The first report of bean common mosaic virus (BCMV) infection of African yam bean (Sphenostylis stenocarpa) in Nigeria

A. O. Dada1 · A. Oresanya2 · S. T. Akinyosoye3 · O. Arogundade3

Received: 5 May 2022 / Accepted: 17 August 2022 / Published online: 26 August 2022
© The Author(s), under exclusive licence to Springer Nature B.V. 2022

Abstract

Background African yam bean (Sphenostylis stenocarpa) is an underutilized crop that has the potential to contribute to sustainable food security. In October 2021, more than 90% African Yam Bean (AYB) plants showed typical virus symptoms of mosaic and necrosis in the grain legumes field of the Institute of Agricultural Research and Training (IAR&T), Nigeria. Methods and results Subsequently, leaf samples were collected and tested by ELISA and PCR to identify the virus species. Anti-BCMV and anti-potyvirus antibodies both gave positive results when symptomatic leaves were tested, and PCR using primers designed to the coat protein gene of BCMV amplified a band of the expected size (469 bp). The sequence of the PCR product was deposited in GenBank with the accession No. OL763314. The nucleotide sequence of the coat protein gene had 99% identity with BCMV isolate TN2 (KY044818). The identities of the nucleotide and amino acid sequence of the partial CP gene of the isolated virus relative to those of other potyviruses were 82.96–99.12% and 87.33–100%, respectively. Phylogenetic analyses of the partial CP-nucleotide sequences grouped the isolate from this study (BCMV-IART-AYB) and BCMV-TN2 in the same cluster with other BCMV strains of the peanut stripe (PSt) and the blackeye cowpea (BIC) strains. Conclusions In this study, we identified Bean common mosaic virus (BCMV) infecting AYB for the first time in Nigeria and show that it has high nucleotide and amino acid identity with an Isolate of cowpea-infecting BCMV in India and China respectively than isolate in Nigeria.

Keywords Antisera · Coat protein · Identities · Nucleotides · Phylogenetic analysis · Potyviruses

Introduction

African Yam Bean (Sphenostylis stenocarpa) is an underutilized crop that is gaining attention as a crop that could contribute to sustainable food security due to its benefits of producing both nutritious leguminous grains and tubers in single plants [1, 2]. African Yam Bean (AYB) has been reported to produce grains and tubers which have protein more than 2 times the amount in potato and higher than in yam and cassava and amino acid value higher than the value in pigeon pea, cowpea, and bambara groundnut [3].

African Yam Bean (AYB) is mostly grown in the Central and West African regions and most extensively in Nigeria [4]. Being a previously underutilized crop, research has focused on exploring the genetic enhancement in breeding programs focused mainly on yield and nutrient content. Another important consideration is the association with pests and disease that may play a significant role in reducing productivity. In Nigeria, several viral and fungal diseases associated with AYB have been documented [5–7]. Bean common mosaic virus in the genus Potyvirus is known to infect many leguminous species including cowpea and soybean [8] and transmission is predominantly through seeds. However, the aphid species Aphis fabae and Myzus persicae can also transmit the virus in a nonpersistent manner [9, 10]. Previously, Ogunsanya et al. [7] in their study to screen 20 accesions of AYB for resistance to viral diseases reported that AYB was susceptible to two viruses, namely, cowpea mild mottle Virus (CPMMV) and blackeye cowpea mosaic virus (BICMV) both of which have been detected using ELISA.
methods in Nigeria. However, there has not previously been reports of BCMV infecting yam bean in Nigeria.

**Materials and methods**

In October 2021, more than 90% African Yam Bean (AYB) plants showed typical virus symptoms of mosaic and necrosis in the grain legumes field of the Institute of Agricultural Research and Training (IAR&T), Nigeria (Fig. 1). Leaf samples were collected from symptomatic and asymptomatic plants (N = 25). The samples were tested using ELISA following the manufacturers protocols. The antisera used included Cowpea mild mottle virus (RT-0907; DSMZ, German Resource Center for Biological Material, Braunschweig, Germany), Bean common mosaic necrosis virus (RT-0239; DSMZ), Bean common mosaic virus (RT-0915-0228/1; DSMZ), Bean yellow mosaic virus (RT-0717; DSMZ), Cowpea aphid-borne mosaic virus (RT-0417; DSMZ), Cucumber mosaic virus (RT-0929; DSMZ), Soybean mosaic virus (RT-00543; DSMZ), Cowpea mottle virus (RT-0212; DSMZ) and anti-potyvirus antiserum (RT-0573/1 DSMZ).

To further confirm virus identity, selected samples from the plants that gave positive results following ELISA were subjected to PCR using primers designed to the coat protein as follows BCMV F1: 5′-ATG TGG TAC AAT GCT GTG AAG-3′/BCMV R1: 5′-TTT CAG TAT TCT CGC TGG T-3′. Total RNA was extracted from AYB leaf samples using modified CTAB method [11]. The extracted RNA was used as template with in reverse transcriptase-polymerase chain reaction (RT-PCR) to amplify 469 bp of the coat protein (CP) gene of BCMV. The RT-PCR consisted of a RT-Phase of 44 °C for 30 min then each cycle in PCR consisted of initial denaturation at 94 °C for 1 min, 35 cycles of denaturation at 94 °C for 60 s, annealing at 54 °C for 60 s, extension at 72 °C for 60 s, and a final extension at 72 °C for 10 min. Products were visualized on a 1.2% (w/v) tris–acetate agarose gel stained with ethidium bromide. Two independent successfully amplified RT-PCR products were sent for Sanger sequencing in both direction (Inqaba biotec, Pretoria, South Africa) using the amplification primers. The nucleotide sequence of one of the isolates was submitted to NCBI GenBank. A phylogenetic tree was constructed by first aligning sequences using Muscle (MEGA 7.0, [12]) with the neighbor-Joining method and bootstrap support was estimated by resampling the data 1,000 times.

**Results and discussion**

Based on the high disease incidence and the symptom severity (Fig. 1), it is probable that BCMV could be an important virus infecting AYB, resulting in significant yield reduction [3, 13]. BCMV could pose a significant threat to AYB cultivation as it could be introduced into crops in new areas by distribution of infected seed [14]. There have been reports of significant yield loss to other legumes especially cowpea [15]. The identity of the virus infecting AYB was initially resolved using ELISA and confirmed using PCR followed by sequencing. The symptomatic leaves were positive using the BCMV and anti-potyvirus antisera, while there were pockets of mixed infections with BCMV and CMV (3 plants) and were negative for the other antisera used (Data not shown). Five samples (Fig. 2) were positive out of seven tested, each giving the expected product size of 496 bp following PCR amplification.

The sequence from two isolates sequenced in this study were found to be identical and had the highest identities (99%) with those of BCMV isolate TN2 (KY044818) and TN-1 (KU761589) reported from infected cowpea (*Vigna unguiculata*) in India. Besides AYB isolates, the

---

**Fig. 1** Healthy (a) and naturally infected African Yam Bean plants showing mosaic and leaf defomation (b, c)
only other Nigerian BCMV isolate sequences that could be retrieved from sequence databases are isolates from Vigna unguiculata [16], which has a 96% nucleotide identity with AYB isolate from this study. The identities of the nucleotide and amino acid sequence of the CP gene of the virus isolated from this study relative to those of other BCMV were 82.96–99.1% and 87.33–100%, respectively (Table 1). Inoue-Nagata et al. [17] after examining several potyvirus sequences, suggested that the cutoff point for optimal CP nucleotide sequence recognition is <76% and CP amino acid sequence recognition is <82% in the same potyvirus species. In this study both the nucleotide and the amino acid sequences of the partial CP of the Nigeria AYB infecting virus vary in their identity by about 0.9–17 per cent with other BCMV isolates (Table 1). In phylogenetic analyses based on the CP-nucleotide sequences, isolates of BCMV were grouped into two major clusters (Fig. 3). Isolates BCMV-IART-AYB and BCMV-TN2 were in the same cluster with other BCMV strains of the peanut stripe (PSt) and the blackeye cowpea (Bic) strains. These results suggest that BCMV-IART-AYB is closely related to a BCMV strain isolated from India. Therefore, more research into determination of the complete sequences of the virus is required as well as its impact on yield and other nutritional factors.

Table 1 The characteristics of the virus isolate/strain used in this study and percentage sequence identity between the coat protein genes of the virus isolated from African yam bean and those of other isolates of BCMV

| Virus isolate/strain<sup>a</sup> | Host plant | Country | Coat protein<sup>b</sup> | GenBank accession |
|---------------------------------|------------|---------|--------------------------|------------------|
| BCMV-Ir-S31                     | Phaseolus vulgaris | Iran    | 93.36 Nt, 97.33 aa       | KU051674         |
| BCMV-L-2                        | Phaseolus vulgaris | India   | 94.14 Nt, 98 aa         | FJ387162         |
| BCMV                            | Macroptilium atropurpureum | Brazil | 92.92 Nt, 96 aa        | DQ897639         |
| BCMV-knxB-1                     | Vigna trilobata | Australia | 93.58 Nt, 94 aa      | JF427624         |
| BCMV-Tz:MVR15-16                | Phaseolus vulgaris | Tanzania | 93.14 Nt, 98 aa       | MF043410         |
| BCMV-p                          | Phaseolus lunatus | Peru    | 93.14 Nt, 96.67 aa     | AM258976         |
| BCMV-EP60                       | Phaseolus vulgaris | Zambia  | 82.96 Nt, 87.33 aa     | MN231644         |
| BCMV-MY15-014                   | Vigna angularis | Republic of Korea | 92.92 Nt, 98.67 aa | MW079241         |
| BCMV-Tohoku2-14                 | Glycine soja | Japan    | 93.58 Nt, 94.40 aa     | LC268996         |
| BCMV-(pst)-Arachi               | Arachi hypogaea | Greece  | 85.40 Nt, 86.33 aa     | LN799597         |
| BCMV-(pst)-Lx2                  | Arachi hypogaea | China    | 91.15 Nt, 98 aa        | HM776083         |
| BCMV-(Bic)-BF-142               | Vigna subterranea | Bukina Faso | 96.91 Nt, 97 aa | MF277042         |
| BCMV-(Bic)-NG-IART-1            | Vigna unguiculata | Nigeria | 96.46 Nt, 98.67 aa | MZ456243         |
| BCMV-(Bic)-R                    | Vigna unguiculata | China    | 96.90 Nt, 100 aa       | AJ312437         |
| BCMV-(Bic)-VN/YBI               | Vigna unguiculata | Vietnam | 96.90 Nt, 98.67 aa     | DQ925424         |
| BCMV-TN2                        | Vigna unguiculata | India    | 99.12 Nt, 99.33 aa     | KY047818         |
| WMV-Z17                         | Zucchini     | Iran     | 75.66 Nt, 82 aa        | KU352744         |

<sup>a</sup> BCMV Bean common mosaic virus, BCMV-(Bic) blackeye cowpea strain, BCMV-(Pst) peanut stripe strain, BCMV-Ir-S31 BCMV isolate Ir-S31, WMV Watermelon mosaic virus

<sup>b</sup> Nt nucleotide, aa amino acid
Fig. 3 Phylogenetic tree showing relationships of BCMV-IAR&T-AYB isolates of African Yam Bean with global BCMV isolates deposited in the GeneBank. The description of the virus isolates used for comparison is contained in Table 1. Cowpea aphid borne mosaic virus sequences was used as out group reference member. The tree (1000 bootstrap replications) was generated based on the MUSCLE alignment using MEGA7 software. BCMV Bean common mosaic virus, BIC blackeye cowpea strain, PST peanut stripe strain, BCMV-Ir-S31 BCMV isolate Ir-S31, CabMV Cowpea aphid-borne mosaic virus, WMV Watermelon mosaic virus

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [AD], [AO] and [OA]. The first draft of the manuscript was written by [AD] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding The authors have not disclosed any funding.

Declarations

Conflict of interest The corresponding author confirms for all authors that there is no conflict of interest.

References

1. Uguru MI, Madukaife SO (2001) Studies on the variability in agronomic and nutritive characteristics of African yam bean (Sphenostylis stenocarpa Hochst ex. A. Rich. Harms). Plt prod Res J 6:10–19
2. Amoatey HM, Klu GYP, Bansa D, Kumaga FK, Aboagye LM, Benett SO, Gamedoaogbao DK (2000) African yam bean (Sphenostylis stenocarpa) a neglected crop in Ghana. West Afr J Apl Eco 1:53–60
3. Afolabi CG, Ogunsanya OM, Lawal OI (2019) Evaluation of some African yam bean (Sphenostylis stenocarpa [Hochst. Ex A. Rich]) accessions for resistance to flower bud and pod rot diseases. Curr Plant Biol 20:1–5
4. Saka JO, Ajibade SR (2004) Survey of underutilized grain legume production systems in the Southwest agricultural zone of Nigeria. J Agric Food Info 6:93–108
5. Ameh GI, Okezie CEA (2005) Pests and diseases of African yam bean. Sphenostylis stenocarpa (Hochst. ex A. Rich) harms. Biol Res 3(1):14–20
6. Adewale BD, Dumet DJ, Vrob-Bi I, Kehinde OB, Ojo DK, Adeghite AE, Franco J (2012) Morphological diversity analysis of African yam bean and prospects for utilization in germplasm conservation and breeding. Genet Resour Crop Evol 59(5):927–936. https://doi.org/10.1007/s10722-011-9734-1
7. Ogunsanya OM, Afolabi CG, Otsusanyo MO, Adebisi MA (2020) Responses of African yam bean (Sphenostylis stenocarpa [Hochst. Ex A. Rich]) accessions to viral diseases and serological identification of the associated viruses. Nig J Biotech 37(1):85–93
8. Wu M, Wu WP, Liu CC, Liu YN, Wu XY, Ma FF, Zhu AQ, Yang YJ, Wang B, Chen QJ (2018) A bean common mosaic virus (BCMV)-resistance gene is fine-mapped to the same region as Rspl-h in the soybean cultivar Suweon 97. Theor Appl Genet 131(9):1851–1860
9. Morales FJ, Chastain RN (1987) Seed transmission characteristics of selected bean common mosaic virus strains in differential bean cultivars. Plant Dis 71:51–53
10. Jordan R, Hammond J (2008) Bean common mosaic virus and bean common mosaic necrosis virus. In: Mahy BWJ, Vanregenmortel MHV (eds) Encyclopedia of virology. USDAARS, Beltsville
11. Ruiz-García AB, Bester R, Olmos A, Maree HJ (2019) Bioinformatic tools and genome analysis of Citrus tristeza virus in Citrus Tristeza Virus. Humana, New York
12. Kumar S, Stecher G, Tamura K (2016) MEGAG: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874
13. Ogah EO (2013) Evaluating the effects of staking and planting dates on the yields of African Yam Bean, Sphenostylis stenocarpa in Nigeria. World J Agric Sci 9(2):196–200
14. Aishwarya P, Rangaswamy KT, Basavaraju S, Achari R, Govin K, Radhawasamy KP, Prameela HA (2020) Evaluation of the seedborne nature of Bean Common Mosaic Virus (BCMV) in cowpea. Int J Curr Microbiol App Sci 9(11):239–245
15. Manjunatha N, Sah RP, Deb D, Shivakumar MS, Archan S (2016) Effect of bean common mosaic virus infection on yield potential and nodulation of cowpea genotypes. Range Mgmt & Agroforestry 37(2):185–191
16. Kareem KT, Oduwoye OF, Adejiji AO, Akinyosoye ST, Adetumbi JA (2021) Isolates of Blackeye cowpea mosaic virus from progenies of Hebrown and IT-95K-193-12 cowpea varieties in Nigeria (Unpublished)
17. Inoue-Nagata AK, Jordan R, Kreuze J, Li F, López-Moya JJ, Mäkinen K, Ohshima K, Wylie SJ. Ictv Report Consortium (2022) ICTV Virus Taxonomy Profile: Potyviridae 2022. J Gen Virol 103(5):001738. https://doi.org/10.1099/jgv.0.001738

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.