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# STATUS AND PRE-EMPTIVE MANAGEMENT STRATEGIES OF MAIZE LETHAL NECROSIS DISEASE IN NIGERIA

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ABSTRACT

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*Maize lethal necrosis disease (MLND) caused by mixed infections of Maize chlorotic mottle virus (MCMV) and Maize dwarf mosaic virus (MDMV) is currently the greatest threat to maize productivity in East African countries. Maize is a major cereal crop in Nigeria but there is no information on the status of MLND in the country. To ascertain this, a survey of maize viruses was conducted in selected States (Akwa Ibom, Cross River, Kano, and Katsina) in May and August 2017. A total of 108 maize leaves sampled from maize farms were tested serologically for MCMV, MDMV and SCMV using Antigen Coated Plate-Enzyme-Linked Immunosorbent Assay (ACP-ELISA). All the samples reacted negatively with MCMV, MDMV and Sugarcane mosaic virus (SCMV) antibodies, whereas 41 samples (38 %) exhibited strong positive reaction with Maize streak virus (MSV) antibody. The incidence of MSV disease was highest (51.2 %) in Kano, followed by Katsina (34.1 %), whereas the lowest incidence was found in Akwa Ibom and Cross River, with about 7.3 % each. This study showed that MLND is not yet in the study area. However, there is a need for continuous survey and surveillance, and pre-emptive breeding for maize germplasms that are resistant to MLND. In addition, farmers and the general public should adhere to preventive strategies in order to prevent severe yield losses.*

**Keywords:** Disease incidence, Maize viruses, Pre-emptive breeding, Survey and surveillance

## INTRODUCTION

Maize (*Zea mays* L.) is a major staple food crop grown in diverse agro-ecological zones and is consumed by people with varying food preferences and socio-economic backgrounds in sub-Saharan Africa (SSA) (Olaniyan, 2015). Maize is intensively cultivated in South Africa, Nigeria, Ethiopia, Egypt, Tanzania, Malawi, Kenya, Zambia, Uganda, Ghana, Mozambique, Cameroon, Mali, Burkina Faso, Benin, DRC, Angola, Zimbabwe, Togo, and Cote d'Ivoire. These countries account for 96 % of the total maize production in sub-Saharan Africa (Nuss and Tanumihardjo, 2011). It may be eaten as a vegetable or processed into various dishes and is regarded as a hunger breaker after a long dry period in developing countries. Maize is extensively traded as feed crop in livestock industries, and about 460 million (65 %) of total world maize production is used for this purpose (Olaniyan, 2015).

In addition to human consumption and livestock feeding, maize has a wide range of industrial applications including manufacturing of ethanol and pharmaceuticals (Gwirtz and Garcia-Casal, 2014). In West and Central Africa (WCA), maize production potential is greatest in the moist savanna where annual rainfall and solar radiation are favourable (Ismaila *et al.*, 2010). In spite of its numerous uses, maize yield in SSA including Nigeria is still very low compared to developed countries such as the United States, China, and Brazil due to many constraints, which may be biotic (weed infestations, insect pests and diseases) or abiotic (e.g. drought and low soil fertility) (Nuss and Tanumihardjo, 2011). Among the several virus diseases of maize, Maize lethal necrosis disease is the most economically important at the moment (Kiruwa *et al.*, 2016). Maize lethal necrosis disease (MLND), first reported in 2011 in Kenya, is a new virus disease in Africa that has devastated maize production and reduced yields by 30 to 100 % in the affected farms (Wangai *et al.*, 2012). This disease has since spread to Rwanda, South Sudan, Tanzania, and Uganda. The great risk exists for further spread within Africa. MLND is a synergistic disease caused by co-infection of *Maize chlorotic mottle virus* (MCMV, genus *Machlomovirus*) a potyvirus such as *Sugarcane mosaic virus* (SCMV, genus *Potyvirus*), *Maize dwarf mosaic virus* (MDMV) and *Wheat streak mosaic virus* (WSMV) (Mahuku *et al.*, 2015). Single infection of any of these viruses does not lead to lethal necrosis. *Maize chlorotic mottle virus* is transmitted by thrips and beetles (Mahuku *et al.*, 2015), whereas the potyviruses are transmitted by aphids (Whitfield *et al.*, 2015). Low rate (1:2500) of MCMV transmission through seed has also been reported by Mahuku *et al.* (2015). Although this is yet to be proven for African MCMV isolates, seed transmission represents a means for long-distance virus spread in the continent.

The first attempt to investigate the presence and distribution of MLND in West Africa was in the year 2014 (Salaudeen *et al.*, 2015). In the study, some Nigerian States sharing the boundary with Eastern African countries were surveyed but the disease was not detected. Because of the huge yield losses (up to 100 %) imposed by MLND, in May, 2015, Alliance for Green Revolution in Africa (AGRA), International Maize and Wheat Improvement Centre (CIMMYT), and the Bill and Melinda Gates Foundation sponsored a workshop attended by leading plant virologists, entomologists, agronomists, geneticists, and plant breeders. Other institutions represented at the workshop were seed companies and seed trade association representatives; regulatory and

phytosanitary authority and donor organizations with the objectives of sharing information that would help prevent the spread of the disease from Eastern Africa to West, Central, and Southern Africa.
Sustainable control of virus diseases requires a thorough understanding of their diversity, strains, and identification of resistant sources. In Eastern Africa, studies are already on-going to understand and curb the MLND menace but such attempt has not received much attention in West Africa. The spread of MCMV can trigger an onset of MLND epidemics in any region due to the ubiquitous occurrence of complimenting potyviruses (especially *Sugarcane mosaic virus* and *Wheat streak mosaic virus*) and insect vectors (thrips). Information on potyvirus diversity in Nigeria, particularly if the pathogen's profile is different from Eastern African countries would facilitate collaboration between MLND workgroups in Africa. Therefore, the need to intensify regular surveys and surveillance in areas where MLND has not been reported and development of resistant maize germplasm is essential. This study was conducted to determine the status of MLND in selected parts of Nigeria.

## MATERIALS AND METHODS

**Collection of samples**

A total of 49 maize fields surveyed in four States (Akwa Ibom, Cross River, Kano, and Katsina) for MLND in May and August 2017. These States were selected because of their proximity to Nigér, Chad, and Cameroon which serve as a link between Nigeria and Eastern African countries, where MLND has been confirmed. The exact coordinates of each farm were captured using the Geographical Positioning System (GPS) equipment (GPS-4300; Ethrex Garmin GPS, Taiwan). Photographs of some sampled plants were also taken. Detailed information including date and time of visit, farm size, cropping system, crops in neighbouring fields, fertilizer applications, insect pest and disease management strategies was recorded. Between 1 and 12 leaves were collected from each field, from maize plants exhibiting virus and virus-like symptoms, cumulating in 108 leaf samples. The leaves were preserved on silica gel to maintain viability before they were transferred to the laboratory for indexing. Each field was assessed for disease incidence and severity. Disease incidence was expressed as a percentage of the total plants exhibiting virus and virus-like symptoms. Disease severity was rated on a scale of 1 to 5 (Gowda *et al.*, 2015), where

1 = no visible MLND symptoms,
2 = fine chlorotic streaks mostly on older leaves,
3 = chlorotic mottling throughout the plant,
4 = excessive chlorotic mottling on lower leaves and necrosis of newly emerging leaves), and
5 = complete plant necrosis

**Virus identification by enzyme-linked immunosorbent assay (ELISA)**

Serological analysis of the sampled leaves was based on Antigen-Coated Plate ELISA (ACP-ELISA) (Kumar, 2009). Each leaf sample was homogenized in carbonate buffer pH 7.4 (0.015 M sodium carbonate plus 0.0349 M sodium bicarbonate per litre of distilled water) at the rate of 100 mg/mL, using cold sterilized mortar and pestle. One hundred microlitres of the sap from each sample was tested in duplicate wells of ELISA plates (Thermo Scientific "Nunc", Milford, MA). Sap sample from a healthy maize plant and a known virus-infected plant were used as a negative and positive control, respectively. After incubating at 37 °C for 1 hour the plate was washed three times with phosphate buffered saline-Tween (8 g NaCl, 1.1 g $Na_2HPO_4$, 0.2 g $K_2HPO_4$, 0.2 g KCl, 0.5 mL Tween – 20, 1 L distilled water, pH 7.4) (PBS-T). This was followed by addition of 200 µL of a blocking solution [3 % (w/v) dried non-fat skimmed milk in PBS – T].

The plates were incubated at 37 °C for 30 minutes and then tap-dried on a paper towel. Each sample was tested with 100 µL of the MCMV, MDMV and MSV polyclonal antibodies diluted (1:2000; v/v) with conjugate buffer [half strength PBS-T containing 0.05 % (v/v) Tween-20, 0.02 % (w/v) egg albumin, 0.2 % (w/v) polyvinylpyrolidone]. The plates were incubated again at 37 °C for 1 hour, washed thrice and 100 µL of the goat anti-rabbit antibody diluted with conjugate buffer (1:15,000) was added to the wells. The plate was incubated at 37 °C for 1 hour, washed and 100 µL of *p*-nitrophenyl phosphate dissolved in substrate buffer (97 mL diethanolamine, 1000 mL $H_2O$, pH 9.8) was added to the wells. The plate was finally incubated in dark at room temperature (37 °C). The absorbance of virus concentration was read at 405 nm ($A_{405}$) using a microplate reader (MRX, Dynex Technologies, Inc., USA) after 1 hour and overnight. The ELISA readings were accepted to be positive when values exceeded two times the negative control (Kumar, 2009).

## RESULTS

**Passport data of the surveyed sites**

In all the agro-ecologies surveyed majority of the farmers grew maize on less than 1 hectare of land. In Akwa Ibom and the Cross River States, although maize is cultivated twice annually most farmers preferred cassava as number one crop. Farmers cultivate maize twice a year owing to bimodal nature of the rainfall. Maize was grown in mixtures with other principal food crops such as cassava, yam, sugarcane, banana and plantain, cocoyam, fluted pumpkin, okra, pepper etc. All the farmers interviewed in Akwa Ibom and the Cross River States stated that they

usually purchased maize seeds from the market. Similarly, they never used pesticides to control insect pests and diseases; they normally replenished unproductive soils through the application of poultry droppings and careful management of plant debris after harvesting. Some farmers confirmed the presence of some disease conditions on yearly basis but attributed such to the low level of soil fertility. All the farmers interviewed were ready to acquire seeds from reliable sources such as Research Institutes, Agricultural Development Projects (ADPs), Ministries of Agriculture etc. In Kano and Katsina States, rainfall is unimodal and maize is the principal cereal crop. In most instances, maize was intercropped with guinea corn or millet. Most farmers stated that maize seeds were sourced from research institutes such as IITA, and ADPs. Some farmers attested to pesticide application only for weed and insect pests in cowpea fields. Soil improvement was mainly through the application of inorganic fertilizers (NPK and Urea). Some farmers also applied cow dung or poultry droppings on the farm in order to boost yield. All the farmers interviewed were ready to acquire maize and sugarcane seeds/cuttings from reliable sources such as Research Institutes, ADPs, Ministry of Agriculture etc.

**Disease incidence and severity**

Symtoms observed on the maize fields were leaf mottling, yellowing, mosaic and streaks along the mid-rib of infected plants (Plate I). All the samples reacted negatively with MCMV and MDMV antibodies (data not shown). However, 38 % (41) of the samples exhibited strong positive reaction with MSV antibody ( Table 1). Of the 41 samples that tested positive, the incidence of MSV disease was highest (51.2 %) in Kano, followed by Katsina (34.1 %). The lowest MSV disease incidence was found in Akwa Ibom and Cross River, with about 7.3 % each (Fig. 1). MSV incidence was observed at Bebeji, Garun Mallam, Kura, Dawakin Kudu, Kumbosto, Ungu and Tofa LGAs in Kano State. In Katsina, MSV was detected at Malumfashi, Musawa, Mutazu, Kankia, Charanchi, and Rimi. In Akwa Ibom State, the MSV positive samples were found at Uyo and Abak LGAs. In Cross River State, MSV was found specifically at Akai and Awi LGAs. Disease severity varied between 2 and 5 across the surveyed sites. Some of the susceptible plants were stunted and failed to produce cobs. In some others, small and deformed cobs were produced. In the late infected plants, disease severity did not affect cob formation.

Plate I: Leaf mottling and streaking symptoms on infected maize plants

## DISCUSSION

Several viruses cause significant reductions of maize yield in sub-Saharan Africa and this worsens food crises. The detection of MSV in all the surveyed sites revealed that the virus is still prevalent in Nigeria. Streak infection has been known in Nigeria since the 1970s. The prevalence of MSV disease could have been facilitated by a number of factors. Notable among them is the mode of spread. *Maize streak virus* is obligately transmitted by leafhoppers in a persistent manner (Alegbejo *et al.*, 2002). Therefore, the infected plants become sources of primary inoculum and virus refuge to infect other plants. Because of the active nature of the vector infection is not usually restricted to the nearby plants but those in the far distance are also at risk. The fact that maize farms were not sprayed with insecticide could have aggravated the MSV disease incidence in the surveyed areas. However, it has been reported that insecticide is partially effective because the recurring influx of migrant hopper populations re-infect the crop after each application (Magenya *et al.*, 2008). Low level of streak incidence was encountered in some maize fields due to late infection. The incidence of streak disease was high in Kano partly because of the intensive maize cultivation. In a study, Iken and Amusa (2004) reported that maize production has increased tremendously in the Savanna agro-ecology of Nigeria. Kano and Katsina States belong to Savanna agro-ecology, which is generally home to grass plants that enable leafhopper vectors to survive between seasons. Besides, the observation that maize is commonly grown in mixture with guinea corn and millet was possibly responsible for high rate of infection because these crops are also important host plants for insect vectors.

Table 1: Serological reactions of maize leaves to *Maize streak virus* (MSV) antibody

| Sample ID | MSV Antibody | Sample ID | MSV Antibody | Sample ID | MSV Antibody |
|---|---|---|---|---|---|
| AKW1.1 | 0.544* | CRS22.3 | 0.187 | KN36.7 | 0.123 |
| AKW1.2 | 0.232 | CRS23.1 | 0.136 | KN36.8 | 0.129 |
| AKW2.1 | 0.563* | CRS24.1 | 0.129 | KN36.9 | 0.130 |
| AKW3.1 | 0.127 | CRS24.2 | 0.171 | KN36.10 | 0.126 |
| AKW4.1 | 0.144 | KN25.1 | 0.137 | KN36.11 | 0.124 |
| AKW5.1 | 0.125 | KN25.2 | 0.191 | KN36.12 | 0.598* |
| AKW5.2 | 0.156 | KN25.3 | 0.363* | KN37.1 | 0.495* |
| AKW6.1 | 0.151 | KN25.4 | 0.269* | KN37.2 | 0.114 |
| AKW7.1 | 0.156 | KN26.1 | 0.370* | KN37.3 | 0.108 |
| AKW7.1 | 0.173 | KN27.1 | 0.185 | KN37.4 | 0.150 |
| AKW8.1 | 0.180 | KN27.2 | 0.447* | KN37.5 | 0.128 |
| AKW9.1 | 0.197 | KN28.1 | 0.795* | KN38.1 | 0.137 |
| AKW9.2 | 0.138 | KN29.1 | 0.608* | KN38.2 | 0.112 |
| AKW10.1 | 0.136 | KN30.1 | 0.145 | KN38.3 | 0.204 |
| AKW11.1 | 0.174 | KN30.2 | 0.163 | KN38.4 | 0.294* |
| AKW11.2 | 0.866* | KN30.3 | 0.498* | KN38.5 | 0.126 |
| AKW12.1 | 0.141 | KN31.1 | 0.129 | KN38.6 | 0.350* |
| AKW13.1 | 0.154 | KN31.2 | 0.774* | KT39.1 | 0.103 |
| CRS14.1 | 0.286* | KN31.3 | 0.368* | KT39.2 | 0.689* |
| CRS15.1 | 0.198 | KN32.1 | 0.133 | KT39.3 | 0.881* |
| CRS15.2 | 0.193 | KN32.2 | 0.300* | KT40.1 | 0.571* |
| CRS15.3 | 0.178 | KN33.1 | 0.893* | KT40.2 | 0.280* |
| CRS15.4 | 0.139 | KN34.1 | 0.113 | KT41.1 | 0.187 |
| CRS16.1 | 0.144 | KN34.2 | 0.122 | KT42.1 | 0.120 |
| CRS16.2 | 0.152 | KN34.3 | 0.119 | KT42.2 | 1.004* |
| CRS17.1 | 0.124 | KN34.4 | 0.122 | KT43.1 | 0.262* |
| CRS18.1 | 0.311* | KN35.1 | 0.463* | KT43.2 | 0.651* |
| CRS18.2 | 0.453* | KN35.2 | 0.380* | KT44.1 | 0.620* |
| CRS18.3 | 0.132 | KN35.3 | 0.137 | KT45.1 | 0.462* |
| CRS18.4 | 0.135 | KN35.4 | 0.894* | KT45.2 | 0.589* |
| CRS19.1 | 0.154 | KN36.1 | 0.541* | KT46.1 | 0.437* |
| CRS19.2 | 0.226 | KN36.2 | 0.121 | KT46.2 | 0.544* |
| CRS20.1 | 0.176 | KN36.3 | 0.117 | KT47.1 | 0.197 |
| CRS21.1 | 0.164 | KN36.4 | 0.121 | KT48.1 | 0.266* |
| CRS22.1 | 0.145 | KN36.5 | 0.359* | KT49.1 | 0.112 |
| CRS22.2 | 0.124 | KN36.6 | 0.453* | KT49.2 | 0.289* |

Diseased control = 1.124; Healthy control = 0.131; Buffer control = 0.127;
Key: *Positive reaction; AKWA=Akwa Ibom State; CRS = Cross River State; KN = Kano State; KT = Katsina State

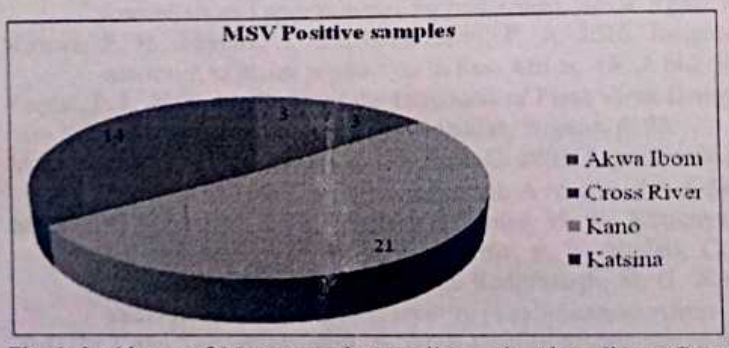


Fig. 1: Incidence of *Maize streak virus* disease in Akwa Ibom, Cross River, Kano and Katsina States of Nigeria

Weather is another factor which enhances leafhopper population. Disease incidence is particularly aggravated under favourable temperature. For instance, Alegbejo and Banwo (2005) reported a significant positive correlation between leafhopper population and MSV disease incidence in the Savanna agro-ecology of Nigeria. Symptom severity was not uniform in the study area due to a number of reasons. As clearly confirmed, by the farmers most of them sourced seeds from the open markets. This practice does not guarantee the use of streak-resistant/tolerant varieties. The differences in MSV disease severity was also variable as a result of differences in the genetic architecture of the cultivars, plants' age at the time of infection, strain, and virulence of the invading.

The fact that MCMV was not found in the tested samples indicated that MLND is not present in the study area. MCMV has been identified as the more devastating component virus resulting in MLND. All the east African countries where the disease has been reported are in close proximity to one another. Therefore, apart from insect transmission, seed exchange between those countries could be implicated in the spread of the disease. The fact that the disease has not been confirmed in Cameroon, Nigér, and Chad which are neighbouring countries to Nigeria may be a reason why it has not been found in the country. In addition, the establishment of several research institutes such as the International Institute of Tropical Agriculture and Institute for Agricultural Research that have maize as their mandate crop could have discouraged seed importation from other countries. Besides, the presence of several reputable seed companies in different parts of Nigeria might have prevented seed importation from other countries. Since MCMV has been reported to be seed transmitted, cultivation of clean seeds might have excluded the pathogen from the country.

## CONCLUSION AND RECOMMENDATIONS

The results of this investigation showed that incidence MSV was higher in Guinea Savanna (Kano and Katsina) than the forest agro-ecological zone (Akwa Ibom and Cross River). Cultivation of maize cultivars that are resistant to the virus and its leafhopper vector is recommended so as to minimize yield losses. Maize lethal necrosis disease is not yet in Nigeria. Although MLND was not detected in the surveyed locations, adequate preventive measures should be put in place in order to guarantee insurance against possible outbreak. Such strategies include regular surveillance of MLND and pre-emptive breeding for resistant maize germplasms.

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