Shakywar RC, Pathak SP, Kumar S, Singh AK. Evaluation of fungicides and plant extracts (botanicals) against *Phytophthora colocasiae Raciborski* causing leaf blight of taro Journal of Plant Disease Science. 2012;7(2):197-200.
28. Lokesh MS, Patil SV, Naik N, Prashantha A, Chandan K. Efficacy of fungitoxicants in the management of leaf blight (*Phytophthora colocasiaeRaciborski)* of taro (*Colocasia esculenta (L.) Schott.)* in Uttar Kannada, Karnataka, India. International Journal of Advanced Research in Biological Sciences. 2014;1(6):185-188.
29. Tarla DN, Fon DE, Takumbo EN, Fontem DA. Economic evaluation of fungicides application on taro (*Colocasia esculenta*)

leaf blight. Journal of Experimental Biology and Agricultural Science. 2014;8694:286-296.

30. Misra RS, Mishra AK, Sharma K. *Characterisation of Phytophthora colocasiae* isolates associated with leaf blight of taro in India. Archives of Phytopathology and Plant Protection. 2011;44(6):581–591.
31. Ellis D, Davis S, Alexiou H, Handke R, Bartley R. Descriptions of medical fungi. Mycology Unit, Women's and Children's Hospital, School of Molecular and Biomedical Science, University of Adelaide, Australia. 2007;188.
32. Brooks FE. Detached-leaf bioassay for evaluating taro resistance to Phythophthora colocasiae. Plant Diseases. 2008;92:126-131.
33. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A guide to methods and applications. Innis, M., Gelfand, D., Sninsky J., and White T., (Eds.). Academic Press, San Diego. 1990;315-322.
34. Tande C, Dorrance AE, Schwarzrock D, Mahecha E, Byamukama E. First report of Phytophthora sansomeana causing rot of soybean in South Dakota. Plant Disease. 2020;104(6):1877.
35. Chang KE, Hwang SE, Ahmed HU, Fu H, Zhou Q, Strelkov SE, Turnbull GD. First report of Phytophthora sanaomeana causing root rot of field pea in Alberta, Canada. Crop protection. 2017;101:1-4
36. Zelaya LX, Ellis ML, Berr SA, Dorrance AE. First report of *Phytophthora sanaomeana* causing wilting and stunting of corn in Ohio. Plant Disease. 2010;94(1):125-125.
37. Hansen EM, Willcox WF, Reeser PW, Sutton W. *Phytophthora rosacearum* and P. sansomeana, new species segregated from Phytophthora megasperma"complex". Mycologia. 2009;101:129-135.
38. Tang QH, Gao F, Li GY, Wang H, Zheng XB, Wang YC. First report of root rot caused by Phytophthora sansomeana on soybean in China. Plant Disease. 2010;94(3): 378-378.
39. Lin MJ, Ko WH. Occurrence of isolates of Phytophthora colocasiae in Taiwan with homothallic behaviour and its significance. Mycologia. 2008;100:727-734.
40. Agrios GN. Plant Pathology. (5th edition). Academic Press. 2005;922.
41. Alexopoulos CJ, Mims CW, Blackwell M. Phylum Oomycota. 1n: Introductory Mycology. John Wiley and Sons Inc. 1996;682-737.
42. Latijnhouwers M, de Wit PJG, Govers F. Oomycetes and fungi: similar weaponry to attack plants. Trends in Microbiology. 2003;11:462-469.
43. Strange RN, Scott PR. Plant disease: A threat to global food Security. Annual Review of Phytopathology. 2005;43:83-116.

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