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Occurrence of cassava mosaic *begomovirus*-associated satellites on cassava in Ghana

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Abstract: Cassava mosaic disease (CMD), arguably the most important viral disease of cassava, an important food security crop has in recent times, been reported to be associated with satellites in some parts of sub-Saharan Africa. We investigated the status of *Cassava mosaic begomovirus* and associated satellites in CMD-affected cassava plants in Ghana using polymerase chain reaction. In all, 110 CMD-affected cassava leaf samples collected from cassava fields in Southern Ghana. *Africa cassava mosaic virus* (ACMV) was detected in 107 (97%) cassava leaf samples, out of which 76 (69%) was in mixed infections with *East Africa cassava mosaic virus*-Cameroon (EACMV-CM). EACMV (CM) alone was detected in three (3%) cassava leaf samples. Out of the 110 cassava leaf samples affected by cassava mosaic begomoviruses, *sat*III and *sat*II were detected in 66 (60%) and 47 (43%), respectively. To the best of our knowledge, detection of CMB-associated satellites in Ghana is the first in the country and possibly in West Africa, and has serious epidemiological implications on the management of CMD, thereby requiring further studies and concerted efforts to safeguard cassava production.

Subjects: Agriculture; Crop Science; Pest Management

Keywords: cassava; cassava mosaic disease; begomovirus; satellites



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ABOUT THE AUTHORS

Stephen K. Torkpo holds a PhD (Crop Science) from the University of Ghana, Legon. He is a Research Fellow at the University of Ghana. His research focuses on diagnosis, epidemiology and management of viral and viral-like diseases of crops using conventional and molecular techniques. He was involved with the laboratory work and drafting of the manuscript.

Emmanuel Amponsah is currently a Masters student at the Department of Crop Science of the University of Ghana, Legon. He contributed to the laboratory work and drafting of the manuscript.

Celestina Danso Arhin holds a Bachelor of Science degree in Agriculture (Crop Science). She contributed to the laboratory work and drafting of the manuscript.

Samuel Kwame Offei is a Professor of Plant Virology and Molecular Biology at the University of Ghana. His research covers all aspect of Plant Virology and Molecular Biology. These include cassava mosaic begomoviruses and satellites..

PUBLIC INTEREST STATEMENTS

Cassava, known as "agbeli" in the Ewe dialect in West Africa, is a major a food security crop in Ghana and much of sub-Saharan Africa. Cassava is a good source of income and an important crop to the vulnerable and disadvantaged. Apart from its uses for food (many local diets), it has several industrial uses as ethanol, biofuel, cassava flour, cassava chips, starch, and wet cake. Despite the importance of cassava, its production is hindered by cassava mosaic disease (CMD), a disease caused by viruses. This disease reduces the yield of cassava significantly. We reported in Ghana, in addition to cassava mosaic begomoviruses, particles that are much smaller than viruses.These particles are known as satellites, and are reported to make cassava plants more susceptible to the disease. This current finding could influence policy leading to finding cassava planting materials that will produce optimum yield in the future.

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1. Introduction

Cassava (*Manihot esculenta* Crantz) is one of the important crops in sub-Saharan Africa (FAO, 2010) and has become a good income source to families, peasant and subsistence farmers all year round. It's a good food security crop in many African countries. Today, cassava has become one of the major staple crops cultivated across sub-Saharan Africa, producing 177 million metric tons (MMT) of the total world production of 284.9 MMT (FAOSTAT, 2017).

Ghana is the third highest producer of cassava in Africa, producing 18 MMT (FAOSTAT, 2017). Cassava is the most important staple in the country, with per capita daily intake of 642 calories, far exceeding maize and rice with 434 and 217 calories, respectively (FAO, 2006).

Despite its enormous importance, cassava cultivation is constrained by cassava mosaic disease (CMD). CMD is arguably described as the most important disease of the crop in Africa (Legg & Fauquet, 2004; Sseruwagi et al., 2004). CMD is caused by several species of single-stranded DNA viruses in Africa and Asia, usually referred to as Cassava mosaic begomoviruses (CMBs), family, *Geminiviridae* (Sseruwagi et al., 2004), and include *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV), *South African cassava mosaic virus* (SACMV) and *Indian cassava mosaic virus* (ICMV) (Brown et al., 2015). These viruses are transmitted by whiteflies [*Bemisia tabaci* (Gennadius)] and perpetuated through infected cuttings, which is the usual method of propagating the crop (Otim-Nape et al., 1994).

Begomoviruses are often associated with sub-viral agents referred to as alphasatellites and betasatellites. Until recently, most of the single-stranded DNA satellites were reportedly associated with monopartite begomoviruses (Patil & Fauquet, 2010), and are about 1.3 kb in size. Alphasatellites encode their own Rep and replicate autonomously (Ndunguru et al., 2016). Betasatellites, on the other hand, depend on begomoviruses for their replication, movement and encapsidation (Mubin et al., 2020). Betasatellites are diverse set of symptom-enhancing molecules that are associated with monopartite begomoviruses (Briddon et al., 2008; Gnanasekaran et al., 2019).

During the last two decades, unusual disease symptoms, such as filiform-shaped leaves, seen on CMB-infected cassava in East Africa, led to the discovery and description of satellites from cassava that are different from both alphasatellites and betasatellites (Ndunguru et al., 2016, 2008). Similar reported occurrence of satellites together with bipartite, cassava mosaic begomoviruses on CMD affected cassava plants in East and Central Africa had also been made (Mwatuni et al., 2015; Ndomba, 2012) with major implications for epidemiology of CMD. Earlier studies on CMD in Ghana has reported the occurrence of species of *Cassava mosaic begomovirus* but not satellites (Torkpo et al., 2017). The present study therefore seeks to investigate the status of satellites and Cassava mosaic begomoviruses that are associated with cassava in the Ghana, a major cassava producer in sub-Saharan Africa.

2. Materials and methods

2.1. Collection of CMD-affected cassava leaves

Young leaves from CMD-affected plants were collected in 2018/19 in parts of Southern Ghana, namely: Eastern, Central, Volta, and Greater Accra regions for the study (Figure 1). At each location, an observation was made of the presence or absence of CMD using modified "Z" fashion (i.e., from two sides of the field and along a diagonal across the field (Otim-Nape et al., 1998). Sample collection was not limited to only major cassava growing areas. Young moderately severe to very severe CMD affected leaves were collected into plastic bags, labelled and kept on ice and transported to the Biotechnology Centre of the School of Agriculture in the

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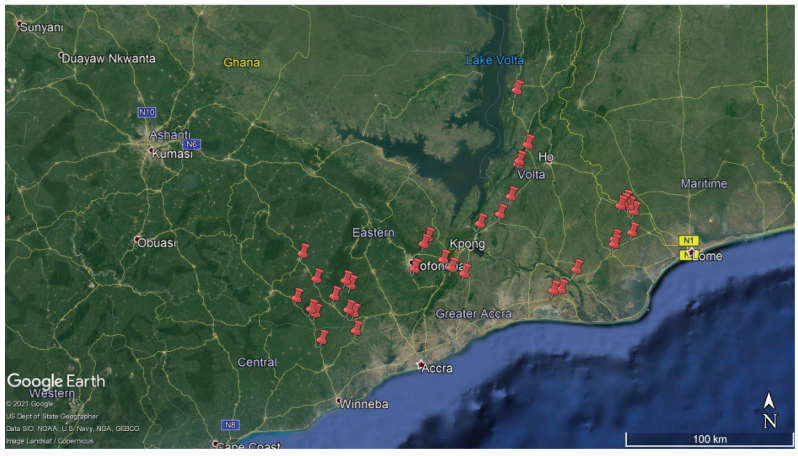


Figure 1. Areas surveyed for cassava mosaic begomoviruses and associated DNA satellites.

Table 1. Primers, primer sequences and their respective target viruses used for the *Cassava mosaic begomovirus* and associated satellites

Target virus, satellite	Primer	Primer sequence (5´- 3´) Reference
ACMV	JSP001	ATGTCGAAGCGACCAGGAGAT Fondong et al., 2000
	JSP002	TGTTTATTAATTGCCAATACT Fondong et al., 2000
EACMV-CM	JSP001	ATGTCGAAGCGACCAGGAGAT Fondong et al., 2000
	JSP003	CCTTTATTAATTTGTCACTGC Fondong et al., 2000
*sat*II	Sat IIF	GCCGCACCACTGGATCTC Ndomba, 2012
	Sat IIR	CAGCAGCCAGTCAGGAAGTT Ndomba, 2012
*sat*III	Sat IIIF	AGGCCTCGTTACTAAAAGTGC Ndomba, 2012
	Sat IIIR	ACCTGACGGCAGAAGGAAT Ndomba, 2012

Synthesized by Inqaba Biotech West Africa Ltd, Ibadan, Nigeria.

University of Ghana. DNA extraction and further laboratory analysis were performed on 110 samples collected.

2.2. DNA extraction and polymerase chain reaction (PCR)

DNA was extracted from the leaf samples using the CTAB method (Doyle & Doyle, 1987) and quality of isolated DNA assessed by electrophoresis prior to PCR (Saiki et al., 1988). PCR was performed using specific primer pairs (Table 1) designed to detect ACMV, EACMV (CM), *sat*II and *sat*III. The reaction mixture comprised of 12.5 µL Master Mix (New England Biolab, NEB), 1.0 µL each of forward and reverse primers (10 pM), 8.5 µL nuclease free PCR water (Inqaba Biotech West Africa Ltd) and 2.0 µL DNA to make up a final volume of 25.00 µL. The cycling regime for the amplification was as follows: initial denaturation at 94°C for 3 min, followed by 30 cycles of 94°C for 1 min, 56°C for 1 min 30 sec and 72°C for 1 min, with a final extension of 72°C for 4 min

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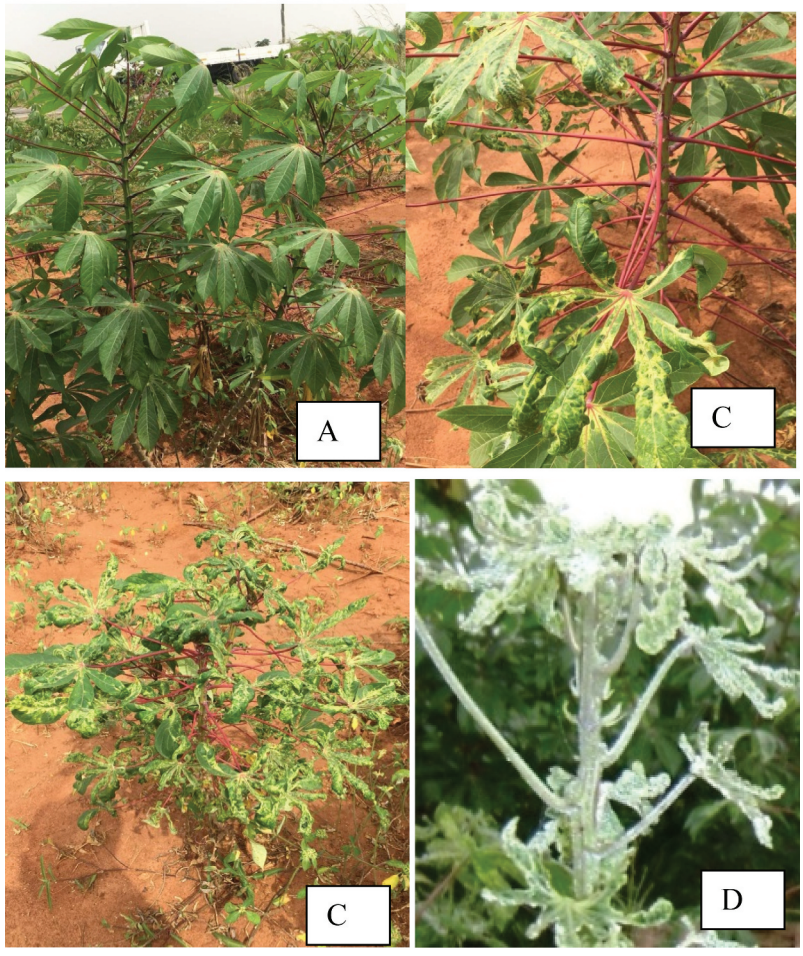


Figure 2. Cassava leaves. (a), Asymptomatic (b), Moderately severe mosaic symptom (c), Severe mosaic symptom (d). Very severe mosaic symptom.

(Mwatuni et al., 2015) in the Applied Bio systems Thermal Cycler. Amplicons were separated by electrophoresis on 1.5% agarose gels (which was stained with ethidium bromide) for 100 V for 1 h. Bands were viewed and the images saved using Syngene Gel documentation system.

3. Results and discussion

Even though asymptomatic cassava plants were recorded on some of the locations, leaves of cassava plants showing moderately severe to very severe cassava mosaic symptoms (Figure 2) were the samples collected and analyzed. DNA was successfully obtained from all the samples collected. ACMV, EACMV-CM, satellite DNA II (*sat*II) and satellite DNA III (*sat*III) were detected from the DNA extracted from the leaf samples in PCR using specific primers

Cassava mosaic begomoviruses were detected in all the cassava leaf samples. The presence or absence of *Cassava mosaic begomovirus* or DNA satellites in a sample was not dependent on the type of symptom. Out of 110 CMD-affected samples tested, 107 tested positive for ACMV (Figure 3), and 76 tested positive for EACMV-CM. This represented 97.0% and 69.0% of samples that tested positive for ACMV and EACMV-CM, respectively. Three (3) of the CMD-affected samples were singly infected by only EACMV. This was equivalent to 2.7% of the samples. Thirty-one (31) of the samples were positive for only ACMV.

Overall, *sat*II and *sat*III were detected in 47 and 66 of the CMD-affected cassava leaves, representing 43% and 60% respectively. The expected PCR products of ~895 bp and ~306 bp were respectively

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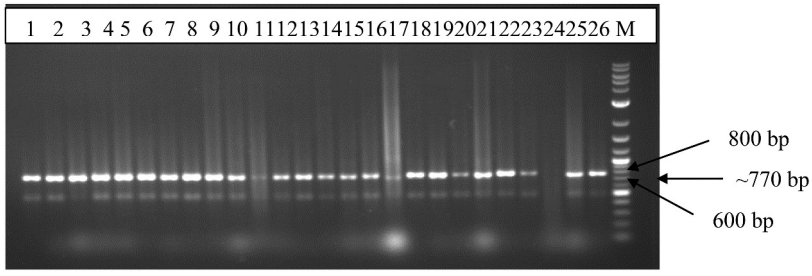


Figure 3. Image of electrophoresis of total PCR product on 1.5% agarose gel generated using primer pair JSP001/002 that detect ACMV. M-1.0 kb molecular marker; Lanes represent CMD-affected leaf samples collected from the various locations in southern Ghana.

obtained with the *sat*II F/R and *sat*III F/R primers. The 1.0 kb molecular marker (GIBCO Life Technologies), which was correctly used, gave the expected PCR product (Figures 4 and 5). Both satellites were widely distributed in southern Ghana. A gel image of severely CMD-affected cassava leaf samples arranged in the same order is presented for *sat*III (Figure 4) and *sat*II (Figure 5).

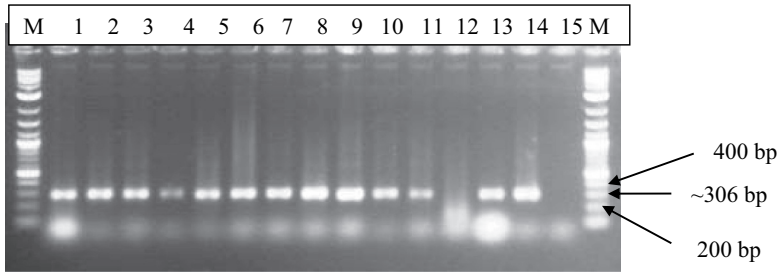


Figure 4. Agarose gel electrophoresis of DNA *sat*III specific PCR products of 306 bp from CMD-affected cassava leaf samples total nucleic acid. Lanes 1 to 14 contained separate cassava samples. M-1.0 kb molecular marker; Lane 15- Negative Control.

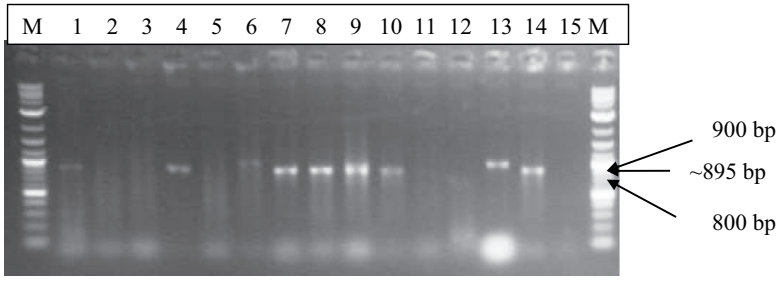


Figure 5. Agarose gel electrophoresis of DNA *sat*II specific PCR products of 895 bp from CMD-affected cassava leaf samples total nucleic acid. Lanes 1 to 14 . Lanes 1 to 14 contained separate cassava samples. M-1.0 kb molecular marker; Lane 15- Negative Control.

ACMV was detected in the majority of CMD-affected cassava leaves, and remain the predominant *Cassava mosaic begomovirus* species in the country, which is consistent with earlier findings in the country (Offei et al., 1999; Oteng-Frimpong et al., 2012; Torkpo, 2009; Torkpo & Offei, 2007) in which they reported ACMV as the frequently detected CMB in the country. ACMV was also the predominant CMB species in Nigeria (Alabi et al., 2007; Ogbe et al., 2006) and Togo (Adjata et al., 2009). The Mixed infection of ACMV and EACMV (CM) as revealed in the present study concurs with that of other parts of Africa in which more the one CMB species were reported from CMD-affected cassava plants. (Adjata et al., 2009; Alabi et al., 2007; Busogoro et al., 2008; Ogbe et al., 2006; Were et al., 2004).

Detection of single infection of cassava by EACMV in the current study agrees with Offei et al. (1999) but not other findings in the country (Torkpo & Offei, 2007; Torkpo et al., 2017), in which no single EACMV infection was detected in the country.

We report, for the first time, the occurrence of DNA *sat*II and DNA *sat*III in Ghana, and in West Africa. More samples tested positive for DNA *sat*III compared to DNA *sat*II, and concured with what occurred in Tanzania (Ndomba, 2012). Mwatuni et al. (2015) reported high occurrence of DNA *sat*III in Kenya though it is less in comparison to what was reported in this present study. Additionally, higher levels of DNA *sat*II reported in the present study contradicts the very low numbers reported by Mwatuni et al. (2015) but not Ndomba 2012. The occurrence of high levels of DNA *sat*II and DNA *sat*III in Ghana presents major challenges to the already serious CMD situation. Sub-viral agents have been reported from CMD-affected plants in Tanzania (Ndunguru et al., 2016, 2008), and the authors stressed that the sub-viral agents have the ability to overcome CMD2 resistance, and their whitefly transmissibility has major implications for the long-term durability of CMD2 resistance. The epidemiological implications of these sub-viral agents reported in Ghana on the country's production of cassava cannot, therefore, be over emphasized.

4. Conclusion

ACMV remains the predominant *Cassava mosaic begomovirus* species and was detected in all the 46 locations in the south of the country. EACMV-CM was detected in 35 locations. Mixed ACMV and EACMV-CM was detected. Three cassava leaf samples were positive for EACMV-CM alone. DNA Satellites, *sat*III and *sat*II were detected in 25 and 17 locations, respectively. Both *sat*III and *sat*II were detected in mixed infections with CMBs. No DNA Satellite was detected alone from any cassava leaf sample. Detection of CMBs, satIII and satII was across sample type. This was the first detection of DNA Satellites from CMD-affected cassava leaves in the country. This undoubtedly would have epidemiological implications that could pose challenges to CMD management and have negative effect on cassava yield and food security.

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