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EVALUATION OF VIRUS DISEASE STATUS ON SUGARCANE GERMPLASM IN WESTERN CAMEROON

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ABSTRACT

Sugarcane cultivation is hindered by several biotic constraints of which viral disease plays a major disaster. This study was carried out to evaluate the virus disease situation of sugarcane germplasm in western Cameroon, consisting of three regions (Southwest, Northwest and West region). A survey was carried out in 66 villages in these regions. Landraces identified included SMU58, SBK36, SNC16, NBfPc48 and NBfAg53. Representative samples of the landraces were collected and grown in an experimental field in the Department of Plant Science, University of Buea. Canes were observed for virus disease symptoms eleven months after planting. Single leaf samples of the symptomatic plants were collected from 10 randomly selected plants constituted a batch sample. A total of 66 and 15 batch samples collected from the field and experimental plot respectively were tested for the detection of Sugarcane Mosaic Virus (SCMV) and Maize Streak Virus (MSV) by direct Double Antibody Sandwich ELISA (DAS-ELISA). Of the 66 composite samples tested for SCMV and MSV, 54 samples tested positive for at least one virus. Maize streak virus was the most prevalent, with an incidence of 11.25%. Mixed infection was also recorded. Sugarcane in western Cameroon is infected with some virus diseases though the prevalence is low. This is a course of concern.

Keywords: Survey, virus diseases, SCMV, MSV, Western Cameroon

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1. INTRODUCTION

Noble sugarcane (*Saccharum officinarum L.*), is widely grown in Western Cameroon for marketing as a chewing fruit. In Africa, the highest productivity is observed in South Africa (49.5tons/ha) followed by Egypt (13 tons/ha). Cameroon ranks 51 in the world amongst the sugarcane producing countries and her production quantities has dropped from 1,450,000 tons in 2009 to 1.200.000 tons in 2013 [1].

The sustainable and consistent production of sugarcane by farmers in the recent past has been adversely hampered by biotic constraints particularly diseases. Many diseases of sugarcane have been reported from different parts of the world. Smut, red rot, leave blast, Sugarcane mosaic, ratoon stunting disease, sugarcane wilt disease among other diseases is affecting sugarcane in some parts of Cameroon[2]. Viral diseases are causing economically significant losses worldwide [3]. The viruses causing sugarcane mosaic disease include *Sugarcane mosaic virus, Sorghum mosaic virus and Sugarcane streak mosaic virus.*Sugarcane mosaic virus disease is rated as one of the world's most important cane disease and potential the most destructive [4]. Sugarcane mosaic disease can reduce the yields of some susceptible sugarcane cultivars by 17% to 50%.These viral diseases are more prevalent than before and are assuming as bottlenecks to sugarcane production [5]. There are no economically feasible chemical agents similar to fungicides and bactericides that are effective against sugarcane viruses. Strategies aimed at plant virus disease management are largely directed at preventing virus infection by eradicating the source of infection to prevent the virus from reaching the crop, minimizing the spread of the disease by controlling its vector, utilizing virus-free planting material, and incorporating host-plant resistance to the virus [6].

The techniques used for virus detection in surveys usually depend on the survey objectives; available infrastructures, resources, facilities, availability of reagents, level of specificity and sensitivity, required expertise and skills available to carry out these assays, type and number of samples to be tested, the amount of information available on the virus to be detected and the cost [7]. Many methods have been developed for the detection and identification of plant viruses. A single diagnostic test or assay may provide adequate information on the identity of methods for the detection of plant virus diseases but a combination of methods is generally needed for unequivocal diagnosis [8].

Visual assessment of symptoms is the simplest method of virus identification in a survey. This method is based on the fact that virus incite morphological changes in the plant. Visual inspection is relatively easy when symptoms clearly are characteristic of a specific disease [9]. Symptoms due to mixed infection could lead to wrong identification while plants with symptomless infections may be considered as being healthy. Usually, it is necessary that visual

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inspection for symptoms in the field is done in conjunction with other confirmatory tests to ensure accurate diagnosis of virus infection.

Serological assay has been used successfully for a number of years for the detection of plant viruses. The enzyme-linked immunosorbent assay (ELISA) has been very popular for detection of viruses in plant materials and vegetative propagules since it was introduced to plant virology by [10]. In practice, DAS-ELISA is highly strain-specific and requires each detecting antibody to be conjugated to an enzyme. DAS-ELISA is widely use in handling large number of samples [11]. The reagents and chemicals required are readily available, and it gives adequate identification of viruses.

In spite of its numerous uses, the production of sugarcane in Western Cameron is hampered by many factors including diseases. There has been little interest on the growing of the crop despite its economic importance and the rich tropical soil of Western Cameroon. However, the incidence and distribution of virus on sugarcane has not been regarded as important in this area. The present study therefore seeks to access virus diseases of sugarcane in Western Cameroon.

2. MATERIALS AND METHODS

2.1 Field survey

The present study was carried out in Western Cameroon, involving the Southwest, Northwest and the west regions of Cameroon. A survey of farmers' fields was carried out to observe virus and virus-like symptoms of sugarcane. Farms sampled were chosen based on earlier reconnaissance survey. The five different landraces identified were recorded and visual assessment of symptoms was the method of virus identification in the survey. This method is based on the fact that virus incite morphological changes in the plant. Representative cane samples of the five landraces identified were collected and grown in an experimental field.

In the farmer's field, plant sampling was done when the canes were mature, in 2 x 2m quadrants 4m apart along the field diagonal or some longest straight line across the field for some irregularly shaped farms. A 2cm^2 single leaf samples of the symptomatic and plants suspected to be infected by virus were collected from 10 randomly selected plants per field. These 10 leaf samples constituted a batch sample. A total of 66 batch samples were collected on the basis of virus and viral like symptoms. Sample collected were placed in grip polythene sample bag and appropriately labeled to indicate location, sample number, date of collection and cultivar name. The samples were temporarily stored in an ice box. These were later carried to the Life Sciences Laboratory of the University of Buea, and stored in the refrigerator at 4^{0}C until they were tested for viruses.

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The occurrence of viral diseases was also assessed on the experimental plot. Fifteen batch samples, three from each of the five landraces were collected from the experimental field eleven months after planting for testing.

2.2.1 Sugarcane virus disease incidence from survey

Disease incidence refers to the number of visibly diseased plants, usually in relation to the total number assessed and so expressed as the proportion or percentage of plants in a stand with symptoms on a scale of 0–100 (%). This technique was used to calculate the incidence of virus symptoms on the field. The various symptoms were observed and recorded.

2.2.2 Sugarcane virus disease Severity

Disease severity usually refers to the degree of symptom expression as assessed visually using an arbitrary scale. Ten sugarcane plants were taken randomly from each landrace along a diagonal and observed for virus symptoms. Phenotypic data on host reaction were recorded in terms of symptom expression following a five-point scoring scale modified after [12]., (Table 1).

Table 1: Desease severity scoring system for virus of sugarcane in western Cameroon

Severity class	Description
0	Healthy.
1	Mosaic not extremely distinct and little yellowed area on any symptomatic leaf.
2	Mild symptoms on one or more leaves, with mosaic.
3	More symptomatic leaves per tiller. Severe mosaic symptoms, widespread on plant
4	Severe symptoms, as in 3 but in addition either noticeable stunting or small to moderate amount of necrosis.
5	Very severe symptoms, severe stunting, obvious and significant amount of necrosis

Adopted from Pipper (1996).

Assessments of sugarcane virus incidence, and severity were all based on the accurate and reliable visual assessment of symptoms.

2.3 Serological diagnosis

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For the screening of each gathered samples, Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay (DAS-ELISA) was used. The technique of direct Double Antibody Sandwich ELISA (DAS-ELISA) was performed following the method of [10]for the detection of Sugarcane Mosaic Virus (SCMV) and Maize Streak Virus (MSV).

The plates were each read at 405 nm in an ELISA reader (EPSON LX-300) 30-35 minutes later. A sample was considered positive when the Optical density (OD_{405}) was at least twice that of the negative control. From the ELISA results, virus incidence was calculated from the formula of [13].

$$Y = \left[1 - \left(\frac{\text{Nt} - \text{Ni}}{\text{Nt}}\right)^{\frac{1}{n}}\right] x\ 100 \text{------------------------------------} (1)$$

Y= % Incidence

Nt = No of samples assayed

Ni = No of positive samples for a virus

n= No of leaves in batch of samples.

3. RESULTS

3.1 Virus diseases symptoms

The diversity of virus-like symptoms observed in the field is shown in Fig 1.

Result shows that mosaic symptom (Fig 1a) was common on NBFAg53. This symptom was either chlorotic areas with leaf malformation on tops of young leaves, marked stunting growth of cane or darker green ‘island’ on lighter green background. This mosaic symptom was commonly observed in fields with intercropping.

Mottling of leaf lamina was also observed in many fields (Fig. 1b). In some plants showing yellowing, there was general yellowing of the leaves (Fig. 1c). In others, there was yellowing of leaf with necrosis on the base of the leaf (Fig. 1d). This situation was common in the NBFPc48. In SBK36, some of the plants’ leaves showed stunting and necrosis, death of ratoon stool and reddish midrib (Fig. 1e).

Knife-cut lesions were also observed although this was not very common in all the fields. This symptom was common in fields intercropped with maize and was mostly found in SNC16 and SMU58. The infected plants had thin leaves. Plants with this symptom had pale yellow colour (Fig. 1f).

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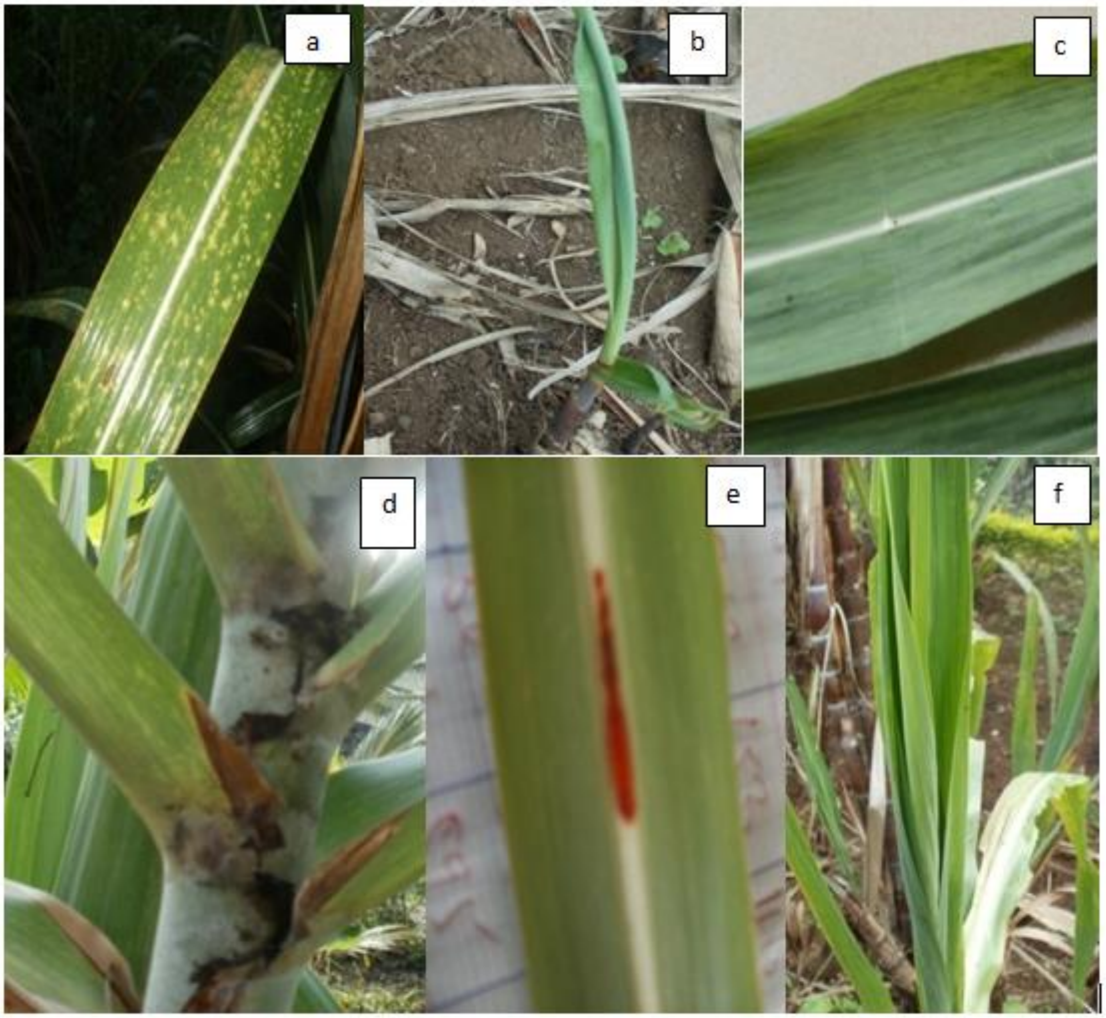

Figure 1: virus induced field symptoms on leaves: a) mosaic symptom, b) mottling c) yellowing d) yellowing with necrosis e) reddish midrib f) knife-cut lesion.

Results showed that mosaic was most common in the Southwest, whereas necrosis occurred most frequently in the Northwest (Table 2). Mosaic symptoms were between 12% in the West to 36% in the Southwest. Apart from the Northwest, reddish midrib, knife-cut lesion and stunting symptoms were very low. Of the observed virus-like symptoms, the Northwest region recorded the highest incidence with the West recording the least. However, Southwest region also had a high incidence of all virus-like symptoms. The incidence of necrosis was the most common observed virus-like symptom type while the least was knife-cut lesion.

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Table 2: Incidence of virus-like disease symptoms on sugarcane in western Cameroon

Regions	Symptom types (%)					
	Mosaic/chlorotic mottle	Yellowing	Necrosis	Reddish midrib	Knife-cut lesion	Stunting lesion
Southwest	36	12	8	6	6	80
Northwest	18	24	14	7	11	94
West	12	14	9	8	7	69
Mean	22	16.7	10.3	7.0	8	81

The incidence of virus-like disease symptoms on five sugarcane landraces in the experimental field is shown on Table 3. The total incidence ranged from 38% in SNC16 to 57% in SBK38. The most common symptom type was mosaic and chlorotic mottle with an incidence of up to 51% while the knife cut lesion type was the least with an incidence of 10%.

Mosaic and necrosis were generally higher in all the landraces. Mosaic symptom was most common on SBK38 and lowest in SNC16. Yellowing was also found in all the cultivars. Its incidence was generally less than 14%. It was least prevalent on NBfPc48 and highest on SBK38.

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Table 3: Incidence of virus- like disease symptom types on five sugarcane landraces in the experimental field.

Landraces	Incidence virus-like symptom types (%)							
	Mosaic/ chlorotic mottle	Yello wing	Necrosis	Reddish midrib	Stunting	Knife-cut lesions	Mixed infection	Total infection
NBfAg 53	9	8	15	8	2	1	4*	47
NBf Pc 48	7	5	18*	4	2	2	3	41
SNC 16	6	8	11	5	3*	2	3	38
SMU 58	13	11	4	13*	2	1	2	46
SBK 38	16*	13*	6	13	3*	4*	2	57
Total	51	45	50	43	12	10	14	

*Symptom- types per landrace with high incidence

The total incidence on NBfAg53, SNC16 and SMU58 were equally. Necrosis was widely distributed in all the cultivars, though the incidence was generally below 19%. The incidence of reddish midrib ranged from 4 to 13%. The highest was on SMU58 and SBK38 and the lowest on NBfPc48.

Symptomatic plants of various degree of severity were observed. The virus disease severity increased from 16% in the Northwest to 19% in the West (Fig 3).

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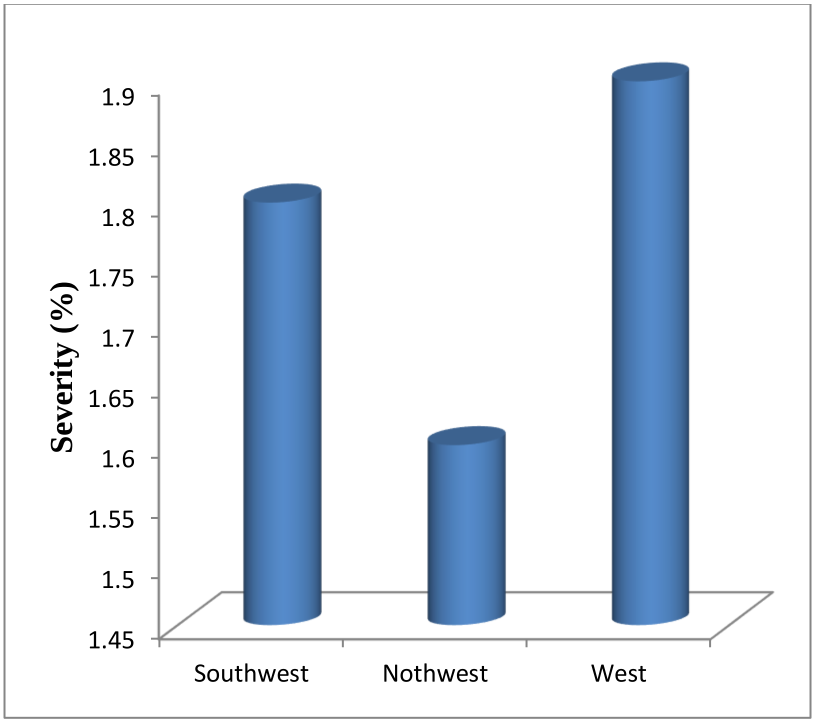

Fig 3: mean virus disease severity for the regions in western Cameroon

The proportion of symptomatic plants in each disease score was different in the three regions surveyed, with the highest recorded in the West region and the lowest in the Northwest region. All the regions recorded all the severity scores i.e., 0 - 5scores.

For the different canes, NBFAg58 and SBK16 recorded no severity score of five. NBFAg58 and NBFPc48 did not record any zero score (Fig 4.).

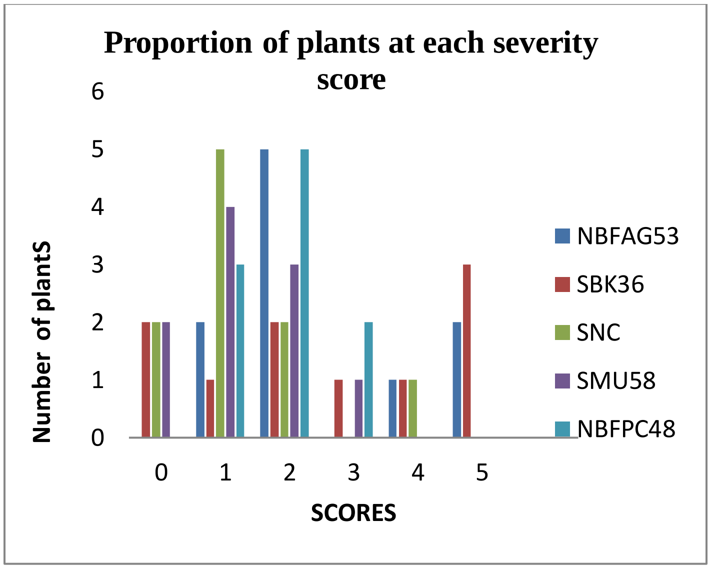

Fig 4: mean virus severity score for five sugarcane landraces from western Cameroon

3.2 Serological detection of viruses

Out of the 66 composite samples tested for SCMV and MSV, 54 samples tested positive for at least one virus (Table 4). Maize streak virus was the most prevalent, with an incidence of 11.25% while SCMV had an incidence of 2.93%. Mixed infections accounted for about 2% (Table 4).

Table 4: Incidence of viruses and virus mixture in western Cameroon

Virus/virus mixture	Total number of samples positive	% Incidence
MSV	46	11.25
SCMV	17	2.93
MSV + SCMV	12	1.99
All viruses	54	15.67

MSV= Maize streak virus, SCMV=Sugarcane mosaic virus

Based on the incidence of the viruses in the villages, a map was developed as shown in Figure 2.

Fig 2: distribution of viruses in western Cameroon

The incidence of single infection was highest in the West region followed by the Southwest and lastly the Northwest region. This ranged from 0.19% to 11.7% (Table 5). There was no incidence of MSV only in the West region but the incidence was 10.07% and 11.77% for Southwest and West regions respectively. The Southwest and Northwest recorded percentages of 0.19% and 8.12% respectively for SCMV only. Results showed that the incidence of mixed infections was generally low, ranging from 1.53% in the Northwest region to 3.31% in the west region(Table 5). The viruses tested for were found in all the regions under survey.

Table 5: Incidence of MSV and SCMV in the three regions of western Cameroon

Region	Incidence (%)			
	SCMVonly	MSVonly	SCMV + MSV	Total
SouthWest	0.19	10.07	1.88	12.14
North West	8.12	0	1.53	9.65
West	0	11.777	3.31	15.08

MSV= Maize streak virus, SCMV=Sugarcane mosaic virus

From the experimental field collections, 12 out of 15 composite samples were found to be positive for either one or two of the viruses tested, having an % incidence of 14.89%. Of the 15 composite samples, 7 were found MSV positive with 6.09 % incidence and 5 out of 15 composite samples for SCMV with 3.97% incidence. The incidence of viruses on landraces ranged from 0.69% (SMU58) to 6.09% (NBFPc48). There was no incidence of mixed infection on SMU58 and SBK16. Equally SMU58 did not test positive for SCMV (Table 6).

Table 6: Incidence of viruses on five sugarcane landraces from western Cameroon

	Incidence (%)			
Landraces	MSV only	SCMV only	SCMV + MSV	Total
NBFPc53	2.21	1.42	1.42	6.09
NBFAg48	0.69	0.69	0.69	2.21
SNC36	0.69	0.69	0.69	2.21
SMU58	0.69	00	00	0.69
SBK16	0.69	0.69	00	1.42

4. DISCUSSION

Diseases caused by fungi, bacteria, viruses and Mycoplasma-like organisms cause considerable damage to sugarcane [14]. Of all the diseases, viruses were assessed. Many of the sugarcane landraces that predominate in farmers' fields in many parts of western Cameroon were susceptible to virus and express obvious symptoms when infected by one or more viruses. No region was found free from viruses' infection. Canes infected by viruses show varied symptoms. These symptoms were typical of those described for the disease [15]. These symptoms were different from those of red rot disease, another common disease of sugarcane. Symptom types were common on all the landraces, ranging from 14 to 51%. The symptoms were variable. Mildly affected plants exhibitedpatchy leaf chlorosis and little or no mottling or impaired

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growth, whereas severely affected plants had smaller leaves, severe chlorosis and stunting. Typical virus symptoms of sugarcane observed were either entirely or on the younger leaves. The result showed that mosaic/chlorotic mottle and necrosis were most common. The incidence of symptom types was generally high on all the landraces ranging from 33% to 51%. The total infection was highest on SBK38 and was least on SNC16. This implies that SNC16 can be tolerant to most of the sugarcane viruses than the other landraces while SBK38 can be susceptible to viruses. The low disease severity observed in the canes was expected. Canes with virus disease severity scores of 4 and 5 constituted only 3.49% of the canes, thus resulting in a low disease severity index observed, though no good management strategy was put in place in the villages surveyed. This severity index can be mitigated in these regions with good management efforts. The results obtained can also be due to the fact that severity measurements are subjective and can sometimes be prone to bias and experimental errors. Also, visual assessment of disease usually tends to overestimate disease severity, especially with low levels of infection [16]. [17]suggested that the visual assessments are made quickly and do not require expensive equipment, chemical analysis or highly trained personnel, but their subjective nature creates concern and determines that the accuracy and precision of the measurement of injuries are questionable. Farmers' awareness on these viral diseases is however poor and is of major concern. In particular, majority of farmers are ignorant of these viral infections in their sugarcane fields. They largely attributed the symptomatology of these viral diseases as manifestation of sugarcane decline due to unknown reasons. However, few farmers attributed unhealthy seed as source of these viral diseases. Despite this drawback, severity of a disease is often considered an important and useful measure of disease intensity in management strategies.

The incidence of the viruses was generally low when compared to reports from other parts of the world on sugarcane. Viruses cause a significant reduction in the number of malleable canes and significantly reduce net carbon dioxide assimilation rate during the grand period of growth. At harvest, canes stalk from virus infected canes significantly has reduced cane diameter, cane weight, stalk height and number of internodes [3]. Similar results were observed in this field survey. The incidence of MSV was generally high (8.55%) while that of SCMV had only 0.78% incidence. The result also showed that MSV is the most common virus disease as it was detected in 49 out of the 66 composite samples. This high incidence of MSV as compared to SCMV suggests its long existence in the area. It was interesting to note that out of 66 samples tested, 05 samples were positive to SCMV only. This indicates that SCMV and MSV are different viruses, although sometimes found together in one infected sample. Hence the two viruses which were tested were found prevalent throughout the surveyed areas. The crop situation observed showed that the farmers were continuously using their own local germplasm, so that the virus was accumulating in the field. This is a major factor in disease development and spread, especially given that sugarcane is vegetatively cultivated [18] The incidence of MSV in this study was low as compared to the 50.77% recorded in Punjab Province [19]

Previous studies [20] showed SCMV on sugarcane in Cameroon and in other parts of the world [21]. Similarly, SCMV was detected in the study. It had an incidence of 0.78% which is lower as compared to the study carried out by [15] and [19] Nigeria and Pakistan respectively.The occurrence of SCMV has been reported in other countries [22, 23]. Maize streak virus strain A (MSV-A), the causal agent of maize streak disease, is today one of the most serious biotic threats to African food security [24].

The incidence of these viruses was generally low, ranging from 2.11% to 17.68%. In spite of the positive results reported above, a total of 12 infected sugarcane samples tested negative for the two viruses in DAS-ELISA. This negative result obtained might be as a result of poor identification of symptoms on the field. Mixed infections by viruses were equally observed with incidence of 0.99%. Mixed infections are common in nature and are known to cause severe yield loses in plants [25]. A number of important virus diseases of plants are the outcomes of interactions between causative agents. It has been reported earlier that the synergetic effect of SCMV and other viruses mostly affect sugarcane growth and yield more than each disease separately [19]. Results of [11] on tomatoes showed that viruses occurring singly or in mixed infection are the most important pathogens of plants. This agrees with the general notion that viruses are very important pathogens of various crops in Temperate and Tropical areas. The prevalence, distribution and the incidence of the two viruses in the sugarcane crop might be due to poor management strategies since most of the fields were dirty and also due to lack of an elaborate sugarcane seed program in the study area.

Generally, all the landraces registered a low virus disease incidence ranging from 0.6 to 3.97%. The incidence was highest on NBFPc53. The fact that virus disease was recorded on the landraces on the experimental field with good management practices implies that the setts were already infected before planting.

This study documents the first comprehensive study on the incidence and severity of virus diseases of sugarcane in western Cameroon. The pattern of incidence, distribution and severity can be attributed to the management practices. Although virus diseases cause reduction in yield, its spread is still limited in the study area. Though the incidence and severity of the viruses were low, there is still need to monitor the disease on a continuous basis. Therefore, there is need for research on new and emerging virus and virus-like disease problems of sugarcane in Cameroon.

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5. CONCLUSION

Results from this work shed some light on the health situation of sugarcane in western Cameroon and highlight the need for further research to determine other viruses that may affect the canes in this part of the country and other regions. In particular, results from this work highlight the potential infection risk to sugarcane, resulting in a drop in productivity, and underscore the need for a better seed source and management practices. The problem of disease does exist in the sugarcane production system in western Cameroon and must be taken into account when planning for any large-scale production.

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