

Full Length Research Paper

Distribution of cassava mosaic begomoviruses in the North-Western provinces of Democratic Republic of Congo

Olivier Likiti Kola^{1*}, Rosine Kisuka Muvirirwa¹, Guy-Roger Diko Eboma¹, Jean-Claude Bulonza Mulimbanya¹, Tony Bakelena Zeyimo³, Onésime Mubenga Kankonda² and Godefroid Monde te-Kazangba¹

¹Laboratoire de Phytopathologie et Biotechnologies Végétales and Central and West African Virus Epidemiology for Food Security (WAVE), Institut Facultaire des Sciences Agronomiques de Yangambi, Democratic Republic of Congo.
²Faculté de Gestion des Ressources Naturelles and Centre de Surveillance de la Biodiversité, Université de Kisangani, Kisangani, Democratic Republic of Congo.
³Central and West African Virus Epidemiology for Food Security (WAVE) and Institut National pour l'Etude et la Recherche Agronomique, Centre de Recherche de Mvuazi, Democratic Republic of Congo.

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This study was carried out for the first time in the North-Western Democratic Republic of Congo (DR Congo) as part of the Viral Epidemiology in West and Central Africa (WAVE) program. Its dual aim was to identify and map the viruses responsible for African cassava mosaic in this part of the country. Cassava leaf samples were collected during a geo-referenced survey conducted from 1st February to 31st March, 2022 in three provinces: Mongala, Nord Ubangi, and Sud Ubangi. Molecular diagnostics were carried out to identify the viral strains associated with Cassava Mosaic Disease (CMD). The results showed the presence of African Cassava Mosaic Virus (ACMV) and East African Cassava Mosaic Cameroon Virus (EACMCV) in the study area. EACMCV was present in all provinces, while ACMV was only reported in the province of Nord-Ubangi. This study recommends good agricultural practices and participatory surveillance as a strategy for managing CMD in the Democratic Republic of Congo.

Key words: Epidemiology, cassava, viruses, East African Cassava Mosaic Cameroon (EACMCV), African Cassava Mosaic Virus (ACMV).

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the most important food crops in sub-Saharan Africa. It is a source of income and a key element of food security for poor

farmers in the eastern and central Great Lakes region (Maruthi et al., 2004; Legg et al., 2017). In the Democratic Republic of Congo (DR Congo), cassava covers the daily

*Corresponding author. E-mail: likitiolivier@gmail.com.



Figure 1. Cassava leaf showing symptoms of CMD.

calorific needs of over 70% of the population (Monde et al., 2013; Akinpelu et al., 2012). Its consumption amounts to 250 kg/person/year, making the country one of the world's leading cassava consumers (FAO, 2018). DR Congo is ranked the second producer of cassava in Africa after Nigeria and fifth in the world, with production estimated at over 45 million tonnes (FAO, 2022).

Despite its ability to withstand extreme growing conditions: low mineral supply (Hillocks et al., 2002), drought, and climatic disturbances (Rey and Vanderschuren, 2017; Jarvis et al., 2012; El-Sharkawy, 2006; De Tafur et al., 1997; Cock et al., 1985), cassava is subject to strong biotic pressure (Legg et al., 2011). Diseases and pests affect its growth and development and consequently reduce its yield in several production zones in Africa (Bakelana et al., 2019; Legg et al., 2011). Cassava mosaic disease (CMD) is one of the major constraints of cassava production in the 21st century (Winter et al., 2010; Alicai et al., 2007). The disease is spread by infected cuttings and by whitefly vectors (*Bemisia tabaci*) (Chi et al., 2020; Njoroge et al., 2017; Maruthi et al., 2005). CMD is widespread in several countries in East, Central, and West Africa and towards Southern Africa (Chikoti et al., 2015; Tresh and Cooter, 2005; Sseruwagi et al., 2004).

CMD is caused by a Cassava Mosaic Begomoviruses (CMBs) complex composed of eleven species of bipartite Begomoviruses, nine of them have been reported in Africa (Crespo-Bellido et al., 2021; Maruthi et al., 2004). The distribution of these begomoviruses associated with CMD symptoms often varies from one country to another and/or from one region to another within the same country (Monde et al., 2010; Patil and Fauquet, 2009; Ndunguru et al., 2005; Ariyo et al., 2005; Were et al., 2004).

Several epidemiological studies carried out in the Democratic Republic of Congo (DR Congo) reported the

presence of CMD in the majority of cassava-growing areas. These studies have focused on incidence and severity, identification and/or characterization of the viruses responsible, as well as assessment of the impact of the disease on certain cassava varieties/accessions, and finally, screening for resistance in different agroecosystems in the country (Biola et al., 2022; Bisimwa et al., 2019, 2015, 2012; Monde et al., 2012, 2010; Tata-Hangy et al., 2007). Despite these various studies carried out in DR Congo, the distribution of African cassava mosaic viruses remains less documented in several provinces. The present study aims to identify and map the distribution of CMD-associated viruses in North-Western DR Congo.

MATERIALS AND METHODS

Cassava leaves sampling

A total of two hundred and seventy-six CMD-symptomatic cassava leaves were collected from cassava fields aged between 3 and 9 months (Figure 1). Fields were prospected at intervals of 5 to 10 km in the three north-western provinces of the Democratic Republic of Congo where any CMD-associated virus survey had been conducted (Figure 3). The leaves were air-dried in herbarium beds and then taken to the WAVE Molecular Diagnostic Laboratory at INERA M'vuazi for analysis. The coordinates of each sample were recorded using GPS Garmin 62s.

DNA extraction

Total DNA extraction was performed using the modified CTAB method (Lodhi et al., 1994). 0.5 g of each sample was ground in 100 ml extraction buffer containing 2 g Cethyl Triethyl Ammonium Bromide (2%), 4.09 g NaCl (1.4 M), 200 µl B-mercaptol-Ethanol (0.2 M), 0.37 mg EDTA 2H₂O (20 mM), 0.6 g Tris-HCl (100 mM), and 100 ml water. The DNA obtained was kept cold (-20°C) before being used for biomolecular analysis.

Table 1. Primers used to identify viruses associated with CMD.

No.	Primers used	Primer sequences	Target region	Size (bp)	References
1	JSP 001/JSP002	5' ATGTCGAAGCGACCAGGAGAT 3' 5' TGTTTATTAATTGCCAATACT 3'	ACMV DNA-A (CP)	783	Pita et al. (2001)
2	ACMV B F/R	5' TCGGGAGTGATACATGCCAAGC 3' 5' GGCTACACCAGCTACCTGAAGCT 3'	ACMV DNA-B (BV1/BC1)	628	Matic et al. (2012)
3	JSP001/JSP003	5' ATGTCGAAGCGACCAGGAGAT 3' 5' CCTTTATTAATTGTCACTGC 3'	EACMV DNA-A (CP)	780	Pita et al. (2001)
4	CMBRepR F/EACMVRe R	5' CRTCAATGACGTTGTACCA 3' 5' GGTTTGCAGAGAACTACATC 3'	EACMV DNA-A (AC1)	650	Alabi et al. (2008)
5	VNF031 F/R	5' GGATACAGGATAGGGTCCAC 3' 5' GACGAGGACAAGAATCCAAT 3'	EACMV-CM DNA-A (AC2/AC3)	560	Fondong et al. (2000)
6	ACMV21 F/R	5' CRTCAATGACGTTGTACCA 3' 5' GGTTTGCAGAGAACTACATC 3'	DNA-A (AC3-AC2-AC1) of ACMV, EACMV, EACMCV, EACMKV, EACMMV, EACMZV, and SACMV	552	Matic et al. (2012)

Polymerase chain reaction (PCR) amplification

PCR amplification was carried out according to the harmonized protocol of the Central and West African Virus Epidemiology Program. The PCR Mix was prepared to a final volume of 25 µl containing 5 µl of extracted DNA, previously diluted 1:50, 11.375 µl of distilled water, 0.5 µl of dNTP, 1 µl of MgCl₂, and 5 µl of colourless green Buffer and 1 µl of each of the specific primer pairs listed (Table 1). The PCR program was performed at 95°C for 4 min, followed by 35 cycles of denaturation at 95°C for 1 min, hybridization at 54°C for 1 min, elongation at 72°C for 1 min, and final elongation at 72°C for 10 min.

Analysis of PCR products by agarose gel electrophoresis

Ten microliters of amplicons (PCR products) from each sample were migrated in electrophoresis under 90 V at 58

mA for 60 min in 1.2% agarose gel: 2.4 g agarose in 200 ml TAE (1x). Bands were visualized on the UV transilluminator at 254 nm. A 1 Kb DNA molecular weight marker was used to determine amplicon size (Figure 2).

RESULTS

Two viral strains are associated with CMD in North-Western DR Congo. PCR detected 2 viral strains of CMD in the cassava leaf samples diagnosed (Figure 2).

CMD is widespread in north-western DR Congo. Two viral strains were identified in the three provinces as mentioned in Table 2. These viral strains are ACMV and EACMCV (Figure 2). The EACMCV strain was identified in all three provinces surveyed, while ACMV was only found

in the province of Nord-Ubangi. The proximity of the province of Nord-Ubangi to the Central African Republic could justify the presence of the ACMV strain in this part of the national territory. Indeed, in his study on the epidemiology of African CMD in the Central African Republic in 2022, Zinga et al. (2012) identified two viral strains. The distribution of EACMCV in the three provinces surveyed is explained by the evolution of CMD from east to west DR Congo. This strain is different from what was characterized in the majority of the various agroecological zones of the DR Congo (Yangambi and South Kivu). The low rate of virus identification is due to the primers used in this study and suggests that they are not specific to the CMD virus in DR Congo that is widely infected by the Uganda variant (Monde et

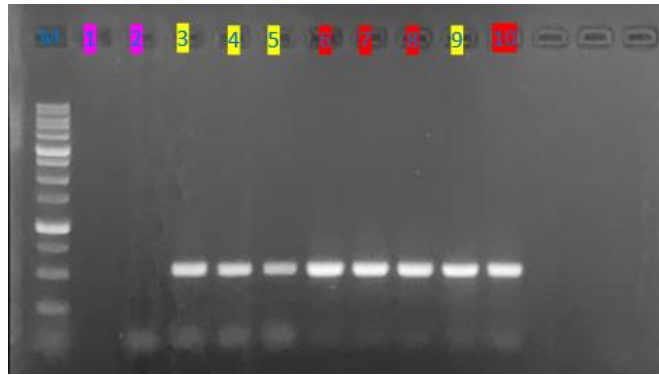


Figure 2. Gel electrophoresis of ACMV (628 pb: 3, 4, 5 & 9); EACMCV (650 pb: 6, 7, 8 & 10), and Negative control (1 & 2).

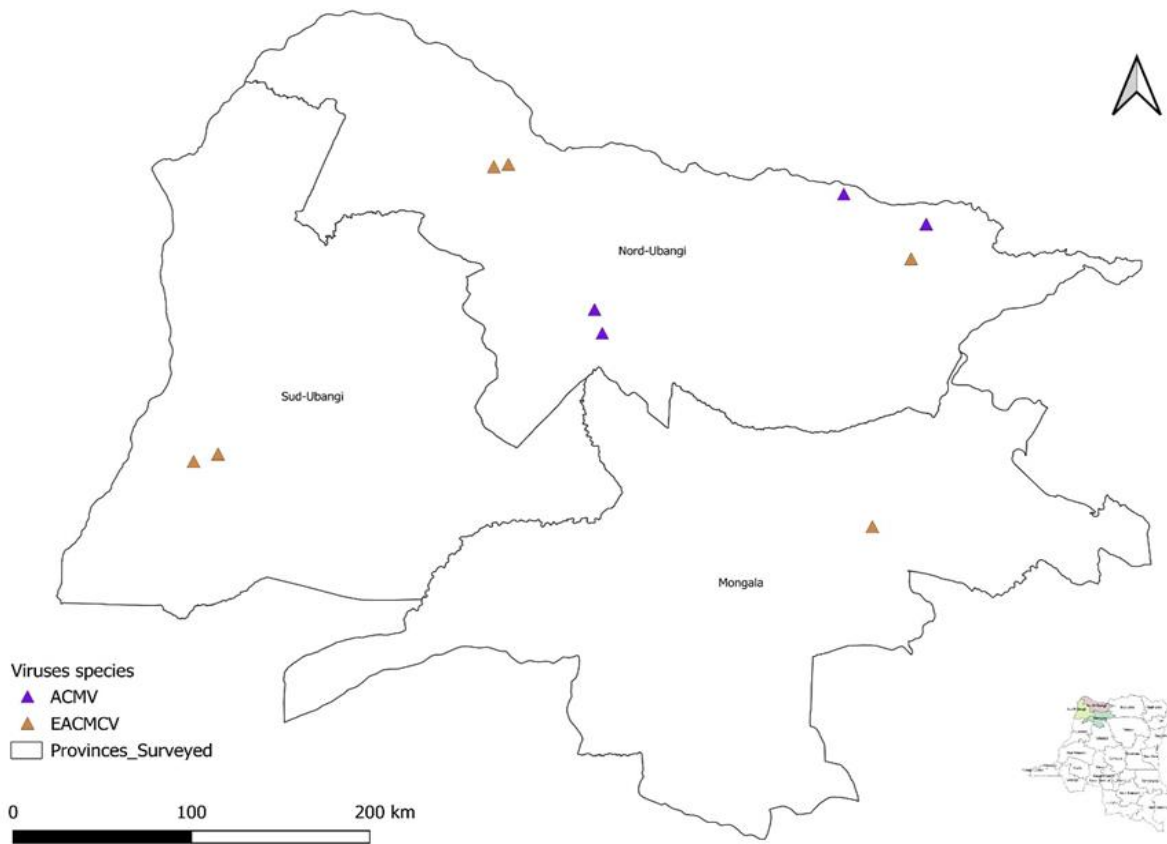


Figure 3. Distribution of cassava mosaic begomoviruses isolates in three surveyed provinces.

al., 2010; Bisimwa et al., 2012).

Distribution of CMD-associated viruses in the Mongala, Nord-Ubangi, and Sud-Ubangi provinces

The CMD-associated virus distribution identified in the

three north-western provinces of DR Congo was mapped using geospatial coordinates (latitude, longitude, and altitude) recorded for collected samples using QGIS software version 3.14.15 (Figure 3). It showed the two strains were differently distributed in the three provinces. ACMV has been identified in the province of Nord-Ubangi, while EACMCV has been identified in all three

Table 2. Two viral strains associated with CMD in North-Western DRC.

Province	Total samples	Positive samples (%)	Negative samples (%)	Virus identified
Mongala	38	1 (2.63)	37 (97.37)	EACMCV
Nord-Ubangi	122	7 (5.74)	115 (94.26)	EACMCV (3), ACMV (4)
Sud-Ubangi	105	2 (1.91)	103 (98.09)	EACMCV

provinces.

DISCUSSION

Two viral strains are responsible for CMD in north-western DR Congo

CMD is caused by eleven cassava mosaic geminiviruses (CMGs), nine of which are present in Africa, singly or in combination (Legg and Fauquet, 2004; Ndunguru et al., 2005; Bull et al., 2006; Patil and Fauquet, 2009; Harimalala et al., 2012; Tiendrébéogo et al., 2012; Zinga et al., 2016). The results of molecular analyses revealed the presence of two viral strains in the three north-western provinces of DR Congo: African Cassava Mosaic Virus (ACMV) and East African Cassava Mosaic Cameroon Virus (EACMCV). These two viral strains are the most widespread in most African countries (Legg et al., 2011). EACMCV is present in all provinces, while ACMV has only been identified in the province of Nord-Ubangi. This province shares borders with the Central African Republic and has been hosting Central African Republic (CAR) refugees for several years. This situation suggests the possibility of the introduction of propagation materials from the CAR, where ACMV has been identified and characterized as a single infection or mixed infection on cassava (Zinga et al., 2012). In contrast, ACMV is thought to come from other eastern provinces of DR Congo neighbouring Uganda and move westwards before reaching the CAR (Tocko-Marabena et al., 2017). Viral diseases of cassava, including CMD transmission, spread from one country to another or from one region to another (De Brun et al., 2012). CMD has evolved from East Africa through Central Africa to West Africa (Legg et al., 2011) and is mostly spread through the infected cuttings that farmers take from their own or neighbouring fields (Armél et al., 2023). In DR Congo, CMD is present in all cassava-growing regions across the country. The EACMV-Ug and ACMV strains prevalent in north-western DR Congo are thought to have originated in areas previously infected by this strain, namely the agroecosystems of mountainous South Kivu (Bisimwa et al., 2012) and the Yangambi region (Monde et al., 2010), where this viral strain was identified and characterized on cassava and other host plants (*Pueraria javanica* and *Centrosoma pubescent*). EACMCV founded in DRC

suggests the expansion of the Cameroon strain in Central and West African countries from Cameroon where it has been characterised (Alabi et al., 2008b). EACMCV were also found in Nigeria on Cassava and alternate host (Alabi et al., 2008a), Burkina Faso (Soro et al., 2021) and in Côte d'Ivoire (Amoakon et al., 2023).

Conclusion

This study aimed to identify and map the CMD viruses in the provinces of Mongala, Nord-Ubangi and Sud-Ubangi in North-Western DR Congo. To achieve this, cassava leaves in fields on plants showing characteristic symptoms of CMD were sampled in three North-Western provinces of DR Congo. These cassava samples were sent to WAVE INERA-M'vuazi for molecular diagnosis. The laboratory results revealed the presence of two strains that are associated with CMD: ACMV and EACMCV. These two strains are distributed across the three provinces in different pathways. The results of the present study suggest the use of various primers to identify cassava viruses that cause the CMD in DR Congo and recommend participatory surveillance as a control strategy against African CMD in DR Congo in general and in the northwest of the country in particular.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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