DETECTION OF *CUCUMBER MOSAIC VIRUS* (CMV) FROM BAMBARA (*VIGNA SUBTERRANEAN* (L) VERDC.)

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ABSTRACT

Cucumber mosaic virus (CMV) has the widest host range of all known plant viruses. A viral and virus-like symptom of yellowing, leaf distortion, mottling, vein clearing and stunted growth were observed on Bambara groundnut undergoing a breeding trial at a research field of University Putra Malaysia (UPM). Ten infected plants were screened for CMV using reverse transcription-polymerase chain reaction (RT-PCR) with CMV coat protein (CP) gene-specific primers. The results showed that the causal organism was CMV from two samples tested. The Bambara seeds were collected and transported to Malaysia from Nigeria. Hence, there is the possibility of seed transmission or infection by other CMV-infected crops around the research field.

Keywords: Virus, infection, *Fabaceae, Cucumber mosaic virus,* Bambara groundnut

INTRODUCTION

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is an indigenous African crop of the *Fabaceae* family and the most important pulses grown in the continent. It is very easy to be cultivated but suffered negligence over the decades (Udeh *et al.*, 2020). It has two botanical varieties, cultivated and wild types, *Vigna subterranea* var. *subterranean* and *V. subterranean* var. *spontanea,* respectively (Aviara and Lawal, 2013; Dike, 1997; Thottappilly and Rossel, 1997). The plant has very high adaptability to harsh environmental conditions and it is grown mostly as a mixed crop predominantly with cowpea, maize, sorghum or groundnut (Thottappilly and Rossel, 1997) and usually cultivated as a subsistence crop by small-scale female farmers (Uddin *et al.*, 2017). Bambara groundnut serves as a good source of nutrients such as carbohydrates (65%), protein (25.2%), lipids (6%) when weighed in its dried form (Rowland, 1993; Amarteifio *et al.*, 1997). The crop has nutraceutical and antimicrobial potentials and serves as a source of livelihood to many rural farmers in Sub-Saharan African countries (Udeh *et al.*, 2020). Like other crops, Bambara groundnut is susceptible and is ravaged by pests and diseases, including Coleoptera, Diptera, and Hymenoptera, among other insect Orders (Dike, 1997; Uddin II *et al.*, 2017). Fungal diseases have been reported to infect the Bambara groundnut crop (Ouoba *et al.*, 2019), as well as viral diseases such as *Cowpea mild mottle virus* (CMMV), *Cowpea mosaic virus* and *Cucumber mosaic virus* (CMV).

CMV, or cucumber mosaic virus, has been na med one of the top ten most economically sign ificant plant viruses. This is due to its capacity to adapt to various environments and new hosts and its possession of the largest host range among all the known plant viruses (Scholthof *et al.*, 2011). The virus is the type species of *Cucumovirus* genus in the *Bromoviridae* family, with single-stranded RNA wrapped in a tripartite genome can cause yield loss of up to 30% (Zitter and Murphy, 2009). CMV infects a wide range of crop species, including monocots and dicots from over 100 plant families, and has a global distribution (Bald-Blume *et al.*, 2017; Roossinck, 2001; Zitter and Murphy, 2009). The most common techniques employed for the CMV identification are serological- and nucleic acid-based methods through ELISA and RT-PCR (Eni *et al.*, 2013). The virus is easily managed by employing sound cultural and agronomic techniques, such as the use of virus-free seeds, the exclusion of weed hosts and other reservoir hosts, vector elimination, rouging of affected plants, and quarantine procedures (Nono-Womdim, 2001). This study was conducted to identify virus(es) inciting virus and virus-like symptoms of yellowing and mottling observed on Bambara groundnut plants in a research field at the University Putra Malaysia (UPM) Serdang, Selangor, Malaysia.

Materials and methods

Ten symptomatic and asymptomatic leaf samples, five from each displaying virus and virus-like symptoms, were collected in a research field in the Faculty of Agriculture of the University Putra Malaysia in 2019. The samples were taken to the Virology lab in the Department of Plant Protection for viral disease diagnosis. The samples were surfaced-sterilized, using 1 % NaOCl and their nucleic acid, ribonucleic acid (RNA) was then isolated using TRIzol™ reagent (Thermo Fisher Scientific, Waltham, USA) according to the manufacturer's instruction. The RNA was converted to complementary deoxynucleic acid (cDNA) using SensiFAST™ cDNA synthesis kit (Bioline, UK) as follows in a total reaction volume of 10 µL, containing 1 µL total RNA, 4 µL 5×TransAmp buffer, 1 µL reverse transcriptase and 14 µL nuclease-free water. The mixture was prepared on ice and briefly centrifuged using a min centrifuge for 5 s. The reaction was performed by heating at 25°C for 10 min, 42°C for 15 min, 48°C for 15 min, 85°C for 5 min and held at 4°C in a thermal cycler, Master Cycler (Eppendorf, Germany). The cDNA was stored at -20°C until further use. Polymerase chain reaction (PCR) was carried out using the CMV-specific primers designed by Wylie *et al.* (1993) (Table 1) using of 2×AmpMaster™ Taq (Korea) PCR master mix. Each reaction tube contained 1 µL each of forward and reversed primers, 10 µL of 2×AmpMaster™ Taq, 3 µL cDNA template and nuclease-free water to a final volume of 20 µL. The reaction mixture was subjected to the following cycling regimes in the aforementioned thermal cycler: initial denaturation at 95°C for 2 min, then 35 cycles of denaturation at 95°C for 20 s, annealing at 60°C for 10 s, extension at 72°C for 30 s, and then a final extension of 72°C for 5 min.

Table 1: Primers designed by Wylie *et al.* (1993) for the detection of all CMV subgroups

Primer	Sequence	Expected amplicon size (bp)	Reference
CMV1F	5'-TATGATAAGAAGCTTGTTTCGCG-3'	482 – 488	Wylie *et al.* (1993)
CMV2R	5'-GCCGTAAGCTGGATGGACAA-3'		

Agarose (2 g) was mixed with 100 mL 1× TBE buffer (2% w/v). Five µL of PCR product (500 bp) was loaded onto each well with a 100 bp DNA ladder (Smobio, Taiwan) and run for 25 min at 90 V. The gel was stained in ethidium bromide solution (10 mg/mL) for 10 min and then de-stained with distilled water for 5 min, visualized using Gel documentation system (Gel Doc XR, BioRad, USA) and the image captured and recorded.

Results

Two of the samples (20 %) were found to be CMV-positive. The symptoms observed on the first one was mottling, stunted growth, vein clearing and chlorosis (Plate 1A). In comparison, the last one had mild mottles and chlorosis on many leaves of the plant (Plate 1B).

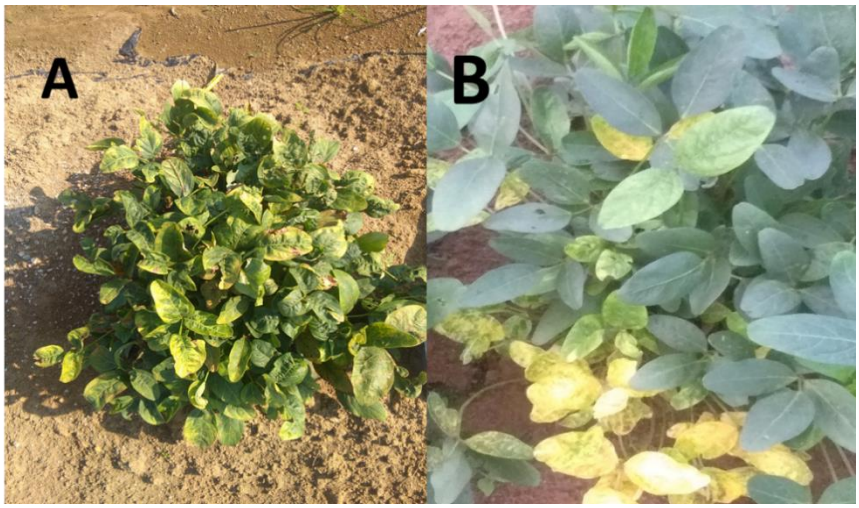

Plate 1: Symptoms of CMV infection in Bambara groundnut (A) Stunting, mottling, chlorosis and vein clearing (B) Mild mottling and chlorosis.

The PCR result showed the expected amplicon of approximately 500 bp when resolved on 2% Agarose gel, confirming the presence of CMV in the test sample as showed by Wylie *et al.* (1993) (Plate 2).

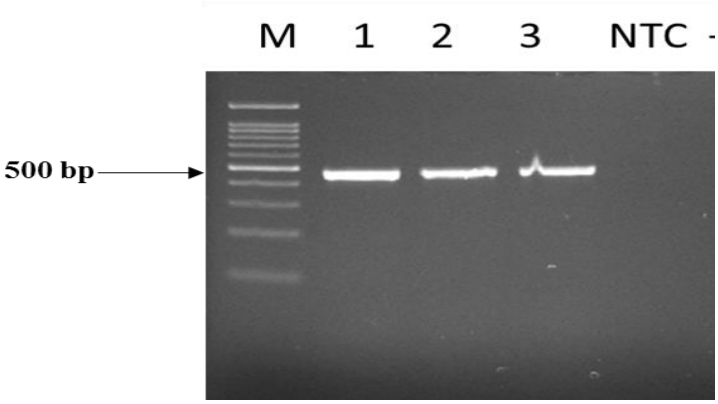

Plate 2: Agarose gel photo showing the two 500 pb of CP gene CMV from Bambara groundnuts M: 100 bp DNA ladder (Smobio, Taiwan) 1: CMV positive control 2: Bambara sample 1 and 3: Bambara sample 2 NTC: No-template control.

Discussion

Cucumber mosaic virus (CMV) was detected from the two samples observed to show virus and virus-like symptoms. Nucleic acid-based technique, PCR with specific primers which amplify 500 bp in the coat protein of the CMV, was used to determine the presence of the virus in the samples. Wylie *et al.* (1993) have used this pair of primers over the decades to detect all CMV subgroups. The symptom characteristics of mottling, stunting and yellowing observed were induced by CMV infection on Bambara groundnuts. The seeds used in the propagation of the Bambara groundnut in this study were imported from Nigeria, and it was found that eight viruses were found infecting the plant in Nigeria by Thottappilly and Rossel (1997) when screened the plant against some viruses thought to infect the plant, CMV was among the viruses identified during their study. If the virus is seed transmissible, it could then be the imported seeds that spread the virus, as the virus was reported to be seed-transmissible (Ali and Kobayashi, 2010). On the other hand, CMV has been very much present in Malaysian Agroecology over the years, ravaging all forms of crops, vegetables, spices, food and ornamental crops Muhammad *et al.*, 2021; (Mazidah *et al.*, 2012; Saad *et al.*, 2019; Saad, 2012) and the nature of the virus itself having the widest host range of all the known plant-infecting viruses to date (Bald-Blume *et al.*, 2017; Eni *et al.*, 2013; Scholthof *et al.*, 2011). More than 80 aphid species could transmit the virus in a non-persistent manner (Bald-Blume *et al.*, 2017); hence, the virus could have been transmitted to the Bambara groundnut by any three methods as mentioned above.

CONCLUSION

Cucumber mosaic virus (CMV) was detected from Bambara groundnut using the technique of RT-PCR from plants showing mottling, yellowing, stunting and chlorosis symptoms.

RECOMMENDATION

Based on the result obtained in this study, it will be very good to conduct a seed transmission experiment to ascertain the possibility of CMV spread in Bambara groundnut found to be CMV-positive in this study and that mixed infection scenario should be investigated in further studies.

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