Nig. J. Biotech. Vol. 36 (1): 152-158 (June 2019)
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and www.biotechsocietynigeria.org
DOI: https://dx.doi.org/10.4314/njb.v36i1.20

# Molecular identification of viruses infecting maize (*Zea mays* L.) in Ekiti State, south-western Nigeria

***[1]Fajinmi, A. A., [1]Bamiteko, M. O., [2]Fajinmi, O. B. and [3]Adediji, A. O.**

[1]Department of Crop Science and Horticulture, Faculty of Agriculture, Federal University Oye Ekiti, P.M.B. 374, Ikole, Ekiti State, Nigeria.

[2]Fruits Research Programme, National Horticultural Research Institute, PMB 5432, Ibadan, Nigeria.

[3]Department of Crop Protection and Environmental Biology, Faculty of Agriculture, University of Ibadan, Ibadan, Nigeria.

[6]Department of Plant Physiology and Crop Production, Federal University of Agriculture, Abeokuta, Nigeria.



## Abstract

**Maize is one of the most commonly cultivated crops in southwestern Nigeria, but its production is constrained by virus diseases. Information on viruses infecting maize within the region is still limited. Therefore, a survey was conducted to identify the viruses infecting maize on randomly selected farmers' cultivated maize fields in 16 local government areas in Ekiti State, southwestern Nigeria using molecular detection tools. Four cultivated maize fields were randomly surveyed in each local government area with an average size of 50 m x 50 m. The sampling was done on fifty randomly selected plants per field. Viral diseases were confirmed on maize plants through visual symptoms observations and serological diagnosis, although severity and incidence varied per location. Visible symptoms observed include chlorotic patches, severe streaking, red pigments and venation along mid rib and vein with stunted growth. Although only *Maize streak virus* (MSV) and *Maize chlorotic mottle virus* (MCMV) were targeted, serological assay indicated the occurrence of only MSV. Polymerase chain reaction (PCR) confirmed the presence of MSV while MCMV was negative. This provides additional information to the prevalence of MSV as an ongoing threat to maize production in southwest Nigeria,**

**Key words:** *Maize streak virus, Maize chlorotic mottle virus,* ELISA, Polymerase chain reaction, Symptoms

Corresponding Author E-mail: ayodele.fajinmi@fuoye.edu.ng

## Introduction

Maize production in Nigeria has been on the decrease since 2008 (USDA 2016). It was observed in Nigeria in 2005 that yield in maize have greatly reduced due to disease infection which in turn affects the cost of production and the returns accruable to the farmers (Fayenuwo et al., 2005). Akinbode et al. (2014) maintained that maize production in Nigeria was constrained by diseases, insects and vertebrate pests. Disease presence and spread is directly or indirectly affected by various factors (environment and weather condition, insect infestation, and other mechanical factors). Also, Fajinmi et al. (2012) reported that the incidence of viral diseases causes great yield loss in crop production in Nigeria.

However, the presence of virus disease on maize and its effects on growth parameters in Ekiti State, Nigeria still remain scanty. Virus and viral diseases of maize as reported by Offei et al. (2001) in sub-Saharan Africa include *Maize streak virus* (MSV), *Maize dwarf mosaic virus* (MDMV), *Maize chlorotic mottle virus* (MCMV), *Sugarcane*

*mosaic virus* (SCMV), *Maize chlorotic dwarf virus* (MCDV), *Barley yellow dwarf virus* (BYDV), *Brome mosaic virus* (BMV), *Maize mosaic virus* (MMV), *Maize stripe virus* (MStpV) and *Guinea grass mosaic virus* (GGMV). While Thottappilly et al. (2007) reviewed the characteristics of viruses occurring in Nigeria and summarized the following as viruses affecting maize: *Maize mottle/chlorotic stunt virus, Maize stripe tenuivirus, Maize dwarf mosaic potyvirus, Maize mosaic rhabdovirus, Maize eyespot virus and Guinea grass mosaic potyvirus*.

Traditional diagnosis of plant viruses as reported by Kamal and Raj (2011) requires bio-assay, an indicator plant, determination of host range, symptomatology, virus particle morphology (size and shape), and vector relations. But progress in molecular biology, biochemistry and immunology has led to the development of many new, accurate, rapid and less labour – intensive methods of virus detection. Polymerase chain reaction is used to make several copies of DNA. The procedure detects the pathogen, in this case a virus, and possible strain variants. It can detect the presence of a pathogen already during the incubation period, even before it produces symptoms (Roder, 2013). However, many samples that are tested in laboratories come from infected plants that are already showing disease symptoms. This makes the use of PCR particularly important to researchers who are investigating the epidemiology and etiology of virus.

Information on the different maize viruses occurring in Nigeria is still scanty, especially on their etiology, diversity and biology. This is important for adequate management measures for better productivity for farmers. Therefore, it is important to undertake investigations to ascertain and identify the specific viruses infecting maize, their symptomatologies and geographic distribution. This will aid researchers in understanding maize viruses and also provide data towards the development of resistant varieties by breeders.

## Materials and Methods

The research was carried out in Ekiti state, Nigeria. Ekiti state, comprising of 16 local government area (Fig 1), is found in the derived savannah region of Nigeria within Longitudes 4º 45' to 5º 45' East and Latitudes 7º 15' to 8º 51' North covering about 7000km² in area (Fig 1). It has an average annual rainfall of about 2000 to 2700mm with an average temperature ranging between 25ºC to 36ºC.

*Sampling techniques and data collection*

Sixteen local government area were surveyed, namely Moba, Ilejemeje, Oye, Ikole, Ekiti east, Gboyin, Ijero, Ekiti west, Efon, Ekiti south west, Ikere, Irepodun/ifelodun, Ado-Ekiti, Ido-Osi, Ise-Orun, and Emure. Four cultivated maize fields were randomly surveyed per local government area with an average size of 50 m x 50 m. The sampling was done on fifty randomly selected plants. This was achieved by walking across a 'W' shaped path in a field with plants spaced at equal distance from each other for the presence of viral symptoms such as streaks, stunting, venation, mosaics. Identification of the diseases was based on visual symptoms as described by CIMMYT (2004).

Leaf samples collected from the fields were adequately labelled, wrapped in aluminium foil and taken to the Virology Laboratory of the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria for serological and molecular identification. All leaves from each local government area were pooled together and composite samples taken for further analyses.

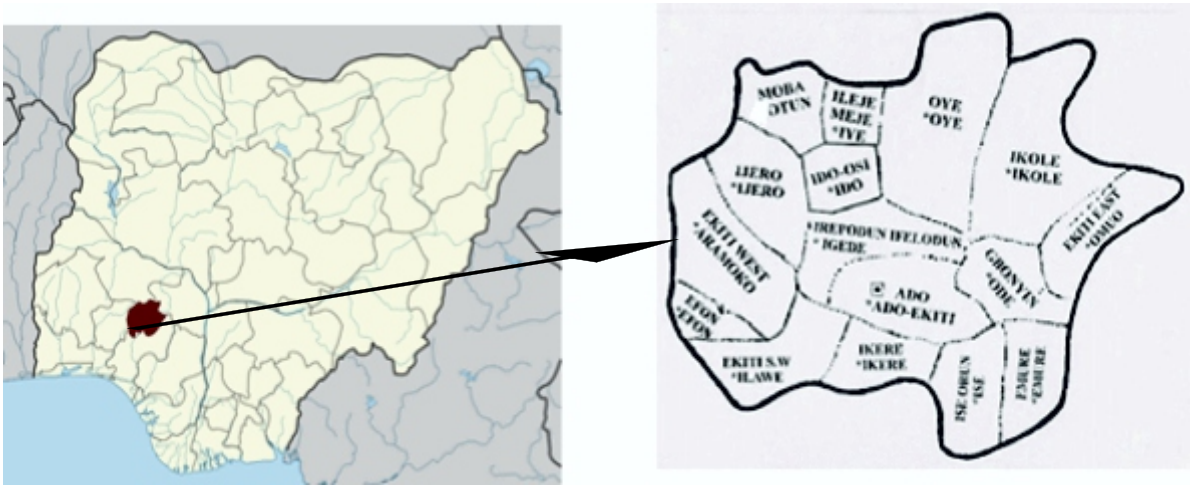


**Fig. 1:** Map of Nigeria showing Ekiti state (insert: Ekiti State 16 local government areas.)

*Virus detection by enzyme linked immunosorbent assay (ELISA)*

Using antigen coated plate enzyme linked immunosorbent assay (ELISA) procedure as described by Fajinmi (2012), the leaf samples were ground in 100 µl of antigen coating buffer with DIECA. The ground sample was loaded with a micro pipette into plate and covered and incubated for 1 hour at 37°C. The plate was washed three times with PBS – Tween solution by flooding for three minutes each time. It was emptied and tap dried. Blocking was done with 200 µl per well of 3% dried skimmed milk in PBS – Tween. The plate was covered and incubated for 30 mins at 37 °C. The plate was tap dried again. Then, 100 µl per well of Polyclonal antibody for MSV, diluted accordingly depending on the titer of the antibody was added in conjugate buffer (50 µl Antibody stock+4950 conjugate buffer= 1/100 Ab dilution) but for monoclonal antibody like Anti Ig G- AP: 1:2000 dilutions were used. The plate was covered and incubated for 1 hour at 37°C. The plate was then washed three times with PBS -Tween by flooding for three minutes each time. Emptied and tap dried. Goat Anti rabbit Alkaline Phosphatase 100 µl / well (as secondary antibody) diluted in 1:15,000 in conjugate buffer (as recommended by manufacturer) was added. The monoclonal Antibody, Goat Anti mouse was used diluted in 1:15,000. The plate was covered and incubated for 1 hour at 37°C.

The plate was washed three times with PBS -Tween by flooding for three minutes each time. Emptied and tap dried. P- nitrophenyl phosphate substrate 100 µl of 0.5-1 mg/ml in substrate buffer was added. The plate was kept in the dark for 1 hr and the optical density values were measured at absorbance of 405 nm, using BioTek ELx800 Universal Microplate Reader**.** The sample was considered virus positive when the optical density value was greater than twice the mean of the negative controls i.e. virus – free plants. For overnight readings, plates were kept at 4 °C till the next day and read.

*Virus detection by polymerase chain reaction*

Extraction of nucleic acid from virus infected maize leaf samples was done by using Dellaporta et al. (1983) protocol. Using a sterile mortar and pestle, about 100 mg of virus infected maize leaf were ground in 1000 µl of Phosphate extraction buffer saline (pH 7.4) prepared by dissolving 8.0 g sodium chloride (0.136 M), 0.2 g Potassium phosphate mono-basic (0.001 M), 1.1 g Sodium phosphate dibasic (0.006 M), 0.2 g Potassium chloride (0.003 M) and 20 ml Tween-20 into one litre sterilized distilled water. The sap was poured into new sterile tube and vortex briefly. Tubes were incubated in water bath at 60 $^{\circ}$C for 10 mins. It was brought to room temperature by placing on ice and equal volume of phenol, chloroform and isoamyl alcohol were added at ratio 25:24:1 respectively. The mixture was vortex and centrifuged at 12,000 g for 10 mins, after which 450 µl of the supernatant was removed into new sterile tube and then 300 µl of cold isopropanol was added into it. It was gently mixed and incubated for 1 hr at -20 $^{0}$C. It was centrifuged again at 12,000 g for 10 mins to sediment the nucleic acid. Decantation was done gently to remove the supernatant in a way that the pellets were not disturbed. To wash the pellets, 500 µl of 70% ethanol was added to the pellets and centrifuged at 12,000 g for 5 mins. The ethanol was decanted and the total nucleic acid was air dried at 37 $^{\circ}$C for 30 min. Pellets were suspended in 50 µl TE buffer (10 mM Tris-HCl, 1mM EDTA) for further use and storage.

Two primers (Table 1) were used to amplify MSV and MCVM in all pooled samples. Each reaction cocktail consisted of 5x Green GoTaq® Flexi buffer (Promega, WN, USA), 1.5 mM $MgCl_2$ (Promega, WN, USA), 0.2 mM dNTPs (New England Biolabs, USA), 10 µM of each primer pair, 0.3 U of Taq polymerase (Promega, WN, USA), 5 – 50 ng/µl of DNA templates and

**Table 1:** Primers used for the molecular detection of maize viruses in Ekiti State, Nigeria

| Primer name | Orientation | Target virus | Sequence (5′ – 3′) | Expected size (bp) | Reference |
|---|---|---|---|---|---|
| 1770-1792 | Forward | MSV | TTGGVCCGMVGATGTASAG | 1, 400 | Palmer & Rybicki (2001) |
| 215-234 | Reverse | MSV | CCAAAKDTCAGCTCCTCCG | 1, 400 | Palmer & Rybicki (2001) |
| 2681F | Forward | MCMV | ATGAGAGCAGTTGGGGAATGCG | 548 | Wangai et al. (2012) |
| 3226R | Reverse | MCMV | CGAATCTACACACACACACTCCAGC | 548 | Wangai et al. (2012) |

MSV: *Maize streak virus*
MCMV: *Maize chlorotic mottle virus*

DNase-free sterile up to a final volume of 12.5 µl. For MCMV reaction, 12 U of *Moloney murine leukemia virus* reverse transcriptase enzyme (Promega, WN, USA) was added for cDNA production. For amplification fo MSV, thermocycling conditions were set as 94° C for 5 mins, 35 cycles of 94 °C for 30 s, 52 °C for 1 min and 72 °C for 1.5 min with final extension at 72 ºC for 7 min. For MCMV detection, conditions were set at 44 °C for 30 mins, 94 °C for 1 min, 54 °C for 2 mins, 72 °C for 3 mins, 35 cycles of 94 °C for 1 min, 54 °C for 2 mins, 72 °C for 1 min and a final extension step at 72 °C for 5 mins. All reactions were carried out in a Veriti thermal cycler (Thermo Fisher Scientific, MA, USA) unit and productes were analysed in 1.5% agarose gel in 1x TAE buffer at 120 V for 1 hr.

## Results

The symptoms observed during survey of cultivated maize field varied per location in the 16 local government areas. Visible symptoms observed included; chlorotic patches, severe streaking, red pigment venation along mid rib and vein and stunted growth. Streaking was a symptom that occurred over a wide range of location and was well distributed within the local governments.

*Serological and molecular detection of Maize streak virus and Maize chlorotic mottle virus*

The ELISA serological assay was able to detect the presence of *Maize streak virus* (MSV) in only 3 local government area: Oye, Ikole and Gboyin (Table 2). No sample tested positive for MCMV. In the PCR test, MSV was detected in 63.15% of the leaf samples areas while none tested positive for MCMV. The occurrence of *Maize streak virus* (MSV) was detected via PCR in 12 local government area out of the 16 local government area surveyed (Figs 2 and 3).

**Table 2:** Serological and molecular detection of *Maize streak virus* and *Maize chlorotic mottle virus* using enzyme linked immunosorbent assay

| | Maize streak virus | | | Maize chlorotic mottle virus | | |
|---|---|---|---|---|---|---|
| LGA[1] | 1 hour | Overnight | ELISA[2] detection | 1 hour | Overnight | ELISA detection |
| Ilejemeje | 0.208 | 0.383 | - | 0.199 | 0.338 | - |
| Ekiti SouthWest | 0.201 | 0.379 | - | 0.212 | 0.379 | - |
| Ikere | 0.232 | 0.412 | - | 0.236 | 0.431 | - |
| Ido/Osi | 0.228 | 0.407 | - | 0.196 | 0.331 | - |
| Moba | 0.245 | 0.436 | - | 0.228 | 0.417 | - |
| Oye | 0.467 | 1.006 | + | 0.211 | 0.363 | - |
| Irepodun/Ifelodun | 0.199 | 0.37 | - | 0.201 | 0.354 | - |
| Emure | 0.232 | 0.435 | - | 0.242 | 0.414 | - |
| Ekiti East | 0.221 | 0.408 | - | 0.235 | 0.401 | - |
| Ijero | 0.201 | 0.372 | - | 0.227 | 0.370 | - |
| Ado-Ekiti | 0.232 | 0.564 | - | | | |
| Ikole | 0.858 | 1.889 | + | 0.199 | 0.365 | - |
| Efon | 0.21 | 0.367 | - | 0.221 | 0.393 | - |
| Gboyin | 0.868 | 1.876 | + | | | |
| Ekiti West | 0.201 | 0.325 | - | 0.184 | 0.316 | - |
| Ise/Orun | 0.212 | 0.367 | - | 0.204 | 0.360 | - |
| Diseased | 0.342 | 0.734 | + | 2.235 | 2.874 | + |
| Healthy | 0.205 | 0.356 | - | 0.215 | 0.384 | - |
| Buffer | 0.14 | 0.177 | - | 0.136 | 0.173 | - |

[1]LGA: Local government area

[2]ELISA: Enzyme linked immunosorbent assay

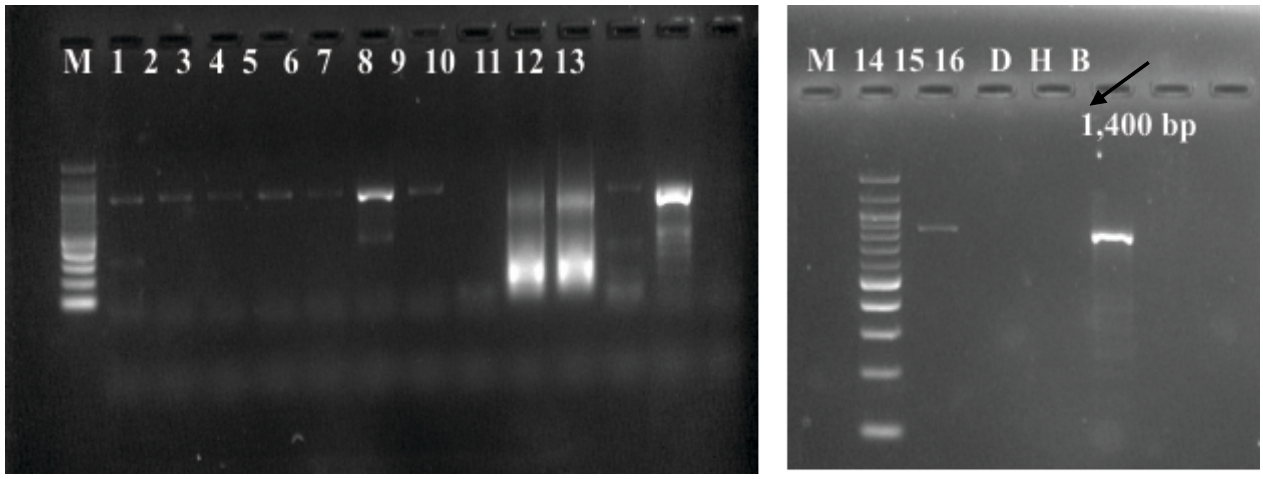


**Fig. 2:** Detection of *Maize streak virus* at expected band size of 1,400 bp from leaf samples from maize fields across Ekiti State, Nigeria.
M: 100 bp molecular marker; 1: Ilejemeje; 2: Ekiti South West; 3: Ikere; 4: Ido/Osi; 5: Moba; 6: Oye; 7: Irepodun/Ifelodun; Gbonyin; 8: Emure; 9: Ekiti East; 10: Ijero; 11: Ado-Ekiti; 12: Ikole; 13: Efon; 14: Gboyin; 15: Ekiti West; 16: Ise/Orun; D: Diseased; H: Healthy; B: Buffer.

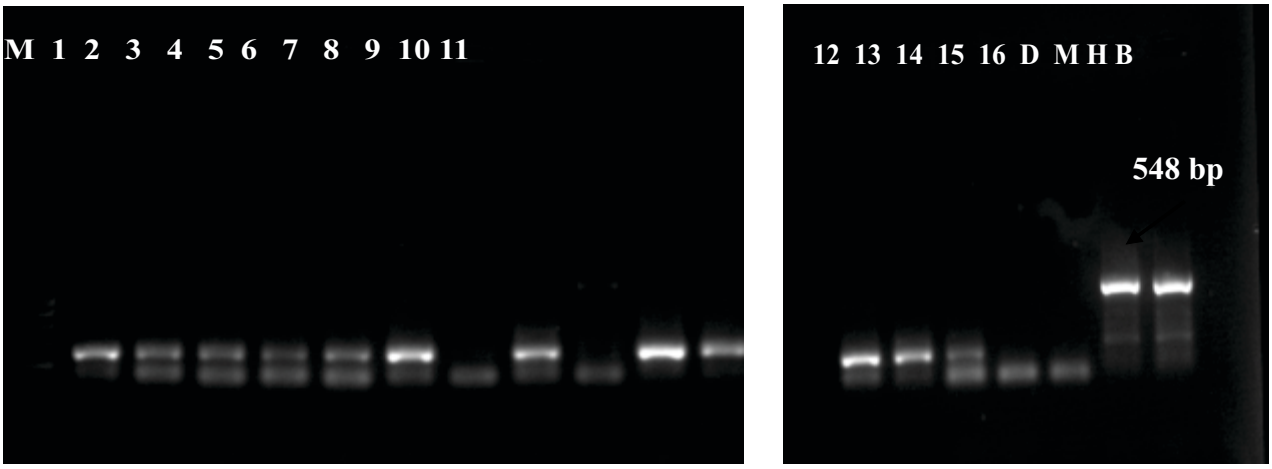


**Fig. 3:** Detection of *Maize chlorotic mottle virus* from leaf samples across maize fields in Ekiti State, Nigeria.
M: 100 bp molecular marker; 1: Ilejemeje; 2: Ekiti South West; 3: Ikere; 4: Ido/Osi; 5: Moba; 6: Oye; 7: Irepodun/Ifelodun; Gbonyin; 8: Emure; 9: Ekiti East; 10: Ijero; 11: Ado-Ekiti; 12: Ikole; 13: Efon; 14: Gboyin; 15: Ekiti West; 16: Ise/Orun; D: Diseased; H: Healthy; B: Buffer.

## Discussion

The ecological zone, farmer's cultivation practices as well as the varietal characteristics (susceptibility to virus infection) are probable contributors to viral disease incidence in Nigeria (Fajinmi and Odebode, 2010). These factors might have affected the incidence, distribution of the *Maize streak virus* (MSV) disease and their symptoms. *Maize streak virus* (MSV) was observed to be widely isolated in most of the farms in Ekiti State, this confirmed earlier observation of its prevalence in Nigeria (Mesfin et al., 1992; Alegbejo et al, 2001). MSV is known to be transmitted by leafhoppers (Oluwafemi and Alegbejo, 2011). Though occurrence of MSV vectors was not evaluated in this study, however, this is also important for future epidemiological studies.

The non-detection of *Maize chlorotic mottle virus* (MCMV) suggest a good note for maize farmers in Nigeria. The maize lethal necrosis (MLN) disease is one of the most devastating diseases affecting maize and it has caused immense yield losses in East Africa (Mahuku et al., 2015). The disease is known to be caused by the synergistic interactions of MCMV with other potyviruses infecting maize (Redinbaugh and Zambrano-Mendoza, 2014). The absence of MCMV shows that the threat of MLN to maize production in Ekiti State is absent. Currently, MLN has not been reported in Nigeria and this information is important to sustain quarantine efforts being made to prevent its entry.

The PCR was designed to detect the pathogen itself, its virulence and the different

strains of the virus (Dellaporta et al., 1983). Its advantage includes that it can detect the presence of a pathogen during the incubation period, even before it produces symptoms (Chen et al. 1993). In this study, PCR has proved as a reliable test kit for further characterization. Further work on sequencing may identify more of the strain of the Maize streak virus. ELISA is a biochemical technique designed to detect the presence of an antibody or an antigen (protein) in a sample, although it was reported to be considered sensitive and specific (Roder, 2013). The presence of MSV has been determined previously by serological and molecular methods (Oluwafemi et al., 2007). Maize streak virus has been identified to cause the most important virus disease of maize and the epidemic of the disease can result to 100% yield loss (Bosque-Perez, 2000). This study has also strengthen this position especially with regards to farmers in Ekiti State.

ELISA tests are good at determining if a plant has been exposed to a pathogen (McLaughlin et al. 1981). Many reviews have laid emphasis on the use of PCR for diagnosis of plant diseases (Arnheim and Erlich, 1992; Bej and Malibulani, 1992; Dong et al, 1992). The PCR is very sensitive and specific but ELISA is sensitive not very specific which makes PCR a better diagnostic tool.

## Conclusion

This study has established the prevalence of MSV in Ekiti State and the absence of MCMV within maize fields. Although additionally, PCR diagnostic analytical method is very sensitive and more specific than ELISA as PCR targets the gene.

## Acknowledgement

The authors are grateful for support received from the virology laboratory of the International institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

## References

Akinbode O. A., Ogunniyan D. J., Olasoji J. O., Ajijola S., Anjorin F. B., Olakojo S. A., Afolabi C. G., Omodele T. and Kareem K. O. (2014) Survey of Resurgence of Maize Diseases in South Western and Kwara States of Nigeria. Int. J. Agricult. Forestry 2014; 4(6): 451-458.

Alegbejo, M. D., Olojede S. O., Kashina B. D. and Abo M. E. (2001). *Maize streak mastrevirus* in Africa: distribution, epidemiology, economic significance and management strategies. Journal of Sustainable Agriculture 19 (4): 35-45.

Anklam E., Gadani F., Heinze P., Pijnenburg H. and Eede G.V.D. (2002). Analytical methods for detection and determination of genetically modified organisms in agricultural crops and plant derived food products. Eur. Food Res. Tech., 214: 3-26.

Arnheim, N., and Erlich, H. (1992). Polymerase chain reaction strategy. Annu. Rev. Biochem. 61: 56-151

Bej, A. J. and Malibulani, M. N. (1992). Applications of the polymerase chain reaction in environmental microbiology. PCR Methods Appl. 1: 59-151

Bock K. R., Guthrie E. J. and Woods R. D. (1974). Purification of maize streak virus and its relationship to viruses associated with streak diseases of sugarcane and *Panicum maximum*. Ann. Appl. Bio., 77: 289-296.

Clark M. F. and Adams A. N. (1977). Characteristics of the micro plate method of enzyme-linked immuno-sorbent assay for the detection of plant viruses. J. Gen. Virol., 34: 475-483.

Clark M. F. (1981). Immuno-sorbent assays in plant pathology. Annu. Rev. Phytopathol., 19: 83-106.

Chen C. C., Tsai J. H., Chiu R. J. and Chen M. J. (1993). Purification, characterization, and serological analysis of *Maize stripe virus* in Taiwan. Plant Dis., 77: 367-372.

Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) International Maize and Wheat Improvement Center (2004). "Maize disease. A guide for identification". 4th edition, Mexico D.F.p.124.

Dekker E. L., Pinner M. S., Markham P. G. and Van-Regenmortel M. H. V (1988). Characterization of *Maize streak virus* isolates from different plant species by polyclonal and monoclonal antibodies. J. Gen. Virol., 69: 983-990.

Dellaporta, S. L., Wood, J. and Hicks, J. B. 1983. A plant DNA mini preparation: version II. Plant Molecular Biology Reporter 4: 19-21.

Dong, L. C., Sein, C. W., Theis, K. l., Luthe, D. S., and Graves, C. H. (1992). Use of polymerase chain reaction to detect pathogenic strains of *Agrobacterium*. Phytopathol. 82: 39-434

Fajemisin J. M. and Soyinka S. A., (1976). "*Maize streak virus* diseases in Africa". In : Williams L. E., Gordon D. T. G., Nault L. R., eds. International Maize Virus disease colloquium and workshop. Wooster: Ohiom Agricultural Research and Development Center. P. 52-61.

Fajinmi, A. A. and Odebode C. A. **(**2010). Evaluation of maize/pepper intercropping model in the management of *Pepper veinal mottle virus*, genus Potyvirus, family Potyviridae, on cultivated pepper (*Capsicum annuum* L.) in Nigeria'. Arch. Phytopathology Plant Protect, 43: (15).

Fajinmi, A. A. (2012). Some selected serological diagnostic techniques in Plant virology. In Serological Diagnosis of Certain Human, Animal and Plant Diseases, ISBN 978-953-51- 0370-7, Edited by Moslih Al-Moslih. pp 59-70. INTECHPublishers. Rejeka, Croatia. Available on line www.intechopen.com

Fajinmi, A. A , Dokunmu A. O., Akheituamen D. O. and Onanuga K. A. (2012). Incidence and infection rate of *Maize streak virus* disease by *Cicadulina triangular* on maize plants and its distribution from the lowest diseased leaf under tropical conditions. Arch. of Phytopathology Plant Protect. pp 1 -8.

Fayenuwo J. O., Olakojo S. A., Akande M., Amusa N. A. and Olujimi O. A. (2005). Comparative evaluation of vertebrate pest damage on some newly developed quality protein maize (QPM) varieties in south-western Nigeria. Afr. J. Agric. Res. Vol. 2 (11), pp. 592-595

Kamal Sharma and Raj Shekhar Misra1 (2011) "Molecular approaches towards analyzing the viruses infecting maize (*Zea mays* L.)" J. Gen. Mol. Virol. Vol. 3(1), pp. 1-17.

Mahuku, G., Lockhart, B. E., Wanjala, B., Jones, M. W., Kimunye, J. N., Stewart, L. R., Cassone, B. J., Sevgan, S., Nyasani, J. O., Kusia, E., Kumar, P. L., Niblett, C. L., Kiggundu, A., Asea, G., Pappu, H. R., Wangai, A., Prasanna, B. M., and Redinbaugh, M. G. 2015. Maize lethal necrosis (MLN), an emerging threat to maize-based food security in sub- Saharan Africa. Phytopathology 105:956-965.

McLaughlin M. R., Barnett O. W., Burrows P. M. and Bavm R. H. (1981) Improved ELISA conditions for detection of plant viruses. J. Virol. Methods. 3:13-25.

Mesfin,T., Bosque-Perez N.A., Buddenhagen I.W., Thottappilly G. and Olojede S. O. (1992). Studies on *maize streak virus* isolates from grass and cereal hosts in Nigeria. Plant Disease 76: 789-795.

Miller S. A. and Martin R. R. (1988). "Molecular diagnosis of plant diseases". Annu. Rev. Phytopathol., 26: 409-432.

Oluwafemi, S., Thottappilly G. and Alegbejo M. D. (2007). Variation among some isolates of *Maize streak virus* from different parts of Nigeria by polymerase chain reactions. African Crop Science Conference Proceedings 8: 899-903.

Oluwafemi, S., and Alebgejo M.D. (2011). Diversity, abundance and host range of *Cicadulina* species (Homoptera: Cicadellidae) vectors of *Maize streak virus* in Nigeria. Moor Journal of Agriculture 12 (1): 11-21.

Offei, S. K. 2001. Virus and viral diseases of sub-Saharan Africa: analysis of responses to questionnaires by scientists in sub-Saharan Africa. In: Plant virology in sub-Saharan Africa. Proceedings of a conference organized by IITA, 4 – 8 June 2001, eds: Jacqueline d'A Hughes and Babajide O. Odu. Pp128- 136.

Redinbaugh, M. G., and Zambrano-Mendoza, J. L. 2014. Control of virus diseases in maize. Adv. Virus Res. 90:391-429.

Roder M. (2013) Detectability between ELISA and PCR may be strongly influenced by potential differences between cultivars. (Retrieved from www.ncbi.nlm.nih.gov )

Thottappilly, G., Bosque-Perez, N. A., Rossel, H. W. 2007. Viruses and virus diseases of maize in tropical Africa, Plant Pathology 42(4):494 - 509

United State Department of Agriculture, USDA (2016). Nigerian corn production by year 2016. Published by Index Mundi. Retrieved from www.indexmundi.com