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Response of some maize (*Zea mays* L.) varieties to natural infection of *Maize streak virus* in the Western Highlands of Cameroon

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Abstract

Viruses are a limiting factor in maize production worldwide. *Maize streak virus* (MSV) significantly reduces maize yield in Africa and causes production losses ranging from 30 to 100%. Previous studies indicate that: in Cameroon, the incidence of maize streak disease ranges from 10 to 60% depending on the agro-ecological zone; and this is due to the susceptibility of the varieties used. A natural screening of 12 maize varieties, including 8 newly introduced varieties and 4 local varieties, was carried out to assess their resistance to MSV. at the Institute of Agricultural Research and Development (IRAD) at Foumbot in 2019 and 2020, during the dry and rainy seasons. Prior to the trial, vector abundance was determined in localities Foumbot, Ndop, Santchou and Dschang of the Western Highlands of Cameroon. The incidence and severity of maize streak disease (MSD) was assessed at symptom onset for all varieties. The presence of MSV in leaf samples was confirmed by the Triple Antibody Sandwich Enzyme-Linked Immunosorbent Assay (TAS-ELISA). Results showed that leafhoppers and the man vector *Cicadulina mbila* were more abundant in the Foumbot locality. MSV infections are higher in the dry season than in the rainy season. The maize varieties KASSAÏ, ACR06, ATP and MADJSYN VAR2 are less susceptible to MSD (5%, 12%, 14% and 13.33%) and the infection rate of MSV with ELISA test (5%, 12%, 15% and 8.3%). These varieties could be used in the control of maize streak disease to limit yield losses in Western Highlands zone of Cameroon.

1. Introduction

Maize (*Zea mays* L.) plays a key role in the global human diet and is the staple food for people in Sub-Saharan Africa [1]. It is grown throughout Cameroon and is the main food of the populations [2]. Maize cultivation is faced with many abiotic and biotic constraints. These include bacteria, fungi and viruses [3]. According to Brian et al. [4], three main virus diseases are reported on maize crops in the tropics caused by *Maize stripe virus* (MStpV), *Maize mosaic virus* (MMV) and *Maize streak virus* (MSV) [5]. The latter is the most dangerous and widespread in the tropics and in Sub-Saharan Africa [6].

MSV contributes to considerable yield losses ranging from 30 to 100 % in maize fields [7]. It is a single-stranded circular DNA virus of the family Geminiviridae, with virus particles of about 2685 bp [8]. Advanced manifestations are elongated yellow chlorotic stripes [9]. The transmission of MSV is persistent, non-propagative and is obligatorily carried out by several species of dipterans belonging to the genus *Cicadulina* (*Cicadelidae homoptera*) of the biting-sucking type [10].

In Cameroon, the main control strategy adopted against MSV is anti-vectorial.

This control is done through the use of chemical insecticides. Unfortunately, this method is harmful to the environment, to beneficial insects such as bees and pollinating insects and to the health of consumers [11].

To address these problems, the use of MSV-resistant varieties would be a particularly interesting method, as it limits the use of pesticides, while preserving the environment [12].

A recent study carried out in Cameroon on the epidemiology of maize streak disease reveals that the incidence of MSD varies from 10 to 60 %, depending on the agro-ecological zone. Mbong *et al.* [13] stated that this variation in incidence is due to the susceptibility of maize varieties used by farmers. Indeed, the MSV-resistant maize varieties introduced in Cameroon more than 25 years ago have degenerated and their resistance to MSV has considerably decreased [14]. In order to reduce maize yield losses in Cameroon, it is important that new maize varieties are tested for resistance to MSV to replace the failing varieties.

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2. Material and methods

2.1. Geographical and climatic description of the Western Highlands zone

The study was carried out between 2019 and 2020 in the Western Highlands zone of Cameroon, which lies between latitudes 4°54" and 6°36" North and longitudes 9°18" and 11°24" East and covers the West and North West regions, with a total area of 3.1 million hectares. It offers a great diversity of relief: from the Bamoun Plateau with an altitude of about 1,240 m to the Bamiléké Plateau which goes from the Bamoun Plateau to Mount Bamboutos (2,740 m) and the volcanic plateaus of Bamenda which are situated at about 1,800 m altitude. The climate is of the "Cameroonian highland" type and is marked by two seasons of unequal length: a dry season, which runs from mid-November to mid-March, and a rainy season which lasts from mid-March to mid-November. Average temperatures are low (19°C) and rainfall is abundant (1500-2000 mm) and falls in a monomodal pattern. The characteristic mid-mountain landscapes are savannah vegetation, stepped plateaus, depressed basins and plains crossed by gallery forests. All kinds of crops are grown: coffee, tea, banana, maize, potato, groundnuts, rice, market gardening [15]. The maize screening was carried out in Foumbot located between latitudes 4° 50" and 6°15 " N and longitudes 9° 55" and 6°15" E with a monomodal rainfall of 2000 mm and volcanic soil (Andosols).

2.2. Assessment of relative leafhopper abundance by locality

In order to assess the abundance of leafhoppers, they were captured using the method described by Dabrowski [16]. These specimens were preserved in 95° alcohol and then transported to the entomology laboratory of the International Institute of Tropical Agriculture (IITA) in Yaoundé-Cameroon for sorting and counting. The identification of Cicadelidae was done using the identification key of Dietrich [17]. The relative abundance of each species was determined by dividing the number of individual species by the total number of species per locality and the result was multiplied by 100 [18].

2.3. Determining the susceptibility of different maize varieties to MSV

2.3.1. Plant materials

All The plant materials used for this study consisted of 12 open-pollinated maize varieties, 8 of which were newly introduced by IITA and 4 from the breeding program of IRAD (Table 1).

Table 1: Maize varieties use for the screening

Origine	Varieties	Characteristics	Average yield (T/ha)	Grains colors
IITA	ACRO 6	Breeze resistant	2 to 4	White
	Aflatoxine	Fungi diseases resistant	2 to 4	Yelow
	Early white	Precocious	2 to 4	White
	MADJSYN VAR2	Breeze resistant	2 to 4	White
	MADJSYN VAR5	Breeze resistant	2 to 4	White
	PVA SYN6	Pro Vitamine A	1 to 3	Yelow
	PVA SYN13	Pro Vitamine A	1 to 3	Yelow
	TZ Comp4	Breeze resistant	2 to 4	White
IRAD	CMS 8704	Resistant to water stress	2 to 4	Yelow
	CMS 8501	Resistant to water stress	2 to 4	White
	KASSAÏ	Resistant to water stress	2 to 4	White
	ATP	Acid Tolerent Pollution	2 to 4	Yelow

2.3.2. Setting up the trial

The trial was set up in 2019 and 2020, at the beginning of the dry and rainy seasons; periods when populations of insect vectors of MSV are more abundant [16]. The maize varieties were sown in a two factors trials design with 3 replications, comprising 12 units of 4.5 m2 (a total of 36 units).

Each maize varieties were planted at the distances of were 75 cm between rows and 50 cm between the patches at 3 seeds per stand and thinned two weeks after planting to 2 plants per stand for a density was 42 plants per unit. Each subplot was separated by 1.5 m.

2.3.3. Monitoring and monitoring of the trial

Two weeding sessions were carried out 15 days apart. The application of NPK fertilizer (20-10-10) at a rate of 200 kg/ha was carried out 2 weeks after sowing. Urea 46 % was added 4 weeks after sowing at the rate of 100 kg /ha [19].

Infestations by the vector and infections occurred naturally in the field. As soon as symptoms appeared, the incidence was assessed from the ratio of the number of attacked plants in each experimental plot to the total number of plants inspected, and this was multiplied by 100 [20].

Disease severity was assessed visually using the semi-quantitative scale of Bello et al. [21] which ranges from 1 to 5 depending on the severity of symptoms. Scores were assigned and each number corresponds to:

1) Less than 10% spots visible on very close inspection;
2) 20 - 40% a slight streak easily visible;
3) 60 % of the plant has a streak;
4) 75 % of the plant has significant streaking with dwarfing;
5) More than 75% of the plant is fully symptomatic with very severe dwarfing.

The infection coefficient was obtained by multiplying the severity with the incidence [22].

At maturity, when all maize cobs were dry, they were harvested and threshed. Grain yield per plot was obtained from the weight of grain at 12.5% moisture content (assuming 80% threshing percentage) and converted to kilograms per hectare [12].

2.3.4. Confirmation test for virus infection and determination of the infection rate

In order to perform the virus infection confirmation test and determine the infection rate, maize leaf samples were collected from 60 randomly selected plants of each variety. These leaf samples were placed in polyethylene paper and stored in a cooler containing ice gels. They were then transported to the Applied Botany Research Unit of the University of Dschang for analysis using the TAS-ELISA test following the protocol described by Clark and Adam [23]. This test was carried out using leaf extracts from the collected samples and serological kits obtained from BIOREBA AG. After 1h incubation at room temperature, the appearance of yellow color indicated the presence of MSV. The optical density value was obtained by connecting a computer to the spectrophotometer. The threshold of positivity (TP) was calculated with the optical densities of the wells containing the negative control by the formula: TP = (average OD at 405 nm of the wells with the negative control) x 2. Thus, samples were declared positive when the average of its optical densities was greater than or equal to two times the TP. The infection rates were expressed by the formula $T_i = (N_i / N_t) \times 100$ where N_i: number of samples tested positive, N_t: total number of samples [24].

2.4. Data analysis

A data matrix was arranged to make the descriptive analysis of the relative abundance of leafhoppers in the different localities.

The incidence and severity data for each maize variety were analyzed using ANOVA with 2 factors represented by variety and seasons, using the SAS PROC-MLG model [25]. The mean values of the different parameters were separated by Tukey's HDS test (α = 0.05, [26]).

In order to test the association between the MSV infection rate data, a cross tabulation was used to generate Pearson Chi-square values.

Correlation analysis was conducted to establish the relationships between incidence, severity, number of cops, number of cops per infected plant and yield.

3. Results

3.1. Relative abundance of leafhoppers

Table 2 presents the relative abundance of leafhoppers in the different localities of the Western Highlands zone of Cameroon. Fourteen species of leafhopper were captured, including *Cicadulina mbila*, which is the most efficient vector in the transmission of MSV. The locality of Foumbot recorded the highest relative abundance of *C. mbila* (6.94%) followed by Ndop (4.34%), Dschang (3.92%) and Santchou (2.72%).

Table 2: Leafhoppers species percentage relative abundance (total) in different localities

Cicadelle	FOUMBOT		SANTCHOU		NDOP		DSCHANG	
Afrosteles distans	109	(24,38)	182	(30,95)	161	(25,88)	414	(45,05)
Maiestas subsirii	34	(7,61)	70	(11,90)	52	(8,36)	34	(3,7)
Nephotettix modulatus	168	(37,58)	210	(35,71)	276	(44,37)	342	(37,21)
Cicadulina mbila	31	(6,94)	16	(2,72)	27	(4,34)	36	(3,92)
Austroagallia sinuata	7	(1,57)	6	(1,02)	10	(1,61)	11	(1,2)
Balclutha jafari	74	(16,55)	90	(15,31)	75	(12,06)	60	(6,53)
Paralimnus taeniatus.	1	(0,22)	5	(0,85)	4	(0,64)	2	(0,22)
Orouis argentatus	0	(0,00)	1	(0,17)	1	(0,16)	1	(0,11)
Maiestas schmidtgeni	1	(0,22)	0	(0,00)	0	(0)	0	(0)
Molopopterus sp.	0	(0,00)	0	(0,00)	6	(0,65)	4	(0,64)
Waita sp.	6	(0,65)	0	(0,00)	4	(0,64)	4	(0,64)
Empoasca sp.	9	(2,01)	5	(0,85)	6	(0,96)	7	(0,76)
Helionides sp.	8	(1,79)	1	(0,17)	3	(0,48)	3	(0,33)
Exitinianus taeniaticeps	5	(1,12)	2	(0,34)	3	(0,48)	3	(0,33)

3.2. Effect of maize varieties on incidence and severity of maize streak disease

Table 3 shows that season had a significant effect on the MSV incidence of the tested varieties. However, it also shows that there are differences between the varieties with regard to disease development. The season had no effect on the different varieties (Table 3).

Table 4 shows the incidence and severity of maize streak disease on maize varieties in the dry and rainy seasons. It shows that the incidence of maize streak disease is higher in the dry season than in the rainy season.

The incidence of maize streak disease (MSD) is higher for the variety AFLATOXINE in both dry and rainy seasons, followed by MADJSYN VAR5 (22.67). The incidence of MSD of ATP in the wet season was higher than that of CMS 8501, CMS 8704 and EARLY WHITE. However, in the dry season, the incidences of MSV of the latter three varieties were higher than that of ATP. (df =21; F = 12.13; P = 0.0001).

The highest severity in the rainy season was noted on the variety CMS 8704 and the lowest on the varieties AFLATOXINE and PVA SYN 13. In the dry season, the highest severity was obtained with the varieties CMS 8704, KASSAÏ, MADJSYN VAR 2, MADJSYN VAR 5 and TZ COMP4 and the lowest with the varieties ACR06 and AFLATOXINE. There was no significant difference between severity, varieties and seasons (df =21; F = 1.36; P = 0.52) (Table 4).

Table 3: ANOVA showing the effect of varieties and seasons on maize streak

Variable: Incidences					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**F**	**P**
Seasons	1	1422.22	1422.22	137.90	< 0.001
Varieties	11	1742.83	158.44	15.36	< 0.001
Seasons. Varieties	11	206.44	18.77	1.82	0.0078
Residual	46	474.42	10.31		
Total	71	3951.50			

Table 4: Incidence and severity of maize streak disease on varieties

Season	Varieties	Incidence (%)	Severity
Dry season	ACR06	$12{\pm}2.52^{de}$	$4.33{\pm}0.33^{a}$
	AFLATOXINE	$29.33{\pm}1.20^{a}$	4^{a}
	ATP	$14{\pm}4.042^{de}$	$4.33{\pm}0.33^{a}$
	CMS 8501	$20{\pm}0.58^{abc}$	$4.67{\pm}0.33^{a}$
	CMS 8704	$17.67{\pm}2.67^{cd}$	5^{a}
	EARLY WHITE	$17{\pm}4.16^{cd}$	$4.33{\pm}0.33^{a}$
	KASSAÏ	$5.33{\pm}0.88^{h}$	$4.67{\pm}0.33^{a}$
	MADTSYN VAR2	$13.33{\pm}3.48^{de}$	$4.67{\pm}0.33^{a}$
	MADTSYN VAR5	$22.67{\pm}1.20^{b}$	$4.33{\pm}0.33^{a}$
	PVA SYN 13	$20.33{\pm}2.67^{bc}$	4^{a}
	PVA SYN 6	$16.33{\pm}2.33^{cd}$	$4.33{\pm}0.33^{a}$
	TZ COMP4	$18.33{\pm}1.77^{bc}$	$4.33{\pm}0.33^{a}$
Rainy season	ACR06	$7{\pm}1.53^{fg}$	3^{a}
	AFLATOXINE	$22.67{\pm}1.45^{ab}$	3^{a}
	ATP	$8.33{\pm}1.45^{f}$	$3.33{\pm}0.33^{a}$
	CMS 8501	$8{\pm}0.58^{f}$	$3.67{\pm}0.33^{a}$
	CMS 8704	$7.67{\pm}0.89^{fg}$	4^{a}
	EARLY WHITE	$7.33{\pm}1.20^{fg}$	$3.67{\pm}0.67^{a}$
	KASSAÏ	$2.33{\pm}0.88^{i}$	$4{\pm}0.57^{a}$
	MADTSYN VAR2	$7.67{\pm}1.85^{fg}$	$4{\pm}0.58^{a}$
	MADTSYN VAR5	$9.67{\pm}0.88^{fg}$	$3.33{\pm}0.33^{a}$
	PVA SYN 13	$6.67{\pm}1.20^{gh}$	$3.67{\pm}0.67^{a}$
	PVA SYN 6	$5.67{\pm}1.20^{gh}$	$3.33{\pm}0.33^{a}$
	TZ COMP4	$6.67{\pm}0.88^{gh}$	$4{\pm}0.58^{a}$
	F	12.13	1.36
	P-value	0.0001	0.52

Values followed by the same letter are not significantly different with at Tukey HDS test at P≤0.05.

3.3. Effect of maize varieties on yield

In the dry season, the highest yield was obtained with the varieties CMS 8501 (2.08 t/ha) and CMS 8704 (1.94 t/ha). However, the lowest yield was obtained with the varieties PVASYN6 (1.13 t/ha) and TZcomp4 (1.03 t/ha) (df =21; F = 6.130; P = 0.0001). In the rainy season, the highest yields were obtained with the varieties CMS 8501 (2.13 t/ha) and CMS 8704 (2.05 t/ha), and the lowest yields were obtained with the varieties PVASYN6 (1.28 t/ha) and TZ COMP4 (1.05 t/ha) (df =21; F = 9.108; P = 0.001) (Table 5).

Table 5: Yields in t/ha of maize varieties used for screening in the dry and rainy seasons

Varieties	Dry season	Rainy seasons
CMS8501	$2.08±0.34^{ab}$	$2.13±0.02^{a}$
CMS8704	$1.94±0.23^{abc}$	$2.05±0.15^{ab}$
ACR06	$1.92±0.19^{abc}$	$1.93±0.1^{abc}$
AFLATOXINE	$1.86±0.2^{abc}$	$1.91±0.27^{abc}$
ATP	$1.8±0.15^{bc}$	$1.85±0.17^{bc}$
EARLY WHITE	$1.65±0.26^{cd}$	$1.68±0.24^{cd}$
KASSAÏ	$1.63±0.06^{cd}$	$1.63±0.21^{cd}$
MADJSYN VAR2	$1.61±0.14^{cd}$	$1.62±0.07^{cd}$
MADJSYN VAR5	$1.51±0.21^{cde}$	$1.51±0.18^{cde}$
PVA SYN 13	$1.38±0.16^{ef}$	$1.48±0.31^{cdef}$
PVA SYN 6	$1.13±0.15^{efg}$	$1.28±0.06^{efg}$
TZ COMP4	$1.03±0.1^{g}$	$1.05±0.21^{g}$
F	9.108	
P-value	0.0001	

Values followed by the same letter are not significantly different with at Tukey HDS test at P≤0.05.

3.4. Correlation between epidemiological parameters and yield

Table 6 shows that there is a negative correlation between incidence, severity and yield of the maize varieties screened. Similarly, there is a negative correlation between the number of cops of maize per infected plant and yield. However, there is a positive correlation between the number of cops per plant, the number of cops per diseased plant and yield of these maize varieties

Table 6: Pearson correlation between disease parameters and yield of screened maize varieties

	Incidence	Severity	Yields	CIP	CP	NC
Incidence	1					
Severity	0.189	1				
Yields	-0.078*	-0.023*	1			
CIP	-0.118*	-0.145*	-0.389*	1		
CP	-0.067*	-0.041	0.267	0.1	1	
NC	-0.111	-0.065	0.448**	0.352*	0.295	1

*. Correlation is significant at the 0.05 level (two-tailed). **. Correlation is significant at the 0.01 level (two-tailed).

CIP: Cop per Infected Plant, CP: Cop per plant, NC: Number of Cops

Table 7 shows the infection rates of the different maize varieties in both seasons. From this figure. It appears that MSV tested positive on all maize varieties tested, regardless of season. Thus, of the 1440 samples tested in each season, 14.6% were found to be infected with MSV during the dry seasons (P<0.0001), in contrast to the rainy seasons when an infection rate of 13.8% (P<0.0001) was recorded.

The AFLATOXINE variety was the most infected during the dry (25%) and rainy (26.7%) seasons. These infection rates of the variety AFLATOXINE were followed by those of the variety ACR06 (20%) during the dry seasons and those of the variety MADJSYN VAR5 (21.7%) during the rainy seasons. However, KASSAI (8.3%) and MADJSYN VAR2 (3.3%) had the lowest infection rates in the dry and rainy seasons, respectively (Table 7).

Table 7: Infection rates of different maize varieties during the seasons

Maize varieties	S T	S I D S (%)	S I R S (%)
ACR06	60	12 (20)	10 (16.7)
AFLATOXINE	60	15 (25)	16 (26.7)
ATP	60	9 (15)	8 (13.3)
CMS8501	60	9 (15)	12 (20)
CMS8704	60	11 (18.3)	8 (13.3)
EARLY WHITE	60	8 (13.3)	6 (10)
KASSAÏ	60	5 (8.3)	2 (3.3)
MADJSYN VAR2	60	5 (8.3)	4 (6.7)
MADJSYN VAR5	60	13 (21.7)	13 (21.7)
PVA SYN 13	60	9 (15)	7 (11.7)
PVA SYN 6	60	3 (5)	5 (8.3)
TZ COMP4	60	6 (10)	8 (13.3)
Total	720	105 (14.6)	99 (13.8)
F		38.066	48.977
P-value		0.0001	0.0001

S T: Sample tested per season, **S I D S :** Sample Infected in Dry Season, **S I R S:** Sample Infected in Rainy Season

4. Discussion

All twelve maize varieties evaluated showed variable responses to MSV infection in both seasons. The severity of the maize streak disease varied from severe to very severe infection. This suggests that these maize varieties would possess partial resistance under varying environmental conditions; indicating a pathogen-host-environment interaction as reported by Olaoye [27]. The differences in resistance levels between these maize varieties can be attributed to genes conferring resistance [28].

The results of this study revealed that maize streak disease incidence and severity were higher in the dry season Olaoye [27]. These results are in agreement with those of Asare-Bediako et al. [29] who reported that maize streak disease incidence and severity are higher in the early dry season. This significant location effect on the epidemiology of maize streak disease is thought to be due to the interaction between the viral pathogen, the host (maize plants) and the environment.

The significant differences in incidence and severity between the two seasons are thought to be due to differences in the population density of wild grasses that are reservoirs of MSV and hosts for leafhoppers [30]. It has been reported by Clemente-Orta et al. [31] those viral diseases of maize and other cereals infect other plant species that become reservoirs of MSV, thus influencing the epidemiology of maize streak disease.

The low grain yield obtained is similar to the report of Bosquez-Perez et al. [32] which indicates that maize streak disease is negatively correlated with plant height and dry weight, grain weight per plot and grain weight. This negative relationship between maize streak disease severity and grain yield is in agreement with Martin and Shepherd [33] who reported that maize streak disease is a major maize disease that causes considerable yield losses in SSA.

The: maize yield of all varieties used and the severity of maize streak disease are strongly related. The highest yielding varieties CMS 8704; KASSAÏ, ATP, ACR006 and AFLATOXINE were found to be resistant to maize streak disease. In fact, yield loss of different maize varieties between seasons could also be related to the genetic potential of each cultivar and the interaction between the viruses and its hosts [34] moreover, differences in MSV symptoms observed in maize plant populations would be due to the ability of each variety to resist [35].

The highest infection rate was recorded on the variety AFLATOXINE. This could be due to its susceptibility to MSV and the infection of this pathogen. In fact, as the earlier the infection, the more time the virus has to multiply in order to reach the detection threshold by the TAS-ELISA test used for this purpose [36]. The high infection rates recorded during the dry seasons are thought to be related to the fact that MSV vectors proliferate more in the dry season than in the rainy season, due to the higher temperatures and lower grass cover during this period, which causes the insects to feed on the little that is present in the shallows [37].

Conclusion

Fourteen species of leafhopper were identified in the agro-ecological zone of the Western highlands of Cameroon. The most efficient vector in the transmission of MSV *Cicadulina mbila* was more abundant in the Foumbot locality compared to the other localities. The incidence of maize streak disease was higher in the dry season for all varieties as opposed to the rainy season. The varieties KASSAÏ, ACR06, ATP and MADJSYN VAR2 were less susceptible to MSV. These varieties could be used in the control of maize streak disease to limit yield losses.

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