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Original Article

Genetic diversity of late blight (*Phytophthora infestans*) populations of potato (*Solanum tuberosum* L.) in Jos Plateau State, Nigeria

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ABSTRACT

Potato (*Solanum tuberosum* L.), grown mainly in Jos, Nigeria is affected by diseases including late blight caused by the fungus *Phytophthora infestans* (Mont.) De Bary. Worldwide, new strains of the pathogen are emerging, limiting its production. This study described the morphology and molecular properties of *P. infestans* isolates occurring in Jos. The pathogen was isolated from leaves of ten infected potato varieties, cultured in Potato Dextrose Agar media, and observed. Extracts of DNA were obtained from fully grown cultures and two primers, internal transcribed spacer 4/5, and INF FW2/Rev primers yielded gene fragments with polymerase chain reaction amplification. The products were sequenced and aligned with other worldwide *P. infestans* isolates. Pairwise identities were calculated and phylogenetic trees determined. Genetic variability determinants and neutrality tests were also determined. The similarities within the *P. infestans* isolates from Nigeria ranged from 91.91% to 96.37%, while phylogeny identified two clustered within the population, indicating varying ancestry. The putative occurrence of balancing selection within the Nigerian *P. infestans* population was also identified and indicated the occurrence of considerable diversity within the *P. infestans* population occurring in Jos, the major potato producing region in Nigeria. To the best of our knowledge, this is the first molecular description of *P. infestans* from Nigeria and is important for the development of potential resistant varieties in the region.

Keywords: *Oomycete,* PCR, internal transcribed spacer gene, Tajima's D, resistant varieties

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INTRODUCTION

Potato (*Solanum tuberosum* L.), is one of the world's major food crops,[1] ranking fourth after rice, wheat, and maize.[2,3] Its production is a major part of the farming systems in Jos, Nigeria resulting in about 92% of Nigeria's annual output.[4] The relative ease of cultivation of potato and its high energy content has made it a valuable cash crop in developing countries.[5] The crop thrives in cool temperate climates, subtropical winters, and mountainous areas in the tropics.[6] Although Nigeria's potato production has increased by over 12% in the past decade, it is still grossly below demand.[7] Low-quality seeds, poor storage facilities, pests, and diseases, especially late blight, are major limiting factors to potato production.[7]

The late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is one of the most devastating diseases of potatoes in regions where the crop is cultivated worldwide.[8] In Nigeria, epidemics have been reported in the major growing areas of Plateau State, resulting in huge damages.[4,9] Losses up to 85% have been reported when susceptible cultivars remain unprotected, resulting in complete defoliation, tuber rot, and subsequent plant death.[10,11] Resistance to the disease remains a crucial management strategy for the disease[12] and has been previously explored in Nigeria.[13-15] However, for host resistance to be effective and durable, it is important to

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characterize the pathogen populations prevalent in such growing areas. Therefore, this study focused on the morphological and molecular properties of *P. infestans* isolates occurring in Jos, the main growing region for potatoes in Nigeria as a strategy in exploring the selection of disease-resistant variants.

MATERIALS AND METHODS

Isolation of *P. infestans* from Infected Samples

Blighted potato leaves were obtained from ten different varieties of potatoes (Marabel, Condor, Caruso, Nicola, Sp, Oceania, Delice, Connect, Famosa, and Yellow) grown at the National Root Crops Research Institute, Kuru. The samples showed an infected single lesion with white cottony growth on the underside which was characteristic of the late blight disease.[16] Tubers of potato variety "Famosa", known to be susceptible to late blight, was also sampled and used as a growth substrate for the pathogen. Isolation of the causal pathogen from the blighted leaves was carried out as described by Zarzycka and modified by Sobkowiak and Sliwka.[17,18] Briefly, the leaf surfaces were disinfected by dipping in 0.05% Sodium hypochlorite for 5 min and rinsed in distilled water before air drying. The tubers from potato variety "Famosa" were rinsed in distilled water to remove soil from the surface of the tubers. These were dipped in 70% alcohol for 30 s, rinsed in sterile distilled water, blotted dried, and cut into slices 5–7 mm thick. Each slice was placed in a petri-dish and blighted leaf samples were cut at the margin of the lesion using disinfected scissors. The samples were placed on the tuber slices in the petri-dish, covered, and incubated at room temperature of 27 ± 8°C. After 5 days, distinct colonies formed on the tubers slices were sub-cultured by point inoculation on the solidified Potato Dextrose Agar medium and was again incubated at room temperature (27 ± 8°C) for 6 days. Sub-culturing was carried out three times before pure cultures were obtained.

Identification of *P. infestans*

Cultural and microscopic identification was carried out as described by Gallegly and Hong.[19] The growth characteristics, sporangial characteristics, and colony characteristics of the cultures were examined and compared with established descriptions for *P. infestans*. Cultures were observed for hyphae appearance, size, and shape of sporangia, and presence of oospores. Microscopic identification was carried out using lactophenol cotton blue while mycelia were observed under the microscope (100 ×) and compared with available descriptions for *P. infestans*.[17,18]

DNA Extraction and Polymerase Chain Reaction (PCR)

Pure cultures of *P. infestans* were transported to the Biotechnology Laboratory of National Veterinary Research Institute, Vom, and DNA extraction was carried out using the Quick-DNA™ Fungal/Bacterial Mini-Prep Kit (Zymo) according to the manufacturer's instructions. Two primers namely, internal transcribed spacer (ITS)5 (5'-GGAAGTAAAGTCGTAACAAGG-3')/ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and INF FW2 (5' TGGGCGAGCCCTATCAAAA-3')/INF REV (5'-CCGATTCAAATGCCAAGCTAA-3') were used to target *P. infestans* isolates for molecular characterization. ITS5/ITS4 are universal primers used to identify fungi-like organisms while INF-FW2/INF-REV are *P. infestans*-specific primers. Both the primers were synthesized in Macrogen. The first PCR was conducted in 50 µl total reaction volume consisting of approximately 50 ng of template DNA, 5 µl 10× PCR buffer, 2 µl 1.25-2.0 mM dNTPs, 2 µl 10 mM $MgCl_2$, 2 µl each of 10 µm ITS4/ITS5 primers (White *et al*., 1990), 0.4 µl Taq polymerase (5 U/µl) and sterile distilled water up to final volume. The second PCR was conducted in 20 µl reaction volumes consisting of 2.0 µl template DNA, 2.0 µl buffer, 2.0 ul dNTPs (2 mM), 2.0 µl primer INF-FW2/INF-REV (5 µM), 0.2 µl Taq polymerase and sterile distilled water up to the final volume.

All reactions were overlaid with sterile mineral oil prior to thermal cycling and parameters were set as initial denaturation at 96°C for 2 min, followed by 35 cycles consisting of denaturation at 96°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min. A final extension at 72°C for 10 min followed. Negative controls (no template DNA) were used in every experiment to check for the presence of contamination. Electrophoresis was performed on 1.0% agarose gels containing 0.5 µg/ml ethidium bromide with 1×TBE running buffer. A 100 base pair DNA ladder (GeneRuler) was included on each gel as a molecular size standard. Amplified products were purified using Gene-Clean (Geneaid Biotech Limited) according to the manufacturer's recommendations and shipped to Inqaba, South Africa for automated DNA sequencing in forward and reverse orientations.

Sequence Analyses

Nucleotides from both strands per sample were assembled and manually edited using the BIOEDIT program.[20] Consensus sequences were obtained for each sample and verified by the nucleotide option of the basic local alignment search tool (BLASTn)[21] to determine similarity match and identification. Pairwise identities were performed using SDT v1.2[22] with pairwise gap deletions. Using the CLUSTAL W alignment program,[23] sequences were aligned with published sequences from 21 other Phytophthora species[24] available in GenBank and downloaded from the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov). A closely related sequence from *Phytophthora palmivora* (Accession number MF446701) was obtained from GenBank as outgroup and included in the phylogenetic analyses. Trees of 1,000 bootstrap replicates were constructed using PHYML[25] and the alignments were used to obtain maximum likelihood trees using the Jukes and

Cantor model as implemented in the Molecular Evolutionary Genetics Analysis Program v6.06.[26,27] Genetic structure and diversity within the *P. infestans* populations in Nigeria were investigated to understand any likely evolutionary dynamics that yield variations. Population structure parameters estimated included the average nucleotide diversity (π), haplotype diversity (Hd), number of polymorphic or segregating sites (S), the statistic estimate of population mutation based on the number of segregating sites (θ-W), total number of mutations (Eta), the average number of nucleotide differences between sequences (k) and the statistic estimate of population mutation based on the total number of mutations (θ-Eta). These were estimated using nucleotide sequences in DnaSP v5.10.01.[28]

RESULTS

The symptoms associated with diseased potato leaves revealed varying levels of infection with varieties such as "Oceania" and "Famosa" showing intense leaf blights [Figure 1a and b] while others such as "Nicola" and "Connect" [Figure 1c and d] were not so intense. The cultures obtained from these leaves revealed white cottony colonies which are typical for *P. infestans* [Figure 2]. When observed under the microscope, the sporangia were lemon or oval shape, with the dimensions of 21–38/12–23 µm[29] [Figure 3].

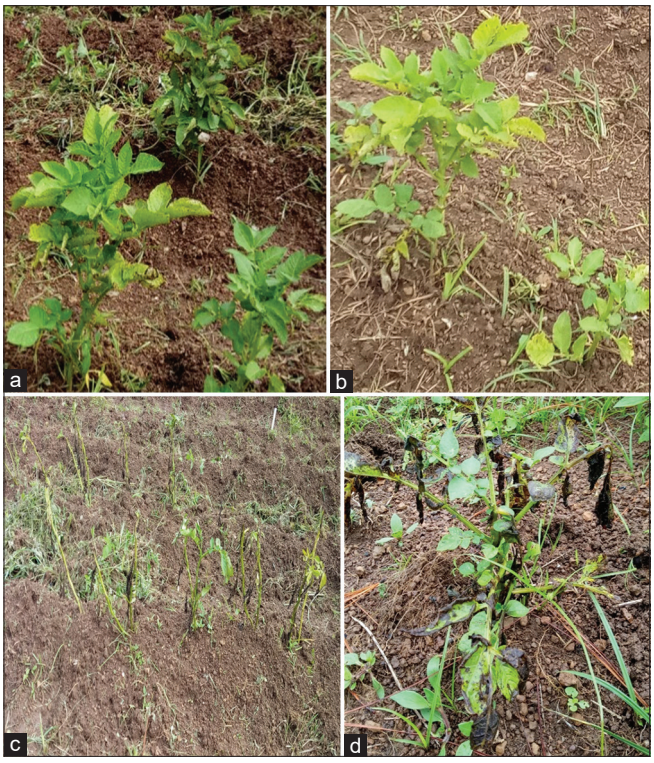

Figure 1: Leaves of potato grown in Jos, Nigeria showing intense blight symptoms on varieties "Ocenia" (a) and "Famosa" and mild symotoms on "Connect" (c) and "Nicola" (d)

After DNA extraction and PCR, the results analyzed under agarose gel revealed positive amplifications for both the general ITS4/5 primers [Figure 4a] and the specific INF primers [Figure 4b]. However, isolates obtained from varieties "Delice" and "Yellow" did not reveal any band or amplification for both primers [Figure 4a and 4b, lanes 7 and 10].The PCR amplification from the INF-FW2/INF-REV specific primers confirmed the isolates as *P. infestans* while the products from the ITS4/5 primers were purified and sequenced. These resulted in sequences which ranged from 606 to 607 bp in length. Using nBLAST, these showed high identities (95.22–97.20%) with published *P. infestans* sequences from the database with query coverage ranged from 99% to 100% [Table 1].

These eight sequences were deposited in the GenBank under accession numbers MW829623-MW829630. Pairwise identity analyses of the eight sequences revealed high similarities among the isolates, as revealed in Table 2.

The lowest pairwise identity was between "02-CON" and "08-FAM" (91.91%) while isolates "02-CON" and "05-OCE" had the highest pairwise identity, at 96.37%. Figure 5 depicts the phylogenetic relationships between the ITS rRNA gene segments of *P. infestans* isolates obtained from Jos, Plateau

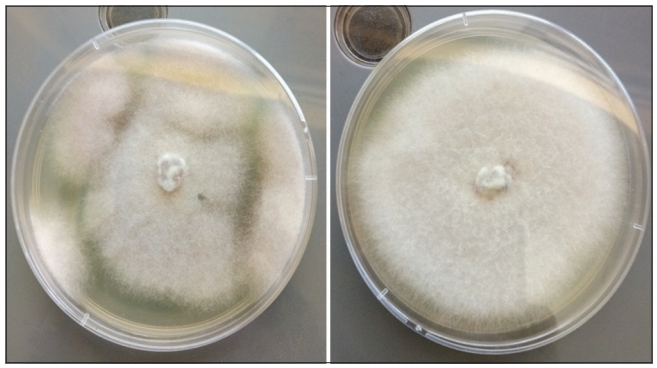

Figure 2: White cottony *Phytophthora infestans* colonies isolated from blighted potato leaves in Jos, Nigeria

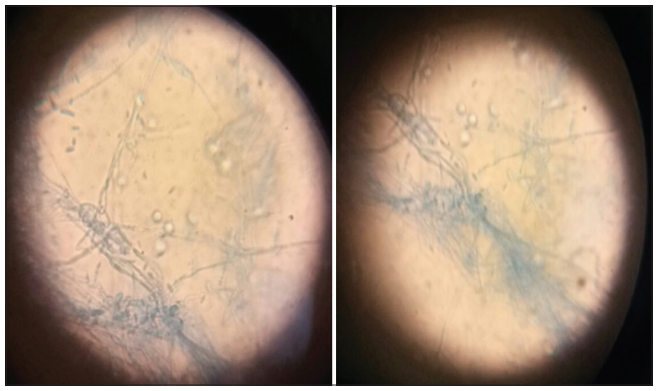

Figure 3: Photomicrograph of *Phytophthora infestans* colonies isolated from blighted potato leaves in Jos, Nigeria

Table 1: Summary of identification of *P. infestans* amplicons from potato samples in Jos, Nigeria by BLAST and their identity with closest database homologs

Isolate	Accession Number	Length (bp)	Highest nBLAST similarity (%) in GenBank	Query cover (%)	Alignment identities	E-value	Alignment score
NG-01-MAR	MW829623	606	95.22, with JF834701	100	578/607	0.0	>200
NG-02-CON	MW829624	607	97.03, with LS479125	99	588/606	0.0	>200
NG-03-CAR	MW829625	607	97.20, with JF834701	100	590/607	0.0	>200
NG-04-NIC	MW829626	606	97.19, with GU258670	99	588/605	0.0	>200
NG-05-OCE	MW829627	607	96.87, with LS479125	100	588/607	0.0	>200
NG-06-DEL	MW829628	607	97.19, with JF834701	99	589/606	0.0	>200
NG-08-FAM	MW829629	606	95.87, with EU200319	99	580/605	0.0	>200
NG-09-YEL	MW829630	606	95.39, with JF834701	100	579/607	0.0	>200

BLAST: Basic local alignment search tool, *P. infestans: Phytophthora infestans*

Table 2: Percentage pairwise identity of *P. infestans* isolates obtained from potato leaves in Jos, Nigeria

Accession Number	MW829623	MW829624	MW829625	MW829626	MW829627	MW829628	MW829629	MW829630
MW829623	–							
MW829624	92.57	–						
MW829625	94.40	94.56	–					
MW829626	94.06	95.38	95.05	–				
MW829627	94.72	96.37	94.89	94.55	–			
MW829628	96.04	95.22	95.88	95.71	94.07	–		
MW829629	94.55	94.72	94.06	94.55	94.39	93.89	–	
MW829630	94.39	94.55	94.22	93.73	95.21	93.56	91.91	–

P. infestans: Phytophthora infestans

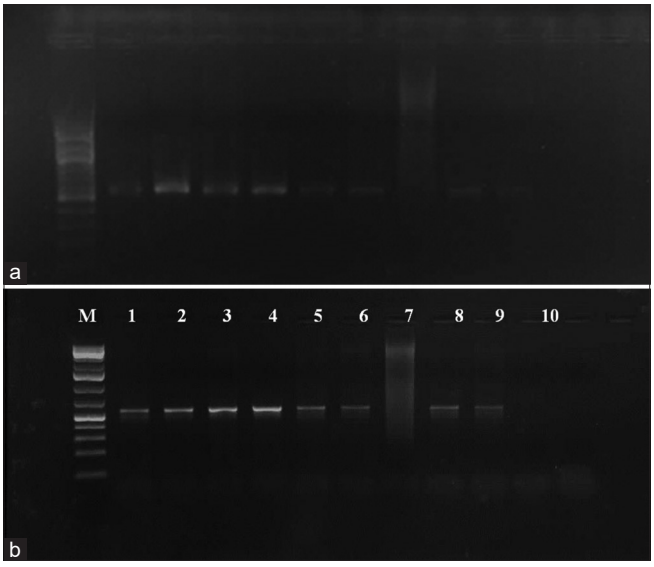

Figure 4: Polymerase chain reaction for the identification of *Phytophthora infestans* in potato leaves from Jos, Nigeria using general ITS4/5 primers (a) and specific INF-F/R primers (b). Both primers amplified a 600bp product size in positive samples. M=100 bp molecular marker, 1="Marabel", 2="Condor", 3="Caruso", 4="Nicola", 5="Sp", 6="Oceania", 7="Delice", 8="Connect", 9="Famosa", 10="Yellow", B="Buffer"

state with others from different parts of the world. The out-group selected for analysis was a *P. palmivora* isolate which belongs to the same taxonomical genus as the amplified pathogen. All the *P. infestans* isolates obtained in this study except "02-CON", "04-NIC" and "08-FAM" clustered into the same sub-group [Figure 5]. Isolates "02-CON", "04-NIC" and "08-FAM" all clustered into separate groups with other worldwide sequences, suggesting different evolutionary origins from the other Nigerian isolates.

Analyses of haplotype number and haplotype diversity, represented by "h" and "Hd", respectively revealed varying patterns among the eight partial ITS rRNA gene of *P. infestans* isolates from Jos, Nigeria, and also among other ITS rRNA gene sequences of *P. infestans* from GenBank [Table 3]. From the Nigerian *P. infestans* isolates (n = 8), haplotypes number "h" was eight while "h" values for the worldwide sequences (n = 28) was 17. Thus, across sequences from Nigeria, each isolate represented a haplotype, revealing high genetic variation. Similarly, Hd values were 1.00 for the Nigerian isolates and 0.825 for the worldwide isolates [Table 3].

Furthermore, genetic distances for each population dataset were calculated, with lower nucleotide diversity (π) values

Table 3: Genetic variability determinants and neutrality tests on the partial ITS ribosomal RNA gene of *P. infestans* isolates from Jos, Nigeria

P. infestans isolates	N	H	S	Hd	Eta	Π	k	θ-W	θ-Eta	Tajima's D	Fu and Li's D	Fu and Li's F
Nigeria (*n*=8)	607	8	78	1.000	86	0.05457	33.0714	33.168	0.05473	–0.0158	0.2152	0.1798
Worldwide (*n*=28)	976	17	57	0.825	76	0.09104	9.0132	19.53	0.19727	–2.0646	–2.7328	–2.9665

N: Number of nucleotide sites, h: Haplotype number, S: Total number of variable or segregation sites, Hd: Haplotype diversity, Eta: Total number of mutations, π: Nucleotide diversity, k: Average number of nucleotide differences between sequences, θ-W: Waterson's estimate of population mutation rate based on the total number of segregating sites, θ-Eta: Waterson's estimate of population mutation rate based on the total number of mutations, *P. infestans: Phytophthora infestans*, ITS: Internal transcribed spacer

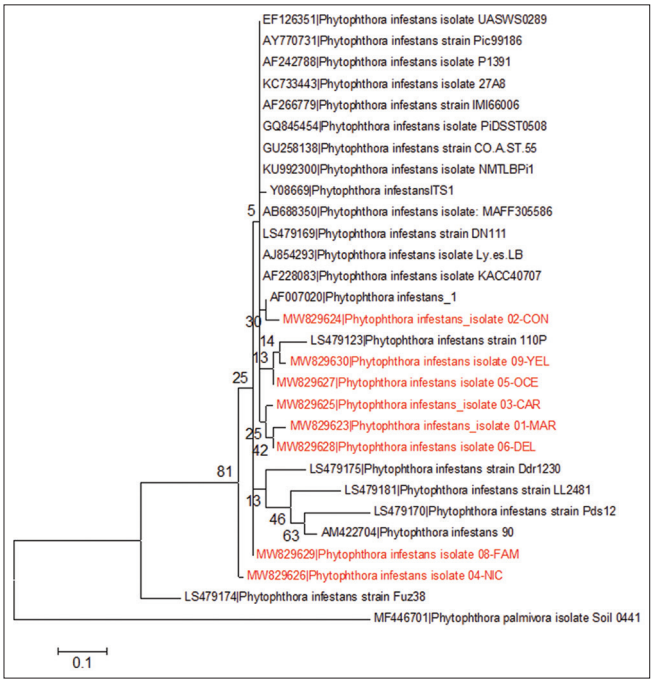

Figure 5: Maximum likelihood phylogenetic tree showing evolutionary relationship between the partial internal transcribed spacer ribosomal RNA gene from eight *Phytophthora infestans* isolates obtained from potato leaves in Nigeria (in red) with other worldwide isolates. The tree was generated based on the Jukes and Cantor model as implemented in MEGA v.6.06. Percentage bootstrap support values (1,000 iterations) are indicated at the branch nodes. The tree is rooted with *Phytophthora palmivora* (MF446701) as the outgroup. The scale bar shows the number of nucleotide substitutions per site. The analysis involved 29 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 74 positions in the final data set

obtained within the Nigerian population, indicating more variation. Collectively, these results show high genetic variability among the Nigerian *P. infestans* population when compared to the rest of the world.

Tajima's D statistical test[30] was used to evaluate the nucleotide polymorphism occurring within the partial ITS rRNA gene within the Nigerian and worldwide *P. infestans* populations. For the worldwide isolates, the Tajima's D, Fu and Li's D, and Fu and Li's F statistic revealed negative values which did not statistically deviate from zero ($P > 0.10$) [Table 3]. These results indicate an excess of low-frequency polymorphism caused by background selection, genetic hitchhiking, or population increases. However, positive values were obtained for the Nigerian isolates except the Tajima's D estimate. These suggest minimal levels of low and high-frequency polymorphisms, indicating a reduction in population size and/or balancing selection within the Nigerian *P. infestans* population.

DISCUSSION

This study has described the morphology and molecular properties of *P. infestans* from Jos, the main producing area of potato in Nigeria. To the best of our knowledge, this is the first molecular description of *P. infestans* isolates from Nigeria. The morphological properties of *P. infestans* in this study are similar to others reported by different authors.[29,31,32]

Two sets of primers were used for the molecular characterization: the ITS4/5 and INF FW2/REV primers. The ITS4/ITS5 is a universal basic primer and conveniently employed region for the molecular identification of fungi.[33] This is due to its variability in length and in nucleotide content among different species of fungi. However, they are not specific to *P. infestans*.[33,34] ITS5 was used as a forward primer, instead of ITS1, as Bertini *et al*.[35] reported ITS5 as preferred because it forms less nucleotide interactions which make it more suitable for PCR in their primer analysis. ITS4 was used as the reverse primer because previous studies have shown that almost all the sequence of primer ITS4 annealed perfectly with corresponding sequences in the gene region being amplified.[35] The PCR results from ITS4/5 did not show clearly, this could be because these primers are not specific to *P. infestans* alone.[36] The INF FW2 and INF REV used in this study are *P. infestans* specific primers and produced good amplicons of about 600 bp. This is in agreement with the findings of Trout *et al*.[36] who also obtained amplicons of about 600 bp when using similar primers to identify *P. infestans*. Furthermore, Hussain *et al*.[37] reported INF FW2 and INF REV to be sensitive and specific to *P. infestans*, detecting as little as 0.5 pg DNA concentration. Extracted DNA from two varieties, "Delice" and "Yellow", did not show up after PCR. This could be as a result of errors

during DNA extraction or degradation of DNA during storage or because the isolates were not *P. infestans*. Knebelsberger and Stoger[38] also reported the degradation of extracted DNA due to long storage and suggested that certain errors during DNA extraction could affect the quality of DNA produced and also the amplicons obtained after PCR.

The ITS rRNA sequences obtained in this study showed low levels of diversity in the pairwise analyses, phylogeny, and genetic diversity studies. First, the intra-population pairwise identity of the eight Nigerian sequences revealed considerable diversity, ranging from 91.91% to 96.37%. This could be as a result of the several years of potato cultivation in the Jos region which has given rise to possible *P. infestans* variants. Potato farming in this region has been practiced for several years and the proliferation to several varieties could give rise to diverse pathogen population. The phylogenetic analyses also confirmed the presence of two possible, distinct populations among the Nigeria isolates as evident in the two separate clusters. All *P. infestans* isolates except "02-CON", "04-NIC" and "08-FAM" clustered together, suggesting varying origins of the pathogen populations in Nigeria. Cooke *et al*.[39] while describing the molecular phylogeny of *P. infestans* reported divergent evolutionary properties within different isolates. This is affirmed in the high genetic diversity obtained in the analyses of variability determinants and neutrality tests. The *P. infestans* population differed in the Tajima's D statistical test, suggesting separate properties from the rest of the worldwide isolates. However, only the ITS region of the *P. infestans* genome was used in this analyses, more information will be revealed with whole-genome sequencing.

There is little information on the molecular identity of *P. infestans* in Nigeria and this preliminary study will contribute an insight toward achieving sustainable solution to the late blight menace in potato-producing regions in the country. To the best of our knowledge, this is the first molecular description of *P. infestans* from Nigeria. This research is a novel molecular identification of *P. infestans* in Jos, Plateau State which will pave way for developing resistant genes that can be incorporated into different varieties of potato. This will confer durable resistance to late blight and will help reduce the dependence on the use of fungicides to control the disease. Whole-genome sequencing is recommended in order to better compare isolates of *P. infestans.* Furthermore, there is a need to identify the specific gene regions that interact with each potato host toward identifying potential potato varieties that are resistant to the late blight disease.

CONCLUSION

There is little information on the molecular identity of *P. infestans* in Nigeria and this preliminary study will contribute an insight toward achieving sustainable solution to the late blight menace in potato-producing regions in the country. To the best of our knowledge, this is the first molecular description of *P. infestans* from Nigeria. This research is a novel molecular identification of *P. infestans* in Jos, Plateau State which will pave way for developing resistant genes that can be incorporated into different varieties of potato. This will confer durable resistance to late blight and will help reduce the dependence on the use of fungicides to control the disease. Whole-genome sequencing is recommended in order to better compare isolates of *P. infestans.* Furthermore, there is a need to identify the specific gene regions that interact with each potato host toward identifying potential potato varieties that are resistant to the late blight disease.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Scott GJ, Rosegrant MW, Ringler C. Global projections for root and tuber crops to the year 2020. Food Policy 2000;25:561-97.
2. Bowen WT. Water productivity and potato cultivation. In: Kijne JW, Barker R, Molden D, editors. Water Productivity in Agriculture: Limits and Opportunities for Improvement. Wallingford, England: Centre for Agriculture and Bioscience International; 2003. p. 229-38.
3. Hussain T. Potatoes: Ensuring food for the future. Adv Plants Agric Res 2016;3:178-82.
4. Chuwang OZ. Recent increased incidences of Potato late blight in Jos, Plateau. A case for intercropping. Agric For Fish 2014;3:363-7.
5. Singh HP, Partasarathy VA, Babu NK. Advance Horticulture Biotechnology-Regeneration Systems-Vegetables, Ornamentals and Tuber Crops. 2nd ed. New Delhi: Westville Publishing House; 2011. p. 442-8.
6. Kumar D, Kumar P, Pandey SK, Singh SV. Processing potatoes in sub-tropical climates. In: Biotechnology in Horticulture: Methods and Application. New Delhi: New India Publishing Agency; 2013. p. 199-214.
7. Ugonna CU, Jolaoso MO, Onwualu AP. A technical appraisal of potato value chain in Nigeria. Int Res J Agric Sci Soil Sci 2013;3:291-301.
8. Fry WE, Birch PRJ, Judelson HS, Grunwald NJ, Danies G, Everts DA, *et al.* Five reasons to consider *Phytophthora infestans* a re-emerging pathogen. Phytopathology 2015;105:966-81.
9. Ibrahim H. Nigeria: Disease Ravages Over 500 Hectares of Irish Potato Farms in Plateau. Daily Trust Newspaper, Abuja; 2014. p. 2.
10. Duncan JM. *Phytophthora*-an abiding threat to our crops. Microbiology 1999;26:114-6.
11. Garelik G. Agriculture: Taking the bite out of potato blight. Science 2002;298:1702-4.
12. Gergely L. Testing for resistance of Potato varieties to late blight agent (*Phytophthora infestans* [Mont.] De. Bary) and the effect

of certain environmental factors on disease resistance. In: PhD Thesis, Veszprem University, Georgikon. Agricultural Faculty, Crop Production and Horticultural Sciences; 2004. p. 1-8.
13. Kwon-Ndung EH. A Preliminary Screening for Field Resistance to Late Blight Disease of Potatoes, *Phytophthora infestans* among Tuber Families and Clones in Vom Area of Jos Plateau, Nigeria. Unpublished M.Sc. Thesis. Nigeria: University of Jos; 1991.
14. Kwon-Ndung EH, Ifenkwe OP. A preliminary screening of potato tuber families and clones for resistance to late blight *Phytopthora infestans* in Jos Plateau, Nigeria. Niger J Bot 1993;6:103-14.
15. Chingle W, Kwon-Ndung E. Managing the menace of late blight disease of potato using field resistance in Jos Plateau, Nigeria. Int J Innov Approaches Agric Res 2019;3:210-6.
16. Kapsa J, Hansen JG. Establishment of monitoring network for potato late blight (*Phytophthora infestans)* in Poland. Plant Breed Seed Sci 2004;50:63-70.
17. Zarzycka H. Isolation, maintenance and preservation of *Phytophthora infestans* isolates. In: Czembor HJ, editor. Vol. 10. The Methods of Evaluation and Selection Applied in Potato Reseaech and Breeding, Monografie Rozprawy Naukowe; 2001. p. 60-6.
18. Sobkowiak S, Sliwka J. *Phytophthora infestans:* Isolation of pure cultures, storage and inoculum preparation. Plant Breed Seed Sci 2017;76:10-5.
19. Gallegly MC, Hong C. *Phytophthora:* Identifying species by morphology and DNA fingerprints. J Plant Prot Res 2008;48:274-5.
20. Hall TA. BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp Ser 1999;41:95-8.
21. Altschul SF, Warren G, Webb M, Eugene WM, David JL. Basic local alignment search tool. J Mol Biol 1990;215:403-10.
22. Muhire BM, Varsani A, Martin DP. SDT: A virus classification tool based on pairwise sequence alignment and identity calculation. PLoS One 2014;9:e108277.
23. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994;11:4673-80.
24. Lee SB, Taylor JW. Phylogeny of five fungus-like protoctistan *Phytophthora* species, inferred from the internal transcribed spacers of ribosomal DNA. Mol Biol Evol 1992;94:636-53.
25. Guindon S, Gascuel O. A Simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 2003;52:696-704.
26. Jukes TH, Cantor CR. Evolution of protein molecules. In: Munro HN, editor. Mammalian Protein Metabolism. New York: Academic Press; 1969. p. 121-32.
27. Tamura K, Stecher G, Daniel, PD, Alan FA, Kumar S. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 2013;30:2725-59.
28. Librado P, Rozas J. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 2009;25:1451-2.
29. Tsedaley B. Late blight of potato (*Phytophthora infestans*)*:* Biology, economic importance and its management approaches. J Biol Agric Healthc 2014;4:215-25.
30. Tajima F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 1989;123:585-95.
31. Forbes G, Perez W, Andrade PJ. Field Assessment of Resistance in Potato to Phytophthora infestans. Lima, Peru: International Potato Center; 2014. p. 35.
32. Binyam T. Late blight of potato (*Phytophthora infestans*) biology. Economic importance and its' management approaches. J Biol Agric Health 2014;4:25-6.
33. Gardes M, Bruns TD. ITS Primers with enhanced specificity for basidiomycetes: Application to the identification of mycorrhizae and rusts. Mol Ecol 1993;2:113-8.
34. Simon L, Lalonde M, Bruns TD. Specific amplification of 18S fungal ribosomal genes from vescicular-arbuscolar endomycorrhizal fungi colonizing roots. Appl Environ Microbiol 1992;8:291-5.
35. Bertini L, Amicucci A, Agostini D, Polidori E, Potenza L, Guidi C, *et al.* A new pair of primers designed for amplification of the ITS region in tuber species. Fems Microbiol Lett 1999;173:239-45.
36. Trout CL, Ristaino J, Madritch M, Wangsomboondee T. Rapid detection of *Phytophthora infestans* in late blight infected potato and tomato using PCR. Plant Dis 1997;81:1043-8.
37. Hussain S, Lees AK, Duncan JM, Cooke DE. Development of species-specific and sensitive detection assay for *Phytophthora infestans* and its application for monitoring of inoculum in tubers and soil. Plant Pathol 2005;54:373-82.
38. Knebelsberger T, Stoger I. DNA extraction, preservation and amplification. Methods Mol Biol 2012;858:311-38.
39. Cooke DE, Drenth A, Duncan JM, Wagels G, Brasier CM. A molecular phylogeny of *Phytophthora* and related oomycytes. Fungal Genet Biol 2000;30:17-32.