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Molecular Identification of Maize Streak Virus in Côte d'Ivoire

Guinagui N'Doua Bertrand[1,2*], Koala Moustapha[3,4], Yéo Gnénakan[5], Tuo Seydou[1,2], Ouattara Bakary[3], Ouattara Bognan Winnie Miyasi[1], Sérémé Drissa[3], Koné Daouda[1,2], Fatogoma Sorho[1,2]

[1]Laboratory of Biotechnology, Agriculture and Valorization of Biological Resources, Pedagogical and Research Unit of Plant Physiology and Pathology, UFR Biosciences, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire
[2]Wascal/African Center of Excellence on Climate Change, Biodiversity and Sustainable Agriculture, Abidjan, Côte d'Ivoire (Wascal/CEA-CCBAD), Abidjan, Côte d'Ivoire
[3]National Center for Scientific and Technological Research, Institute of the Environment and Agricultural Research, Crop Protection and Defense Laboratory, Ouagadougou, Burkina Faso
[4]National Biosafety Agency, National Biosafety Laboratory, Ouagadougou, Burkina Faso
[5]National Center for Agricultural Research, Ferkessedougou Research Station, Ferkessedougou, Côte d'Ivoire
Email: *guinagui.bertrand@ufhb.edu.ci, guinaguibertrand@gmail.com

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Abstract

Maize (*Zea mays* L.) is the world's leading cereal crop, with production estimated at over 1 billion tonnes in 2022. In Côte d'Ivoire, maize is one of the most widely consumed foods, with national production exceeding 1 million tonnes in 2022. Despite its importance, this crop is subject to numerous biotic constraints, including Maize streak virus (MSV). The objective of this study was to characterize MSV isolates from Côte d'Ivoire using molecular biology techniques. To achieve this, maize leaf samples displaying characteristic symptoms of MSV were collected from different agro-ecological zones (AEZs) of Côte d'Ivoire. Total DNA was extracted from the collected samples using the CTAB method and quantified with a Nanodrop spectrophotometer. The C2 region of the MSV genome's open reading frame (ORF) was amplified through polymerase chain reaction (PCR) using MSV-specific primers. The resulting PCR products were sequenced using the Sanger method. Bioinformatics analysis was performed using MSV sequences from other African countries (retrieved from NCBI) alongside sequences obtained in this study. The analysis was conducted using MEGA X version 10.05 software. The results showed that the main symptoms observed in the field included the presence of longitudinal light green streaks on leaves, stunted plant growth, and incomplete seed development in infected plants. Phylogenetic analysis of the sequences from Côte d'Ivoire revealed three monophyletic groups, with sequences of some isolates collected from the same plot belonging to different groups. The MSV

sequences from Côte d'Ivoire are very close to those from Rwanda. This study underscores the need for further investigation into the genetic diversity of MSV strains to enhance the diagnosis and management of this viral disease, which is particularly prevalent in maize crops in Côte d'Ivoire.

Keywords

Characterization, Molecular Biology, MSV, Maize, Côte d'Ivoire

1. Introduction

Maize (*Zea mays* L.) is the world's leading cereal crop, with an estimated production of over one billion tons [1]. Maize is one of the most consumed cereals in sub-Saharan Africa [2] and represents an important source of minerals and vitamins [3]. It provides more than 20% of human energy and up to 30% of proteins, 60% of energy, and 90% of starch for animals [4]. In Côte d'Ivoire, maize ranks second in terms of consumption after rice, with a production of over one million tons and a cultivated area of more than 575 hectares [1]. This production represents a significant source of income for local populations but remains insufficient to meet national needs [5]. Despite its importance, maize cultivation is subject to numerous biotic constraints, including viruses that limit its production by causing losses ranging from 30% to 100% per year [6]. More than 50 phytoviruses affect maize worldwide [7]. In Africa, three main viruses have been associated with maize cultivation: Maize stripe virus (MStpV), Maize mosaic virus (MMV) and Maize streak virus (MSV) [8], which is the subject of our study. Maize streak virus, belonging to the genus Mastrevirus and the family Geminiviridae, infects many cereals worldwide, such as millet, wheat, and maize [9]. It is obligately transmitted by as many as six leafhopper species in the Genus Cicadulina, but mainly by *C. mbila* [10]. Maize streak virus can lead to significant maize production losses of up to 100% in sub-Saharan Africa [8]. The streak caused by Maize streak virus is characterized in maize by yellowish streaks on the leaves, which can progress to a general discoloration of the plant [10]. In Côte d'Ivoire, due to climate variability, an increase in symptoms characteristic of Maize streak virus has been reported in several maize plots in agro-ecological zones I, V, and VI. To implement a sustainable control strategy against this emerging virus and minimize its impact on maize production in Côte d'Ivoire, rapid detection is essential. Therefore, the objective of this study was to identify, using molecular biology techniques, the Maize streak virus isolates originating from the agro-ecological zones (ZAE) of Côte d'Ivoire.

2. Materials and Methods

2.1. Materials

The plant material used in this study consisted of maize leaf samples from various varieties exhibiting characteristic symptoms of Maize streak virus (MSV).

Study Area

The study was conducted in four localities distributed across agro-ecological zones (AEZ) I, V, and VI of Côte d'Ivoire, as indicated in Figure 1. An agro-ecological zone is a land resource mapping unit based on climate, soil, geology, and/or vegetation cover. Based on these parameters, seven AEZs have been identified in Côte d'Ivoire [11].

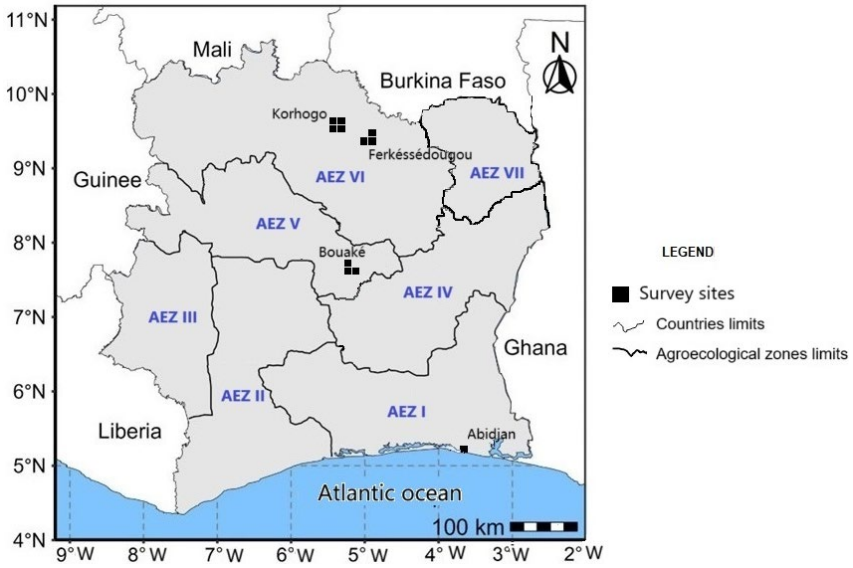

Figure 1. Map of Côte d'Ivoire indicating the survey sites.

2.2. Methods

2.2.1. Collection Maize Leaf Samples

Surveys were conducted in August 2022 and 2023 across three agro-ecological zones (AEZs) in Côte d'Ivoire in particular AEZ I, IV, and VI. The survey covered 11 plots. Upon arrival at the plot, GPS coordinates were recorded using a Garmin Global Positioning System (GPS) device, and the farmers provided information on previous crops and technical itineraries. Sampling was conducted using the "X" method described by Kouakou *et al.* (2024) [11]. This method involves collecting samples along the two diagonals of the plot. In the field, symptomatic leaf collection was carefully performed using plastic bags to avoid direct contact with the leaves. The collected samples were immediately labeled and transported to the laboratory, where they were finely chopped, preserved in calcium chloride following Bos's technique, and then stored at 4˚C in a refrigerator [12].

2.2.2. Molecular Characterization of MSV Isolates

Total DNA from symptomatic maize leaves was extracted using the cetyltrimethylammonium bromide (CTAB) protocol recommended [13]. Symptomatic leaf samples were ground and homogenized in the presence of DNA extraction buffer. Total DNA was isolated by adding chloroform and precipitating using isopropanol. The detection and quantification of MSV in collected maize leaf samples were performed by amplification and sequencing of the C2 gene in the MSV ORF [14].

2.2.3. Quantification of Total DNA

The extracted DNA was quantified using a NanoDrop spectrophotometer (NanoDrop One/OneC). A small volume of distilled water was first used to test the device surface. After calibration, 1 µl of each DNA sample was analyzed.

2.2.4. Polymerase Chain Reaction (PCR)

PCR was performed on the extracted DNA using Go-Taq polymerase (Promega, USA). The reaction mixture consisted of 5 µl of cDNA, 10 mM dNTP (1 µl), 1 µl of each primer pair (sense P1: 5'CCAAAKDTCAGCTCCTCCG3'; antisense P2: 3'TTGGVCCGMVGATGTASAG5'), 0.1 µl of Go-Taq enzyme, and 10 µl of Go-Taq 5x buffer. Sterile water was added to obtain a final volume of 50 µl. The PCR protocol included an initial denaturation cycle at 94˚C for 1 min, followed by 33 denaturation cycles at 94˚C for 1 min, annealing at 50˚C for 1 min, and elongation at 72˚C for 2 min. The final elongation step was conducted at 72˚C for 7 min. The PCR products were stored at 4˚C until electrophoresis.

2.2.5. Agarose Gel Electrophoresis

The amplification products were verified on a 1% (w/v) agarose gel prepared in 0.5x TAE buffer and melted by microwave heating. After cooling, approximately 2 µl of ethidium bromide (EtBr) was added to the agarose solution. The gel was then poured into pre-prepared casting plates. After polymerization, the gel-containing support was placed in an electrophoresis tank filled with 0.5x TAE buffer.

The PCR products (10 µl) were mixed with loading buffer and loaded into gel wells. A 1-kb molecular weight marker was loaded in parallel to estimate the band size. The electrophoresis was conducted at 100 V for 30 min. Amplicons were visualized under UV light using a transilluminator and photographed using a digital camera (Samsung ES74; 14.2 megapixels).

2.2.6. Sequencing and Bioinformatics Analysis of Sequences

All PCR products of the correct size were sent to Macrogen for sequencing [15]. Bioinformatics analysis involves aligning among sequences. The obtained sequences were edited and aligned using the Molecular Evolutionary Genetics Analysis (MEGA X) software, version 10.05. The obtained sequences were blasted in the NCBI database. Ten sequences from the C2 region of the ORF representing MSV diversity in Africa were downloaded from the NCBI database using accession numbers to study the phylogenetic relationships among isolates.

2.2.7. Sequence Alignment

Nucleotide sequence alignments were performed using the ClustalW alignment algorithm [16] in MEGA X version 10.05. A multiple alignment was generated to produce a distance matrix. From this matrix, a phylogenetic tree was constructed using the appropriate method for the dataset [17]. Progressive sequence alignment was performed according to the branching order.

2.2.8. Phylogenetic Tree Construction

The evolutionary history was inferred by using the Maximum Likelihood method

and Kimura 2-parameter model [18] with 1000 bootstrap repetitions. The tree with the highest log likelihood (−15134.52) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.8188)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 20 nucleotide sequences. There were a total of 2909 positions in the final dataset. Evolutionary analyses were conducted in MEGA X version 10.05 [19]. Only nodes with bootstrap values equal to or greater than 60% were retained.

3. Results

3.1. Distribution of Samples According to Agro-Ecological Zones

A total of 20 maize leaf samples, including 15 symptomatic of streak disease and 5 asymptomatic samples, were collected. The main symptoms observed in the plots included light green longitudinal streaks on the leaves and stunted plant growth. Poor grain filling was also noted in infected plants during the fruiting stage (**Figure 2**).

A: stunted plants in Korhogo; **B**: incomplete inflorescence development in Ferkessédougou; **C**: incomplete grain filling.

Figure 2. Symptomatology of MSV in Côte d'Ivoire.

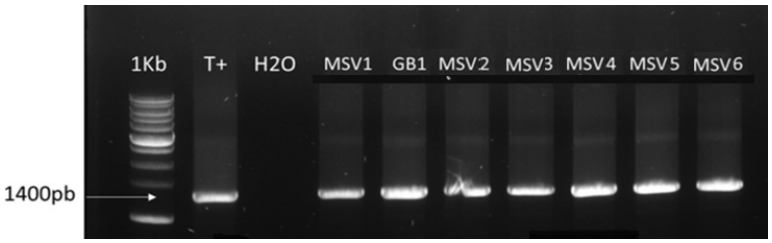

Figure 3. Agarose gel electrophoresis (1%) showing amplicons from several samples. 1kb: Molecular marker; **T+**: positive control; **H_2O**: water used as a negative control; **MSV1** to **MSV2**: MSV-infected maize leaf samples collected in Korhogo; **MSV3** to **MSV5**: MSV-infected maize leaf samples collected in Ferkessédougou; **MSV6**: MSV-infected maize leaf samples collected in Bouaké; **GB1**: sample collected in Abidjan but not part of the study.

3.2. Amplification of the MSV Capsid Protein

RT-PCR amplification demonstrated that the symptoms were indeed due to MSV. Figure 3 shows a sample image of the C2 zone of the ORF gene amplification gel from the collected MSV isolates.

3.3. Structural Study of MSV Populations in Côte d'Ivoire

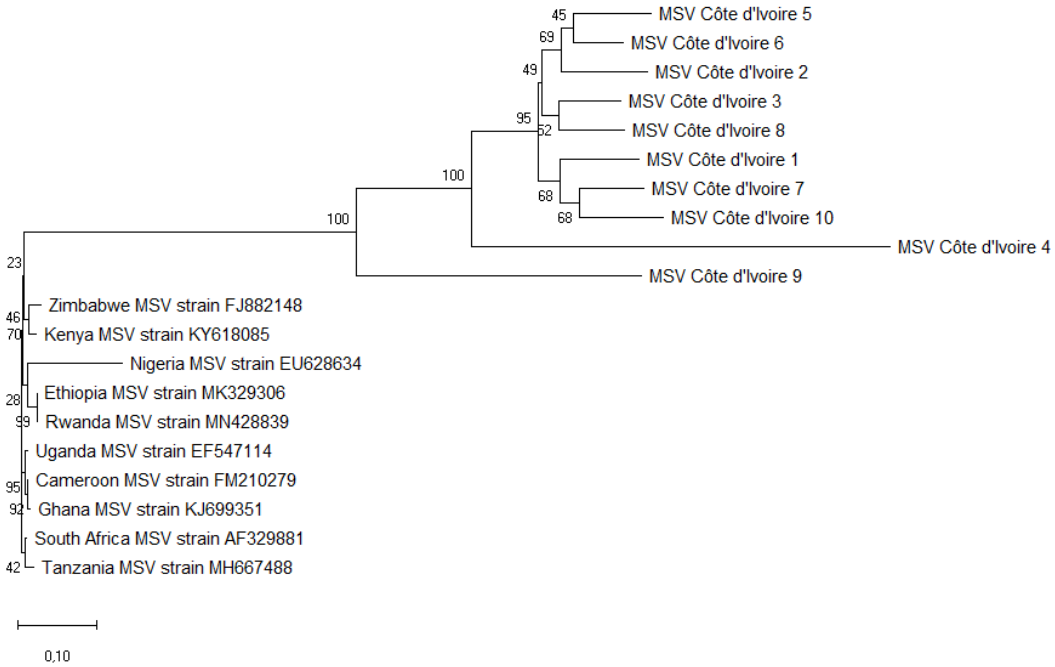

Figure 4. Phylogram of MSV isolates from Côte d'Ivoire and MSV strains from Africa.

The comparison of MSV sequences from Côte d'Ivoire with those from other countries present in the NCBI database showed that the sequences from Côte d'Ivoire are 98% identical to those from Rwanda the phylogenetic tree analysis revealed three monophyletic groups, with Bootstrap values of 100%, 100%, and 95% for groups 1, 2, and 3, respectively (Figure 4). Group 1 consists of MSV 9 Côte d'Ivoire isolates originating from agro-ecological zone I. Group 2 is composed of MSV 4 Côte d'Ivoire isolates from Ferkessédougou (ZAE VI), and Group 3 includes MSV 1 Côte d'Ivoire, MSV 2 Côte d'Ivoire, MSV 3 Côte d'Ivoire, MSV 5 Côte d'Ivoire, MSV 6 Côte d'Ivoire, MSV 7 Côte d'Ivoire, MSV 8 Côte d'Ivoire, and MSV 10 Côte d'Ivoire isolates. MSV 1 Côte d'Ivoire and MSV 2 Côte d'Ivoire isolates originate from Korhogo (ZAE VI), while MSV 3 Côte d'Ivoire, MSV 5 Côte d'Ivoire, and MSV 6 Côte d'Ivoire isolates come from Ferkessédougou (ZAE VI). However, MSV 6 Côte d'Ivoire, MSV 7 Côte d'Ivoire, and MSV 8 Côte d'Ivoire isolates are from Bouaké. Within Group 1, some subgroups were observed. However, these subgroups are not related to the geographical origin of the collection (Figure 4). Moreover, this grouping could be linked to the pathogenic properties of the isolates. It is noteworthy that, in some cases, isolates collected from the same plot are distributed into two different groups. This is the case for

MSV 4 Côte d'Ivoire isolates and MSV 3 Côte d'Ivoire, MSV 5 Côte d'Ivoire, and MSV 6 Côte d'Ivoire isolates, which belong to two different monophyletic groups and were collected from the same plot. The tree topology shows a rake-like structure, indicating a lack of phylogenetic structuring. This phylogenetic tree topology of Côte d'Ivoire isolates shows relatively short branches for Group 3 isolates (MSV 1 Côte d'Ivoire, MSV 2 Côte d'Ivoire, MSV 3 Côte d'Ivoire, MSV 5 Côte d'Ivoire, MSV 6 Côte d'Ivoire, MSV 7 Côte d'Ivoire, MSV 8 Côte d'Ivoire, MSV 10 Côte d'Ivoire), whereas the branches of MSV 4 Côte d'Ivoire and MSV 9 Côte d'Ivoire isolates are somewhat longer.

4. Discussion

The molecular characterization of Maize streak virus (MSV) has been extensively studied throughout the world. However, this study is the first of its kind in Côte d'Ivoire and serves as an initial exploration of the molecular structruration of this virus within the country. The evolutionary potential of a viral population, which is controlled by various evolutionary forces such as population size, gene flow, reproductive system, mutations, and selection, is also influenced by ecological factors [20]. The impact of climate change on pathogens is undeniable. Environmental instability modifies the epidemiological components of pathogen populations, subsequently influencing their structure and pathogenic properties of plant virus populations [21]. MSV was first identified in Côte d'Ivoire in the 1980s [22]. However, research on this pathogen has not progressed beyond serological detection, leaving a significant gap in the field of molecular characterization within the country. The results confirmed the presence of MSV as a causative agent of maize crop damage in Côte d'Ivoire, corroborating earlier findings by [22]. This disease has been present in West Africa for several decades, yet few studies have been conducted on this virus. In the context of intensified global climate variability, particularly in Africa, plant disease epidemiology is evolving. It is crucial to adopt new, rapid, and reliable identification methods, such as PCR, NGS, and metagenomics, to implement effective control strategies for these emerging diseases. The structure of the phylogenetic tree is in the form of a rake, indicating the adaptation of MSV in Côte d'Ivoire. Analysis of the results relating to the symptoms observed in the collection areas shows that the main symptoms observed are light green longitudinal striations on the leaves and stunted plant growth. Poor grain filling was also observed in infected plants during the fruiting phase in the plots visited. These results are in line with those observed in Cameroon [5], Nigeria [14], in Ethiopia [23] and other African countries. However, this study is a preliminary study that has made it possible to amplify the C2 zone of the ORF of the maize stripe virus genome in Côte d'Ivoire. However, more in-depth studies will enable the genetic diversity of MSV in Côte d'Ivoire to be investigated so that sustainable strategies can be put in place to combat this disease, which causes enormous damage to maize crops in Côte d'Ivoire, particularly among small-scale growers.

5. Conclusion

The study enabled molecular characterisation of MSV in Côte d'Ivoire. The samples were collected in three maize production areas, divided into three agro-ecological zones. This is a preliminary study that will enable the genetic diversity of MSV in Côte d'Ivoire to be investigated, and will help to put in place sustainable control strategies for this virus, which is so prevalent in maize crops in Côte d'Ivoire.

Author's Contributions Statement

The project's conception, laboratory analysis and sample collection were carried out by GNB, MK and OB. YG carried out one part of the sample collection. GNB and OBWM took charge of bioinformatic analysis and manuscript writing. TS and YG read and re-read the draft article. SD, KD and FS wrote the discussion section and proofread the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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