



Detection and characterization of cucumber mosaic virus (CMV) infecting passion fruit in Nigeria

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Abstract

Several viruses have been reported to affect the productivity of passion fruit (*Passiflora edulis* Sims.), a perennial herbaceous and wood plant, including cucumber mosaic virus (CMV). In this work, a *Cucumovirus* isolated from passion fruit plants was characterized. Passion fruit leaves showing virus-like symptoms were collected from the experimental field of the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria and analyzed using ELISA. The infected leaf samples were used as virus source for inoculating healthy *Nicotiana benthamiana*, cowpea, and pepper plants in the screen house. Symptom evaluation revealed that the virus showed severe mosaic symptoms on *N. benthamiana* 7 days after inoculation but no observable symptoms were seen on cowpea and pepper and indexing with ELISA confirmed the presence of CMV on sampled plants. For virus characterization, RT-PCR was used to amplify 500 bp of the CMV coat protein, which was then purified and sequenced. Sequence analysis revealed that the CMV isolate from this study share a similarity of 98% with CMV isolated from Yam with accession number LC066509, having values in the range of 98–99% with subgroup IA, 92–95% with subgroup IB and below 80% with subgroup II. Also, comparisons of the deduced amino acid sequences showed 97.3 to 100% sequence identity with coat protein ORF gene of CMV subgroup I, while below 89% with subgroup II. This study identifies CMV infecting passion fruit in Nigeria as member of subgroup IA.

Keywords Detection · *Cucumovirus* · ELISA · Inoculation · Symptoms · Subgroup IA

Introduction

Passion fruit (*Passiflora* spp.) is a perennial herb and woody plant belonging to the *Passifloraceae* family, with great genetic variability (about 530 species), native to southern Brazil, Paraguay, and Northern Argentina (Fischer and Resende, 2008; Joy and Sherin 2012). The leaves of some

varieties are used as a tea substitute, while the leaves and roots of others have medicinal properties. The fruit can be eaten alone or combined with fruit salads, ice cream, jams and certain sweet products, and is often added to other juices to enhance aroma (Fischer and Rezende 2008). It is rich in vitamins and minerals, provides fiber, and is low in calories (Li et al. 2016). In Nigeria, the fruit is grown as a relatively underutilized crop in the North-Central and Southeastern parts of the country, and is also grown in backyards and farms across the country. Its cultivation is also gaining popularity in the national juice industry (Arogundade et al. 2018). However, the quantity and quality of *P. edulis* are severely affected by several diseases which are caused by fungi, bacteria, phytoplasma-associated diseases, nematodes, and viral pathogens (Joy and Sherin 2012).

Several viruses, including numerous Potyviruses, cucumber mosaic virus (CMV), passion fruit latent virus (PLV), lemon mosaic virus, passion fruit vascular virus (PaYMV), purple granadilla mosaic virus (PGMV), passion fruit green spot virus (PGSV), Geminivirus, Maracujá mosaic virus (MarMV), tomato green spot virus (ToRSV) are known to

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infect passion fruit (Fischer and Rezende 2008). Among them, passion fruit woodiness virus of the genus *Potyvirus* (Arogundade et al. 2018) was reported in Nigeria.

Cucumber mosaic virus (CMV) is a member of the genus *Cucumovirus* in the family *Bromoviridae* and is one of the most common viruses that infects more than 1,200 species in more than 100 monocotyledonous and dicotyledonous plant families, causing varying disease symptoms (Palukaitis and Garcia-Arenal 2003; Ouedraogo and Roossinck 2018). It is transmitted mechanically through plant sap, transmitted non-persistently in nature by more than 80 species of aphids, and is known to be transmitted through seeds of various plant species (Palukaitis and Garcia-Arenal, 2003; Arogundade et al. 2019). According to a study carried out by Longe et al. (2022), which detected CMV on tomato seedlots, seeds of plants infected with CMV can serve as a ready source of the virus inoculum, and they tend to produce asymptomatic seedlings which make it important to detect CMV on possible host plants such as *P. edulis*. CMV is a positive-sense single-stranded RNA virus with a tripartite genome in descending order of size (1, 2, 3) encoding five ORFs. ORF1a and 2a are viral RNA replication proteins, and ORF 2b encodes a viral suppressor important for RNA silencing, ORF 3a encodes movement protein, 3b encodes coat protein (Roossinck 2002; Palukaitis and Garcia-Arenal 2003). Many previously reported CMV isolates have been classified into two major subgroups I and II. Subgroup I is further subdivided into subgroups IA and IB based on serological relatedness and nucleotide sequence similarity (Roossinck et al. 1999; Palukaitis and Garcia-Arenal 2003).

Among the three main subgroups, IA, IB, and II, nucleotide identity is 72–94% for subgroup II compared with subgroups IA/IB and subgroup IA compared with subgroup IB respectively (Pavithra et al. 2019).

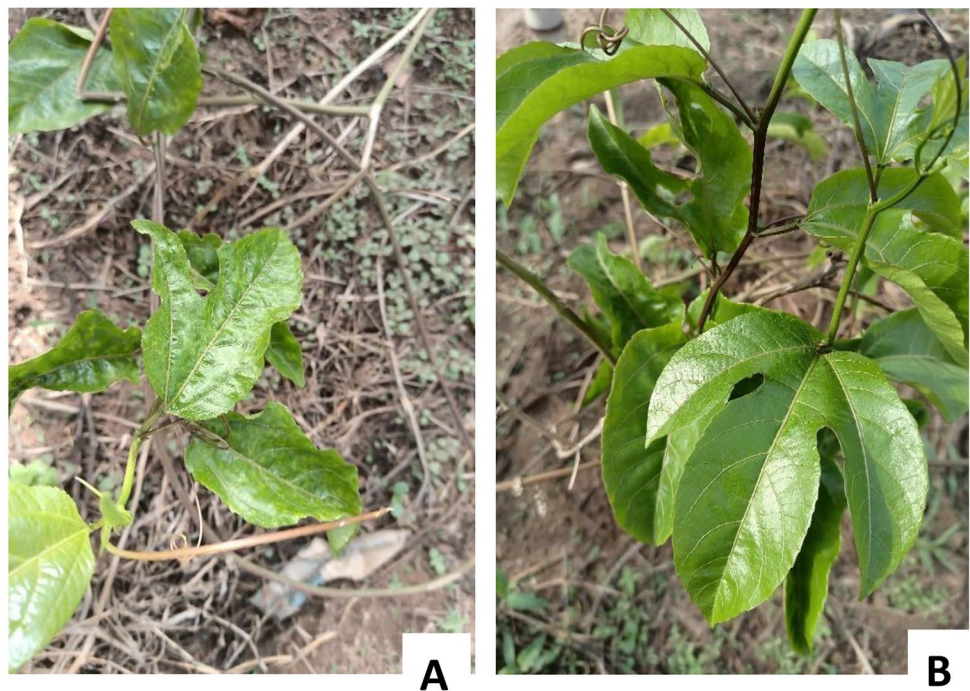
CMV infection of *P. edulis* plants was reported first in Taiwan in 1981, later in Brazil in 1986, in Guangdong in 1987, in Hainan in 1995, and in Fujian in 1996 (Lan et al., 2020). In Nigeria, CMV isolates have been reported in various crops such as leafy greens, tubers, legumes and spices (Arogundade et al. 2012, 2015; Eni et al. 2013; Ayo-John and Hughes 2014; Odedara and Kumar 2017; Adediji 2019). Although reported in South Africa (Brand and Wechmar 1993), there is no report of CMV infection on passion fruit from Nigeria. In this article, we reported the occurrence of CMV in *Passiflora* species in Nigeria and investigated the biological and molecular properties of the CMV coat protein sequence. To our knowledge, this is the first report of CMV in the species of *Passiflora* in Nigeria.

Materials and methods

Source and maintenance of the virus

During the year 2017, passion fruit plants were observed exhibiting virus-like symptoms on the experimental field of the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria. The naturally infected plants exhibited mosaic and leaf mottling (Fig. 1). The leaves from the infected passion fruit plants were collected and used for

Fig. 1 CMV-infected passion fruit plant showing mosaic with chlorotic spots, and mottling in the field (A), and symptom-free passion fruit (B)



mechanical inoculation to healthy plants of *Nicotiana benthamiana* and healthy passion fruit.

Serological detection of CMV using enzyme linked immunosorbent assay

To initially detect the associated virus, 30 samples randomly collected from the diseased field were subjected to enzyme linked immunosorbent assay (ELISA) using a commercial specific CMV (DAS ELISA) and general *Potyvirus* antibodies (Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany) according to manufacturer's instructions. For every test, negative and positive controls were included. The double antibody sandwich-ELISA (DAS-ELISA) procedure was performed according to standard DSMZ protocol (Clark and Adams 1977; Hosseinzadeh et al., 2012). Microtiter plates were coated with 100 ml of 1:1000 IgG in carbonate coating buffer (1.59 g Na₂CO₃, 2.93 g NaHCO₃, and 0.20 g NaN₃, pH 9.6) and incubated at 37 °C for 2 to 4 h. Samples were extracted in PBS extraction buffer, and wells were washed three times, at 3 min intervals, with washing buffer (PBST). 100 ml of plant extract was added to each well and incubated overnight at 4°C. Plate wells were washed three times with washing buffer; then 100 ml of alkaline phosphatase-conjugated IgG diluted in conjugate buffer was added and incubated for 4 h at 37 °C. Wells were washed three times with washing buffer; then 100 ml of the substrate (10 mg p-nitrophenyl phosphate, dissolved in 10 ml substrate buffer, pH 9.8) was added and incubated at room temperature; and absorbance was determined at 405 nm by an ELISA-reader after 60 min. A well was classified as positive if its optical density value was greater than twice the plate's healthy control value plus the standard deviation.

RNA extraction and RT-PCR of the coat protein gene of CMV

For molecular detection of the virus isolates, total RNA was isolated from two representative symptomatic passion fruit samples using modified CTAB protocol as described by Abarshi et al. (2010). Extracts were evaluated by RT-PCR using CMV specific forward and reverse primers of coat protein gene. RT reaction was carried out at 42 °C for 30 min, followed by a 35-cycle, 3 steps PCR. Each cycle included a denaturing step at 94 °C for 30 s, an annealing step at 55 °C for 30 s, and an extension step at 72 °C for 90 s, and finally kept at 72 °C for 10 min for final extension. Extracted and amplified cDNA fragments were visualized on a 2% agarose gel in TAE buffer, stained with ethidium bromide at 60 V for 50 min and visualized under a UV transilluminator.

Sequencing and phylogenetic analysis

The purified amplicon was sequenced bi-directionally using a DNA-analyzer (Genetic Analyzer 3500, Applied Biosystems) at Inqaba Biotec sequencing facility (Pretoria, South Africa). The sequence generated in this study was compiled and edited using Bio-Edit sequence alignment editor (version 7.2.5) and compared with sequences from the GenBank database using the NCBI basic alignment search tool (BLAST). Multiple alignments of nucleic acid and amino acid sequences were performed using CLUSTALW (Thompson et al. 1994). Nucleic acid and amino acid pairwise identity comparison was obtained as a colored matrix using Sequence Demarcation Tool, version 1.2.

(Muhire et al. 2014). The Phylogenetic relationships of CMV coat protein sequences were constructed by the Maximum likelihood method as implemented in MEGA v10.1.8 (Kumar et al. 2018) with 1000 random bootstrap replications. Peanut stunt virus (PSV) (Accession number NC 002040.1) was used as an out-group.

Result

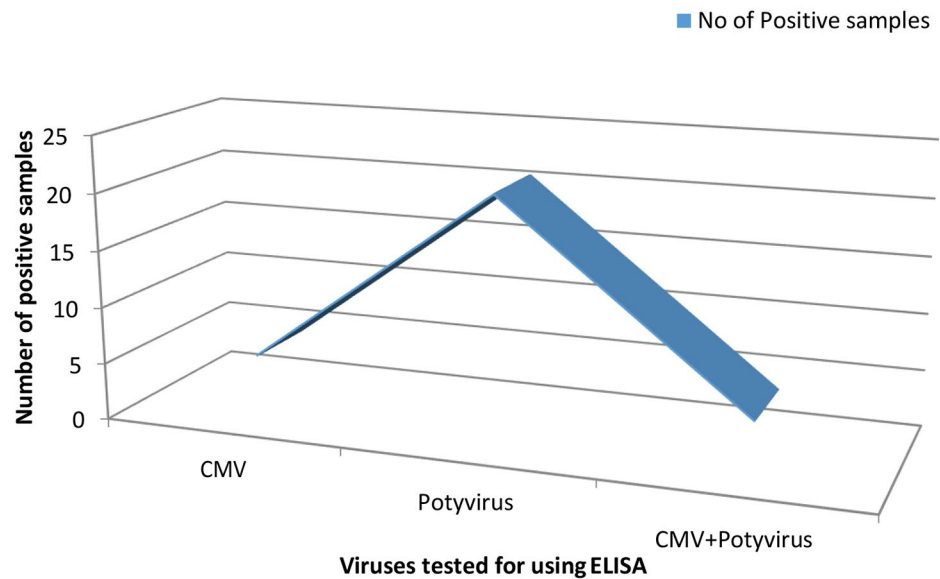
Symptom observation and CMV detection

Of the 30 samples tested by ELISA, only 5 (16.67%) were positive for CMV singly. The majority 21 (70%) of the positive samples were positive for potyvirus, while 4 (13.3%) had mixed infection of potyvirus and CMV (Fig. 2). The virus from CMV infected passion fruit was mechanically sap transmitted to healthy *N. benthamiana* used as diagnostic host for CMV. On *N. benthamiana*, a systemic mosaic was observed 7 days after inoculation. Fragment of approximately 500 bp was amplified with CMV coat protein gene primers from virus infected plants, but not from a healthy control when tested using RT-PCR (Data not shown).

Analysis of nucleotide sequence of CMV coat protein

To confirm the identity of the CMV detected in passion fruit, the partial nucleotide sequence of the CMV coat protein gene from this study (GenBank accession number MW684293) shared 99% similarity with CMV isolated from yam in the republic of Benin. The sequence (CMV P-NGR) encoding 120 amino acids were compared with 23 other CMV sequences from the GenBank (Table 1). Percentage similarities of these nucleotide and amino acid sequences are reported as a color matrix (Figs. 3 and 4). The sequence generated from this study has similarity values in the range of 98–99% with subgroup IA, 92–95% with subgroup IB and below 80% with subgroup II. Similarly, comparisons

Fig. 2 ELISA results of diseased samples obtained from the field showing differential reactions to antibodies used



of the deduced amino acid sequences showed 97.3 to 100% sequence identity with coat protein ORF gene of CMV subgroup I, while below 89% with subgroup II. The “CMV P-NGR”, coat protein ORF showed highest sequence identity at nucleotide level with the CMV Yam isolate from the Republic of Benin, at amino acid level it has the highest sequence identity with a turmeric isolate for Malaysia and a CMV subgroup 1 A isolate infecting *Yucca aloifolia* in Italy (100%).

Phylogenetic analysis of CMV coat protein showed that 24 CMV isolates were divided into three subgroups such as subgroup IA, IB and II. CMV isolate in this study belonged to subgroup IA and was closely related to the CMV-Yam and -Cameroon isolate in analysis with the RNA 3b sequence. Analysis of nucleotide sequences with RNA 3b showed that CMV P-NGR was most closely related to the members of subgroup IA (Fig. 4). Therefore, results of phylogenetic analyses of the RNA 3b segments suggested that CMV P-NGR belongs to subgroup IA of CMV.

Discussion

CMV has been identified as the virus with the widest host range and most likely to cause large economic losses in important food and ornamental crops (Karanfil 2021). In this study, the presence of *Cucumber mosaic virus* in Nigerian passion fruit was confirmed by a biological, ELISA assay using anti-CMV polyclonal antibodies and molecular phylogenetic analysis. The incidence of plant samples that were positive for a single CMV was low compared to other samples that were positive for potyvirus either singly or in mixed infection with CMV. Indeed, both wild and

cultivated plant species are susceptible to disease, and in the wild plants can be attacked by several pathogens simultaneously, the incidence and prevalence of the disease in plants can vary greatly. The incidence of diseases can also change seasonally due to many factors (Arogundade et al. 2015; Pelczar et al. 2021).

However, the detection of CMV in passion fruit in Nigeria makes it very important to know how the strain is related to other CMV strains reported from other parts of the world and to other strains of CMV reported from the same geographic area. The low CMV rate in this study may be due to the sample size. The low incidence of the disease can also be attributed to many factors, including the time of sampling, the availability of aphid vector and the hygiene measures applied (Karanfil 2021). Therefore, sampling across different seasons and location is recommended for further characterization studies on passion fruit in Nigeria. However, disease detection requires knowledge of the normal growth habits and normal variability of plants within a species. In a host range study using *N. benthamiana* as an indicator plant, the characteristic leaf mosaic and spotting symptoms occurred after mechanical inoculation with virus from infected passion fruit, consistent with the results of other studies (Kim et al. 2014; Mokbel et al. 2020).

The Phylogenetic trees constructed from the sequences of the CP gene revealed that our isolate (MW684293) is positioned in a subgroup IA and cluster with CMV from Yam and an isolate of CMV from Cameroon which forms a clade. This conclusion is supported by a review of other studies that reported that subgroup IA CMV isolates were the most frequently reported isolates in different hosts and locations (Çaglar 2006; Ergün et al. 2013; Ohshima et al. 2016). The deduced amino acid sequences for the CMV

Table 1 Nucleotide and amino acid sequences of CMV coat protein stored in the GenBank used for comparison in this study

Accession no	Iso- late name	Country of origin	Host	Sub- group	aa accession no
D10538.1	Fny	United State	<i>Vigna</i> spp.	IA	BAA01397.1
U22821.1	Ny	Australia	Unknown	IA	AAA87194.1
Y18137.1	I17F	FRANCE	Unknown	IA	CAA77065.1
D42079.1	C7-2	Japan	Unknown	IB	BAA07675.1
U20219.1	Ixora	United State	<i>Lycop- ersicon esculen- tum</i>	IB	AAC54619.1
D00463.1	WL	United State	<i>Lycop- ersicon esculen- tum</i>	II	BAA00358.1
AB006813.1	m2	Japan	Unknown	II	BAA22168.1
Z12818.1	Kin	United Kingdom	Unknown	II	CAA78279.1
KM091954.1	SWN- IW	Nigeria	<i>Solanum lycopersi- cum</i>	IB	AIY68673.1
EU428827.1	Cam- eroon	Cameroon	<i>Musa</i> spp.	IA	ACA00205.1
EU274471.1	Yam	Benin	<i>Dioscorea</i> spp.	IA	ABX80306.1
KU976471.1	PV- 0533	Nigeria	<i>Capsicum</i> sp.	IB	APB09218.1
KU976478.1	PV- 0434	Nigeria	<i>Musa</i> spp.	IB	APB09225.1
FJ896160.1	Nige- ria	Nigeria	<i>Vigna</i> spp.	IB	ADA63484.1
KM091958.1	SWN- ORI	Nigeria	<i>Solanum lycopersi- cum</i>	IA	AIY68677.1
KM091956.1	SWN- NH	Nigeria	<i>Solanum lycopersi- cum</i>	IB	AIY68675.1
KM091957.1	SWN- OLV	Nigeria	<i>Solanum lycopersi- cum</i>	IA	AIY68676.1
KM091955.1	SWN- IY	Nigeria	<i>Solanum lycopersi- cum</i>	IA	AIY68674.1
KM091952.1	SWN- ID	Nigeria	<i>Solanum lycopersi- cum</i>	IB	AIY68671.1
M21464.1	Q	Australia	Unknown	II	AAA46416.1
AF198103.1	LY	Australia	<i>Lupinus angustifo- lius</i>	II	AAM10540.1
KM091953.1	SWN- IF	Nigeria	<i>Solanum lycopersi- cum</i>	IA	AIY68672.1

isolate in this study showed 97.3 to 100% sequence identity with coat protein ORF gene of CMV subgroup I, while below 89% with subgroup II which is similar to the pattern of relationship observed in the nucleotide sequence comparison between the CMV isolates in this study and other CMV isolates in the different sub-groups. Previous studies have revealed a high degree of coat protein gene sequence homology between the same subset of CMV isolates and their global populations (Roossinck et al. 1999).

The results of this study show that CMV isolated from passion fruit in Nigeria is closely related to CMV isolated from yam and banana. This inference is similar to what was observed by Rivera-Toro et al. (2020) on chili peppers in Colombia, where they reported a close relationship between CMV isolated from pepper and CMV isolated from banana. In their study, they show that the scenario is likely due to the close proximity of fields where banana is currently or were previously grown, implying that the host is likely to switch from banana to pepper. A similar pattern was observed with the CMV strain from our study, as the passion fruit orchard, where the samples used for this study were collected was close to banana plantations. In conclusion, this study confirms the first CMV report on passion fruit in Nigeria and recommends that a broader nationwide sampling and characterization be performed to determine the diversity of strains since other CMV groups have been reported in crops in Nigeria. This will help develop appropriate management techniques to support crop production.

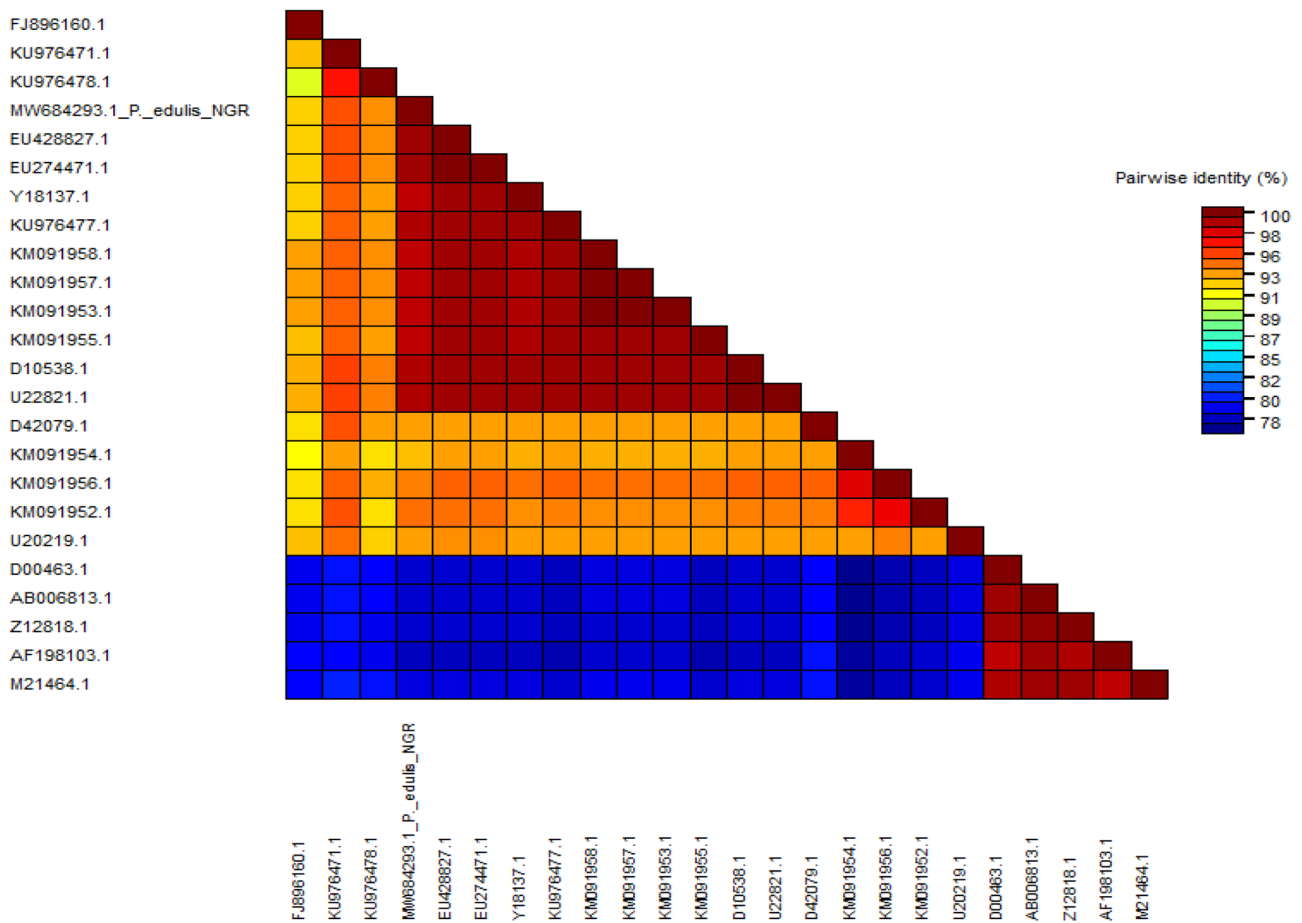


Fig. 3 Colour-coded Nucleotide pairwise identity matrix generated from CMV coat protein gene sequences. Each coloured cell represents a percentage identity score between two sequences (one between pair-

wise identities and the colours displayed in the matrix, indicated horizontally to the left and the other vertically at the bottom). A coloured key indicates the correspondence

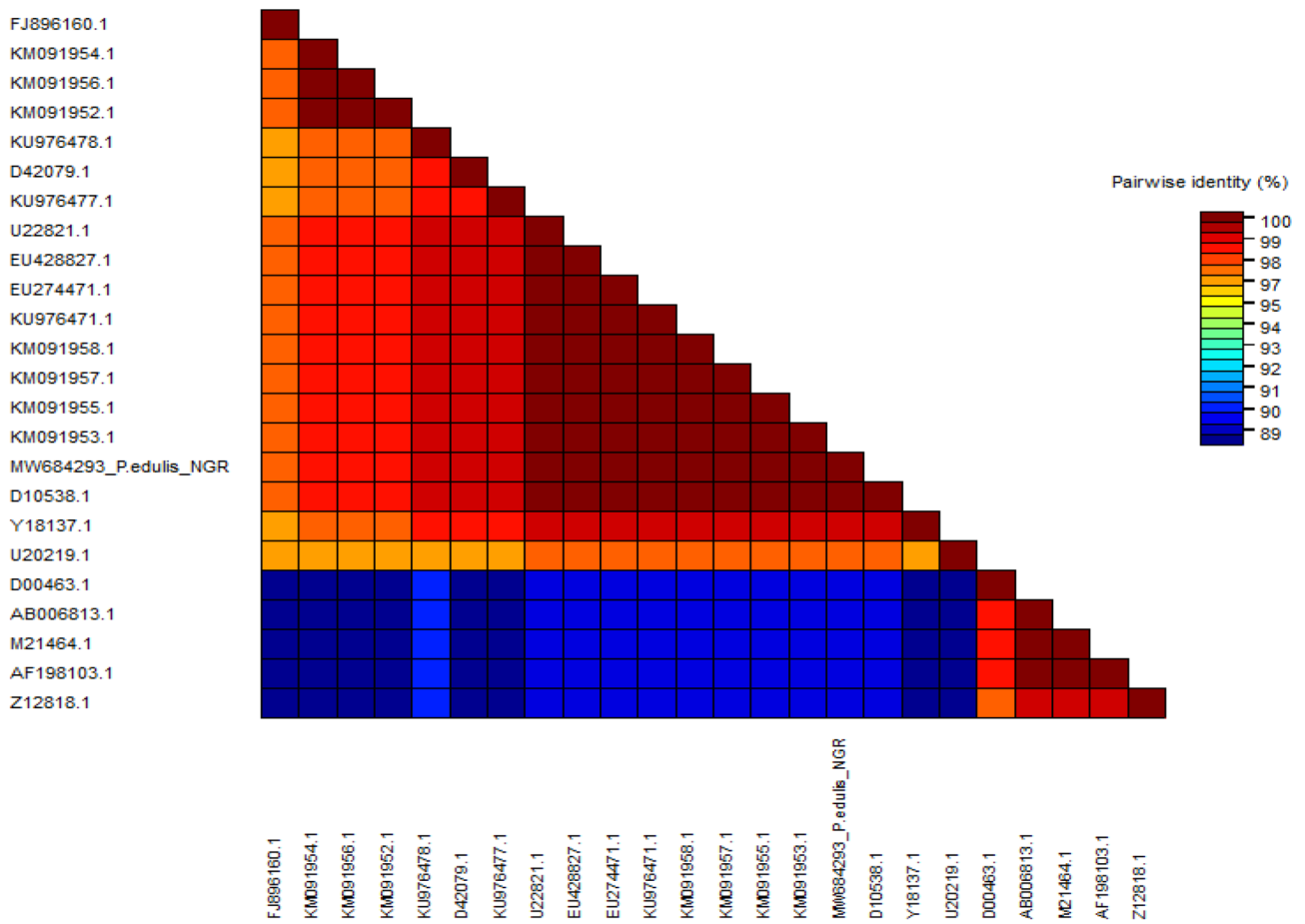
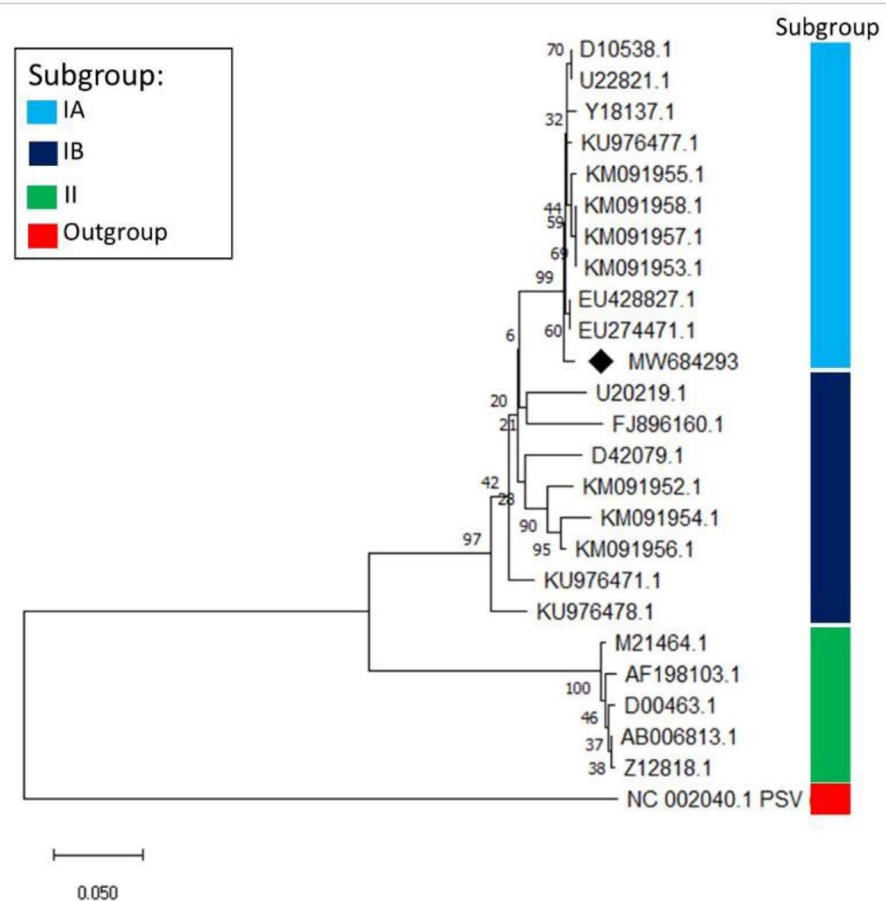


Fig. 4 Pairwise amino acid sequence identity of *P. edulis_NGR* isolate to other CMV. The colored cell represents the pairwise identity score for two sequences. The scale represents percentage amino acid identity obtained with SDTv1.2

Fig. 5 Maximum-likelihood phylogenetic trees obtained from alignment of nucleotide sequences of coat protein (CP) gene from cucumber mosaic virus (CMV). The comparison made was between 22 CP gene sequences obtained from GenBank with one new CP gene sequence obtained in this study. The tree was created in MEGA v10.1.8 using ClustalW with 1,000 replicates. Bootstrap values are percentages with values shown at the nodes. Peanut stunt virus (NC 002040.1) was used as an outgroup. Sequence label: from this study (Diamond sign (◆))



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Authors contribution [Olawale Arogundade] and [Taiye Hussein] contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Olawale Arogundade], [Olayinka Adegbite] and [Vincent Ebre]. The first draft of the manuscript was written by [Habeeb Atanda] and revised by [Abisola Banji]. All authors commented on previous versions, read and approved the final manuscript.s.

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Data availability There is no supplementary data.

Declarations

Conflict of interest All authors confirm for all authors that there is no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate All authors are given their consent for participation.

Consent for publication All authors are given their consent for publication.

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