Full Length Research Paper

Identification of virus isolates inducing mosaic of sugarcane in Makarfi Local Government Area of Kaduna State, Nigeria

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Sugarcane mosaic disease caused by sugarcane mosaic virus (SCMV), Johnsongrass mosaic virus (JGMV), maize dwarf mosaic virus (MDMV) and sorghum mosaic Virus (SrMV) is an economically important viral disease of sugarcane worldwide. Field survey was conducted to assess the presence of the viruses involve in mosaic disease of sugarcane in Makarfi Local Government Areas of Kaduna State (Northern Guinea Savannah), Nigeria. A range of symptoms were observed on the infected land races from the pale green stripes to yellow chlorotic stripes on a dark green background. The purple land race ("Bakarkwama") was highly susceptible followed by green land race ("Bahausa") and the least infected was the white land race ("fararkwama"). 63 symptomatic and asymptomatic sugarcane leaves and stem juice extract from 14 villages of Makarfi L.G.A. were screened for the four viruses using DAS and TAS ELISA methods. SCMV, MDMV and SrMV were detected from both symptomatic and asymptomatic sugarcane leaf samples whereas JGMV was not detected in the locations sampled. SCMV isolate has the highest incidence (83%) from all the locations followed by SrMV (10%) and MDMV (5%) isolates. Mixed infections of the three viruses were also detected in some samples. This is the first report of identification of virus isolates inducing sugarcane disease in one of the major sugarcane producing areas of the Northern Guinea Savannah of Nigeria.

Key words: Sugarcane, SCMV, MDMV, SrMV, TAS-ELISA, DAS-ELISA.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.), belongs to the family Poaceae, it is a high value cash crop in many parts of the world. It is an old energy source for humans, produced on commercial basis and is either chewed or use in the production of brown and refined sugar as well as ethanol. Virus is one of the major limiting factors in many sugarcane growing areas of the world (Grisham et

al., 2013). Mosaic in sugarcane is a member of potyvirus genus of the family potyviridae consisting of four distinct viruses based on serological properties; coat protein and genome sequence (Shukla et al., 1992) and is one of the most common and economically important viruses in sugarcane cultivars causing severe effect on sugarcane production worldwide. Forty (40) percent yield loss due to

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mosaic has been reported in Australia (Croft et al., 2000).

In South Africa, SCMD brought sugar industry to its knees (Anon, 1980). Under conditions of severe SCMV infection, reduction in sucrose yield up to 42% has been reported in susceptible varieties in South Africa (Bailey and Fox, 1987). Makarfi lies at 11° 22' N, 7° 52' E, it has 2, 520 sq miles and has a long history of sugarcane cultivation in northern Nigeria. Makarfi is blessed with abundant farmland and the major cash crop is sugarcane which is exported outside the state and country. Sugarcane is cultivated in 80% of the villages and three land races of chewing cane are grown. As common to areas where sugarcane is cultivated, sugarcane mosaic disease has caused great economic losses to farmers in all the areas *vis* reduced palatability, marketability and yield. Yang and Mirkov (1997) used genome-based technique to differentiate strains of SCMV and SrMV in Texas, USA. Balamuralikrishnam et al. (2004) used both genome-based and antibody-based techniques to detect SCMV in India. Mohammad and Behzad (2009) used antibody based technique and detected SCMV in Iran. However, in Nigeria, little work has been done on identification of sugarcane mosaic disease. Wada et al. (1999), reported SCMV incidence of 6% based on sap inoculations on susceptible maize varieties from the Southern Guinea Savannah Zone of Nigeria.

As these viruses are transmissible through infected seed canes, they pose the risk of accidental introduction into previous disease-free regions. The four distinct viruses induce similar pale green and yellow chlorotic stripes symptoms on leaf blade and white stripe on stem in infected sugarcane and are indistinguishable based on the visible symptoms. Furthermore, symptom expression may also be confused for environment disorders, as they both cause disruption in plant metabolism. These necessitate the use of antibody-based technique to diagnose the disease. The present paper reports for the first time that, the identification of the virus isolates causing mosaic disease of sugarcane in Makarfi, which is the major sugarcane producing areas of Kaduna State, (Northern Guinea Savannah) Nigeria.

MATERIALS AND METHODS

Survey for sugarcane viruses in sugarcane growing areas of Makarfi Local Government Area (L.G.A)

Survey for sugarcane viruses was conducted in 14 major sugarcane growing villages in Makarfi Local Government (Figure 1). The range of symptoms observed on both leaves and stem were recorded on the three main land races of chewing type sugarcane. A total of 63 samples comprising of both symptomatic and asymptomatic leaves were collected from ratoon and seedcane fields employing systematic sampling methods from May to October 2012. In each case, both symptomatic and asymptomatic leaves were collected from the youngest leaves and put in polythene bag, placed on icebox and stored at -20°C in freezer and some under calcium chloride. Coordinates were also recorded at each site using GPS. Each sample was later tested for presence of SCMV and

JGMV by DAS-ELISA and for MDMV and SrMV by TAS-ELISA.

DAS ELISA for the detection OF SCMV and JGMV

Antibodies to SCMV, JGMV and positive control were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Germany. 20 µl of purified IgG was diluted (1:1000) in 20 ml coating buffer (1.59 g/dm^3 sodium carbonate + 2.93 g/dm^3 sodium bicarbonate + 0.2 g/dm^3 sodium azide). Wells of microtitre plates (Nunc) were coated with 200 µl of IgG and incubated at 37°C for 4 h. Plates were then washed thrice in PBST and tapped dry on tissue paper. Leaf tissues were homogenized separately using sterilized pestle and mortar at ratio of 1 g leaf tissue per 4 ml extraction buffer [PBST+2% PVP (Polyvinyl pyrrolidone)]. 200 µl aliquots of the test sample was added to duplicate wells and incubated at 4°C overnight. Plates were washed thrice as above with PBST and 200 µl anti-virus conjugate was added to each well, incubated at 37°C for 4 h and washed as above. Freshly prepared substrate [10 mg p-nitrophenyl phosphate in 10 ml substrate buffer (97 ml/dm^3 diethanolamine + 600 ml/dm^3 distilled water + 0.2 g/dm^3 sodium azide)] was added (200 µl) to each well and incubated at room temperature. Colour change was recorded by visual observation. Absorbance (A_{405nm}) values were recorded in a microplate reader (optic ivymen system 2100 c) after 1 h at room temperature and overnight at 4°C. A_{405nm} values greater than two times that of the healthy control and were considered positive.

TAS ELISA for the detection of MDMV and SrMV

Plates were coated with antisera to MDMV and SrMV in coating buffer as recommended by the manufacturer (1:1000) and incubated at 37°C for 4 h. Plates were then washed thrice in PBST. Unbound spaces were blocked with 2% skimmed milk (sigma U.S.A.) in PBST and incubated at 37°C for 30 min. Test samples were prepared as described for DAS ELISA above and 200 µl were added to the wells and incubated at 4°C overnight. Monoclonal antibody at 1:1000 in conjugate buffer was added to each well after washing as above and incubated at 37°C for 4 h. Plates were washed and 200 µl anti-virus conjugate was added to each well and incubated at 37°C for 2 h. The plates were washed in PBST. Freshly prepared substrate [10 mg p-nitrophenyl phosphate in 10 ml substrate buffer (97 ml/dm^3 diethanolamine + 600 ml/dm^3 distilled water + 0.2 g/dm^3 sodium azide)] was added (200 µl) to each well and incubated at room temperature. Colour change was recorded by visual observation. A_{405nm} values were recorded in a microplate reader (optic ivymen system 2100c). Absorbance values greater than two times that of the healthy control and were considered positive. Data recorded were analyzed using analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) was used to separate the mean.

RESULTS

Survey for sugarcane mosaic disease

SCMD symptoms was observed in all the villages visited exhibiting a range of symptoms especially on the leaves and stem. The symptoms range from pale green stripes to yellow chlorotic stripes on a dark green background on the leaves while white stripes were observed on the stem Figure 2. The farmers locally call it "Mamar" or "Maizabuwa" based on the symptoms. The Bakarkwama was found to be the most susceptible (76% disease incidence) and the
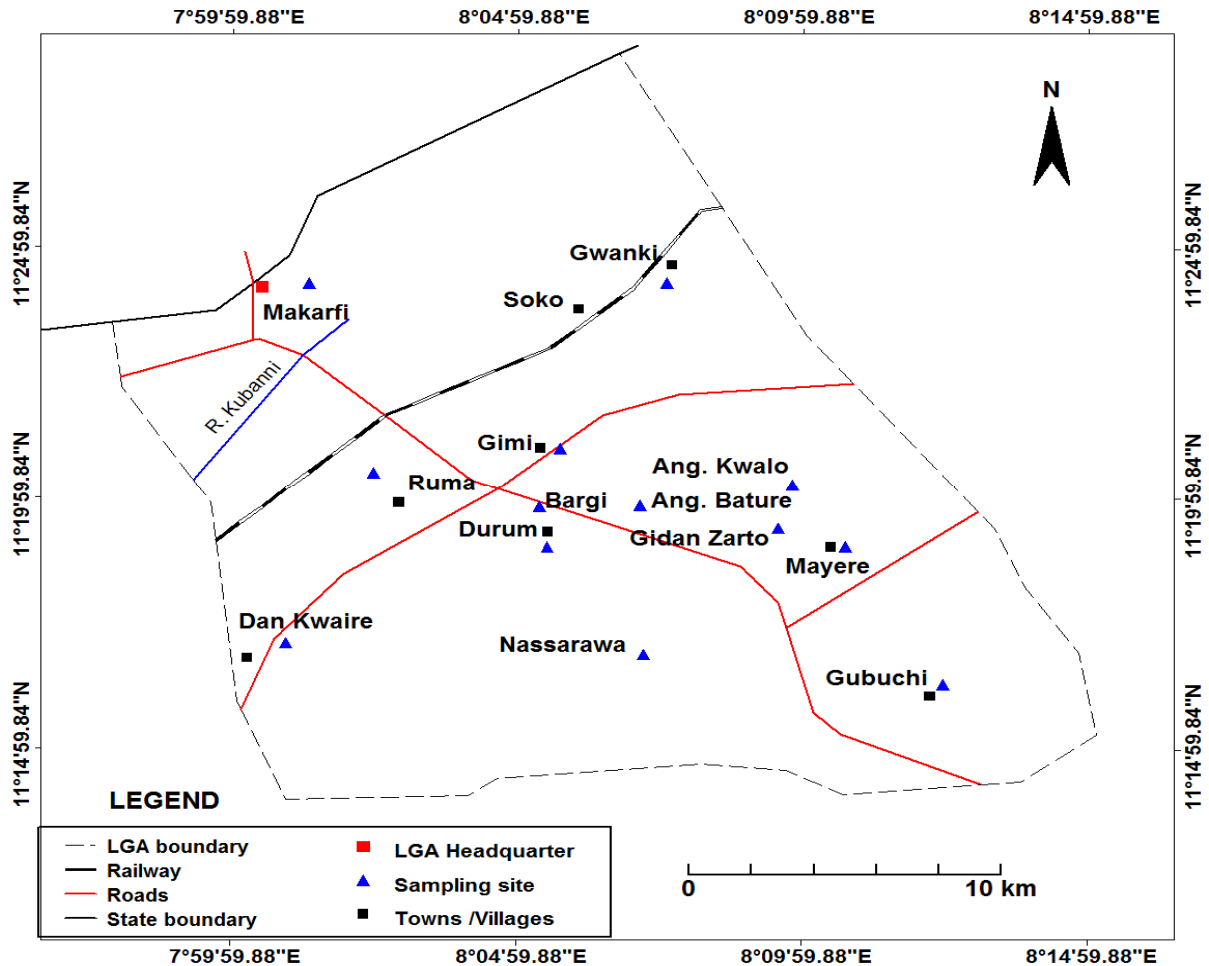
Figure 1. Map showing location of samplng areas. Source: Modified from administrative map of Kaduna State.

least was the Fararkwama (18% disease incidence).

Identification of the virus isolate involve in SCMD in Makarfi Local Government

Enzyme-linked immunosorbent assay (ELISA) analysis of samples obtained showed that SCMV was the most common type detected (Table 1) followed by SrMV and MDMV (Table 2). Out of the 63 samples tested, 52 (83%) samples reacted positive to SCMV antiserum, six (0.1%) were positive to MDMV and three (0.01%) positive to SrMV antisera. JGMV was not detected in the samples obtained from the 14 villages. This showed that SCMV have the highest occurrence. SCMV was detected in 49 purple cane and in three white cane fields from all the fourteen villages visited, while SrMV was detected in 3 (2 white canes and 1 purple cane) from Ruma and 3 (all of the purple cane) from Makarfi town and MDMV was detected in (one white cane) from Bargi and (2 purple cane) from Makarfi town.

However, mixed infections of SCMV and SrMV were recorded in two white canes from Ruma and one purple cane from Makarfi village. Mixed infections of the three viruses: SCMV, MDMV and SrMV were also recorded in two purple land races in the field from Makarfi village (Table 3). A range of ELISA values for the 63 survey samples, healthy controls and negative controls for the four different viruses inducing Sugarcane Mosaic Disease are shown in (Table 4). The mean values for SCMV, JGMV, MDMV and SrMV positive controls were 3.378, 3.317, 3.535 and 3.116 nm while their negative controls were 1.256, 1.190, 1.024 and 0.779 nm, respectively. The absorbance values show that SCMV has the highest concentration followed by MDMV and SrMV.

DISCUSSION

Makarfi has a long history of sugarcane cultivation, the weather and soil conditions support the extensive cultivation of the crop. As such there is a popular saying that Makarfi is the home of sugarcane. Three land races
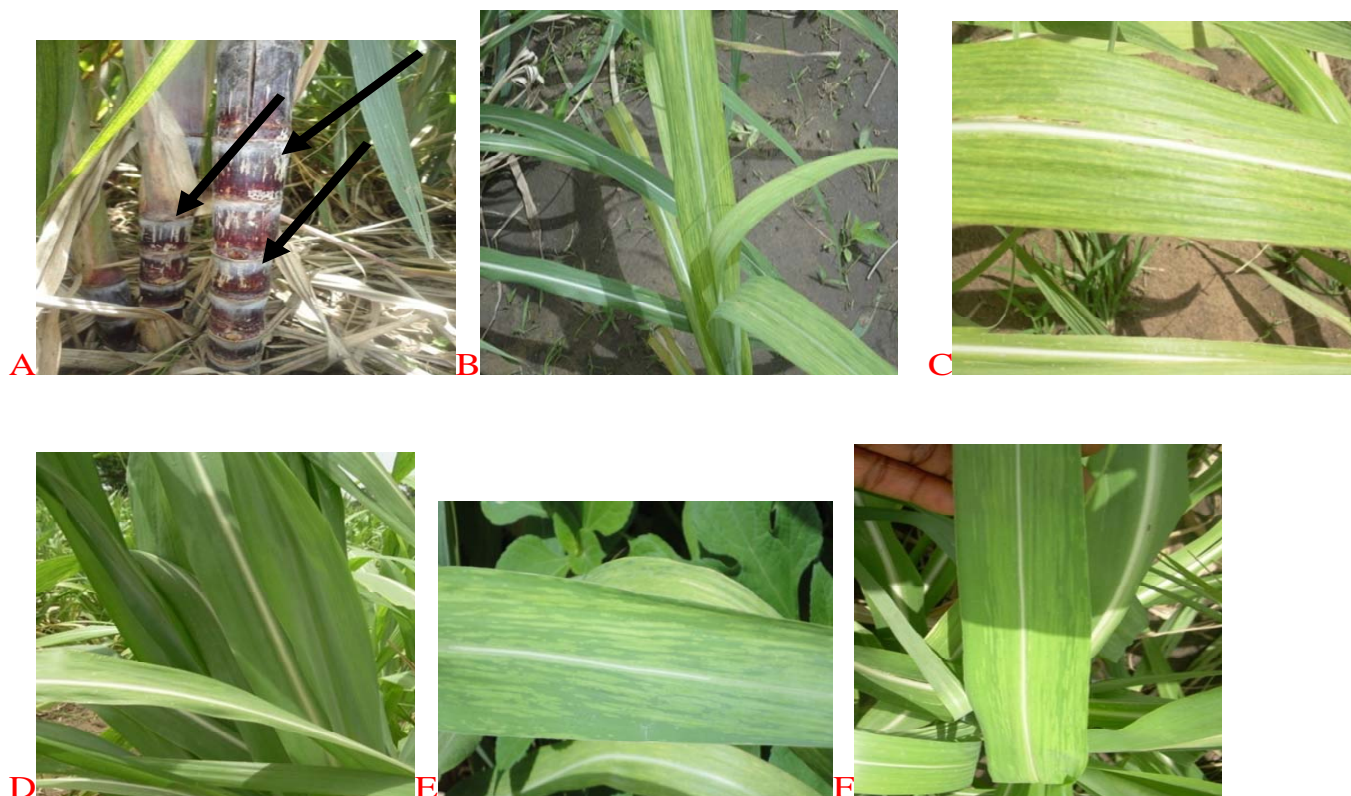
Figure 2. A- White Stripe and short internodes symptoms on infected stem. **B, C-** Yellow chlorotic stripes. **D-** Healthy leaves. **E, F-** Pale green stripes on sugarcane leaves.

Table 1. Numbers of DAS-ELISA positive samples from symptomatic and asymptomatic cultivated sugarcane leaves samples from Makarfi local government areas.

Location	Land race	Number of samples tested	Virus detected	
			SCMV	JGMV
Mayere	purple cane	4	4	0
Ruma	purple cane	6	5	0
	green cane	2	2	0
Nassarawa	purple cane	1	1	0
	white cane	1	0	0
Gimi	purple cane	2	2	0
GidanZarto	purple cane	5	5	0
Ang.kwalo	purple cane	4	3	0
Dankwaire	purple cane	4	3	0
Makarfi	purple cane	8	7	0
Durum	white cane	2	1	0
Durum	purple cane	5	3	0
Gubuchi	purple cane	5	4	0
Bargi	purple cane	3	3	0
	white cane	2	1	0
Gwanki	purple cane	5	5	0
Ang.Bature	purple cane	4	3	0
Total		**63**	**52**	**0**

Table 2. Numbers of TAS-ELISA positive samples from symptomatic and asymptomatic cultivated sugarcane leaves samples from Makarfi Local Government areas.

Location	Land race	Number of samples tested	Virus detected MDMV	Virus detected SrMV
Mayere	purple cane	4	0	0
Ruma	purple cane	6	0	1
	Green cane	2	0	2
Nassarawa	purple cane	1	0	0
	white cane	1	0	0
Gimi	purple cane	2	0	0
GidanZarto	purple cane	5	0	0
Ang.kwalo	purple cane	4	0	0
Dankwaire	purple cane	4	0	0
Makarfi	purple cane	8	2	3
Durum	white cane	2	0	0
Durum	purple cane	5	0	0
Gubuchi	purple cane	5	0	0
Bargi	purple cane	3	0	0
	white cane	2	1	0
Gwanki	purple cane	5	0	0
Ang.Bature	purple cane	4	0	0
Total		**63**	**3**	**6**

Table 3. Natural occurrence of double and triple infections of sugarcane virus isolates of SCMD in Makarfi L.G.

Location	Land race	Detection of mix infections of SCMV and SrMV	Detection of mix infections of SCMV, MDMV and SrMV
Makarfi	purple cane	1	2
Ruma	white cane	2	0

Table 4. Mean SCMD complex enzyme-linked immunosorbent assay (ELISA) absorbance (A_{405nm}) values for all samples tested.

Antibodies	Positive control	Negative control	Field sample (range)
SCMV	3.378	1.256	1.218-3.717
JGMV	3.317	1.190	0.476-2.091
MDMV	3.535	1.024	0.847-3.125
SrMV	3.116	0.779	0.398-3.106

are grown there, BakarKwama", "fararKwama" and BaHausa, however the distribution of these cultivars vary within the local government. SCMD has been very devastating in all the areas visited. Initially, the farmers associated the disease to urea fertilizer application but they later confirm that the symptoms manifested even in fields were urea is not applied as such they named it "Mamar" or "Maizabuwa". The SCMD is the most devastating disease of cane in the areas especially where the "Bakarkwama" (the most susceptible land race) dominates. However, the farmers were not certain of their source of infection. The source of infection might be in weeds or cereals intercropped with sugarcane for example, *Zea mays* and *Sorghum bicolor*. Xu et al. (2008) reported SCMV and SrMV infections in maize and sorghum in China. Sharma and Misra (2011) reported high incidence of MDMV in China, South Africa and United States of America in maize. *A. gossypii* may be responsi-

ble for the spread but to a larger extent contaminated cutlass may be responsible. Singh et al. (2005) confirmed four species of aphids to transmit SCMV in sugarcane in India. The results of DAS and TAS ELISA indicate the occurrence of the three viruses (SCMV, MDMV and SrMV) causing mosaic of sugarcane and the non detection of JGMV in all the samples tested. Different immunological techniques have been used to distinguish the four SCMD complexes (Tosic, 1990). For example, Yasmin et al. (2011) reported the occurrence of SCMV and MDMV in two provinces of Pakistan while SCMV and SrMV were found to be the causal agents of sugarcane mosaic disease in South China (Xu, 2005). However, Mohammad and Behzad (2009) reported the detection of SCMV but not MDMV and SrMV in Tehran province of Iran. However, in another study using ELISA, Selfer et al. (2005) detected the presence of JGMV from sorghum in Nigeria, where the isolate induces necrosis in sorghum but fail to infect Johnsongrass and oat. The information on SCMD subgroup present in a particular area will be of great economic importance in establishing the losses caused by SCMD in the area.

The results show that SCMV type is the most common of the SCMD viruses as it was detected in all locations visited in Makarfi Local Government Area. SCMV is followed by SrMV and MDMV. The occurrence of SCMV has also been reported in other countries like Huckett et al. (1998) who confirmed the presence of SCMV in South Africa using genome based technique. Also, Saleem et al. (2011) confirmed the presence of SCMV from naturally infected sugarcane crop in Pakistan. Of interest are the detection of the three viruses and the non detection of JGMV in sugarcane. The non detection of JGMV in all the samples tested may be because MDMV, SCMV and SrMV are closely related to each other than they are to JGMV (Shukla et al., 1992, Seifers et al., 2005).

The highest incidence of SCMV suggests its long existence in the areas, the crop situation observed showed that the farmers are using continuously their own germplasm so that virus is accumulating in the field and this is a major factor in disease development and spread. The possible reasons for the high incidence of viral infection may be due to susceptibility of sugarcane varieties, lack of a viral screening system (Zhou and Xu, 2005), high densities of aphid populations, which transmit sugarcane mosaic disease (Luo et al., 2003), and also the presence of viral inoculum reservoirs available near sugarcane-growing areas (Zhou et al., 2007). Samples with mixed infections were also observed, one purple cane from Makarfi and two white canes from Ruma were co-infected with SCMV and SrMV while two other purple canes from Makarfi were co-infected with SCMV, MDMV and SrMV. This is in agreement with findings of Xu et al. (2008) in which a high incidence of SCMV and SrMV co-infection was revealed in both hybrid and noble sugarcanes, all co-infected plants showed mosaic symptom. With the introduction of new improved commercial cultivars of sugarcane and other cereals like maize and sorghum, these triple and double infections might lead to more devastating disease. The detection of SCMV in asymptomatic plants suggests that latent infection occur or a mild strain of the virus.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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