PAT December, 2018; 14 (2): 68-77 ISSN: 0794-5213
Online copy available at www.patnsukjournal.net/currentissue
Publication of Nasarawa State University, Keffi

Surveillance of Maize Lethal Necrosis Disease in Nigeria

***Salaudeen[1], M. T., Gana[1], A. S., Bello[1], L. Y., Daudu[2], O. A. Y. and Oyewale[1], R. O.**

[1]Department of Crop Production, School of Agriculture and Agricultural Technology, Federal University of Technology, P. M. B. 65, Minna, Niger State, Nigeria
[2]Department of Plant Biology, School of Life Sciences, Federal University of Technology, P. M. B. 65, Minna, Niger State, Nigeria
**Corresponding author: mtsalaudeen@futminna.edu.ng*

Abstract
Maize is a multipurpose crop and a staple food for millions of people in Nigeria but its potential yield is constrained by several viruses. Maize lethal necrosis disease (MLND) is a new virus disease ravaging maize farms in East Africa with attendant field abandonment and huge financial losses. Therefore, this study was conducted to determine the status of the disease in Nigeria. Maize farms in 25 Local Government Areas (LGAs) selected from Akwa Ibom, Cross River, Kano and Katsina were surveyed between May and August, 2017. A total of 108 symptomatic and asymptomatic leaf samples were collected and tested for Maize chlorotic mosaic virus (MCMV), Maize dwarf mosaic virus (MDMV) and Maize streak virus (MSV). None of the samples was positive for MCMV and MDMV but about 62 % of them reacted positively with MSV polyclonal antibody (PAb). The incidence of MSV was highest in Kano State (41.8 %), followed by Katsina State (23.9 %), whereas incidence of 20.9 and 14.9 % was found in Cross River and Akwa Ibom State, respectively. The negative reaction of all the samples to MCMV and MDMV polyclonal antibodies implied that the surveyed areas were free of MLND. The symptomatic samples that tested negative were probably infected by pathogens other than those investigated in the present study. Despite this positive result, there is need for continuous surveillance, information sharing and awareness campaign within Nigeria and with neighbouring countries. Plant breeders should also intensify research programme for the development of MLND resistant maize varieties.
Keywords: *Breeding; Disease incidence; Maize; Maize Lethal Necrosis Disease; Surveillance*

Introduction

Food insecurity is a common and perennial crisis in sub-Saharan Africa (Ilaboya *et al.*, 2012). Although agriculture is a means of livelihood for millions of people in the region, achieving food sufficiency has been a mirage for most households. Maize (*Zea mays* L.) is one of the staple food crops for human consumption in developing countries (Badu-Apraku *et al.*, 2013). In addition to its use as food, maize has several other domestic and industrial applications in developing countries such as processing into livestock feed and pharmaceutical products. According to FAO (2016), about 1.06 billion tonnes of maize was produced globally in 2016. Of this, the greatest output came from Africa (70.6 million tonnes). Among the African countries, the highest maize output was produced in Nigeria (10.4 million tonnes). Despite continuous land area cultivated to maize in Nigeria, its productivity remains generally low (1.6 t/ha) (FAO, 2016). In the past, maize production in Nigeria was concentrated in the southern part of the country. However, available record has revealed much more production in the northern part of the country in recent times (Anonymous, 2017).

Maize yield is constrained by abiotic and biotic factors. Among the biotic factors, insect pests and diseases have been implicated as impediments to potential maize yield. Viruses are one of the major pathogens limiting crop yields in sub-Saharan Africa (Tadele, 2017). Majority of the maize viruses are transmitted by insect pests, a phenomenon responsible for widespread transmission and epidemic of some plant virus diseases. *Maize chlorotic mottle virus* (MCMV, genus *Machlomovirus*), *Maize dwarf mosaic virus* (MDMV, genus *Potyvirus*) and *Maize streak virus* (MSV, genus *Mastrevirus*) are among the economically important viruses of maize in the region. Recently, an outbreak of Maize lethal necrosis disease (MLND) was reported in some East African countries (Kiruwa *et al.*, 2016). Maize lethal necrosis disease is a synergistic disease induced by MCMV and a *Potyvirus* such as *Sugarcane mosaic virus* (SCMV) or MDMV. Maize lethal necrosis disease can also be caused by mixed infections of MCMV and *Wheat streak mosaic virus* (WSMV, *Tritimovirus*) but it is frequently induced by double infections of MCMV and SCMV. Virus diseases arising from synergistic double infections are usually much more devastating on susceptible crop varieties. Maize lethal necrosis disease epidemiology has been reported in many East African countries due to poor understanding of its ecology by smallholder farmers. Consequently, losses of up to 100 % have been reported in some countries including Kenya and Tanzania. Maize lethal necrosis disease naturally infects maize varieties resulting in chlorotic mottling of the leaves, severe stunting and necrosis which hinder plants' growth and development (Wangai *et al.*, 2012; Adams *et al.*, 2014).

Single infection of MCMV or SCMV causes only mild mosaic or mottling symptoms and a moderate reduction of growth. In mixed infections of both viruses, early infected plants appear stunted and show a general chlorosis, leaf bleaching and necrosis. Both MCMV and SCMV are transmitted through mechanical means. In addition, MCMV can be transmitted by thrips and beetles while SCMV is vectored by aphids (Cabanas *et al.*, 2013). Consequently, MLND has been identified as the most devastating foliar disease responsible for considerable yield loss. According to Ochieng *et al.* (2012), severely affected areas may experience a huge yield loss exceeding 90 % which may worsen food crisis in a country. In order to develop appropriate control and preventive measures against MLND, there is need for regular surveillance in places where it has not been reported. Therefore, the objective of this study was to determine the status of MLND in Nigeria.

Materials and Methods

Field survey and sample collection

Maize fields were surveyed between May and August 2017. The States (Akwa Ibom, Cross River, Kano and Katsina) surveyed (Table 1) served as a link with the East African countries where MLND outbreak has been reported. Abak, Eket, Esit-Eke, Ikot-Ekpene, Nsit-Atai, Okobo and Uyo Local Gvernment Areas (LGAs) in Akwa Ibom State, and Atampa, Biase, Calabar Municpal, Calabar South and Odukpani LGAs in Cross River State were surveyed. The LGAs surveyed in Kano State were Bebeji, Dawakin-Kudu, Garun-Mallam, Kumbosto, Kura, Tofa and Ungu. In Katsina State, Charanchi, Kankia, Malumfashi, Matazu, Musawa and Rimi LGAs were surveyed. In each LGA, between two and 16 maize farms were visited. Information about each farm was captured using data collection sheet. The coordinates of each farm were recorded using Geographical Positioning System (GPS-4300; Ethrex Garmin GPS, Taiwan). Symptomatic and asymptomatic leaf samples were collected from each farm and preserved in vial bottles over self-indicating silica gels. Depending on the symptom types, between one and 12 leaves were collected from each field.

Serological analysis of leaf samples

The leaf samples were subjected to Enzyme-Linked Immunosorbent Assay (ELISA) according to the protocol of Kumar (2009). Samples were ground in sterilized mortar at the rate of 100 mg/mL using cold carbonate buffer pH 7.4 (0.015 M sodium carbonate plus 0.0349 M sodium bicarbonate per litre of distilled water). One hundred microlitres of the leaf extract was added to each well of the microtitre plates (Thermo Scientific "Nunc", Milford, MA). The plates were incubated at 37°C for 1 hour and washed three times with phosphate buffered saline-Tween (8g 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). A blocking solution [3 % (w/v) dried non-fat skimmed milk in PBS – T] was applied at the rate of 200 µL per well. This was followed by incubation of the plates at 37°C for 30 minutes. The plates were tap-dried on a paper towel.

Table 1: States and Local Government Areas where farms were surveyed

State	Local Government Area	Longitude (°E)	Latitude (°N)	Altitude (masl)
Akwa-Ibom	Abak	4.98884	7.78392	52
	Eket	4.66567	7.97544	12
	Esit-Eke	4.66502	8.02646	41
	Ikot-Ekpene	5.16881	7.71965	92
	Nsit-Atai	4.83321	8.04094	20
	Okobo	4.83583	8.12141	44
	Uyo	4.96362	7.99600	64
Cross River	Atampa	5.23776	8.36157	116
	Biase	5.42030	8.18274	123
	Calabar Municipal	5.07703	8.34645	89
	Calabar South	4.92242	8.31795	18
	Odukpani	5.18995	8.26525	11
Kano	Bebeji	11.56243	8.31782	533
	Dawakin-Kudu	11.92800	8.63478	474
	Garun-Mallam	11.65029	8.41760	498
	Kumbosto	11.91547	8.52781	457
	Kura	11.83954	8.51671	430
	Tofa	11.97777	8.33225	490
	Ungu	11.96009	8.41143	448
Katsina	Charanchi	12.51374	7.74421	562
	Kankia	12.40325	7.72016	533
	Malumfashi	11.98185	7.69575	598
	Matazu	12.31316	7.68504	505
	Musawa	12.10579	7.73728	601
	Rimi	12.83870	7.67264	538

Note: masl=metres above sea level

Polyclonal antibodies (PAbs) for MCMV, MDMV and MSV were 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] and 100 µL each was tested against extract of each sample. 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 plates were incubated at 37°C for 1 hour and washed. Afterwards, 100 µL of *p*-nitrophenyl phosphate dissolved in substrate buffer (97 mL diethanolamine, 1000 mL H_2O, pH 9.8) was added to the well. The plates were finally incubated in the dark at room temperature (37°C) overnight. Absorbance of virus concentration was recorded at 405 nm (A_{405}) using a microplate reader (MRX, Dynex Technologies, Inc., USA). Values of ELISA readings twice those of the healthy control were considered to be virus positive.

Results

A total of 67 (approximately 62%) samples out of 108 reacted positively with MSV polyclonal antibody (Table 2), whereas none was detected by MCMV and MDMV. Of the total MSV positive samples, 14.9% were collected from Akwa Ibom State. Among the LGAs surveyed in Akwa Ibom State, the highest incidence (4.5%) of MSV was encountered in Uyo LGA and the samples were collected from Uyo town. Some of the severely infected plants exhibited marked stunting and poor cob production. In such plants, more than 70% of the leaf surface was covered with whitish or chlorotic streaks. Moreover, severity of MSV infection was more acute on the early infected plants than those infected at advanced growth stage. The leafhopper vector of MSV was frequently observed on young maize plants in most fields. However, in Esit-Eke, Nsit-Atai and Okobo LGAs, a uniform MSV disease incidence of 3% was found. Conversely, the lowest MSV disease incidence (1.5%) was observed in Abak LGA. In this LGA, the MSV positive samples were collected from Utu-Abak. In Esit-Eke LGA, the samples that reacted positively with MSV PAb were collected from Ekpene-Obo. For Nsit-Atai LGA, the MSV infected maize plants were collected from Odot 2. However, in Okobo LGA, the MSV positive samples were collected from Nung-Atai-Etta and Nung-Udom-Odobo.

The incidence of MSV was higher in Cross River State (20.9%) than Akwa Ibom State (14.9%) but the phenotypic changes in morphology and general performance of susceptible maize plants were as reported for Akwa Ibom State (Table 2). Majority of the samples collected from Odukpani reacted positively with MSV PAb and consequently accounted for the highest (7.5%) among the LGAs visited in the State. In Atampa and Biase LGAs, a similar proportion (4.5%) of MSV positive samples was found. This was followed by an incidence of 3 % observed in Calabar Municipal while the lowest (1.5%) MSV incidence was found in Ikot-Ekpene LGA. The virus positive samples from Atampa LGA were collected from Awi while those of Biase were found at Iwuru-Obio-Ntan. Moreover, in Calabar Municipal LGA the samples that tested positive for MSV were collected from Bacoco. For Ikot-Ekpene LGA, the MSV positive sample was found at Akai. The virus positive samples from Odukpani LGA were collected at Ikot Nyong.

Of the four States surveyed, the proportion of samples that reacted positively with MSV PAb was highest in Kano State (41.8%) (Table 2). Among the LGAs visited in the State, the highest proportion of positive samples was encountered in Tofa (11.9%). All the virus positive samples from Tofa LGA were collected at Lambu-Dantsu. In Ungu LGA of Kano State, about 10.4 % of the overall samples reacted positively with MSV PAb and all were collected from Rimin-Gata. In

Bebeji, Dawakin-Kudu and Kumbosto LGAs of the State, a uniform (4.5%) level of MSV incidence was observed. The samples that tested positive for MSV in Bebeji LGA were collected from Ranka (3%) and Wiak (1.5%). For Dawakin-Kudu LGA, all the MSV positive samples were obtained from Jido. Conversely, in Kumbosto LGA all the samples that reacted positively with MSV PAb were collected from the same community (Umara). Garun-Mallam and Kura LGAs of Kano State exhibited a comparable level of MSV incidence (3%) and all the positive samples came from Kadawa and Kura township.

Katsina State ranked second based on proportion (23.9%) of the samples that tested positive for MSV among the four States visited (Table 2). Matazu LGA accounted for the highest incidence of MSV in Katsina State with about 7.5% positive samples collected from Karaduwa (4.5%) and Kurku-Jambi (3%). In Kankia and Malumfashi LGAs which exhibited similar (6%) level of MSV incidence, all the virus positive samples were obtained from Jaza and Deyi. The incidence of MSV in Charanchi and Rimi LGA was 3 and 1.5% of the overall, respectively. In Charanchi, the MSV positive samples were obtained from Gangara, whereas that of Rimi LGA was collected from Lambar-Rimi community.

Table 2: Reactions of maize leaves to *Maize streak virus* polyclonal antibody in Enzyme - Linked Immunosorbent Assay (ELISA)

State	Local Government Area	Location	MSV Antibody	Status
Akwa Ibom	Abak	Utu-Abak	0.355	-
	Abak	Utu-Abak	2.629	+
	Abak	Utu-Abak	0.204	-
	Eket	Atairon	0.254	-
	Eket	Atairon	0.240	-
	Eket	Ata-Idung Afahu	0.300	-
	Esit-Eke	Ekpene-Obo	0.393	+
	Esit-Eke	Ekpene-Obo	0.410	+
	Esit-Eke	Ukwa-Isi- Edho	0.232	-
	Ikot-Ekpene	Akai	0.308	-
	Nsit-Atai	Odot 2	0.422	+
	Nsit-Atai	Odot 2	0.446	+
	Nsit-Atai	Idiaba-Afhana	0.265	-
	Okobo	Nung-Atai- Etta	0.675	+
	Okobo	Nung-Udom-Odobo	0.542	+
	Uyo	Uyo	1.974	+
	Uyo	Uyo	0.685	+
	Uyo	Uyo	0.546	+
Cross River	Atampa	Awi	0.178	-
	Atampa	Awi	0.898	+
	Atampa	Awi	1.315	+
	Atampa	Awi	0.193	-

	Atampa	Awi	0.632	+
	Biase	Iwuru-Obio-Ntan	0.467	+
	Biase	Iwuru-Obio-Ntan	0.395	+
	Biase	Iwuru-Obio-Ntan	0.521	+
	Calabar Municipal	Ikot-Ekpo	0.211	-
	Calabar Municipal	Bacoco	0.199	-
	Calabar Municipal	Bacoco	0.411	+
	Calabar Municipal	Bacoco	0.391	+
	Calabar South	Abitu	0.208	-
	Calabar South	Abitu	0.219	-
	Calabar South	Abitu	0.228	-
	Ikot-Ekpene	Akai	0.856	+
	Odukpani	Ikot Nyong	0.765	+
	Odukpani	Ikot Nyong	0.543	+
Cross River	Odukpani	Ikot Nyong	0.445	+
	Odukpani	Ikot Nyong	0.512	+
	Odukpani	Ikot Nyong	0.811	+
	Odukpani	Ikot Nyong	0.251	-
	Bebeji	Ranka	0.205	-
Kano	Bebeji	Ranka	0.205	-
	Bebeji	Ranka	0.932	+
	Bebeji	Ranka	0.748	+
	Bebeji	Wiak	1.160	+
	Dawakin-Kudu	Jido	0.216	-
	Dawakin-Kudu	Jido	2.938	+
	Dawakin-Kudu	Jido	1.264	+
	Dawakin-Kudu	Jido	0.204	-
	Dawakin-Kudu	Jido	0.915	+
	Garun-Mallam	Kadawa	0.283	-
	Garun-Mallam	Kadawa	1.389	+
	Garun-Mallam	Samawa	2.676	+
	Kumbosto	Umara	2.610	+
	Kumbosto	Umara	0.151	-
	Kumbosto	Umara	0.170	-
	Kumbosto	Umara	0.411	+
	Kumbosto	Umara	0.521	+
	Kura	Kura	2.143	+
	Kura	Kura	0.206	-
	Kura	Kura	0.229	-
	Kura	Kura	1.817	+
	Kura	Lambu-Dantsu	1.514	+
	Tofa			

	Tofa	Lambu-Dantsu	0.145	-
	Tofa	Lambu-Dantsu	0.148	-
	Tofa	Lambu-Dantsu	0.423	+
	Tofa	Lambu-Dantsu	0.433	+
	Tofa	Lambu-Dantsu	0.521	+
	Tofa	Lambu-Dantsu	0.457	+
	Tofa	Lambu-Dantsu	0.523	+
	Tofa	Lambu-Dantsu	0.858	+
	Tofa	Lambu-Dantsu	0.199	-
Kano	Ungu	Rimin-Gata	1.041	+
	Ungu	Rimin-Gata	1.580	+
	Ungu	Rimin-Gata	1.380	+
	Ungu	Rimin-Gata	0.232	-
	Ungu	Rimin-Gata	2.633	+
	Ungu	Rimin-Gata	1.687	+
	Ungu	Rimin-Gata	0.167	-
	Ungu	Rimin-Gata	0.152	-
	Ungu	Rimin-Gata	0.166	-
	Ungu	Rimin-Gata	1.031	+
	Ungu	Rimin-Gata	1.393	+
	Ungu	Rimin-Gata	0.169	-
	Ungu	Rimin-Gata	0.207	-
	Ungu	Rimin-Gata	0.230	-
	Ungu	Rimin-Gata	0.189	-
	Ungu	Rimin-Gata	0.184	-
	Ungu	Rimin-Gata	1.841	+
Katsina	Charanchi	Gangara	0.580	+
	Charanchi	Gangara	0.826	+
	Kankia	Jaza	1.391	+
	Kankia	Jaza	1.868	+
	Kankia	Jaza	1.392	+
	Kankia	Jaza	1.986	+
	Malumfashi	Deyi	0.134	-
	Malumfashi	Deyi	2.123	+
	Malumfashi	Deyi	2.739	+
	Malumfashi	Deyi	1.903	+
	Malumfashi	Deyi	0.898	+
	Matazu	Karaduwa	0.704	+
	Matazu	Karaduwa	2.040	+
	Matazu	Karaduwa	1.940	+
		Kurku-Jambi	0.481	+

Musawa	Kurku-Jambi	0.162	-
Musawa	Kurku-Jambi	3.073	+
Rimi	Lambar-Rimi	0.163	-
Rimi	Lambar-Rimi	0.890	+

Diseased Control = 4.000; Healthy Control = 0.193

Discussion and Conclusion

Maize is a staple food for millions of people in Nigeria and is cultivated in all agro-ecological zones of the country. Since the outbreak of MLND in some East African countries, the disease has continued to spread within and to neighbouring countries with attendant food shortage and economic hardship in the affected areas. In the face of a rapidly growing population and the need to reduce poverty among the smallholder farmers, pest-induced losses due to MLND must be tackled. The symptomatic samples that tested negative were probably infected by pathogens other than those investigated in the present study. Although MSV was detected in all the States surveyed, disease incidence was generally low. The negative reaction of all the samples to MCMV and MDMV polyclonal antibodies implied that the surveyed areas were free of MLND. This is similar to the findings of Salaudeen *et al.* (2015).

The need for regular surveillance cannot be over-emphasized because the costs associated with a pest or disease incursion can be very high for farmers, industry, the community, the environment and the economy. Vigilant surveillance based on scientifically proven techniques plays a major part in managing incursions and preventing outbreaks (National Plant Biosecurity, 2013). Cross-border pest and diseases are better controlled in collaboration with other countries which is a policy thrust in tackling plant diseases in Nigeria (Ojo, 2015). Therefore, absence of MLND in the country may be partly attributable to surveillance, information sharing and awareness campaigns being undertaken by the neighbouring countries. Moreover, since the outbreak of MLND in East Africa, there has been intensive campaign and sensitization by agricultural extension agents, Agricultural Development Programmes (ADPs), non-governmental organizations (NGOs), National Agricultural Seeds Council (NASC), Research Institutes and Nigeria Agricultural Quarantine Services (NAQS) in Nigeria.

Maize lethal necrosis disease is transmitted by seed and insects. This underscores the use of clean seeds in order to keep uninfected ecologies free of the disease. Seed companies are expected to play a major role in this regard. In East Africa where the disease has been reported, many seed companies had their seeds rejected at the international markets. Therefore, seed companies need to train key staff on internal seed testing of seed materials for the presence of MLND before distributing or exporting them (Owino *et al.*, 2015). The pattern of spread of disease offers clue for intensification of surveillance at certain locations in the country. Monitoring of possible entry points of diseases exotic to the country is a strategy employed to prevent introduction of new disease (Ojo, 2015). Increased surveillance, restricted movement of seeds from endemic areas, sharing of information and stepping up collaboration among African countries would help to contain the spread of the disease.

Virus diseases are much more difficult to manage than diseases induced by bacteria, fungi and nematodes because there are no chemicals for direct control. Plant breeding is an effective tool for adapting to new challenges of biotic and abiotic stresses in agriculture. Breeding for disease resistance or tolerance is a priority in the national breeding programme for controlling emerging diseases. Therefore, there is need for development of MLND resistant maize varieties as a

preventive measure against the disease in the country. Additionally, there is need for efficient public private partnership because it enables sustainable outcomes that no single party could achieve alone and its output is more than the sum of its parts. The agricultural based NGOs should continue to complement government policy and actions targeted at excluding MLND from Nigeria.

Acknowledgements
Authors are highly indebted to Tertiary Education Trust Fund (TETFund) for the Institutional Based Research Intervention (IBRI) grant provided for this study. The technical support offered by the Virology and Molecular Diagnostics Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria is also appreciated.

References

Adams, I. P., Harju, V. A., Hodges, T., Hany, U., Skelton, A., Rai, S., Deka, M. K., Smith, J., Fox, A., Uzayisenga, B., Ngaboyisonga, C., Uwumukiza, B., Rutikanga, A., Rutherford, M., Ricthis, B., Phiri, N. and Boonham, N. (2014). First report of Maize lethal necrosis disease in Rwanda. *New Dis. Rep.* 29, 22.

Anonymous (2017). Maize: Enhancing the livelihood of Nigerian farmers. *Sahel Rep.*, 14: 1 – 4.

Badu-Apraku, B., Oyekunle, M., Menkir, A., Obeng-Antwi, K., Yallou, C.G., Usman, I. S. and Akinwale, R. O. (2013). Comparative performance of early-maturing maize cultivars developed in three eras under drought stress and well-watered environments in *West Africa. Crop Sci.* 53, 1298–1311.

Cabanas, D., Watanabe, S., Higashi, C. H. V. and Bressan, A. (2013). Dissecting the mode of *Maize chlorotic mottle virus* transmission (*Tombusviridae*: *Machlomovirus*) by *Frankliniella williamsi* (Thysanoptera: Thripidae). *J. Economic Entomol.* 106, 16-24.

FAO (Food and Agriculture Organization). (2016). Maize. http://www.fao.org/faostat/en/#data/QC

Kiruwa, F. H., Feyissa, T. and Ndakidemi, P. (2016). Insights of maize lethal necrotic disease: A major constraint to maize production in East Africa. *Afr. J. Microbiol. Res.* 10, 271 – 279.

Kumar, P. L. (2009). Methods for the Diagnosis of Plant Virus Diseases: Laboratory Manual. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Pp. 90.

Ilaboya, I. R., Atikpo, E.,Omofuma, F. E., Asekhame, F. F. and Umukoro, L. (2012). Causes, Effects and Way Forward to Food Insecurity. *Irania J. Energy Environ.* 3, 180-188.

National Plant Biosecurity. (2013). Surveillance. Available at :http://www.pbcrc.com.au/impact/surveillance

Ochieng, J., Wangai, A., Miyogo, S., Karanja, T., Oduor, H., Kimani, E., Irungu, J., Sikinyi, E., Kinyua, Z., Ngaruiya, P., Ligeyo, D. and Kipkemboi, S. (2012). Status of maize lethal necrosis disease and general maize performance stakeholders' maize tour. 2 – 12 July, 2012 report: pp 1-34.

Ojo, P. O. (2015). Current phytosanitary policies to control the incidence and spread of maize lethal necrosis disease (MLND) in Africa (Nigeria), Paper presented at the MLN diagnostics and management in Africa. Held at InterContinental Hotel, Nairobi, Kenya, 12 – 15 May, 2015.

Owino, K., Mbogo, P. and Mito, J. (2015). MLN free seed production and movement in Africa: Private sector perspective. Paper presented at the MLN diagnostics and management in Africa. Held at InterContinental Hotel, Nairobi, Kenya, 12 – 15 May, 2015.

Salaudeen, M. T., Kumar. P. L. and Menkir, A. (2015). Maize lethal necrosis disease: Investigating risks and pre-emptive management in West Africa. A technical report submitted to International Maize and Wheat Improvement Center (CIMMYT), Texcoco, Mexico, p.19.

Tadele, Z. (2017). Raising crop productivity in Africa through intensification. *Agron.* 7: 1 -30.

Wangai, A. W., Redinbaugh, M. G., Kinyua, Z. M., Miano, D. W., Leley, P. K., Kasina, M., Mahuku, G., Scheets, K. and Jeffers, D. (2012). First Report of *Maize chlorotic mottle virus* and Maize lethal necrosis in Kenya. *Plant Dis.* 96: 1582.