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Assessment of Yield in Maize Streak Virus (MSV) Infected Maize (*ZEA MAYS L.*) in Kebbi State, Nigeria

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Abstract

The research was conducted to assess the yield in maize streak virus infected maize consisting of six treatments (Ba'ara (White), Ba'ara (yellow) Gwado (Yellow), Gwado (White), Doguwa (Yellow) and healthy seed maize. The experiment was laid out in a Randomize Complete Block Design with three replications. The maize was planted in a spacing of 30 x 60 cm on plots of 3 x 4.8m. Information was recorded on plant height, numbers of leaves, cobs weight and grain yield. The data were subjected to Analysis of variance (ANOVA). The results obtained showed that Ba'ara white infected with MSV have the highest yield ((0.18 t/ha)) compared to Ba'ara yellow (0.17t/ha), dogowa (0.17 t/ha), Gwado yellow (0.16t/ha) and Gwado white (0.11 t/ha). Ba'ara white shows less incidence to MSV among the varieties tested using PCR. This is a highly valuable finding and could be exploited for developing strategies to control the current MSVD epidemic in Africa.

Keywords: *Maize, Maize varieties, Incidence, Disease, MSV, Loose, Virus*

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Background to the Study

Maize (*Zea mays* L.) belongs to the family *Poaceae* and is one of the most important cereals grain after wheat and rice which is cultivated worldwide (De Lannoy, 2001). The importance of maize cannot be over emphasized in the developing world, it is a staple food for over 900 million people (CIMMYT and IITA. 2010). Maize is not only use as food for human consumption, but also as basic element of animal feeds and raw material for the manufacture of many industrial products (IITA, 2015). The use of maize varies in different countries. In USA, UK, Israel, Canada and other developed countries, maize is used mainly to feed animals directly (Milind and Isha., 2013). Maize silk has a lot of benefits, in many countries like India, China, Spain, France and Greece it is used to treat kidney stone, urinary tract infection. Decoction of silk, leaves and roots are used for bladder problems, nausea and vomiting, while decoction of cob are used for stomach complaints (Kumar and Jhariya., 2013) Maize has always been preferred to any other food staples, including cassava because most of the world's civilization is developed around grains rather than tuber crops (Fakorede, 2001). Preparation and uses of maize into local dishes, alone or in combination with other foodstaplein Nigeria includes; Ogi, Tuwo, Kunu, Masa, Couscous, Akpu, Gwate, Nakia, Egbo, Abari, Donkwa, Ajepasi, Aadun, Kokoro, Elekute etc (Abdulrahman and Kolawole, 2006). Industrially, maize is being useto generate ethanol for fuel, which is the same type of alcohol found in alcoholic beverages (HLPE, 2013). Maize is produced in all agro-ecological zones of Nigeria and the production in Nigeria is 10.4 metric tons (FAO, 2013). In 2014, the total estimated land area devoted to maize production in Nigeria was about 5.7 million (NAERLS, 2013).

The major biotic constraints to maize production are pests and diseases. Maize yield losses due to disease ranges between 56% to 100% and it varied with the time of infection and varietal resistance (Guthrie, 1978; van Rensburg, 1981; Bosque-Perez *et al.*, 1998). In sub-Saharan Africa maize is grown as a subsistence crop and consequently no estimates have been made of the financial losses due to streak disease (Kamara *et al.*, 2003). With the increasing importance of maize to agriculture in this region more detailed information on the economic impact of maize streak virus (MSV) is needed. Food security is the priority for farming households in Africa, Nigeria and Kebbi State in particular. This security however, is being threatened by disease such as maize streak virus disease (MSVD). Little information is available on how Maize Streak Disease (MSD) affects maize yields in the study area. The symptom severity of MSD on different maize varieties has not been studied in Kebbi State. Identifying the effect of MSVD on yield of different maize varieties will be of great benefit to maize breeders, maize vector entomologists, virologists and other researchers. Identifying the symptom diversity and severity of MSVD on different maize verities will aid decisions in variety selection for resistance to MSV.

Materials and Methods

Experimental Site

The research was conducted in Fadama Teaching and Research Farm of the Kebbi State University of science and technology, Aleiro, located at Jega (Lat. 12°12'N and Lon. 4°22'S), in Sudan savannah agro-ecological zone of Nigeria, during the 2014/2015 dry season. The climate of the area is semi-arid characterized by erratic and scanty rainfall that last for about

four months (May - September) and long dry period of about seven months (October - April). The average rainfall of the area is about 550–650 mm per annum. The relative humidity ranges from 21–47% and 51 – 79% during the dry and rainy seasons, respectively. The temperature averages between 27–41°C during dry season and 14–30°C during rainy season (Raji, 2012). The soil type and fertility vary from place to place, ranging from sandy-loam to clay-loam.

Materials and Methods

Maize varieties: Seed of five local maize varieties that are affected by MSVD were collected from farmer's field and seed from an unknown healthy plant was used as control as follows:

$\mathbf{V_0}$ = Control (healthy seed)
$\mathbf{V_1}$ = Ba'ara MSV affected seed (Yellow)
$\mathbf{V_2}$ = Ba'ara MSV affected seed (White)
$\mathbf{V_3}$ = Gwado MSV affected seed (Yellow)
$\mathbf{V_4}$ = Gwado MSV affected seed (White)
$\mathbf{V_5}$ = Doguwa MSV affected seed (Yellow)

Treatments and Experimental Design

The treatments consisted of six (6) maize varieties described above, laid out in Randomized Complete Block Design (RCBD) with three replicates. Total land areas of 22m x 18m (396 m^2) were made into 18plots of 4.8m x 3m each. The soil samples of the area were evaluated for physical and chemical properties before planting.

Data Collection

Maize Streak Virus Disease symptom diversity

For each treatment, plants showing symptoms of MSVD were counted and recorded. Total maize plants per plot were examined for the effect of MSVD infection on the establishment of maize plants. The effect of MSVD on maize growing leaves, disease symptom diversity and severity were recorded. Number of maize plants that died as result MSVD was recorded to measure the effect of the disease on yields. Leaves symptom was scored on 3-month old plants using a five-point scale where 1 = no visible MSVD symptoms, 2 = mild foliar symptoms on some leaves, 3 = pronounced foliar symptoms but no die-back, 4 = pronounced foliar symptoms which might include slight die-back of terminal branches, and 5 = severe foliar symptoms and plant die-back (Mohammed *et al.,* 2012, 2016, 2017). Plants grown from healthy plots were scored as control.

DNA extraction

DNA was extracted from MSV-infected maize leaf samples using DNeasy® Plant Mini Kit (Qiagen, UK) according to the manufacturer's Instructions. DNA extracted with the DNease® Plant Mini Kit was eluted in 25 µl of DSW and quantified using the Nanodrop DN1000 spectrophotometer.

PCR Amplification of MSV

PCR was prepared in a 25 µl, containing 10X buffer (100 mM Tris-HCl, 500 mM KCl, pH 8.3), MgCl2 (75 mM), dNTP mix (4 mM), 0.275 µM of each primer (V1forward/C2 reverse) (Van-

Antwerpen *et al.*, 2011), 1µl of Taq DNA polymerase and 2 µl of the DNA template. The amplification profile consisted of initial denaturation at 94 °C for 2min, followed by 30 cycles of denaturing at 94 °C for 1 min, primer annealing at 53 °C for 30 seconds and primer extension at 72 °C for 1 min and a final extension cycle at 72 °C for 5 minutes. PCR products were visualized in agarose gels (2%) stained with ethidium bromide and viewed under UV light.

Result

MSVD Symptom type and Development on Maize

Severe leaf spots and uniform clearing symptoms were observed on maize. Initial symptoms of this type appeared as faint green spots (Figure 1) which later turned into yellow and eventually became clear streak. The spots were distributed throughout the leaf. This is followed by the development of yellowing of all the leaves. The yellowing of leaves is mostly even, spreading throughout the affected leaf which later develops into clear spots. The spots developed into discontinuous yellow streaks, several millimeters in length along the infected leaf blades like what was described in the literature. The streak cause reduction in photosynthetic area of the leaves (Figure 1).

MSVD Incidence and Symptom Severity Score

MSVD incidence and symptoms severity score are presented in (table1). Result indicates that Ba'ara white has significantly high incidence both 7 WAP (23%) and 10 WAP (29) at $P<0.05$than Ba'ara yellow (22 and 27%) and Gwado yellow (17 and 21%).In all the varieties tested higher disease incidence was recorded at 10 WAP than at 7 WAP. All MSV-infected leaf samples were amplified by PCR using primer pair V1 forward/C2 reverse targeting 1200 base pair (bp) (Figure 2).

Weight of cobs (g) and Yield t/ha

The results show that there was significant difference ($P<0.05$) in weight of affected maize cobs and grain yield among the varieties (Table 2). Average weight per cob ranges from 0.10kg to 0.24kg. The highest weight was obtained in V_0control (0.24kg) while the lowest weight was obtained fromV_3Gwado Yellow (0.1kg) the yield of V0 (healthy control maize) showed a significant difference from all the four varieties infected with MSVD. The highest yield 2.70t/ha was obtained in (V_0), followed by$V_2$with (1.46t/ha) which were statistically the same with other varieties of affected maize plant. The lowest yield was recorded inV_4(0.20t/ha) and V_3(0.11t/ha) (Table 2).

Plant height and number of leaves (cm)

Result revealed that MSVD had a significant effect ($P<0.05$) on both Plant height and number of leaves at 10 WAP. The result showed no significant different among the MSV affected plant as shown in (Table 3). At 10 WAP, plant height of V5 was significantly low(143.93cm) as compare to varieties; V1, V2, V3, V4 and V0 which are (156.83), (160.73), (143.00), (144.53) and (204.70) respectively. The highest value was obtained from V2 (160.73) of all the five varieties tested as well as highest number of leaves (13 no.) was obtained from variety V2. V0 control shows significantly higher number (205 no.) than the remaining five varieties infected with MSV.

Discussions

Viral diseases are a major constraint to global food production. MSV causes maize streak disease (MSD), the most important disease affecting maize production, and has been known to cause yield losses of up to 100%. Currently, the main strategies employed to protect crops from viruses are based on either pathogen-derived resistance (PDR) or RNA interference (RNAi). However, both these suffer from well-known drawbacks including losses of pathogen resistance over time, food quality and safety issues related to high-level expression of virus-derived transgenes in modified crops, and are yet to be proved sufficiently robust for full virus immunity. Until recently, research on MSVD diversity/severity has largely been restricted to observations in the field on maize plants of different age, genetic makeup, and grown in different agro ecological zones with varying environmental conditions and possibly infected with different virus strains, all of which can independently or in combination influence symptom development, severity and diversity. From the results obtained in this study, it indicates that Maize streak virus disease (MSVD) is currently present in Kebbi State.

The results show that currently MSVD in Kebbi State is mainly caused by MSV and disease incidence varied according to varieties and the age of the plant. The highest cobs weight (0.24kg) was obtained in the healthy control maize plants, while the lowest weight was obtained from the variety Gwado Yellow (0.1kg). Similarly,2.70t/ha was obtained from the healthy variety followed by Ba'ara white (1.46t/ha). The findings of this study were like the work of (Guthrie, 1977), who recorded yield losses of between 33 and 56% due to MSVD in a field trials. Bosque- Perez *et al*. (1998), stated that yield loss attributed to MSVD is variable but losses of up to 70% have been reported in susceptible maize varieties. Other researchers too found that MSVD affected maize plants may be shorter, have less vigor and produce smaller grains and ears (Okoth*et al*, 1987). The MSV detected during PCR analysis in each DNA samples is not enough; further studies are recommended to determined full genomes by deep sequencing and analyzing the sequence results. The results will tell the actual species (MSV-A_1, A_2, A_3, A_4 etc.) of viruses present in Kebbi and that will be helpful in determining the control measures for MSVD. Based on this experiment it is recommended that the use of Ba'ara White seed as a planting material is effective as it result in higher yield compared to other varieties tested despite been affected by MSVD. However higher MSVD incidence was recorded in B'ara white. The need for a strict quarantine measures to prevent farmers from planting MSVD affect maize is also recommended to prevent further spread of MSV between towns and regions. The results obtained in this study have also identified the need for developing maize varieties with broad spectrum resistance to MSV.

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a   b

Figure 1: Maize healthy plant(a) and MSV inffected plant showing symptoms of SVD (b)

Table1: MSV incidence and symptom mean severity score

TREATMENTS	7WAP (%)	10WAP (%)	Mean severity score
V_0(control)	0.0^{a}	0.0^{a}	1.0
V_1(Ba'araYellow)	0.22^{bc}	$0.27b^{ac}$	1.0
V_2(Ba'araWhite)	0.23^{b}	0.29^{ba}	2.0
V_3(GwadoYellow)	0.17^{cd}	0.21^{bd}	3.0
V_4(GwadoWhite)	0.14^{de}	0.19^{dc}	4.0
V_5(Dogowa Yellow)	0.10^{e}	0.13^{d}	5.0
S.E±	**0.15**	**0.25**	

a b, c, and d Means with the same letter(s) along the column are significantly different ($p<0.05$) as analysed by DMRT. WAP=Weeks After Planting

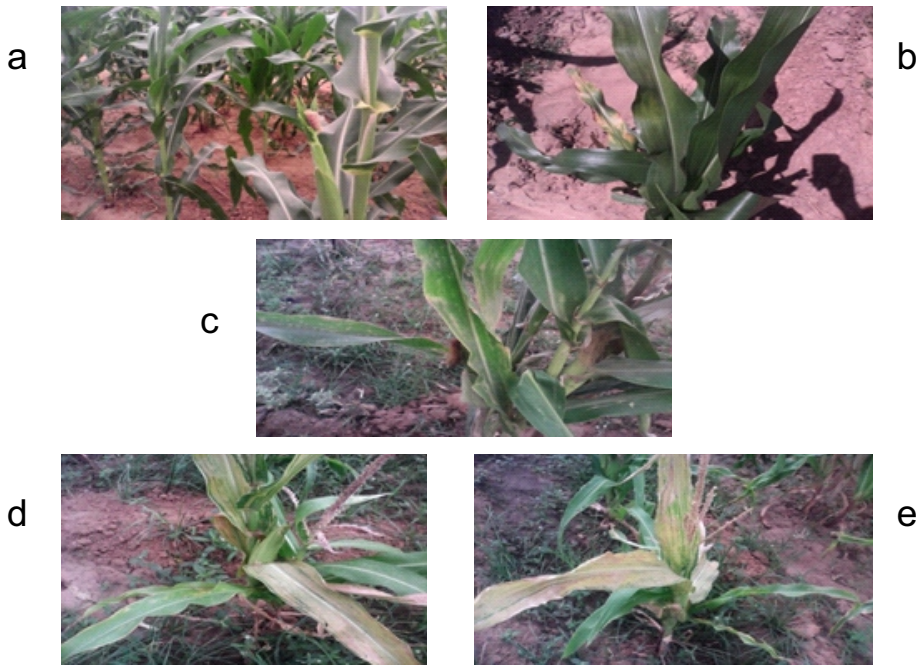

Figure 2: Leaves symptom was scored on 3-month old plants using a five-point scale where 1 = no visible MSVD symptoms, 2 = mild foliar symptoms on some leaves, 3 = pronounced foliar symptoms on leaves, 3 = pronounced foliar symptoms but no die back, 4 = pronounced foliar symptoms with slight die-back of terminal branches, 5 = severe foliar symptoms and plant die-back.

Table 2: Weight of the cobs (kg) and yield t/ha

TREATMENTS	WTC (kg)	YLD t/ha
V_0(control)	2.43^a	2.70^a
V_1(Ba'araYellow)	0.14^b	0.17^b
V_2(Ba'ara White)	0.16^b	0.18^b
V_3(Gwado Yellow)	0.10^b	0.11^b
V_4(GwadoWhite)	0.14^b	0.16^b
V_5(Dogowa Yellow)	0.15^b	0.17^b
$LSD_{0.05}$	**0.422**	0.4691

Means with the same letters along the column are not significant different ($p<0.05$) as analysed by DMRT. WTC=Weight of the cobs and YLD= Yield t/ha.