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Phytosanitary Situation of *Maize streak virus* in the Main Maize Production Zones of Cameroon

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Abstract

Maize streak caused by the *Maize streak virus* (MSV, genus *Mastrevirus*) is transmitted by *Cicadulina* spp., and is responsible for considerable maize yield losses in all maize production zones in Africa, including Cameroon. A survey was conducted in 3 agro-ecological zones (AEZ) of Cameroon (Sudano-Sahelian: zone I, Western Highlands: zone III and Bimodal Rainforest: zone V) between November 2017 and November 2019 to determine the status of streak disease in maize farms. The incidence and severity were determined in 90 maize fields, 30 fields per AEZ; the effect of lightning on the disease was also assessed using 15 fields under shade and 15 opened fields per AEZ. The highest streak disease incidence (60%) was found in AEZ I, whereas the lowest incidence was 10% in AEZV. The highest disease incidence and severity (80% and 4.5 respectively) were observed in maize fields under shade as compared to open fields (70% and 4.5 respectively). The phylogenetic analysis of MSV sequences from symptomatic plants indicated it as MSV-A strain identical to be previously reported to determine the virus diversity in relation to the other characterization isolates. This information is important for the development of control strategies to limit yield losses due to MSV.

Keywords

Maize streak virus, Incidence, Severity, Agro-Ecological Zone, Cameroon

1. Introduction

Maize (*Zea mays* L.) is an important staple food for millions of people and is an

important source of carbohydrates in sub-Saharan Africa [1]. It is also recognized as a staple food ingredient that can provide up to 30% protein, 60% energy and 90% starch in the diet of animals [2]. It is cultivated in all 5 agro-ecological zones of Cameroon. It is the most consumed cereal in Cameroon ahead of rice and sorghum [3]. Among the growing crops in Cameroon, maize occupies the first place. Its increasing demand for animal nutrition and agro-industries has led farmers to take a greater interest in it. Although the number of producers over the years has been increasing and more than one million farmers grow maize as their main crop, its production remains insufficient to satisfy the demand. Maize demand in Cameroon for animal feed and human consumption is estimated at 1,350,000 tons [4]. The annual national demand is estimated at 2.8 million tons for a production of 2.2 million tons in 2019, *i.e.* a deficit of 600,000 tons covered by imports [5] [6]. Although maize is the most important cereal in Cameroon, its production faces abiotic and biotic constraints such as viral diseases. Three main viruses are reported on maize crops in tropical regions: *Maize Stripe Virus* (MStpV); *Maize Mosaic Virus* (MMV) [7] and *Maize streak virus* (MSV) [8] [9]. MSV is the most widespread, and is transmitted by several species of the *Cicadulina* insects, the most widespread being *Cicadulina mbila*. The advanced symptoms are yellow and elongated chlorotic streaks. These viruses contribute to considerable yield losses in tropical Africa [10]. An epidemic of maize streak disease (MSD) has been reported in many countries in tropical Africa and causes yield losses ranging from 30% to 100% [11]. The increase in production in Cameroon is due to the increase in cultivated areas and not to the increase of productivity. However, the control of disease parameters such as *Maize streak virus* in the different agro-ecological zones of Cameroon could allow the development of control strategies to improve productivity.

Molecular identification of *Maize streak virus* in Cameroon revealed the first evidence for a subtype A1 isolate; however, this work was performed only in a part of AZE I [12]. Knowledge about MSV disease parameters in different agro-ecological zones of the country is required for the development of control strategies.

The objective of this study was to determine the status of *Maize streak virus* in different agro-ecological zones of Cameroon in order to develop control strategies.

2. Materials and Methods

2.1. Description of Study Areas

A survey was carried out between November 2017 and November 2019 in three localities of each of three agro-ecological zone (AEZ) of Cameroon which are the main maize production zones of Cameroun: AEZ I (Mogodé, Figuil, Garoua), AEZ III (Foumbot, Dschang, Ndop) and AEZ V (Ntui, Mbalmayo, Obala) (Figure 1). The characteristics of each AEZ are presented in Table 1.
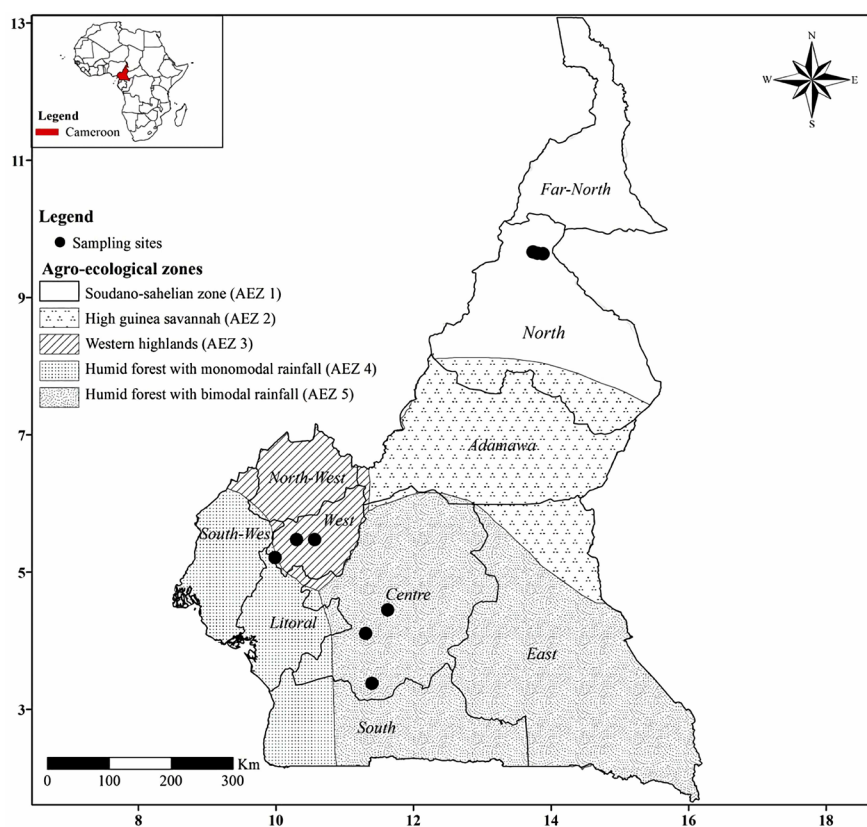
Figure 1. Presentation of agro-ecological zones of Cameroon [13].

Table 1. Characteristics of agro-ecological areas.

Features	Geographic Situation	Altitudes (m)	Precipitation (mm)	Temperature ˚C	Maize status
AEZ I	8˚36" to 12˚54"N 12˚30" to 15˚42"E	200 - 600	500	28 - 45	Main crop
AEZ III	4˚54" to 6˚36"N 9˚18" to 11˚24"E	1000 - 2800	2000	12 - 26	Main crop
AEZ V	2˚6" to 5˚48"N 10˚30" to 16˚12"E	500 - 1000	1600	20 -35	Main crop

2.2. Evaluation of the Incidence and Severity of Maize Streak Disease in the Three Agro-Ecological Zones of Cameroon

The incidence and severity of maize streak disease (MSD) were assessed at the flowering time in 90 fields of at least 0.5 ha. The work was performed in 10 fields randomly selected in each locality during dry and rainy seasons in 2018 and 2019. The fields were separated from each other by a distance of 2 km.

1) Evaluation of MSD incidence

Within each field, strings were used to draw 100 m^2 (10 m × 10 m) quadrats so that the "W" pattern was used for the impact assessment. The number of plants attacked in each quadrat were recorded and the disease incidence was calculated using the following formula:

$$\text{Disease incidence}(\%) = \frac{\text{number of plants attacked}}{\text{total number of plants inspected}} \times 100$$

2) Evaluation of MSD severity

The disease severity was assessed visually using the semi-quantitative scale of Bello *et al.* [14] Donson *et al. [15]* which ranges from 1 to 5 depending on the severity of symptoms:

1: the plant presents a few chlorotic spots visible during a very detailed inspection;

2: the plant shows an easily visible but slight streak;

3: 60% of the plant shows a striation;

4: 75% of the plant shows a significant streak with dwarfism;

5: more than 75% of the plant is fully attacked in a very severe manner with very important dwarfism.

2.3. Sample Collection and Molecular Analyses

Symptomatic young leaves without fungal lesions of approximately 2 inches were collected, wrapped in hydrophilic paper, inserted in labelled envelopes and kept for molecular analysis.

2.4. Serological Analysis of Leaves Sample for Maize Streak Virus Detection

ELISA test was performed on maize leaf samples to confirm the presence of MSD before proceeding with molecular analysis using the protocol described by Clark, and Adams [16] and Thottappilly *et al.* [17].

2.5. DNA Extraction

DNA extraction was carried out at the IITA in Ibadan from fresh MSV infected leaf samples using a modified CTAB method [18].

30 to 50 mg dry corn leaves were crushed using a Vortex. To do so, the dry leaves were put in a 2 ml tube then 2 to 3 beads were added and placed on the vortex rack for grinding until a very fine powder was obtained. Then, after removing the beads, lysis buffer preheated to 60°C in a water bath was added. The cell lysis buffer consists of the following components: Cetyl Trimetyl Ammonium Bromide (CTAB) 2%, NaC l 1.4 M, Glucose 0.5 M, Etylene Diamine Tetra acetic Acid (EDTA) 20 mM and Tris-HCl or Trizma base pH8 100 mM. We used chloroform-isoamyl alcohol in the ratio 24:1 for the precipitation of cellular proteins, then isopropanol for the precipitation of nucleic acids and finally 70% ethanol for their washing. The extracted DNA was suspended in 100 µl sterile distilled water and stored at −20°C for analysis.

2.6. Amplification

The extracted genomic DNA was amplified by PCR using the following primers: MSV 1770-1792 (C1): MSV 215-234 (C2): 5'-TTGGVCCGMVGATGTASAG-3';

and 5'CCAAKDTCAGCTCCTCCG-3' where the ambiguity codes are M = A or C; K = G or T; S = C or G; V = A or C or G; and D = A or G or T. These primers for C2/C1 ORF regions are described by Van Antwerpen and Rutheford [19]. The primers amplified the fragments to 900 bp. PCR was performed using an AMP PCR System 9700. Amplification was performed in a reaction of 25 μl containing 2 μl of DNA; 3.5 μl of primer; 0.625 μl of 10 mM dNTP mix; 0.16 μl of taq DNA polymerase; 2.5 μl of buffer 3.5 μl of 25 mM Mg Cl2. The PCR was performed with 35 cycles of 94˚C for 5 minutes, 60˚C for 1 minute, 72˚C for 2 minutes, 72˚C for 10 minutes. The PCR products were evaluated by electrophoresis in 1.2% agarose gel in TEB buffer soaked with ethyl bromide and visualized under ultraviolet light.

2.7. Purification and Sequencing

The reaction medium to be purified was subjected to an extraction with phenol-Tris/chloroform/isoamyl alcohol (respective proportions: 25/24/1) then the DNA was precipitated by addition of 2 volumes of absolute ethanol in the presence of 200 mM NaCl and 5 μg glycogen. After centrifugation at 4˚C for 15 min at 15,000 rpm, the DNA pellet was washed with 70% ethanol, dried and resuspended in water.

Sequences were assembled and edited using MEGA 6, MSV genome sequences were obtained from GenBank. Sequence sub-sequence realignment tool implemented in MEGA. Phylogenetic trees were constructed by the neighbor-joining (Jules-Cantor distances, 1000 bootstrap replicates) and maximum-likelihood (Hasegawa-Kishino-Yano model, transition/trans version ratio inferred from the data and 100 bootstrap replicates) methods implemented in MEGA respectively. Recombination was analyzed using the RDP, Default settings were used throughout and only potential recombination events detected by the above method coupled with phylogenetic evidence of recombination were considered significant. Also, the severity of Bonferroni correction during detection was minimized by only searching for recombination signals in a single sequence within groups of sequences sharing 99.7% sequence identity. Composite likelihood estimates (CLEs) of population-scaled recombination rates and estimates of population-scaled mutation rates were inferred using the PAIRWISE component of LDHAT. Site-to-site variation in the CLE of the population-scaled recombination rates was assessed using the INTERVAL component of LDHAT.

2.8. Data Analysis

Generalized linear models (GLMs) were used to analyze data on the incidence and severity of MSV collected in agro-ecological zones as described by Wichura [20] using JMP version 8 software. Mean values among different agro-ecological zones, and localities were separated by the Tukey-Kramer test at 5% probability threshold ($P \leq 0.05$).

To understand the implication of any new virus strain, the complete genomes

of isolates from 6 Cameroonian MSV (MSV-CAM) were compared to the reference MSV isolates available at IITA, Ibadan and those available in the gene bank of the National Center for Biotechnology Information (NCBI).

The phylogenetic tree of 6 MSV isolates compared to the IITA reference isolate MSV was constructed using the maximum likelihood method based on the model of Kumar & Nei [21]. The branches corresponding to partitions reproduced in less than 50% of the bootstrap replicates (N = 1000) are reduced. All positions containing gaps and missing data were removed.

3. Results

3.1. Incidence of MSD

The incidence of MSV in the different agro-ecological zones during the years 2018 and 2019 is presented in Figure 2(a). Agro-ecological Zone V had the lowest incidence in both seasons, while AEZ I had the highest incidence, although there were seasonal differences. In 2018, the highest incidence was observed in the dry season in all AEZ zones (29%, 44% and 58% in AEZ V, AEZ III and AEZ I respectively) compared to the wet season (7%, 26% and 46% in AEZ V), In 2019, the dry season had the highest incidence in all AEZs (17%, 42% and 50% in AEZ V, AEZ III and AEZ I respectively) compared to the rainy season (7%, 31% and 42% in AEZ V, AEZ III and AEZ I respectively) (Figure 2(b)).

Plot lightning had a significant effect on the MSD incidence in all agro-ecological zones. Shaded plots had significantly higher incidences than opened plots (Figure 3).

3.2. MSD Severity

The severity of MSD in the different agro-ecological zones is shown in Figure 5. For both years the severity did not vary at all. The highest severity on the rating scale used was noted in Western highland in the dry season (4), which is the Sudano-Sahelian zone. The lowest severity was recorded in the rainy season in agro-ecological zone V (Figure 4).
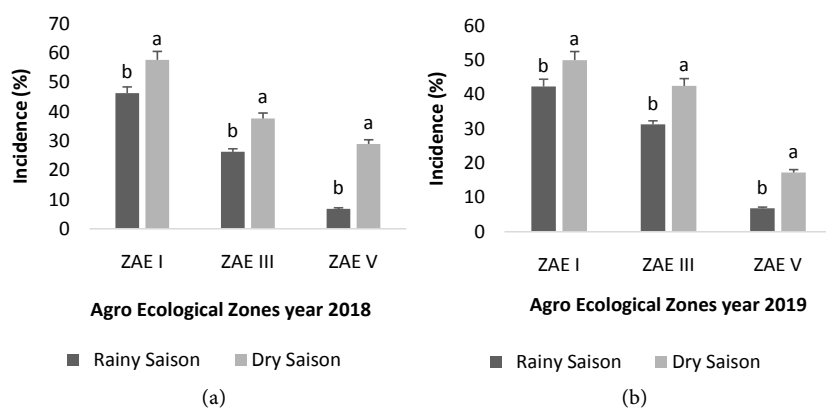

Figure 2. Incidence of maize streak disease in different agro-ecological zones during dry (a) and rainy seasons (b).
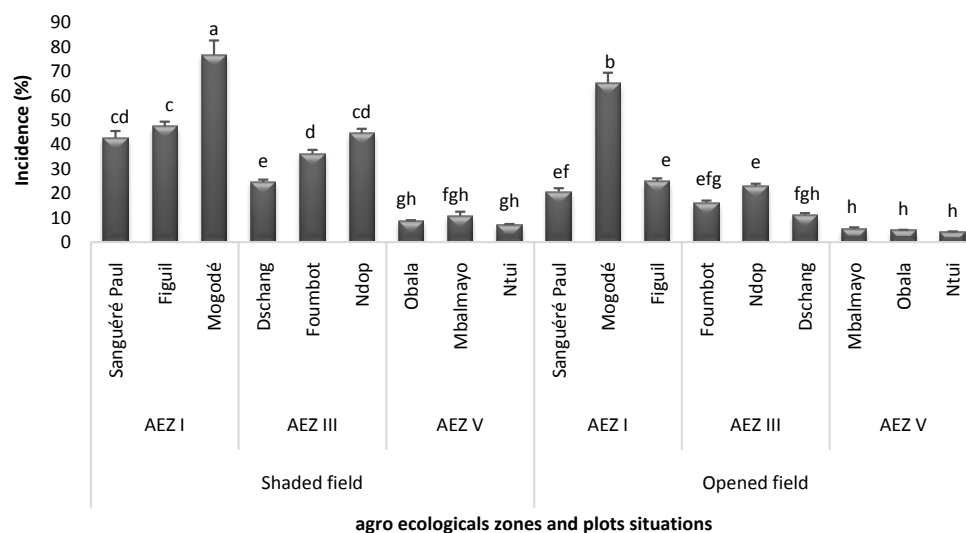
Figure 3. Incidence of maize streak disease in different localities of each agro-ecological zones according to plot lightning. Sample means, and error bars are standard errors of the mean. Mean followed by the same letter are not significantly different (Tukey's HSD test, P = 0.05). **AEZ**: Agro Ecological Zone.
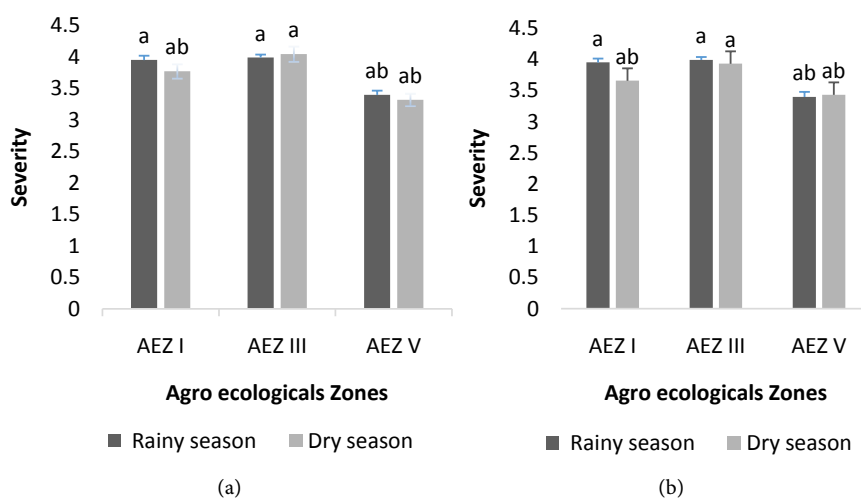

Figure 4. Severity of maize streak disease in less different agro-ecological zones in 2018 (a) and 2019 (b). Sample means, and error bars are standard errors of the mean. Mean followed by the same letter are not significantly different (Tukey's HSD test, P = 0.05). **AEZ**: Agro-Ecological Zone.

Figure 5 shows that the location of the plots has a significant effect on the severity of MSD in all agro-ecological zones. The plots that were in the shade had high severity compared to the plots that were in the open (**Figure 5**).

3.3. Epidemiological Status of *Maize streak virus* in Cameroon

The investigation realized during the two last year allowed to list 12 varieties in 2018 and 17 varieties in 2019 all coming from IRAD.

Figure 6 shows the effect of maize varieties on MSD incidence and severity of
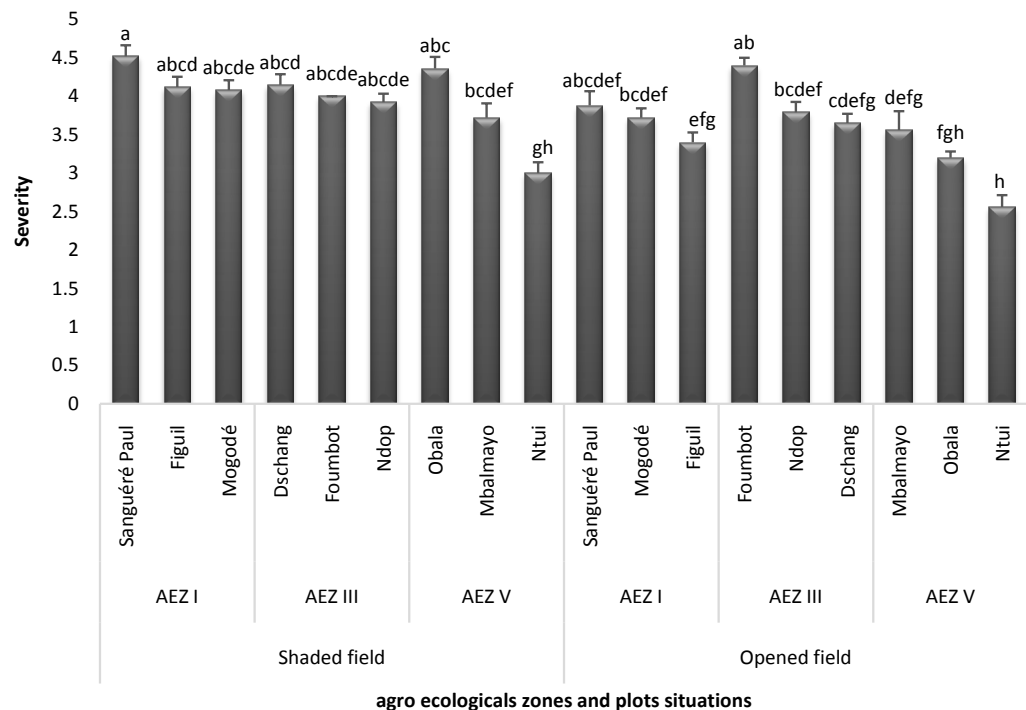
Figure 5. Severity of maize streak disease in different localities of each agro-ecological zones according to plots situations. Sample means, and error bars are standard errors of the mean. Mean followed by the same letter are not significantly different (Tukey's HSD test, P = 0.05). **AEZ**: Agro-Ecological Zones.

during the dry and rainy season survey in 2018. It can be seen that, out of the 12 varieties identified during the year, Varieties CMS8704, CMS9501 and ATP had the highest incidence (60%, 50% and 55% respectively) whereas CMS8501, Pop Corn, PVASYN13 had the lowest incidence (20%, 19% and 21% respectively). The highest MSD severity was recorded on varieties PVASYN6, ACRO and CMS9501 (score 4; 4 and 3.5 respectively).

Figure 7 shows the effect of maize varieties on MSD incidence and severity of during the dry and rainy season survey in 2019. It can be seen that, out of the 17 varieties identified during the year, Varieties CMS8704, Doux local, Shaba and ATP had the highest incidence (65%, 58%, 45% and 45% respectively) whereas Kassai, ACRO6, MADJSYN13 had the lowest incidence (15%, 16% and 18% respectively). The highest MSD severity was recorded on varieties Shaba, TZComp4 and ATP (score 4; 4 and 4 respectively).

3.4. Serological Analysis of Leaves Sample

Table 2 shows the reaction of maize leaf samples to antibodies *Maize streak virus*. It can be seen from this table that, all Cameroonian maize leaf samples tested positive for MSV in the polyclonal antibody ELISA test.
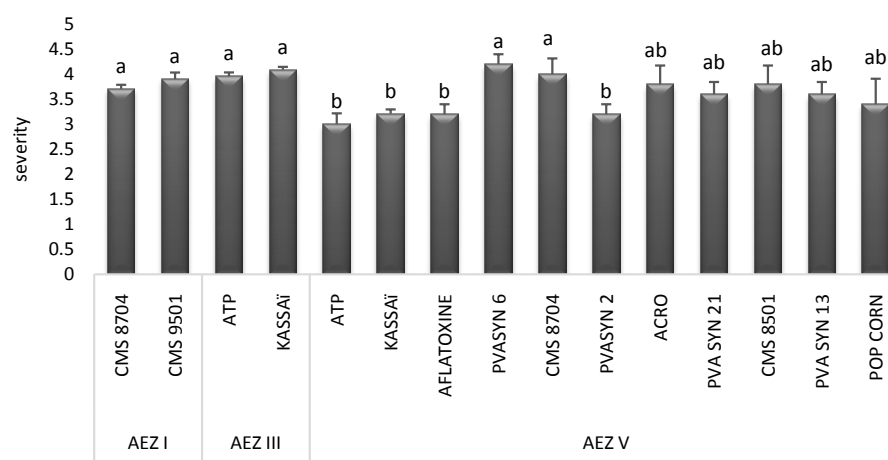
3.5. Molecular Analysis of Maize Leaves Sample

Table 3 shows that the MSV-CAM isolates were 99% identical to the dominant
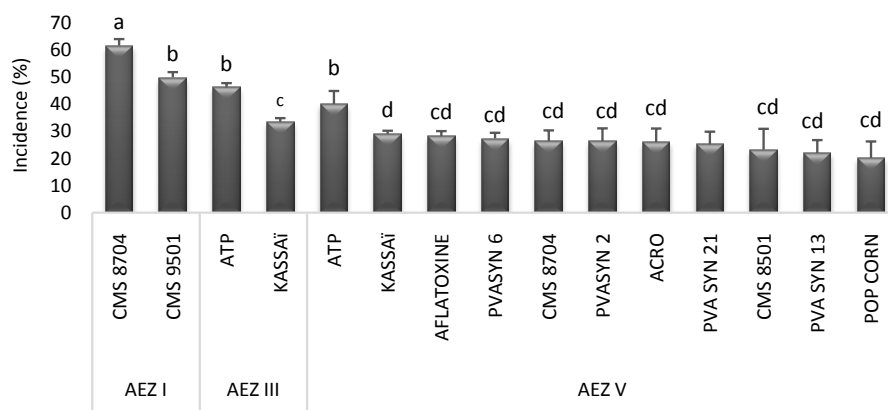
(a)

(b)

Figure 6. Incidence (a) and severity (b) of maize streak disease during the year 2018 according to maize varieties. Sample means, and error bars are standard errors of the mean. Mean followed by the same letter are not significantly different (Tukey's HSD test, P = 0.05). **AEZ**: Agro-Ecological Zones.
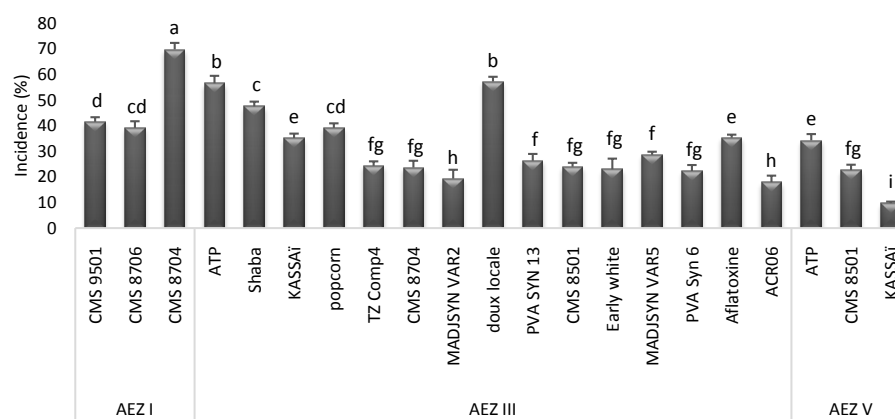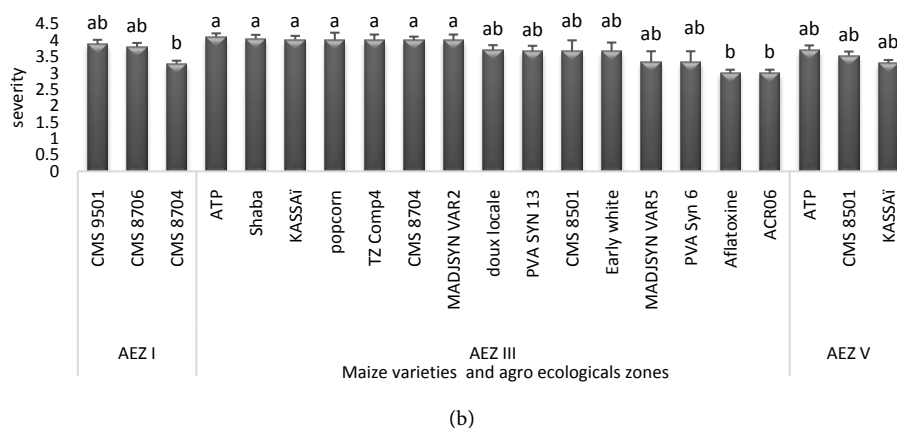

(a)

(b)

Figure 7. Incidence (a) and severity (b) of maize streak disease during the year 2019 according to maize varieties. Sample means, and error bars are standard errors of the mean. Mean followed by the same letter are not significantly different (Tukey's HSD test, P = 0.05). **AEZ**: Agro-Ecological Zones.

Table 2. Reaction of maize leaf samples to antibodies *Maize streak virus*.

Samples	A_{405nm}
West Noun ATP	1.04
M 1216-2 maroua	1.11
TZL COMP 1-WC6 maroua	1.2
EVDT maroua	2.85
Nord-Ouest Ngoketunjia ATP	1.91
M 0926-8 Garoua	1.45
M 1226-2 Mbalmayo	1.13
TZL COMP1/ZDP-SYN 1 Maroua	2.01
CMS 9015 Garoua	2.32
Obatampa soukoundou	2.53
Obatampa soukoundou	1.87
Dongamantung ATP	2.55
Ouest Menoua Kassaï	1.1
MSV Positive control	3.39
Healthy maize	0.19
Buffer control	0.18

reference isolates in the association of each variant sequence or recombinant strain in the samples tested.

Table 4 shows that there is a positive correlation between Cameroonian MSV isolates and IITA reference isolates. However, there is no correlation between Kassaï from Menoua and ATP from Dongamantung isolates, nor correlation

Table 3. Details on the MSV genes used for analysis.

Genes	Countries
MSV-IITA (reference)	Nigeria
MSV-CAM West Menoua ATP	Cameroon
MSV-CAM West Noun ATP	Cameroon
MSV-CAM West Menoua Kassaï	Cameroon
MSV-CAM North-West Dongamantung ATP	Cameroon
MSV-CAM North-West Ngoketunjia ATP	Cameroon
MSV-CAM Obatampa Soukoundou	Cameroon

Table 4. Estimation of the evolution of divergences between genomics sequences of MSV analyzed base on the model of composite *Maximum Likelihood* using MEGA6.

	1	2	3	4	5	6
MSV-CAM West Menoua ATP (1)	0.01					
MSV-CAM West Noun ATP (2)	0.01	0.01				
MSV-CAM West Menoua Kassaï (3)	0.01	0.00	0.01			
MSV-CAM North-West Ngoketunjia ATP (4)	0.01	0.01	0.01	0.01		
MSV-CAM Obatampa Soukoundou (5)	0.00	0.00	0.00	0.00	0.01	
MSV-CAM North-West Dungamatung ATP (6)	0.01	0.01	0.01	0.01	0.01	0.01
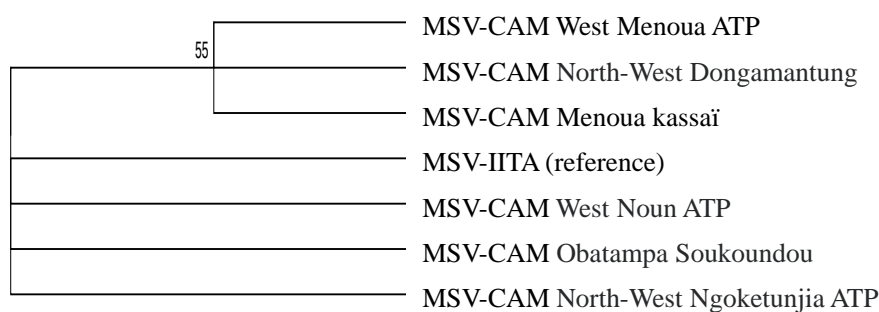
Figure 8. Phylogenetic tree of Cameroonian isolates of MSV from model of *Composite Maximum Likelihood* using MEGA6.

between Obatampa from Soukoundou and ATP from Dongamatung and Ngoketunjia isolates.

Figure 8 shows the Cameroon isolates and the IITA reference isolates in two groups. The isolates MSV-CAM Northwest Ngoketunjia ATP, MSV-CAM Northwest Dongamantung ATP and MSV-CAM Menoua kassaï belong to the same group. The isolates MSV-CAM West Noun ATP, MSV-CAM West Noun ATP and MSV-CAM North-west Ngoketunjia ATP are similar to the reference isolate (MSV-IITA) and belong to the same group.

4. Discussion

The incidence and severity of maize streak disease varied across the different

agro-ecological zones of Cameroon and were higher in the dry season and in shaded field. Temperatures are generally higher in the dry season than in the raining season, which would have favoured the development of the disease in the dry season. These results are similar to those of Matthew *et al.* [22] who reported that several viral diseases are more virulent when temperatures are high. In addition, in the dry season the cultivated areas are reduced and are located in lowlands where grasses belonging to the poaceae family are reservoirs for the vectors of MSV. During dry season, populations of grasses and maize decrease, causing the reduction in vectors food; as a result, the vectors migrate to the only grasses they can find in order to survive and transmit the disease to the only maize and grass population. The higher incidence and severity of shade-borne MSV is believed to be due to the relatively high temperatures and humidity. This is because in the open fields the heat is dry, whereas in shaded fields there is always moisture that allows the development of the *Cicadulina* spp. Temperature is one of the determining environmental parameters in vector-borne viral diseases. It affects the vector and the process of disease transmission. Temperature and humidity have led to geographical restriction and variation in the incidence and severity of MSD [23].

The incidence of disease in corn fields is proportional to the abundance of reservoirs; it is not until 3 to 4 weeks after the start of the rainy season that the number of reservoirs becomes significant [10]. In Cameroon, especially in agro-ecological zones I and III characterized by a very abundant grass flora of the poaceae family, the incidence of MSV is higher. The natural hosts of MSV play an important role in the epidemiology of maize streak. In maize-growing areas, maize fields favor a temporary increase in the host range of MSD [24] [25]. Maize plants first infected from reservoirs then greatly increase the source of inoculum. Leafhoppers migrating from fields to wild Poaceae will inoculate the virus to new hosts [26]. This explains the particularly high incidence in the localities of Mogodé, Figuil and Ndop. Quantitative variation in the grass flora at different times of the year could provide an explanation for the observed spatial distribution of MSV. This distribution is closely dependent on rainfall. According to the agro-ecological zones, the first weeks of the rainy season are in March for zone III and May for zone I where the grass flora is established on the non-irrigated plots allowing vectors to migrate from old plants in the market gardens to young plants in these non-irrigated plots. Since most farmers wait 2 to 3 weeks after the rains before planting, *Cicadulina* spp. have time to establish themselves in the grassy herbaceous plants and then migrate to the maize fields after emergence. Between May, August and September, the herbaceous flora is at its peak. In October most grasses reach the end of their cycle and only perennial and long-cycle species are found.

Maize varieties had different responses to *Maize Streak Virus*. Bua *et al.* [27] Shepherd & Martin [28] reported that: the genetic potential of each maize variety confers disease resistance or not. According to Bosque-Perez [29] varieties

have a great influence on the expression of viral diseases. Viruses multiply rapidly in susceptible varieties and slowly in tolerant varieties. Obatampa maize varieties are known to be resistant to *Maize streak virus* in the transition zone in Ghana [30] [31]. In this study, this variety does not seem to be as resistant in the Sudano-Sahelian agro-ecological zone of Cameroon where it is grown.

5. Conclusion

Among the five agro-ecological zones of Cameroon, Zones I and III had the highest incidence and severity of MSV due to their agro-ecological conditions which favored growing of considerable population of MSV host plants. It is in these maize-growing zones of Cameroon that the risk of streak epidemics is greatest. Shaded fields are the most attacked, hence it is necessary to avoid planting maize under trees or in the shade, as these later provide suitable conditions for development of Maize streak virus. It is important to develop a control strategy to limit yield losses due to maize streak disease. However, early planting, while the source of primary inoculum is relatively low, could reduce the most dreaded early infections and thus minimize the effect of streak disease on maize yields.

Acknowledgements

Conflicts of Interest

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

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