Original Research Article

Virus infecting forest tree (*Treculia africana* Decne) and its characterisation in Oban Forest Cross River State.

ABSTRACT

Treculia africana Decne also called African breadfruit is a forest tree in the family Moraceae. The tree crop has been reported to play significant role in medicine, nutrition and agroforestry. A visit to Oban forest in Cross River State revealed symptoms of mosaic on the leaves. This study was aimed at characterising and identifying virus infecting the tree crop, employing RT-PCR and gene sequence analysis as diagnostic tools. The infected leave samples were tested against RT-PCR using virus specific primer. The result of the study revealed that the amplified PCR generated a single product sequence which tested positive against RT-PCR. Sequence comparison with other virus sequences using BLASTn available at NCBI showed that the sequence has 92 % sequence identity with Alfafa mosaic virus (AMV) with accession number of MK607974.1 confirming that the virus under study is AMV. This is the first report of virus infection on tree crop in Cross River State, Nigeria.

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Keyword: *Treculia africana*, Mosaic, sequence, RT-PCR, Alfafa mosaic virus (AMV),

INTRODUCTION

Treculia africana Decne also called African breadfruit is a forest treet in the family Moraceae with a large, slow-growing, evergreen tree with a dense, spreading crown usually growing 15 - 30 metres tall but with some specimens up to 50 metres. The tree crop is a very valuable food crop in Africa, providing a nutritious protein and oil rich food. It is often grown in and around African villages where it is commonly harvested for its edible seeds and is sold in local markets. The tree is often protected when land is cleared for agriculture. It is usually found growing near streams or in swampy areas in forests at an altitude up to 1,500 metres [5]. The seeds are cooked and eaten as dessert nuts after roasting or boiling, they are also ground into a meal, used in soups and to produce a variety of baked foods such as bread and paste. The seeds are extracted after macerating the fruit in water. The grains have an excellent polyvalent dietetic value of proteins exceeding even that of soybeans. A non-alcoholic beverage, almond milk, can be prepared from the powdered seeds, which is recommended as a breakfast drink in Nigeria. An edible oil can be extracted from the seed [2].

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bread and pasta. The seeds are extracted after macerating the fruit in water. The grains have an excellent polyvalent dietetic value of proteins exceeding even soybeans. A non-alcoholic beverage, almond milk, can be prepared from powdered seeds, recommended as a breakfast drink in Nigeria. An edible oil can be extracted from the seed [2].

In Ghana, a root decoction is used as an anthelmintic and febrifuge. The caustic sap of male African breadfruit is applied on carious teeth. A decoction of the bark is used in the treatment of cough and whooping cough. The ground bark, mixed with oil and other plant parts, is used in the treatment of swellings. It is also used in the treatment of leprosy and as a laxative [2]. The tree has been used in soil conservation programmes. The leaf fall is a good source of mulch. It has also been recommended as a promising species for use in home gardens, and for intercropping systems in agroforestry [1]. The heartwood is yellow with very narrow pale sapwood; very dense, fairly elastic and flexible, rather heavy, with fine, even structure. It is suitable for furniture, carving, turnery and inlay wood. It is suitable for pulp and papermaking. The wood is used for fuel and making charcoal [1]

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There are about 22 established groups of higher plant viruses that have been detected in trees and shrubs [10]. Because of their longevity. woody perennials trees like Treculia Africana are vulnerable to infection for protracted periods. Very few of the many pests and pathogens that colonize sometimes transitorily, trees and shrubs have been tested for their abilities to spread viruses. Even if most of them were inefficient, the feeding damage done to plant cell walls might allow incidental transmission

of viruses. For ex-ample, it has been reported that aphids can inoculate plants with viruses when clawing (scratching) leaf surfaces [8]. Interestingly, many virus-infected trees show symptoms for only a few weeks in a 'year and not all parts of the foliage are affected. In many instances viruses have been isolated from superficially healthy trees and shrubs, however, not much has been done in terms of isolation and characterisation of tree viruses. Symptoms of virus infection were observed during visit to Oban Forest in Cross River State, Nigeria. This study is aimed at characterising virus causing infection in Treculia Africana

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Interestingly, many virus-infected trees show symptoms for only a few weeks in a 'year, and not all foliage parts are affected. Viruses have been isolated from superficially healthy trees and shrubs in many instances. However, not much has been done regarding the isolation and characterization of tree viruses. Symptoms of virus infection were observed during visit to Oban Forest in Cross River State, Nigeria. This study is aimed at characterizing virus causing infection in *Treculia Africana.*

MATERIALS AND METHODS

Virus extraction extraction

Approximately, 100 mg of fresh young leaves of Treculia Africana showing symptoms of infection were collected for virus extraction using a modified Cetyltrimethylammonium bromide (CTAB). The young leaves were collected and weighed to obtain approximately 150–200 mg before grinding

thoroughly with 200 µl of CTAB buffer using clean and sterilized pestles and mortars. Each was later made up to 700 µl with CTAB buffer and the mixture was transferred to 1.5 ml micro centrifuge tube for proper mixing and vortexing. The mixture was incubated at 60 °C for 15 min after which it was brought to room temperature for addition of equal volume of phenol, chloroform and isoamyl alcohol in the ratio of 25:24:1. It was thoroughly mixed and centrifuged at 13, 000 revolutions per minute (rpm) for 15 min. After the centrifugation, 450 µl of the supernatant was transferred into a new and sterile 1.5 ml micro centrifuge tube followed by addition of 400 µl of ice-cold isopropanol for precipitation of the virus particles. It was mixed by gentle inversion and incubated at − 20 °C overnight. At the end of the overnight incubation, it was centrifuged at 14,000 rpm for 15 min to sediment the virus particles. The supernatant was well decanted without disturbing the pellet. The pellet was washed by adding 700 µl of 70% ethanol and centrifuging at 13, 000 rpm for 5 min. The ethanol was decanted followed by air-drying the pellet and suspension in 100 µl of nuclease-free water.

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Polymerase chain reaction and agarose gel electrophoresis

PCR amplification of the extracted RNA samples was done with specific primer consisting of 2.0 µl of 100 ng/ul RNA, 2.5 µl of 10 × buffer, 1.5 µl of 50 mM $MgCl_2$,2.0 µl of 2.5 mM dNTPs, 0.2 µl of 500U RNA Taq polymerase, 1.0 µl of 10 pm each of the ACMV JSP001/F: 5'-ATGTCGAAGCGACCAGGAGAT-3'and JSP002/R: 5'-TGTTTATTAATTGCCAATACT-3', 0.1 µl Mgcl2 (100 Mm), 2.5 µl PCR buffer (10x), 18.8 µlSDW, 0.5 µl dNTPs (2.5 Mm), 0.5 µl JSP001/F (10 µM), 0.5µl JSP002/R (10 µM ACMV), 0.5 µl JSP003/R (10 MmEACMV), 0.1 µl of 5 U/ µl Taq polymerase and 2.0 µl of theDNA template. The PCR cycling profile for the reaction consisted of an initial step at 94 °C for 3 min., 30 cycles of 94 °C for 1 min, 45 °C for 1 min, and 72 °C for 2 min., and 5 min final extension at 72 °C. Five (5) µl of the PCR products were electrophoresed in 1.5% agarose gel containing 0.5 mg/ml ethidium bromide and photographed on Trans illuminator UV light (Fotodyne Incorporated, Analyst Express, USA).

Purification of PCR amplicons and RNA sequencing

The amplified PCR products that generated a single product were purified using ethanol protocol with slight modifications. Briefly, 40 µl of 100% ethanol was added to 20 µl of the PCR products, incubated at room temperature for 15 min and centrifuged at 12,000 rpm for 15 min. The ethanol was carefully decanted and 100 µl of 70% of ethanol was used to wash, maintaining the same centrifugal speed and time. The ethanol was discarded, and the DNA dried at room temperature for resuspension using 20 µl of DEPC-treated water. The purified samples were sequenced at International BioTec West Africa (IBWA), Ibadan, Nigeria.

Data analysis

The raw sequence obtained was carefully edited to remove impurities using BioEdit software version 7. 2. 5. The Basic Local Alignment Search Tool (BLAST) version 2.0, of the National Center for Biotechnology Information found at https://blast.ncbi.nlm.nih.gov/Blast.cgi was used to search for species identification, sequence similarity or homology and other bit scores.

RESULTS

RT-PCR and sequencing

The amplified PCR generated a single product sequence presented in Fig 1. Sequence comparison with other virus sequences using BLASTn available at NCBI showed that the sequence has 92 % sequence identity with Alfafa mosaic virus (AMV) with accession number of MK607974.1

TTTTTATCTTACACACGCTTGTGTAAGATAGTTAATCCATTTATTTTTCCATGCTCTTTCCACAGCATT
ACGTTCATTCAATACTGTGAAGATTTCACTATGAATGCTGACGCCCAATCCACCGATGCCAGCCTTAGTA

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TGCGAGAACCTTTATCTCATGCCTCCATTCAGGAGATGCTTCGACGTGTAGTCGAAAAGCAAGCTGCAGA
CGACACAACTGCAATCGGAAAAGTTTTTTCCGAAGCGGGTCGTGCCTATGCCCAGGATGCTCTCCCTTCA
GACAAAGGTGAAGTCTTGAAGATATCCTTTTCCCTGGACGCCACGCAACAAAACATACTACGCGCCAACT
TTCCTGGTCGACGCACTGTATTTTCAAACAGTTCGAGTTCATCTCACTGTTTTGCGGCTGCCCATCGTCT
ACTAGAAACCGATTTTGTTTACCGATGTTTCGGTAATACGGTTGATAGTATTATAGACCTTGGAGGAAAT
TTTGTTTCCCATATGAAGGTGAAGCGGCATAATGTACATTGCTGCTGTCCCATATTGGATGCTAGAGACG
GAGCTAGGCTCACGGAGAGAATATTGTCTCTAAAGTCGTACGTCCGAAAACACCCGGAAATTGTGGGTGA
AGCAGATTACTGCATGGACACGTTTCAGAAATGCTCAAGGCGAGCTGACTATGCTTTTGCCATCCATTCT
ACTAGCGATCTCGACGTGGGAGAGTTGGCATGTAGTTTGGACCAAAAAGGCGTTATGAAATTCATTTGCA
CCATGATGGTTGATGCAGATATGTTAATTCATAACGAGGGGGAAATTCCTAACTTTAATGTTAGATGGGA
GATCGATCGTAAGAAAGATCTCATTCATTTCGACTTCATCGACGAGCCCAATTTGGGATATAGTCATCGG
TTTTCATTGTTAAAACACTATTTGACTTACAATGCCGTTGATTTGGGTCATGCTGCTTATCGAATCGAAC
GTAAGCAAGATTTTGGAGGTGTGATGGTTATTGACTTAACTTATTCCCTTGGATTTGTCCCCAAGATGCC
ACACTCCAATGGGAGGTCCTGCGCCTGGTATAATAGAGTCAAAGGACAAATGGTAGTGCACACCGTTAAC
GAGGGGTACTATCATCATTCATACCAGACAGCAGTGAGGCGGAAAGTACTTGTCGATAAGAAAGTGCTTA
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FIG. 1 Gene sequence of Treculia Africana virus isolate

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Query 1 TTTTTATCTTACACACGCTTGTGTAAGATAGTTAATCCATTTATTTTTCCATGCTCTTTC 60
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 2 TTTTTATCTTACACACGCTTGTGTAAGATAGTTAATCCATTTATTTTTCCATGCTCTTTC  61

Query61 CACAGCATTACGTTCATTCAATACTGTGAAGATTTCACTATGAATGCTGACGCCCAATCC  120
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct62 CACAGCATTACGTTCATTCAATACTGTGAAGATTTCACTATGAATGCTGACGCCCAATCC  121

Query121ACCGATGCCAGCCTTAGTATGCGAGAACCTTTATCTCATGCCTCCATTCAGGAGATGCTT  180
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct122ACCGATGCCAGCCTTAGTATGCGAGAACCTTTATCTCATGCCTCCATTCAGGAGATGCTT  181

Query181CGACGTGTAGTCGAAAAGCAAGCTGCAGACGACACAACTGCAATCGGAAAAGTTTTTTCC  240
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct182CGACGTGTAGTCGAAAAGCAAGCTGCAGACGACACAACTGCAATCGGAAAAGTTTTTTCC  241

Query241GAAGCGGGTCGTGCCTATGCCCAGGATGCTCTCCCTTCAGACAAAGGTGAAGTCTTGAAG  300
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct242GAAGCGGGTCGTGCCTATGCCCAGGATGCTCTCCCTTCAGACAAAGGTGAAGTCTTGAAG  301

Query301ATATCCTTTTCCCTGGACGCCACGCAACAAAACATACTACGCGCCAACTTTCCTGGTCGA  360
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct302ATATCCTTTTCCCTGGACGCCACGCAACAAAACATACTACGCGCCAACTTTCCTGGTCGA  361

Query361CGCACTGTATTTTCAAACAGTTCGAGTTCATCTCACTGTTTTGCGGCTGCCCATCGTCTA  420
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct362CGCACTGTATTTTCAAACAGTTCGAGTTCATCTCACTGTTTTGCGGCTGCCCATCGTCTA  421

Query421CTAGAAACCGATTTTGTTTACCGATGTTTCGGTAATACGGTTGATAGTATTATAGACCTT  480
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct422CTAGAAACCGATTTTGTTTACCGATGTTTCGGTAATACGGTTGATAGTATTATAGACCTT  481

Query481GGAGGAAATTTTGTTTCCCATATGAAGGTGAAGCGGCATAATGTACATTGCTGCTGTCCC  540
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct482GGAGGAAATTTTGTTTCCCATATGAAGGTGAAGCGGCATAATGTACATTGCTGCTGTCCC  541

Query541ATATTGGATGCTAGAGACGGAGCTAGGCTCACGGAGAGAATATTGTCTCTAAAGTCGTAC  600
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct542ATATTGGATGCTAGAGACGGAGCTAGGCTCACGGAGAGAATATTGTCTCTAAAGTCGTAC  601

Query601GTCCGAAAACACCCGGAAATTGTGGGTGAAGCAGATTACTGCATGGACACGTTTCAGAAA  660
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct602GTCCGAAAACACCCGGAAATTGTGGGTGAAGCAGATTACTGCATGGACACGTTTCAGAAA  661
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Query661TGCTCAAGGCGAGCTGACTATGCTTTTGCCATCCATTCTACTAGCGATCTCGACGTGGGA  720
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct662TGCTCAAGGCGAGCTGACTATGCTTTTGCCATCCATTCTACTAGCGATCTCGACGTGGGA  721

Query721GAGTTGGCATGTAGTTTGGACCAAAAAGGCGTTATGAAATTCATTTGCACCATGATGGTT  780
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct722GAGTTGGCATGTAGTTTGGACCAAAAAGGCGTTATGAAATTCATTTGCACCATGATGGTT  781
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Query: sequence of virus isolated from Treculia Africana

Subject: Sequence of Alfafa mosaic virus (AMV)

DISCUSSION

Gene sequencing as tool for virus identification and characterization has become the ultimate in recent times [12]. A virus identity will become unassailable if the degree of homologue of it sequence is established after comparison with sequences of previously characterised members of the genus to which the virus in question belongs. The results obtained from this study revealed that the virus sequence when compared with other sequences in the GenBank using the BLASTn available in NCBI showed 99 % sequence identity with African cassava mosaic virus.

Judith, *et al*. [9] proposed a demarcation criteria for the classification of viruses into genus taxon. A genome-wide pairwise identities of 91 %, 94 % and 99 % sequence identity are the demarcation threshold for viruses belonging to different isolates, strains and species respectively. The virus under study has a sequence identity of 92 % and is therefore considered Alfafa mosaic virus.

The result obtained in this study is similar to the report by [6] and [7] who reported the identification of CMV and *Algerian watermelon mosaic* virus through molecular characterisation. [4] have also employed molecular tools in the detection of plant viruses. This result further support the work of [13] who reported AMV using gene sequence analysis as diagnostic tool. This further corroborate the report by [3] who detected AMV in forest tree in Kenya and [11] who reported same virus in Northern Nigeria.

CONCLUSION

This study was conducted to characterise and identify virus isolated from Treculia Africana in Oban Forest of Cross River State. The infected leaf samples with mosaic were collected and tested against RT-PCR.

The result obtained from this study revealed that the virus tested positive against RT-PCR primer. The amplified PCR generated a single product sequence. Sequence comparison with other virus

sequences using BLASTn available at NCBI showed that the sequence has 92 % sequence identity with AMV with accession number of MK607974.1. This is one among several other discoveries of viruses infecting forest trees worldwide.

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