Vol. 15(1), pp. 13-20, January-June 2023
DOI: 10.5897/IJGMB2022.0215
Article Number:30AE0C770617
ISSN 2006-9863

http://www.academicjournals.org/IJGMB

International Journal of Genetics and Molecular Biology

Full Length Research Paper

In silico analysis of mutations associated with genetic variability of the strain African cassava mosaic virus (ACMV) in three departments of Côte d'Ivoire

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Received 13 January, 2022; Accepted 4 May, 2022

Cassava (*Manihot esculenta* Crantz) is an important crop that constitutes staple food and income for 800 million people worldwide. Cassava yield in Côte d'Ivoire is reduced due to a variety of factors, including cassava mosaic disease. Despite the impact of the pathogen Cassava Mosaic Virus (CMV) on production, genetic diversity of this virus is rarely studied in Côte d'Ivoire. This study aims to assess the molecular variability of CMV occurring in three of large cassava production area of Côte d'Ivoire. Symptomatic and asymptomatic cassava leaves were collected for genomic DNA extraction and molecular identification was performed by polymerase chain reaction (PCR). Amplified DNA was sequenced and analyzed *in silico*. 68% of infections were identified as African Cassava Mosaic Virus strains. Sequences alignment to Genbank sequences showed high similarity with sequences of virus from Côte d'Ivoire, Ghana, Kenya, Cameroun, Madagascar, and Nigeria. The virus's rapid evolution was demonstrated by a high mutation rate at the protein level. A phylogenetic analysis also revealed seven new genotypes of ACMV strain. This result reflects a progressive genetic evolution of the virus strains, which could impact negatively on cassava crop in Côte d'Ivoire. This study proposed selecting resistant traditional cassava genotypes to control virus spread.

Key words: Cassava mosaic disease, ACMV, *in silico* analysis, mutation, resistant genotype, Côte d'Ivoire.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is the largest staple food consumed by an estimated 800 million people worldwide (Alamu et al., 2019). It is grown almost everywhere in Côte d'Ivoire and is the country's second most important root crop after yam (Mobio et al., 2021). Although cassava has high agronomic potential, the fields are affected by pests and diseases, mainly Cassava Mosaic Disease (CMD), that hinders the productivity.

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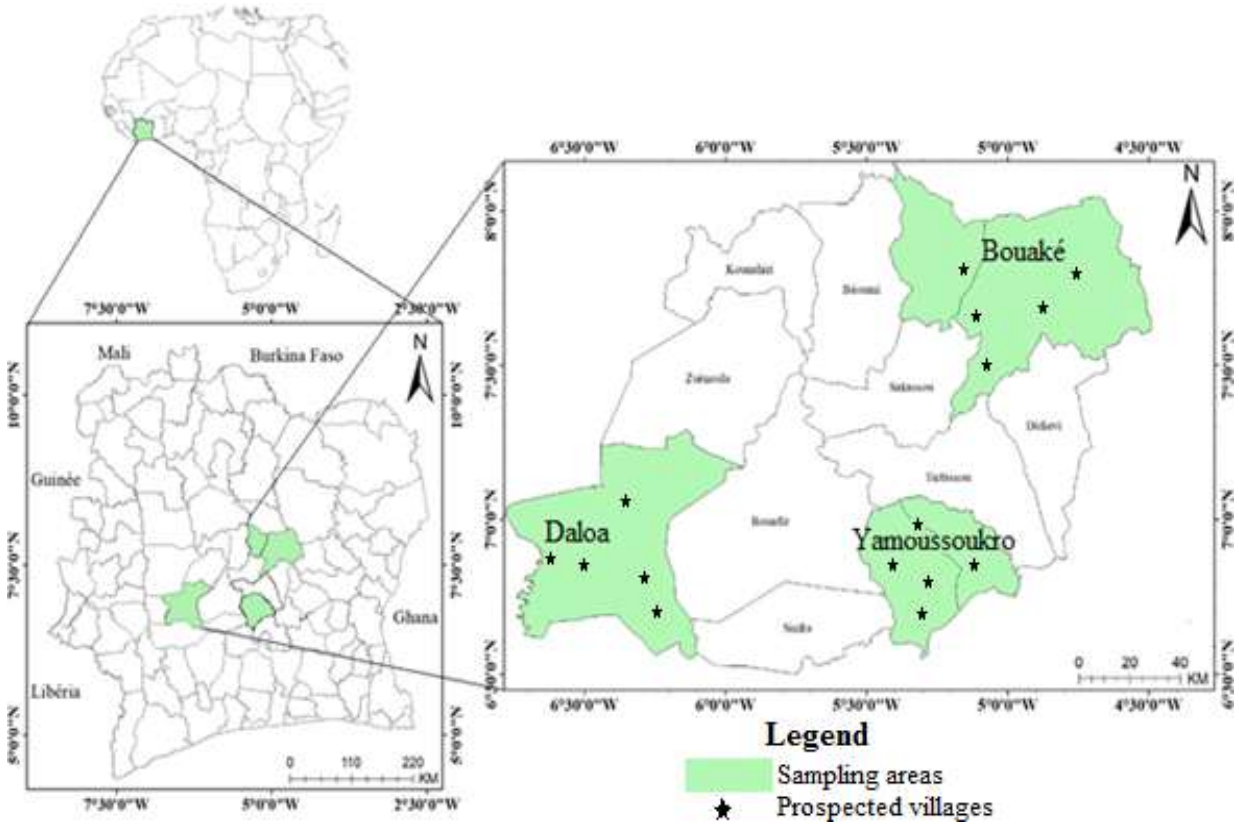

Figure 1. Geographic location of sampling sites.
Source: Authors

CMD is caused by Cassava Mosaic Geminiviruses (CMGs) which are transmitted by infected cuttings or whitefly *Bemisia tabaci* (De Bruyn et al., 2016). Cassava losses caused by Cassava Mosaic Viruses (CMV) can reach 90% (Yéo et al., 2020). Several strains of CMV have been identified in various countries and released in a public database (Elegba, 2018). Among these strains, nine have been described in Africa such as African cassava mosaic virus (ACMV), East African cassava mosaic Cameroon virus (EACMCV), East African cassava mosaic Kenya virus (EACMKV), East African cassava mosaic Malawi virus (EACMMV), East African cassava mosaic virus (EACMV), East African cassava mosaic Zanzibar virus (EACMZV), South African cassava mosaic virus (SACMV), African cassava mosaic Burkina Faso virus (ACMBFV), and Cassava mosaic Madagascar virus (CMMGV).

Two strains of the virus have been reported in Asia such as Indian cassava mosaic virus (ICMV) and Sri Lankan cassava mosaic virus (SLCMV). Among these viruses, studies conducted in Côte d'Ivoire have identified only two strains including ACMV and EACMV (Toualy et al., 2014). However, there is no information on genetic variants and intragenic mutations encoding these virus strains. Thus, the main objective of this study is to assess the molecular variability of ACMV strain in three of the large cassava production area of Côte d'Ivoire.

MATERIALS AND METHODS

Study area and sample collection

Survey for cassava leave sample collection were conducted from November, 2019 to August, 2020 in 191 small-holder farmer fields located in three of large cassava production departments of Côte d'Ivoire, from which 15 villages were selected according to the diversity of cassava varieties (Figure 1). These include N'Djebonoua, Diabo, Kongodekro, Kouakouyebouekro and Kekrekouakoukro villages from Bouaké department, Lolobo, Assanou, Oufouediékro, N'gbessou, and Akpessèkro villages from Yamoussoukro department, and Zakoua, Kibouo, Zaguiguia, Bribouo, and Zakaria from Daloa department. A total of 200 cassava symptomatic and asymptomatic leaves were collected and stored in labeled envelopes kept in a freezer at -80°C for subsequent DNA extraction.

Molecular characterization of virus strains

CMV genome amplification by PCR

Total nucleic acids were extracted from 2 g of each cassava leaf sample, as described by Yao et al. (2019). This extracted DNA was resuspended in 100 µl of elution buffer containing Tris-EDTA, and quality was tested on 1% agarose gel electrophoresis. DNA

solutions were stored at -20°C until amplification. PCR assays were performed to identify different virus strains using primers JSP001/JSP002 and ACMV/ACMV for African Cassava Mosaic Virus (ACMV) and JSP001/JSP003 for East African Cassava Mosaic virus (EACMV). PCR mix of each sample contained 5 µl of 10X buffer with $MgCl_2$ (Qiagen), 3.2 µL of deoxyribonucleoside triphosphates (dNTPs, 200 µM), 2.6 µL of each primer (10 pmol/µl, Eurogentec), 0.1 µL *Taq* polymerase (5 U/µL, Qiagen), 31.5 µL of pure water, and 5 µl of DNA. Reaction conditions were initial denaturation for 5 min at 94°C followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 58°C for 1 min, and extension at 72°C for 1 min, followed by final extension at 72°C for 10 min. After electrophoresis, PCR products were visualized by UV trans-illuminator. Known negative and positive control samples were included in all assays with 100 bp DNA Ladder to identify viruses according to allele sizes. The amplified products were scored (+) indicating the presence of the virus tested or (-) indicating the absence of the virus tested.

Sequencing of CMV strains

After amplification, 22 randomly selected CMV-positive PCR products were sent to Hong Kong BGI TECH SOLUTIONS for forward and reverse sequencing in ABI PRISM 3730 (Applied Biosystem) according to Sanger method (Sanger et al., 1977).

Bioinformatics and phylogenic analysis

The ACMV nucleotide sequences were compared to reference sequences available in Genbank genomic database of National Center for Biotechnology Information (NCBI) using the BLASTN local alignment search tool available online (http://www.ncbi.nih.gov). Protein sequences derived from nucleotide sequences were aligned and analyzed subsequently. These alignments were carried out in order to identify similarities between the CMGs variants obtained in this study and those from Genbank, as well as mutations between the aligned sequences. The Genbank sequence with the highest identity percent was chosen for each alignment. Finally, all sequences were compared using multiple alignments. Bioinformatic analyses were performed using the software Chromas Lite® 2.01 and Geneious prime 2021.1.1. CMV strain isolates were clustered based on their genetic relationships from phylogenetic trees using the Neighbour Joining (NJ) method with 1000 replicates. This analysis was performed using MEGA X software (Kumar et al., 2018). The evolutionary distances were generated by the Jukes-Cantor method based on the number of base substitutions.

RESULTS

Molecular detection and occurrence of the CMV strains

CMV amplification revealed the presence of ACMV and EACMV strains in the surveyed areas. Electrophoretic profile is characterized by DNA fragments of 783 and 1030 bp for ACMV and 780 bp for EACMV (Figure 2). Among 154 samples affected by CMD, 63.64% were due to ACMV while only 19.48 and 16.88% of these infections were caused by EACMV and coinfection ACMV/EACMV respectively, reflecting the predominance of ACMV strains.

Bioinformatic and phylogenetic analysis of ACMV sequences

Nucleotide sequence alignment

Nucleotide sequence analysis revealed genetic diversity in the ACMV strain which reflects the evolution of this strain in Côte d'Ivoire. Indeed, apart from a single sequence similar to a variant already identified in Côte d'Ivoire, isolated viruses were similar to variants already identified in five other countries available in the Genbank genomic database with identity percent between 96.5 and 98.9%. Out of the 22 sequences analyzed, 6 (27.27%) were identified to be homologous to the variant ACMV_GH:AK4A13 from Ghana with accession number MG250093, 5 (22.73%) sequences were similar to the variant ACMV_CM/YA under accession number AY211463 from Cameroun. Three sequences (13.64%) were similar to the Cameroun variant ACMV_CM/AK with accession number AY211461, and 2 (9.10%) were similar to the variant of Cameroun ACMV_CM/39 under accession number AY211462. Two others were comparable to the variant ACMV_CF:CF4AB from Madagascar, one of these sequences (4.54%) was similar to the variant ACMV_CF:CF72AB from Madagascar, one was identified to be similar to the variant ACMV_GH:FM14A from Kenya, one other was similar to the variant ACMV-[NG:So:03] from Nigeria and finally, one variant was similar to the variant from Côte d'Ivoire ACMV-[Ivory Coast] with accession number AF259894 (Table 1). All these variants were detected on the DNA-A of African cassava mosaic virus genome and encode the gene AV2 except two variants: ACMV_CM/YA and ACMV_CM/AK from Cameroon which encode the gene AV1.

Mutation's impacts on the evolution of virus variants

The nucleotide sequence alignments showed low mutation rates between 1.1 and 3.5%. ACMV_Dal1 nucleotide query sequence alignment with reference sequence of accession number MG250093 shows deletion (in green) at position 7 in the query sequence, transversions (in blue) at positions 115 (G115T), 304 (T304G), 640 (T640G) and 705 (G705T) and transitions (in yellow) at positions 147 (C147T), 190 (G190A), 209 (C209T), 276 (A276G), 328 (T328C), 367 (C367T), 646 (C646T) and 670 (C670T) (Figure 3). These mutations have resulted in nucleotide sequence modifications that have caused changes in amino acids codons and therefore of the protein sequences reflecting new variants (Figure 4).

Phylogenetic relationship

Based on the 99% clustering threshold, seven variants of

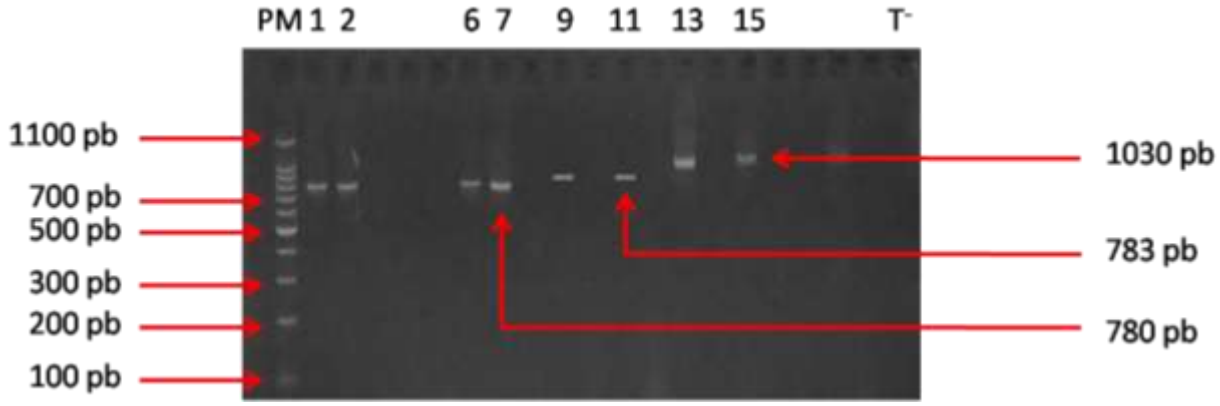

Figure 2. Electrophoretic profile on 2% agarose gel for cassava mosaic viruses' identification.
Source: Authors

Table 1. Characteristics of the Genbank sequences corresponding to the requested nucleotide sequences of ACMV strain.

Isolate	Variants	Accession No.	% Identity	Origin	Sequence length (bp)
ACMV-Bke1	ACMV CM/AK	AY211461	98.1	Cameroun	728
ACMV-Dal1	ACMV_GH:AK4A	MG250093	97.8	Ghana	719
ACMV- Dal2	ACMV_ CF:CF4AB	KJ887756	98.6	Madagascar	730
ACMV- Dal3	ACMV_CF:CF4AB	KJ887756	98.5	Madagascar	723
ACMV-Yak1	ACMV_CF:CF72AB	KJ887780	98,6	Madagascar	708
ACMV-Dal4	ACMV CM/AK	AY211461	98.1	Cameroun	728
ACMV_Bke2	ACMV_GH:AK4A:13	MG250093	98.7	Ghana	720
ACMV_Yak2	ACMV-[NG:So:03]	EU685322	98.3	Nigeria	708
ACMV_Dal5	ACMV_CM/YA	AY211463	97.3	Cameroun	708
ACMV_Yak3	ACMV_GH:AK4A	MG250093	98.9	Ghana	708
ACMV-Yak4	ACMV_ CM/AK	AY211461	98.3	Cameroun	708
ACMV-Dal6	ACMV_ CM/39	AY211462	98.4	Cameroun	741
ACMV_Yak5	ACMV_GH:AK4A	MG250093	98.3	Ghana	708
ACMV_Bke3	ACMV_GH:FM14A	MG250159	98.3	Kenya	708
ACMV_Dal7	ACMV_CM/YA	AY211463	97.3	Cameroun	708
ACMV_Bke4	ACMV_GH:AK4A:13	MG250093	98.4	Ghana	749
ACMV_Yak6	ACMV_GH:AK4A:13	MG250093	98.6	Ghana	732
ACMV_Bke5	ACMV-[Ivory Coast]	AF259894	96.5	Côte d'Ivoire	707
ACMV_ Bke6	ACMV_CM/YA	AY211463	98,3	Cameroun	719
ACMV_ Bke7	ACMV_CM/YA	AY211463	98.3	Cameroun	708
ACMV_ Dal8	ACMV_CM/YA	AY211463	97.3	Cameroun	707
ACMV_ Yak7	ACMV_ CM/39	AY211462	98.4	Cameroun	741

Source: Authors

the ACMV strain could be defined from the 22 isolates studied. Three clusters and four single ACMV isolates protein sequences were identified regarding the phylogenetic tree. While cluster 1 comprises isolates from all the departments surveyed, clusters 2 and 3 included only isolates from the departments of Daloa and Bouaké, respectively (Figure 5). Three out of the four single ACMV isolates were from Daloa department and one from Bouaké. All isolates from Yamoussoukro department are in cluster 1.

DISCUSSION

The study showed the predominant of ACMV strain among Cassava Mosaic Viruses in Côte d'Ivoire. This result is in agreement with Toualy et al. (2014). This virus has been shown to be the predominant virus in several

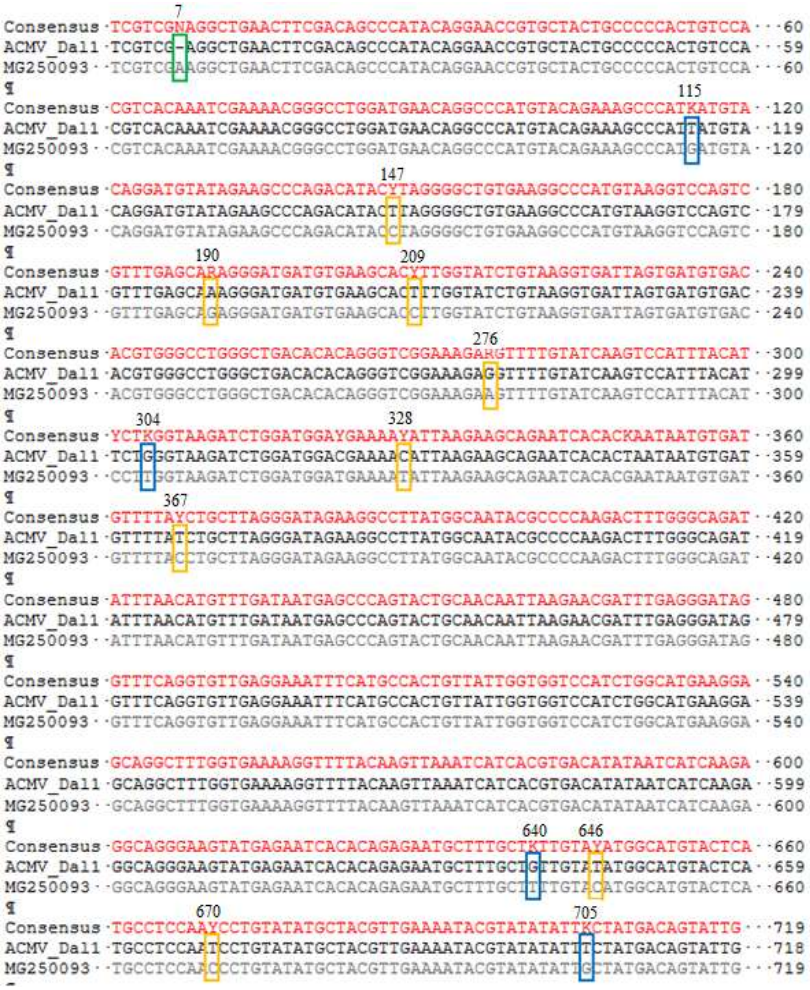

Figure 3. Alignment of nucleotide sequence of ACMV_Dal1 (in black) on the reference sequence of accession number MG250093 (in grey).
Source: Authors

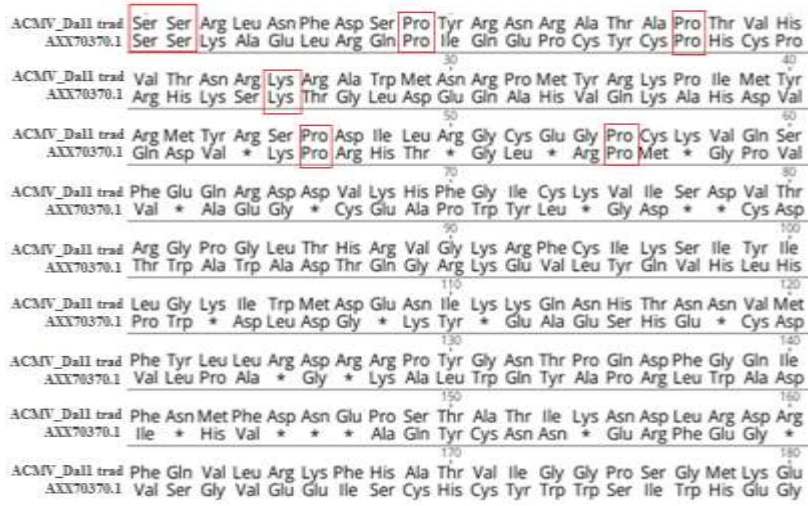

Figure 4. Mutations observed by alignment of the protein query sequence of ACMV_Dal1 with its reference sequence of accession number AXX70370.1.
Source: Authors

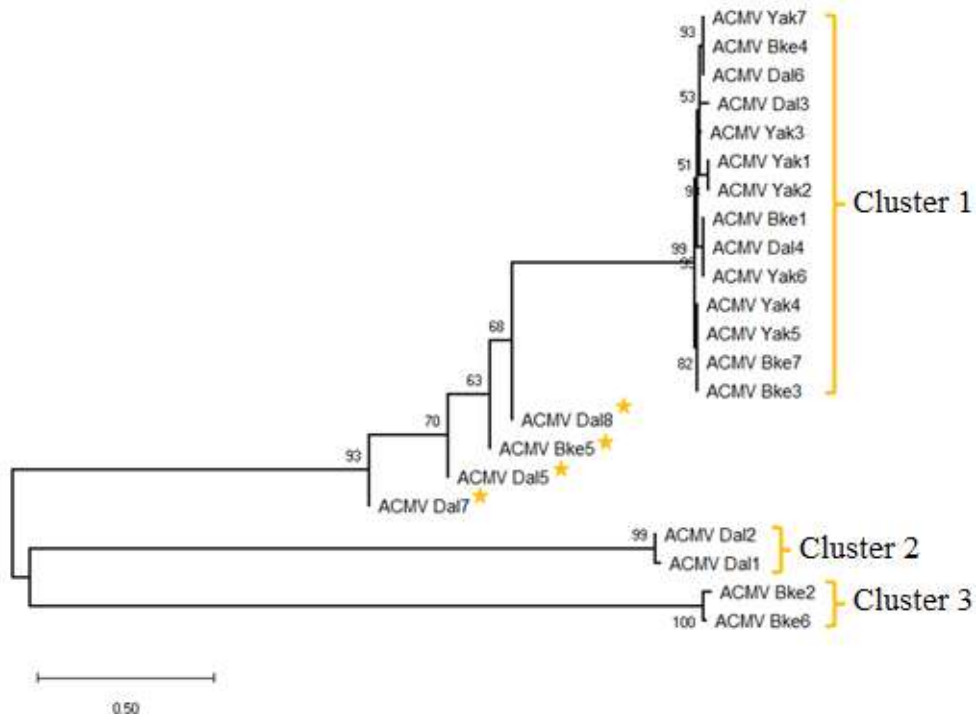

Figure 5. Cladistic structure of the protein sequences of ACMV isolates according to the neighbour joining tree.
Source: Authors

other countries where CMD occur such us Burkina-faso and Madagascar (Tiendrébéogo et al., 2012; Harimalala et al., 2012). The results of this study revealed an important genetic diversity within ACMV strain. The variants isolated using 22 full-length nucleotide sequences of ACMV from farmers' fields were similar to variants of six African countries including Côte d'Ivoire according to the Genbank genomic database. These are variants ACMV_GH:FM14A from Kenya, ACMV_GH: AK4A:13 from Ghana, ACMV_ CF:CF4AB and ACMV_CF:CF72AB from Madagascar, ACMV_CM/YA and ACMV_CM/AK; ACMV_CM/39 from Cameroun, ACMV-[NG:So:03] from Nigeria and ACMV-[Ivory Coast] from Côte d'Ivoire with similarity percentage between 96.5 and 98.9%. These high similarities may indicate that viruses originate from these different countries. The low mutations detected in the nucleotide sequences indicate that isolates are derived from existing variants from these countries with some mutations due to environmental. This result is supported by Mulenga et al. (2016) who showed that CMV diversity in Zambia is caused by cuttings exchange with other countries. Also, cutting, which is the main means of cassava production, has an important role in the spread of viruses. This is the main factor of CMD development (Harimalala et al., 2015). In fact, anthropogenic activities such as exchange of planting material has played a major role in the spread of CMGs outside of their previously reported geographic ranges, facilitating the colonization of new niches (Legg et al., 2014). Missing awareness of the farmers to the risk posed by uncertified plant material, the difficult access to virus-free plant material, and the preference of some varieties by some farmers are among reasons of CMD propagation in addition to whiteflies *Bemisia tabaci* contribution (Legg et al., 2015).

According to Crossley and Snyder (2020), the insects *B. tabaci* provide long-distance flights that can carry them from one area to another. Moreover, underlying mechanisms such as mutation have been reported to play a role in the evolution of geminiviruses (Ramesh et al., 2017). In this study, although there were very few mutations in the nucleotide sequences, they favored the evolution of the viruses which is reflected in the very high mutations in protein sequences. According to Elegba (2018), mutations constitute the diversification engine of viruses because Geminiviruses are single stranded DNA viruses that replicate quickly with proofreading and mismatch repair capacity. These processes strongly help virus acquiring great genetic variability and thus creating new arrangements within the genome (Lefeuvre and Moriones, 2015). Thus, mutant gradually becomes a new virus that is often more dangerous than the initial one. When a mutation in a coding region results in an amino acid change, it can be deleterious to its host plants.

Phylogenetic analysis realized using the protein sequences of the 22 ACMV isolates revealed three

clusters and four singles, reflecting new variants of this strain and high genetic diversity of the virus in Côte d'Ivoire. However, Asare et al. (2014) contend that the viruses' high genetic variability may contribute to the new development of CMD and have serious implications for production. The genetic variability of the strain ACMV observed in this study represents an ideal condition for emergence of others severe variants through numerous possibilities of intra- or intergenic recombination and presents a major epidemiological risk for cassava crop (Elegba, 2018). As a result, this study should be able to challenge all relevant actors regarding CMD control measures. Cassava cultivars that are virus-resistant may be the most effective control measure (Elegba et al., 2020; Houngue et al., 2019).

Conclusion

Molecular genetics and *in sillico* analyses on Cassava Mosaic Virus highlights its perfect evolution in Côte d'Ivoire, with several variants identified. These variants are highly similar to some variants discovered in African countries such as Ghana, Kenya, Cameroon, Madagascar, Nigeria, and Côte d'Ivoire. Genetic variations are reflected by various mutations observed involving natural selection, human activities, and environmental factors. Seven ACMV genotypes represented by three genetic clusters and four single isolates were identified and could be considered as new variants of CMV in this study. As a result, it appears critical to seek cassava cultivars that are resistant to Cassava Mosaic Viruses for effective control.

As a precaution, genomic sequencing of the other mosaic virus strains should be performed in order to identify the different variants and their distribution in Côte d'Ivoire for effective control. Finally, traditional mosaic-resistant varieties would need to be identified for the proposed control methods.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors are grateful to the head of URGEM-Daloa laboratory, Professor KOFFI for having authorized molecular analysis in his laboratory.

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