Molecular Characterisation of viruses infecting selected vegetables grown within University of Cross River State

ABSTRACT

Vegetable crops play very important role in human nutrition and medicine. The production of these crops is seriously under threat due to virus infection. Field survey within gardens and farms located within the University of Cross River State, Nigeria during the 2023 planting season revealed evidence of virus infection of selected vegetable crops like Cucurbita pepo, tomatoes and Trichosanthescucumerina. Infected leaf samples were obtained and total RNA extracted using CTAB protocol and further tested using RT-PCR and gene sequence analysis. The results obtained revealed that *Cucurbit aphid-borne yellows virus* (CABYV) was detected in *Cucurbita pepo* virus while *Tomato spotted wilt virus* (TSWV) was detected in tomatoes and *Trichosanthes mottle potyvirus* (TMPV) was detected in *Trichosanthescucumerina.* This is one among other reports on virus infection on vegetables worldwide. This is the first detection and molecular characterization of CABYV, TSWV, and TMPV in Nigeria

Keyword: molecular, characterization, virus, RNA, RT-PCR

INTRODUCTION

"V*egetable*s usually refers to the fresh edible portions of certain herbaceous plants which can be consumed byroots, stems, leaves, flowers, fruit, or seeds. These plant parts are either eaten fresh or prepared in a number of ways, usually as a savory, rather than sweet, dish. Examples of these vegetables include leafy green like lettuce, spinach and silverbeet, onion, garlic, shallot, pumpkin, cucumber and zucchini, cabbage, cauliflower, Brussels sprouts and broccoli. These crops play very important roles in nutrition and medicine" [1]

"At least nine different families of fruits and vegetables exist, each with potentially hundreds of different plant compounds that are beneficial to health. Virtually all of the more important vegetables

were cultivated among the ancient civilizations of either the Old or the New World and have long been noted for their nutritional importance. Most fresh vegetables are low in calories and have a water content in excess of 70 percent, with only about 3.5 percent protein and less than 1 percent fat'' [1]"Vegetables are good sources of minerals, especially calcium and iron, and vitamins, principally A and C. Nearly all vegetables are rich in dietary fibre and antioxidants'' [2].

"Reports have revealed that the production of these crops is greatly constrained by plant pathogens including viruses. Viruses are ranked among the major causal agents of plant disease reducing the quality and quantity of yield produced" [3].

Several viruses have been implicated and emerged as economically important viruses which have been isolated and characterised globally. For example Eyong, et al. [5] have reported "*Papaya ringspot virus*-W (PRSV-W), *Squash leaf curl virus* (SLCuV), *Soybean mosaic virus* (SMV) infecting selected cucurbits in Southern Nigeria". *Squash mosaic virus* (SqMV) and *Squash vein yellowing virus* (SqVYV). Others include *Tobacco ringspot virus* (TRSV), *Watermelon mosaic virus* (WMV), *Watermelon silver mottle virus* (WSMoV), *Alfalfa mosaic virus* (AMV) have also been characterised by [6] in southern Nigeria.

Other viruses reported to infect vegetables include *Bean pod mottle virus* (BPMV), *Cucurbit aphid borne yellows virus* (CABYV), *Cucurbit yellow stunting disorder virus* (CYSDV), *Cucumber green mottle mosaic virus* (CGMMV), *Cucumber mosaic virus* (CMV), *Melon necrotic spot virus* (MNSV), *Zucchini yellow mosaic virus* (ZYMV), and *Zucchini green mottle mosaic virus* (ZGMMV). These viruses can be transmitted mechanically and by several aphid species in a non persistent manner [6]inducing symptoms such as mosaic, shoestring, chlorosis, leaf deformation, chlorotic spotting, vein clearing and banding, curling and yellowing of the leaves, stunting of the plants and deformation of fruits[4]. This research is therefore aimed at isolating, characterising and identifying viruses infecting selected vegetables within University of Cross River State

MATERIALS AND METHODS

Sources and isolation of viruses

Field survey of vegetables during the 2023 planting season revealed severe virus infection on selected crops. Infected leaf samples from Trichosanthescucumerina, tomatoes, and Cucurbita pepo with typical virus-like symptoms were obtained from different three locations within the University of Cross River State farms (C. pepo from staff quarters, tomatoes from new science block and Trichosanthescucumerinafrom the pavilion). This crop was found growing on farms and gardens in the location named above. Infected leaf samples were collected into Ziploc air tight polyethylene bags to keep them fresh to ensure the viability of the virus particles and later transported to the molecular laboratory of National Institute of Horticulture (NIHORT) Ibadan for the molecular diagnosis.

RNA extraction from infected leaf samples

"Total RNA was extracted from the infected leaf samples using the cetyltrimethylammonium bromide (CTAB) protocol as described" by [9]. One hundred milligrams of infected leaf sample was grounded in sterile mortar and pestle in 1 ml extraction buffer (100 mM Tris-HCl, pH 8.0; 1.4 M NaCl; 20 mM EDTA; 2 % CTAB) (hexadecetyltrimethylammonium bromide); and 0.4 % β- mercaptoethanol, added just before use. "Each of the homogenates was poured into a new 1.5 ml Eppendorf tube. The tubes were vortexed briefly, incubated in a 60^{o}C water bath for 10 minutes and allowed to cool to room temperature. Then 0.75 ml of phenol choloform isoamyl (25:24:1) was added to each tube containing the homogenate. Each tube was then vortexed vigorously to form an emulsion and then centrifuged at the speed of 12000 rcf for 10 minutes. The supernatant was then transferred to a clean 1.5 ml tube. Three hundred of cold isopropanol was added to the supernatant to precipitate the nucleic acid (RNA) and the mixture was kept at -80^{o}C for 10 minutes. The mixture was centrifuged at 12,000 rcf for 10 min to precipitate the nucleic acid. The supernatant was discarded and the nucleic acid pellet washed in 500 µl of 70 % ethanol and centrifuged at 12,000 rcf for 5-10 minutes. The supernatant was decanted and the resultant nucleic acid pellet was air-dried at room temperature. Nucleic acid pellet was then re-suspended in 50 µl sterile distilled water and used as a template source for reversed transcriptase polymerase chain reaction (RT-PCR). Nucleic acid extracts from the leaves of healthy plants were used as negative control". [18]

Reverse transcriptase polymerase chain reaction (RT-PCR)

"Virus-specific complementary DNA (cDNA) fragments were amplified from total nucleic acid derived from the infected leaf samples by a RT-PCR method as described" by [10]. "RT-PCR was performed

using the cylindrical inclusion (CI) primers forward 5'- TGGTCGTCCAACTATTAACCAC-3', Reverse 5'-TACTGATAAACCAGTACCGGTGA -3' as described" by [11]. The RT-PCR reaction mixture (50 µl) consisted of I µl each of CICP 5' and CICP 3', 5x Go Taq green buffer (10.0 µl), $MgCl_2$ (3.0), dNTPs (1.0 µl), Reverse transcriptase (0.24 µl), Taq DNA polymerase (Promega) (0.24 µl), sterile distilled water (30.52 µl) and nucleic acid from infected leaf sample (1:10 dilution) (3.0 µl).

"Amplifications were carried out in a GeneAmp 9700 PCR system thermalcycler (Applied Biosystem Inc., USA) using the following thermocyclic conditions; 42^o C for 30 min for reverse transcription, 94^o C for 3 min for initial denaturing, followed by 40 cycles of denaturing at 94^o C for 30 sec, an annealing step at 40^o C for 30 s, an extension at 68^o C for 1 min and a final extension at 72^o C for 10 min ended the RT-PCR reaction. The PCR reaction products were separated on 1.5 % agarose gel, subsequently stained with ethidium bromide, visualized in UV light and photographed". [19]

Amplicon purification and sequencing

"The RT-PCR amplicon for each sample was purified by adding 95 % ethanol to 40 µl of the amplicon in a new 1500 µl Ependorff tube and the solution was kept in – 80^o C for 10 minutes. The tube was centrifuged for 10 min and the supernatant discarded. Five hundred of 70 % ethanol was added and centrifuged at maximum speed for 5 min. The supernatant was discarded and the tube was left at room temperature to dry after which the purified cDNA was dissolved in 30 µl of sterile distill water. The product was sequenced at Bioscience Laboratory of the International Institute of Tropical Agriculture (IITA) Ibadan". [19]

Sequence analysis

"The sequence identity between the virus under study was established by comparison with known virus sequences in the GenBank available at National Centre for Biotechnology Information (NCBI) using the basic local alignment search tool (BLAST) program (http://www.ncbi.nlm.nih.gov/BLAST/)". [19]Sequence identities were calculated from the sequence identity matrix option in MEGA 6 window software.

RESULTS and DISCUSSION

Nucleic Acid Sequencing andSequence Analysis

The result revealed a fragment of predicted size of 700 bp which wasobtained by RT-PCR. Gene sequenceanalysis using BLASTn available in NCBI database showed that the *Cucurbita pepo* virus isolate sequence had 92 % sequences identity with Cucurbit aphid-borne yellows virus (CABYV) while Tomatoes virus isolate sequence revealed 91 % sequences identity with Tomato spotted wilt virus (TSWV) and *Trichosanthescucumerina*virus isolate sequence showed 89 % sequence identity with Trichosanthes mottle potyvirus (TMPV) (Table 1).

Table 1. Gene sequence analysis of virus isolates using BLASTn

Host/Virus isolate	Viruses	Location	% Identity
Cucurbita pepo	Cucurbit aphid-borne yellows virus (CABYV)	Staff quarters	92
Tomatoes	Tomato spotted wilt virus (TSWV)	New science block	91
Trichosanthescucumerina	Trichosanthes mottle potyvirus (TMPV)	Pavilion	89

Field survey for virus infected plant samples was carried out during the 2023 planting season. Areas visited included garden within the University of Cross River State Staff quarters where *Cucurbita pepo* was obtained, new science block for the collection of Tomatoes infected sample and the Pavilion for the collection of *Trichosanthescucumerina*infected sample.

Infected samples were analysed using molecular method such as RT-PCR and gene sequence analysis. The application of molecular method such as RT-PCR and gene sequence analysis in plant virus diagnosis has become the more reliable in recent time [8]. This is due to its nuclear-derived ability for detecting the presence of specific genetic material in any pathogen, including a virus [12].

In a study titled occurrence, distribution and identification of viruses infecting some cucurbits growing areas in Cross River, Nigeria, [13]deployed the RT-PCR and gene sequence analysis in detecting viruses infecting cucurbits. Some of the viruses detected included Morroccan watermelon mosaic virus, Algerian watermelon mosaic virus, Potato virus Y, Zucchini watermelon mosaic virus and

Soybean mosaic virus. In a separate study by [14]molecular tools was deployed in the detection of Yambean mosaic virus.

The result obtained in this study is similar to the report by [15]who reported the identification of CMV and *Algerian watermelon mosaic* virus through molecular characterisation. Desbiez et al., [8]have also employed molecular tools in the detection of plant viruses.

These resultsfurther corroborate the report by [16]who detected ACMV in cassava field in Kenya and [17] who reported same virus in Northern Nigeria.This study showed that CABYV, TSWV and TMPV are now present in Nigeria

Conclusion

Cross River State Nigeria is a home to vegetables, local formers engaged in the production of these crops to raise income to sustain the families. The important of vegetable consumption to health and nutrition cannot be overemphasised as reports have shown the numerous benefits derived in their consumption. However, this trade and the production of these crops is being threatened by pathogens especially viruses which has been reported to reduce vegetable production in quality and quantity. To this end, field survey was carried out to obtained and characterised virus samples responsible for poor quality production of selected vegetables using RT-PCR and gene sequence analysis.

Cucurbit aphid-borne yellows virus (CABYV) was detected in *Cucurbita pepo* virus while Tomato spotted wilt virus (TSWV) was detected in Tomatoes and Trichosanthes mottle potyvirus (TMPV) was detected in *Trichosanthescucumerina.* This is one among other reports on virus infection on vegetables.This is the first detection and molecular characterization of CABYV, TSWV, and TMPV in Nigeria

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