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*Article*

# Identification and Distribution of Begomoviruses Infecting Cassava Fields in Sierra Leone

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**Abstract:** A dearth of knowledge exists on identifying the begomoviruses and distributing cassava mosaic viruses across key cassava-growing regions of Sierra Leone. The study aimed to identify and map the distribution of cassava mosaic disease (CMD)-associated viruses in farmers' fields in Sierra Leone. Cassava leaf samples were collected in 109 smallholder farms during a geo-referenced survey conducted from 10th May to 5th June 2024. Molecular diagnostics were carried out to identify the viral strains associated with CMD. Findings revealed that infection by stem cutting was more predominant in the south, east, north, and northwest regions than in the west region. In contrast, infection by whitefly was predominant in the west, north, and north-west regions. The PCR screening of 426 samples coupled with sequence analysis revealed the presence of African cassava mosaic-like (ACMV-like) viruses, and East African cassava mosaic-like (EACMV-like) viruses as single infections at 78.1% and 1.3%, respectively. Co-infections of ACMV-like and EACMV-like viruses were detected in 20.6% of the tested samples. In addition, 70.6% of the samples positive for EACMV-like virus (single and mixed infections) were found to be positive for East African cassava mosaic Cameroon virus (EACMCMV). The ACMV and co-infection of ACMV and EACMV viruses were present in all regions, while EACMCV was detected in all regions except the western area. The results indicate more prevalence of EACMCMV variant in Sierra Leone. This study suggests utilization of participatory surveillance and good agronomic practices to manage CMD in Sierra Leone.

**Keywords:** epidemiology; viruses; East African Cassava Mosaic (EACMV); African Cassava Mosaic Virus (ACMV); *Manihot esculenta*

## 1. Introduction

Cassava (*Manihot esculenta* Crantz) is traditionally cultivated using the stem-cutting technique. Cassava propagation through stem cuttings is among the constraints of producing disease-free planting materials due to unnoticeable visual latent disease status [1]. Cassava botanical seed materials are generally exhibit slow germination due to dormancy. Cassava producers often use the conventional stem-cutting propagation technique for cultivation of the crop. This method leads to cumulation of viral, fungal, and bacterial diseases in the stem cuttings, contributing to decreased

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cassava production and productivity and loss of superior genotypes [2]. Moreover, diseases and pests affect the growth and development of cassava, consequently contributing to reduced economic yields in several production zones in Africa [3,4].

Cassava mosaic disease (CMD) is one of the key biotic constraints of cassava production in the 21st century [5,6]. The disease causes significant cassava fresh storage root yield loss in Africa [7]. In West Africa, high CMD infection can reduce cassava storage root yield by up to 90% if control intervention is not implemented [7]. The CMD is spread by whitefly vectors (*Bemisia tabaci*) and infected cuttings [8–10]. The disease is widespread in several East, Central, and West African countries and towards Southern Africa [11–13].

Cassava mosaic disease is caused by a Cassava Mosaic Begomoviruses (CMBs) complex, which constitute 11 species of bipartite Begomoviruses, of which, nine have been detected in Africa [14,15]. The distribution and spread of these begomoviruses often differ from one country to another and/or from one region to another within the same country [16–20]. Cassava Begomovirus, known as alternate hosts, can infect the primary and secondary host, cassava, and other weed species that grow around cassava fields. This makes managing the virus and eradicating it highly challenging.

Several epidemiological studies in Sierra Leone noted CMD's presence in most cassava-growing areas [7,21–23]. These studies have focused on screening for resistance in different agroecosystems, incidence, and severity of CMD, and evaluation of the impact of the disease on cassava genotypes in the country. Despite these various studies in Sierra Leone, the identification and/or characterization of the begomoviruses and distribution of cassava mosaic viruses remains less documented in several provinces. The present study aimed to identify and map the distribution of CMD-associated viruses in farmers' fields in Sierra Leone.

## 2. Results

### *2.1. Phenotypic Detection and Distribution of Cassava Mosaic Disease*

Generally, the mean incidence and severity of CMD, percent of plants infected by cuttings or whiteflies, and number of whiteflies per plant significantly ($p<0.05$) varied among the regions and districts of Sierra Leone (Figures 1 and 2). The CMD symptoms were found in all the 109 cassava fields surveyed, with the highest incidence and severity recorded in the west region. In contrast, the south region recorded the lowest values (Figure 1a and b). Percent of infected plants through stem cutting was more predominant in the south, east, north, and north-west regions than in the west region, where the whitefly mode of infection was higher than the stem cutting (Figure 1c). The highest whitefly abundance was recorded in the western region, with the lowest proportion recorded in the southern region (Figure 1d)

At the district level, the highest CMD incidence scores of almost 100% were recorded in Bombali, western rural, and western urban districts, with the lowest of 70% CMD incidence found in Bonthe district. The western rural and western urban districts exhibited the highest severity values of 4.8 (high infection), with the lowest of 3.2 (low infection of the disease) recorded in the Bonthe district (Figures 2a and b). The percentage of infected plants through stem cutting was more predominant in all the districts except western rural and western urban districts, where the whitefly mode of infection was higher than the stem cutting (Figure 2c). The highest whitefly abundance was captured in the western urban district, followed by the western rural district, with the lowest of 2 whiteflies per plant recorded in Bonthe district (Figure 2d)

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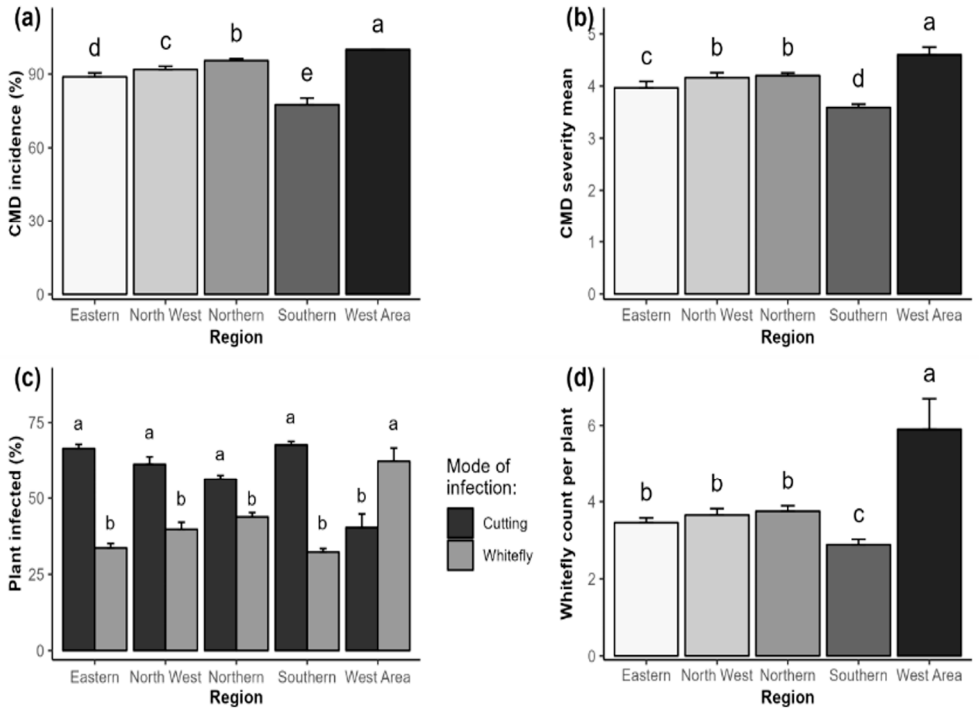


**Figure 1.** Epidemiological assessment of cassava mosaic disease (CMD) across regions of Sierra Leone. (a) mean incidence of CMD, (b) mean severity of CMD, (c) percent of cuttings or whitefly infected plants, (d) mean whiteflies per plant. Error bars represent standard error (SE).

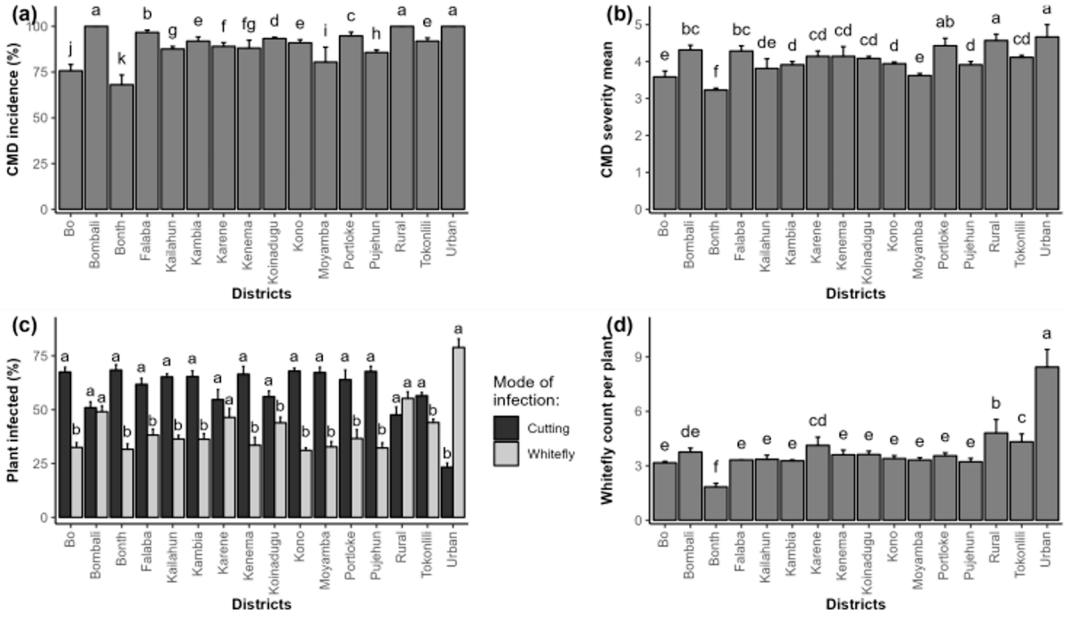


**Figure 2.** Epidemiological assessment of cassava mosaic disease (CMD) per district in Sierra Leone. (a) mean incidence of CMD, (b) mean severity of CMD, (c) percent cuttings or whitefly infected plants, (d) mean whiteflies per plant. Error bars represent standard error (SE).

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The distribution of CMD infection in cassava fields of Sierra Leone is also shown in distribution maps (Figure 3). The results reveal that the incidence of CMD ranged from 25-50% in the south region (Moyamba, Bonthe, and Bo districts) and some parts of the Bombali district, followed by some farms with 51-75% incidence of CMD found in the north and north-west regions. The east, north, north-west, and west areas recorded the highest incidence of CMD, ranging from 76-100%. The south region recorded the lowest severity scores of 2.0-3.0 (mild) and 3.1-4.0 (severe) infection of the disease. Kailahun and Kono districts in the east region had severity scores of 3.1-4.0 and 4.1-5.0, respectively. In the north region, cassava farms in Falaba and Bombali districts recorded severity scores of 3.1-4.0 and 4.1-5.0, respectively. Karene in the northwest region and the western rural and western urban districts in the west region had the highest severity damage, ranging from 4.1 to 5.0.

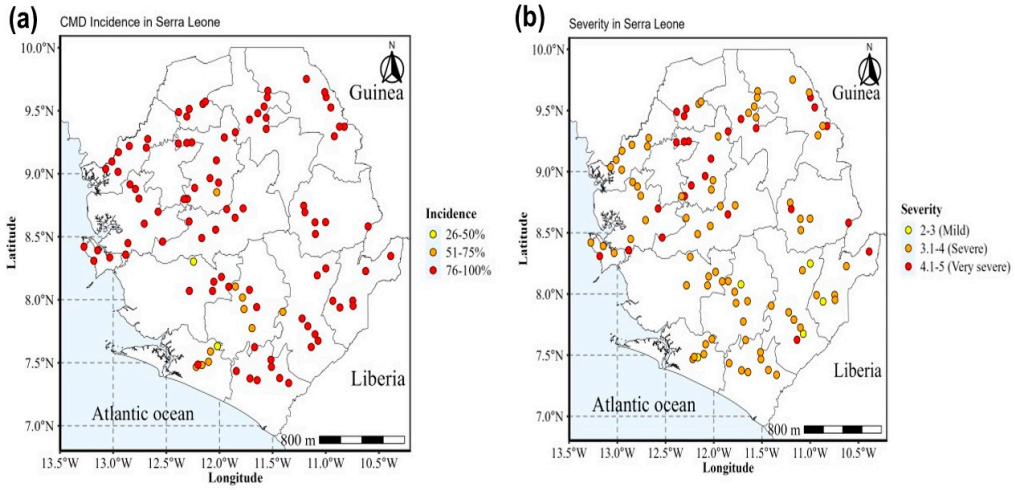


**Figure 3.** Map of cassava mosaic disease (CMD) distribution across Sierra Leone.

*2.2. Molecular Detection and Distribution of Cassava Begomoviruses in Sierra Leone*

For PCR analysis, four hundred twenty-six cassava leaf samples were collected from 320 plants with and 106 without symptoms in 2024. Among the samples with observable symptoms, 5.6% (28/320) tested negative for ACMV-like and EACMV-like viruses. On the other hand, 12.3% (13/106) and 5.7% (6/106) of symptomless samples tested positive for ACMV-like virus and mixed infection of ACMV-like virus and EACMV-like virus, respectively. Of the 426 samples, 311 (73.0%) were found positive, with 115 (27.0%) recorded as unfavorable (Tables 1 and 2). Among the positive samples studied, the single ACMV-like virus infection was the most frequent, accounting for 78.1% (243/311) of all CMD begomovirus infections, followed by mixed infections of ACMV-like virus and EACMV-like virus with 20.6% (64/311), and single infection of EACMV-like virus with 1.3% (4/311).

**Table 1.** Viral strains associated with CMD in Sierra Leone.

| Number of samples | Status of samples | Positive samples | | | | Negative samples |
|---|---|---|---|---|---|---|
| | | Detection of ACMV | Detection of EACMV | Detection of ACMV/ EACMV | Detection of Cameroon variant | |
| 426 | 320 CMD | 230 | 4 | 58 | 43 | 28 |
| | 106 Symptomless | 13 | 0 | 6 | 5 | 87 |
| | Total | 243 | 4 | 64 | 48 | 115 |

Values in brackets are positive single infection of ACMV after delineating for EACMV, EACMV-Cam, and mixed infections. Total positive samples=311 and total negative samples=115.

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The typical gel electrophoresis photo of ACMV begomoviruses detected in the study is presented in Figure 4.

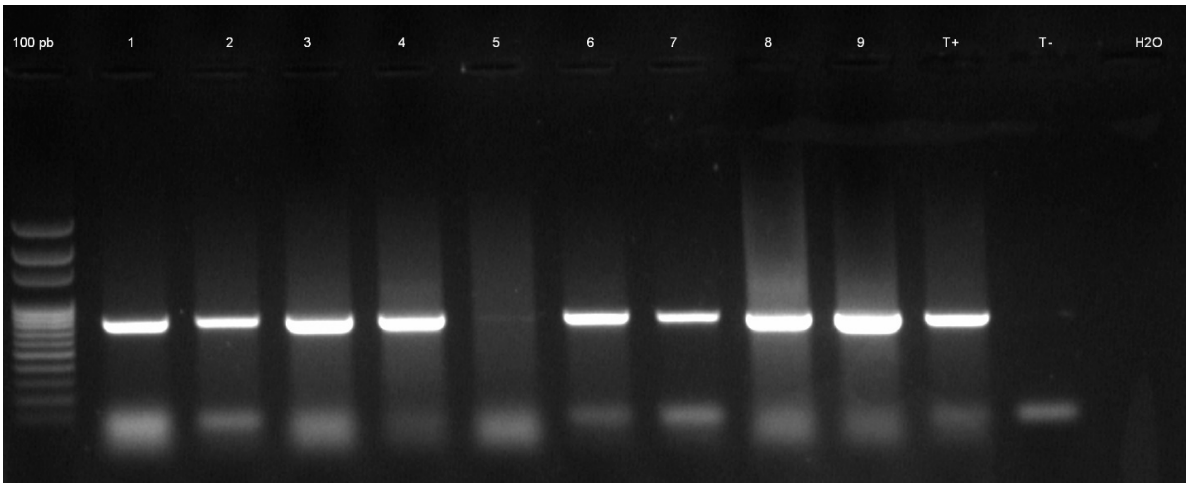


**Figure 4.** PCR amplification of cassava DNA using JSP001/JSP002. ACMV M=DNA ladder, ACMV detection at 783 bp=lanes 1-4, and 6-9; positive control=lane $T^+$; negative control=lane $T^-$; and water=lane $H_20$.

The single infection of ACMV-like virus predominated in all surveyed regions, with the highest proportion (93.1%, 27/29) in the western area, followed by the north region (87.7%, 71/81), whereas the south had the lowest of 64.4% (47/73). The mixed infection occurred in all the regions, with the highest proportions in south (35.6%, 26/73) and the lowest of 6.9% (2/29) was detected in the western area. The single infection of EACMV-like virus was found in the northwest (5.0%, 3/60), and east (13.2%, 1/68) regions, whereas single infection of EACMV-like virus was not detected in the regions of south, north and western area (Table 2). Of the 68 EACMV-like virus positive samples (single and mixed infections), 70.6% (48/68) tested positive for EACMVCM variant using the primer pair VNF031/VNF032.

**Table 2.** Distribution of begomoviruses across regions of Sierra Leone.

| | | | ACMV-single infection | | EACMV-single infection | | Mixed infection (ACMV/EACMV) | | EACMV Cameroon variant | |
|---|---|---|---|---|---|---|---|---|---|---|
| Region | Tested samples | Positive samples | n | % | n | % | N | % | n | % |
| South | 112 | 73 | 47 | 64.4 | 0 | 0.0 | 26 | 35.6 | 26 | 100.0 |
| East | 81 | 68 | 53 | 77.9 | 1 | 1.5 | 14 | 20.6 | 8 | 53.3 |
| North | 110 | 81 | 71 | 87.7 | 0 | 0.0 | 10 | 12.3 | 6 | 60.0 |
| Northwest | 79 | 60 | 45 | 75.0 | 3 | 5.0 | 12 | 20.0 | 8 | 53.3 |
| West | 44 | 29 | 27 | 93.1 | 0 | 0.0 | 2 | 6.9 | 0 | 0.0 |
| **Total** | 426 | 311 | 243 | 78.1 | 4 | 1.3 | 64 | 20.6 | 48 | 70.6 |

n=sample size; %=percent.

Figures 5 and 6 show the distribution of cassava mosaic begomoviruses detected in smallholder cassava farmers' fields of Sierra Leone. The ACMV single infection is widely spread across the districts of Sierra Leone represented by green geo-reference points, followed by ACMV/EACMV mix infection (with red geo-reference points) which is widely spread particularly in the south and north regions. The EACMV Cameroun virus infection, illustrated by a grey color triangular shape, is widely

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spread in the northwest region and some parts in the south and east regions, while the EACMV single infection is only found in the east and the northwest regions with geo-reference points in yellow.

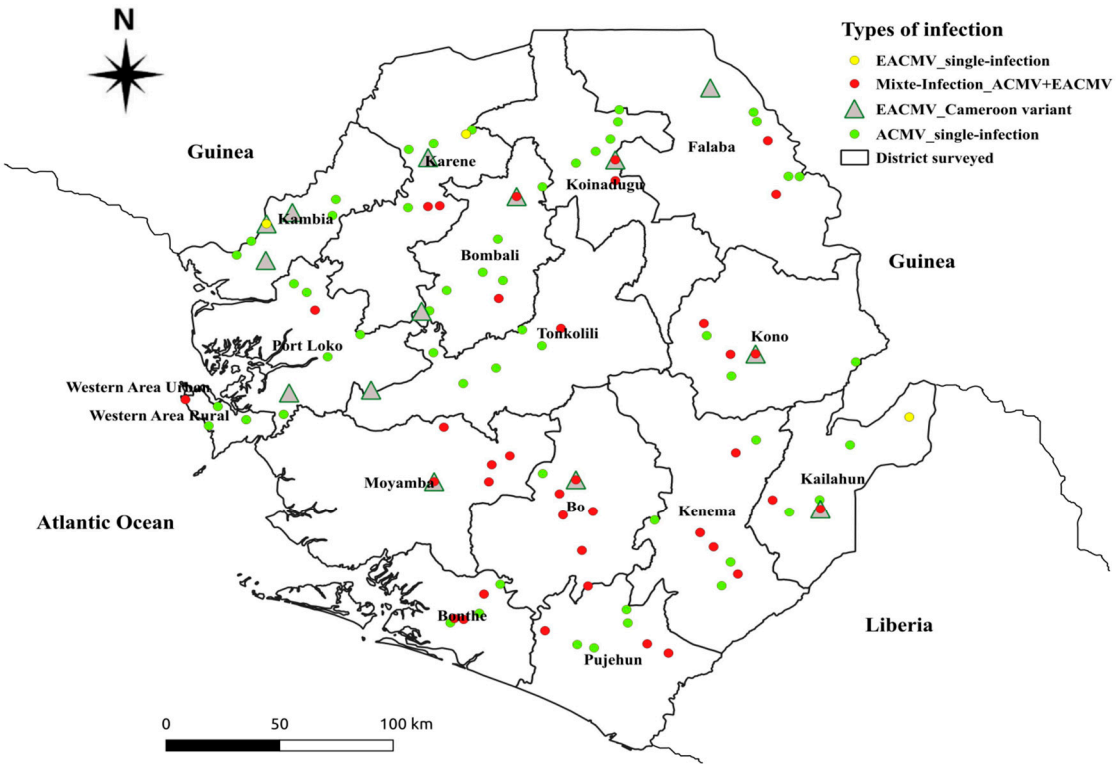


**Figure 5.** Distribution of cassava mosaic begomoviruses isolates in study areas of Sierra Leone.

The distribution map of the CMBs (ACMV, EACMV, EACMCMV, and EACMV-Ug) detected in this study is shown in Figure 6. Results revealed that five out of the 426 samples screened were infected by EACMV-Ug. These EACMV-Ug infected samples were detected in cassava farms in Moyamba, Port Loke, Falaba, and Kono districts. These districts share a border with Guinea, except the Moyamba district.

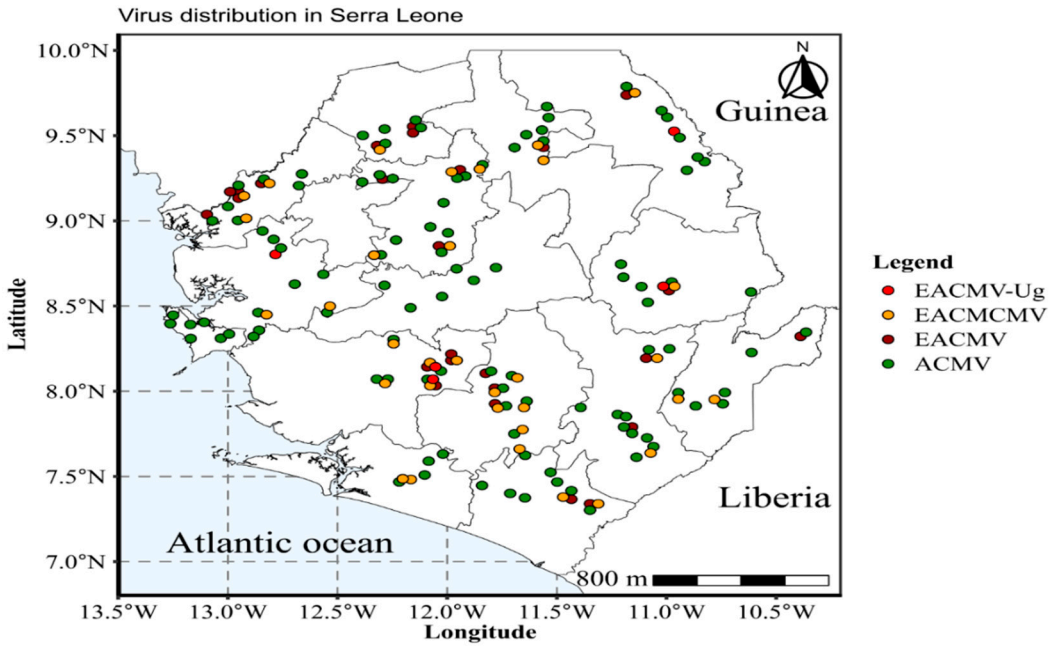


**Figure 6.** Type of infection cassava mosaic disease across Sierra Leone during 2024.

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*2.3. Confirmation of CMGs Identity by Sequencing*

A search for related sequences in the GenBank database (NCBI, BLASTN) revealed the existence and spread of Ug-variant in four districts with red-like star geo-reference points (Figure 7). Sequences of 40 cassava samples detected as positive for the ACMV-like virus were most closely related to ACMV, EACMV_Ug, EACMV_Cameroon variant, EACMV_Nigeria variant, and EACMV_Ghana variant. The EACMV Cameroon virus is widely spread across the country, with geo-reference points in yellow, and is distributed mainly in the south and north regions of Sierra Leone. The blast results showed few EACMV Nigeria, Ghana, and Kenyan variants.

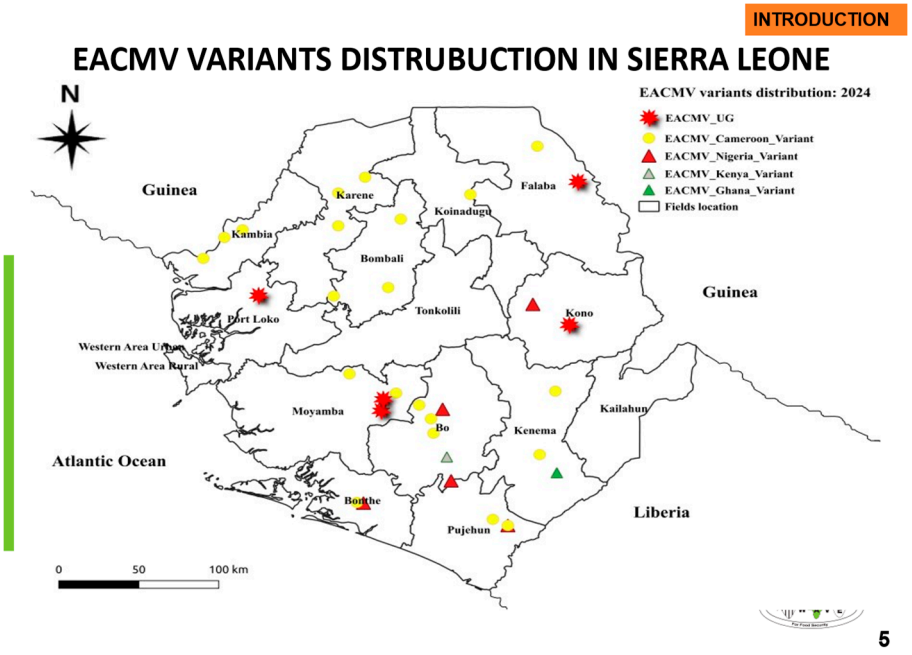


**Figure 7.** Distribution of East African cassava mosaic virus variants in Sierra Leone.

## 3. Discussion

The detection and confirmation of EACMV-Ug and its spread in four different districts in Sierra Leone seriously threaten cassava production in the country. This virus was first detected in Forécariah, Guinea, located near the border of Sierra Leone and in Kambia district, north region of Sierra Leone [24]. Kambia is about 11 km from the Guinean border, whereas Forécariah is 34.8 km from the Sierra Leone border. Considering trade and high traffic activities between this border town of Forécariah, Guinea and Sierra Leone, it is probable that EACMV-Ug was introduced into the country from Forécariah through the exchange of cassava planting materials. The EACMV-Ug virus was found in single and mixed infections with ACMV. Several authors opined that co-infection of ACMV and EACMV-Ug could result in a synergistic interaction that cause epidemics and a severe impact on cassava as typified by the incidence in East Africa during the 1990s [25–28]. Accordingly, the blast *analysis of the full-length nucleotide sequences of EACMV-Ug revealed a trans-replication between the DNA molecules circulating in Guinea and Sierra Leone, which are very similar to EACMV-Ug2 DNA-A and EACMV-Ug3 DNA-B described in Uganda* [25]. The reassortant virus resulting from this association is a severe EACMV-Ug variant favored over the existing less severe EACMV-Ug1 strain. This reassortant virus causes very severe CMD symptoms. The analytical observations depicted close relatedness of the EAMCV-Ug strain from Guinea and Sierra Leone to the one discovered during the Ugandan epidemic. This emerging biotic constraint is a threat to the economy and resource-poor cassava growers of Sierra Leone, considering the devastation caused by EACMV-Ug in cassava fields in Uganda during the 1990s epidemic. The present epidemiological study also revealed a high incidence of CMD in the country, which could be attributable to the continuous growing of

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susceptible cassava genotypes or using diseased planting materials [29]. Findings showed that CMD single incidence varied greatly amongst regions. The highest CMD single infections, 87.6%, were observed in four districts in the north region, which indicates the spreading risk potentialities of the CMD to new areas. This assertion is in conformity with [30], who opined that disease spread is largely attributable to the planting material exchange by cassava producers without following due procedures of ascertaining their phytosanitary status. The lowest CMD detection, 64.45%, was found in the south region involving four districts, possibly attributable to the high adoption and utilization of improved cassava varieties resistant to CMD [31]. The east region recorded the highest of 78% ACMV-single infection for three districts compared to the northwest region, which recorded 75% ACMV-single infection for three districts, and the western area, which had two districts, recorded the highest of 93.1% ACMV. For EACMV-single infection across the five regions, the northwest region recorded the highest of 5%, followed by the east region, which recorded about 1.4%. All the other regions recorded no EACMV-single infection. Report on mix-infection across the five regions of Sierra Leone the south region recorded the highest rates of EACMV-single infection with 35.6% in four districts, where the Uganda virus was found in Moyamba, followed by the east region with 20.6% while the northwest region recorded third highest rates of EACMV-single infection with a recorded of 12.3% and western area recorded the lowest EACMV-single infection across Sierra Leone with a 6.9%. The Cameroon virus infection across the five regions was widespread across the country, with the southern region recording 100% of all cassava fields visited, followed by the north region, which recorded about 60% and 53.3% for both east and northwest regions (0%). Findings of the molecular detection analytical technique based on the samples collected during the 2024 survey in Sierra Leone showed that the country is increasing its hotspot potentiality of cassava begomoviruses diversity in the West African region. Indeed, four begomoviruses, including ACMV, EACMV, EACMCMV, and EACMV-Ug, were previously found in single, double, and triple infections in Guinea and Sierra Leone [24]. This is the second report on such multiple associations of cassava mosaic begomoviruses in cassava plants in Sierra Leone because a recent publication has shown the Uganda virus incident in Sierra Leone [24]. It reflects the importance of CMD pressure on cassava production in Sierra Leone and raises serious concerns about the origin of the diverse CMBs detected in this country. The ACMV was the most detected disease in all the five regions studied in Sierra Leone, as reported for almost all the sub-Saharan African countries where CMD occurs [32,33]. Although triple infections were found in all the regions in Sierra Leone, the southeast and northwest regions registered the highest number of plants infected by ACMV+EACMCMV+EACMV-Ug associated with very severe CMD. The synergistic action among the three viruses involved in this triple co-infection probably contributed to the increased symptom severity, as mentioned by Harimalala et al. [31]. In Burundi, triple infections ACMV+EACMV+EACMV-Ug were also detected [34]. In Guinea, EACMV-Ug was detected in four regions, including the one sharing border with Sierra Leone [24]. An alarming southeast and northwest spread of EACMV-Ug in four different districts in Sierra Leone is similar to the southward spread in Uganda during the CMD epidemic mentioned in several studies [25,34,35]. The blast analysis of EACMV-Ug DNA-A sequences from Sierra Leone showed close relatedness to those from East and Central Africa. Moreover, the EACMV-Ug sequences from Guinea and Sierra Leone contain the same ACMV recombinant fragment in their coat protein (CP) indicating that it is the same reassortant virus EACMV-Ug2 DNA-A + EACMV-Ug3 DNA-B that is circulating in these countries. Findings suggest that this virus was probably introduced into Guinea from East or Central African countries via infected cassava planting materials. During the 2024 field survey, most of the cassava varieties cultivated in Sierra Leone were local varieties, and these cassava varieties were all found susceptible to CMD with ACMV detected in all varieties. However, some were more susceptible than others. These results advocate for the region's urgent deployment of CMD management strategies and CMD-resistant varieties.

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## 4. Materials and Methods

### *4.1. Surveyed Experimental Sites*

Sierra Leone, a country located on the west coast of Africa between latitudes 7° and 10° N and longitudes 10° and 13° W, offers a rich and complex field of study encompassing disciplines such as history, political science, anthropology, economics, environmental studies, and public health. Covering a total land area of approximately 71,740 km$^2$, Sierra Leone boasts a diverse landscape with a wide array of geographical features important for survey studies. The western region features flat coastal plains extending inland for about 100 km, characterized by mangrove swamps and river deltas. As one moves inland, the terrain becomes more rugged, with the central and eastern parts of the country dominated by hills and mountains. The Loma Mountains, including the highest peak, Mount Bintumani, at 1,945 m, provide significant elevation. Dense tropical rainforests cover much of the eastern region, contributing to biodiversity and ecological studies. Sierra Leone borders Guinea to the north and northeast, with a border length of approximately 652 km, and Liberia to the southeast, with a border length of about 306 km. The Atlantic Ocean forms the western boundary, providing a coastline of about 402 km, critical for marine and coastal surveys. The country experiences a tropical climate with a wet season from May to November characterized by heavy rains, receiving annual precipitation ranging between 2,000 and 3,000 mm, with the coastal areas receiving the highest amounts. From December to April, the dry season is marked by lower precipitation and the harmattan winds, affecting visibility and survey conditions. The map of study area showing regions, districts and cassava farms is presented in Figure 8.

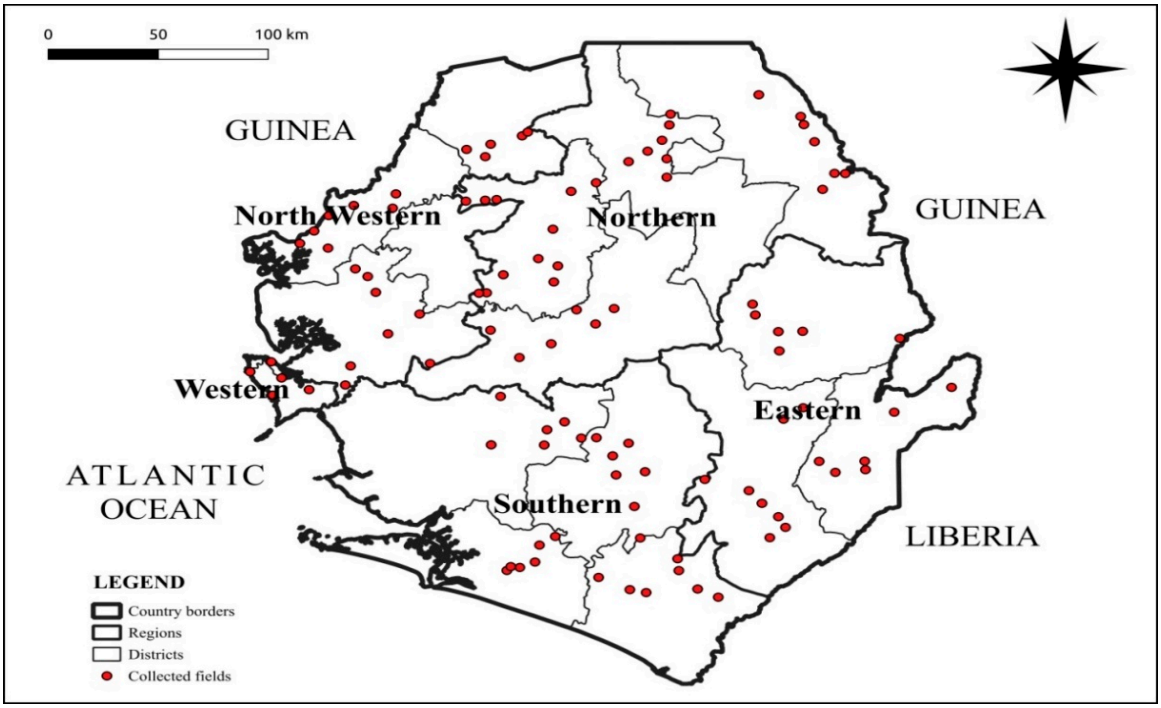


**Figure 8.** Map of study area showing country borders, regions, districts and fields.

### *4.2. Survey Design*

Cassava mosaic disease assessment survey was conducted in 2024 using the Central and West African Virus Epidemiology (WAVE) harmonized sampling and diagnostic protocols [33,36]. Assessments were done in 109 farms covering five regions, sixteen districts, and agro ecologies (rain forest, coastal plains, savannah lowlands, and savannah highlands) of Sierra Leone. The Cassava fields surveyed were 10 km apart. At each farm, 30 cassava plants were randomly selected and

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evaluated along two diagonals. The geolocation coordinates of each field were recorded using a global positioning device (Garmin Dakota TM 10).

*4.3. Field Data Collection and Storage*

Tablet with iForm Zerion (version 9.1.6) software developed by Cambridge, UK's Epidemiology modelling group for survey was used for data collection in all West African virus epidemiology participating countries. Data collected included the name of the village or town, the district, region, whitefly counts, cassava mosaic disease symptom observed, geographical coordinates (latitude and longitude), mode of infection, and altitude. Data was collected on variety, date and time, field size, planting types, and distance between survey sites. The recorded data were uploaded to iForm's cloud-based database and integrated into the WAVE Cube. Data collected were CMD severity, whitefly abundance, and mode of infection (i.e. either cuttings or vector). According to Sseruwagi et al. [13], a distinction between cutting-borne and whitefly-borne infections is possible from three to six MAPs. Symptoms found only on the upper leaves of plants were considered to have resulted from whitefly-transmitted infection, whereas symptoms on the lower leaves or on all leaves were taken as having been infected through cassava cuttings.

The cassava disease severity evaluation was calculated using the 1-5 disease rating scale (Figure 9); where 1 = symptom-less plants; 2 = mild chlorotic patterns affecting most leaves, mild distortions at the bases of most leaves and remaining part of the leaves are typical; 3 = pronounced chlorosis on most leaves, narrowing and distortion of the lower one-third of the leaflets; 4 = severe chlorosis and distortion of two-thirds of most leaves and general reduction of leaf size and some stunting; and 5 = most severe symptoms (severe chlorosis, leaf distortion, twisting, misshapen leaves, severe reduction of most leaves and severe plant stunting) [37]. The CMD incidence was calculated as the quotient of CMD symptomatic plants over the total number of plants expressed as a percentage following the formula by Sseruwagi et al. [13].

Mean incidence (%) = $\frac{\sum \text{Infected plants}}{\sum \text{plants}} \times 100$. The percent incidence was scored using the diseases rating scale in which fields with 0% incidence were recorded as healthy; >0–25% as low incidence; >25–50% as medium incidence; >50–75% as high incidence; and >75–100% as very high incidence. Whitefly abundance was calculated by counting whiteflies on top five fully opened leaves. The mean of whiteflies per plant was calculated as the total number of whiteflies recorded on 30 plants divided by 30.


**Figure 9.** Cassava showing (a) a healthy-looking plant; (b-e) cassava mosaic disease (CMD) infected plants.

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*4.4. Molecular Detection*

Cassava leave samples were utilized for the total DNA extraction using the CTAB protocol [38]. The DNA concentration was carried out using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) by adjusting the equipment to 150 ng/µl. Previous studies noted that the most cumbersome CMBs in smallholder cassava cultivation systems in Sierra Leone were ACMV and East African cassava mosaic virus (EACMV) [23]. For detection of the ACMV-like and EACMV-like viruses, the DNA samples were subjected to PCR using specific primers (Table 3). The positive samples for EACMV-like virus were subjected to another round of PCR using specific primers to detect EACMVCM. The PCR mix was prepared in a final volume of 25 µl using 20.9 µl of molecular biology grade water, 2.5 µl of 10× reaction buffer, 0.5 µl of 10 mM dNTPs, 0.5 µl of 10 µM of each primer, 0.1 µl of 5 U/µl of Maximo Taq DNA polymerase (GeneON), and 150 ng DNA template of each sample. The DNA amplification was carried out in a SimpliAmp thermal cycler (Life Technologies Holdings Pte Ltd). The PCR temperature profile was set at 94°C for 4 or 5 min for initial denaturation, followed by 35 cycles of amplification at 94°C for 45 or 60 s, 55°C for 45 or 60 s, and 72°C for 55 or 60 s (depending on primers). The final elongation step was performed at 72°C for 7 or 10 min. The PCR-amplified products were subjected to 1% agarose gel electrophoresis and then stained with ethidium bromide. The electrophoresis was performed at 100 V and the gel was visualized using a Compact Digimage System, UVDI series (MS major science).

**Table 3.** Details of primer pairs utilized for detection of virus species.

| Primer | Sequence (5′-3′) | Target region | Expected size (bp) | Virus species | Reference |
|---|---|---|---|---|---|
| JSP001 | ATGTCGAAGCGACCAGGAGAT | DNA-A (CP) | 783 | ACMV | Pita et al. [25] |
| JSP002 | TGTTTATTAATTGCCAATACT | | | | |
| ACMBVF | TCGGGAGTGATACATGCGAAGGC | DNA-B (BV1/BC1) | 628 | ACMV | Matic et al. [39] |
| ACMBVR | GGCTACACCAGCTACCTGAAGCT | | | | |
| WAVE-508F | AAGGCCCATGTAAGGTCCAG | AV1/AC3 | 800 | ACMV | WAVE |
| WAVE-1307R | GAAGGAGCTGGGGATTCACA | | | | |
| WAVE-177F | GATCTGCGGGCCTATCGAAT | BV1 | 800 | ACMV | WAVE |
| WAVE-197R | TTCACGCTGTGCAATACCCT | | | | |
| WAVE-370F | ACAGCCCATACAGGAACCGT | AV1/AC3 | 1000 | ACMV | WAVE |
| WAVE-1369R | CGACCATTCCTGCTGAACCA | | | | |
| WAVE-982F | TTCGTGTCATCTGCAGGAGA | BV1/BC1 | 800 | ACMV | WAVE |
| WAVE-1781R | GTACCATGGCAGCTGCTGTA | | | | |
| JSP001 | ATGTCGAAGCGACCAGGAGAT | DNA-A (CP) | 780 | EACMV | Pita et al. [25] |
| JSP003 | CCTTTATTAATTTGTCACTGC | | | | |
| CMBRepF | CRTCAATGACGTTGTACCA | DNA-A (AC1) | 650 | EACMV | Alabi et al. [40] |
| EACMVRepR | GGTTTGCAGAGAACTACATC | | | | |
| WAVE-EA1875F | TGTACCAGGCGTCGTTTGAA | AC1 | 800 | EACMVCM | WAVE |
| WAVE-E2674R | TGTCCCCCGATCCAAAACG | | | | |
| WAVE-EB1869F | TTCCAAGGGGAGGGTTCTGA | BC1 | 800 | EACMV | WAVE |

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*4.5. Data Analysis*

4.5.1. Phenotypic Analysis

The phenotypic recorded were subjected to analysis in R software v. 3.6.1 (R Development Core Team). Normality of phenotypic variables was estimated through the Shapiro–Wilk test. The generalized linear model was used for variables that were not distributed according to the normal distribution. The difference in the number of whiteflies per plant between regions and in the severity score of CMD between regions were assessed using the generalized linear model. The map of Sierra Leone showing the regions where surveys were done in 2024 was developed using QGIS software v. 2.18.26 (https://qgis.org/downloads/).

4.5.2. Molecular Analysis

About 50 EACMV-like positive sample PCR products were sequenced in forward and reverse orientations at the Macrogen Meibergdreef 57 1105 BA, Amsterdam, the Netherlands, Europe, using the Sanger et al. [41] method. This was followed by assembling and editing of contigs through the Geneious Prime® 2022.2.1. (Biomatters Ltd, Auckland, New Zealand.) software. Consensus sequence obtained from forward and reverse sequences for each sample was subjected to BLASTn in NCBI for preliminary species assignment and pairwise sequence comparison [42]. Sequence alignments with representative isolates of begomoviruses was done through the ClustalW alignment method in MEGA X software [43].

## 5. Conclusions

This study identified and mapped the distribution of CMD-associated viruses (begomoviruses) in farmers' fields in Sierra Leone that could be exploited for effective phytosanitation strategies to mitigate their effect on crop yield loss. The cassava farms in the east region, north region, northwest region, and western areas were more susceptible to the disease attack due to heavy utilization of infected cutting planting materials. The spread of the disease is linked to the use of infected planting materials, mainly through stem cuttings. This is the first report on the spread of EACMV-Ug infecting local cassava varieties in smallholder farms across south, north and northwest regions of Sierra Leone. Identification of EACMV-Ug in four districts, three of which sharing border with Guinea, presents a serious threat to cassava production in the country. Findings suggest timely implementation of rapid mitigating strategies by national and regional cassava stakeholders in Sierra Leone and the subregion. Routine cassava farm disease status assessment should be exploited to ascertain the spread of EACMV-Ug. Awareness campaigns should be done to various stakeholders, including farmers, extension officers, and policymakers, on the importance of CMD, identification of the disease symptoms, and best practices to mitigate its spread. Efficient disease response and phytosanitation (i.e. seed certification and deployment of resistant genotypes) strategies should be implemented to limit the spread of EACMV-Ug in Sierra Leone and the ECOWAS region.

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**Conflicts of Interest:** The authors of this study have no conflicts of interest to declare.

## Abbreviations

This study utilized the following abbreviations:

| | |
|---|---|
| ACMV | African Cassava Mosaic Virus |
| CMD | Cassava Mosaic Disease |
| CTAB | Cetyltrimethylammonium bromide |
| DNA | Deoxyribonucleic acid |
| EACMV | East African Cassava Mosaic Virus |
| PCR | Polymerase chain reaction |

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