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DISTRIBUTION AND INCIDENCE OF VIRUSES INFECTING YAM (*DIOSCOREA* SPP.) IN NIGERIA

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ABSTRACT
A survey was conducted during the months of July and August 2009 and 2010 to determine the incidence and distribution of the viruses infecting yam in five major yam-producing States and the Federal Capital Territory (FCT) in the Guinea Savanna zone of Nigeria. Yam leaf samples were collected from 54 fields and indexed for Yam mosaic virus (YMV), Cucumber mosaic virus (CMV), Yam mild mottle virus YMMV and Badnavirus by enzyme-linked immunosorbent assay (ELISA), immunocapture polymerase chain reaction (PCR) and IC-reverse transcription-PCR (IC-RT-PCR). Fifty one percent (51%) and 61% of leaf samples tested in 2009 and 2010 respectively, were infected with YMV, CMV, YMMV and badnavirus. Yam mosaic virus was the most prevalent virus detected in 26.2% of the total leaves sampled followed by CMV (13.1%), badnavirus (10.9%) and YMMV (0.5%) in 2009, while in 2010 YMMV was the most prevalent virus detected in 21.4% followed by Badnavirus (16.3%), YMV (9.2%) and CMV was not detected. Yam mosaic virus was the most prevalent virus detected and the occurrence of the virus in *D. rotundata* was highest in Kwara State (20.5%) and least in Abuja (2.7%) in 2009. In 2010, Abuja (27.3%) recorded the highest incidence of YMMV while Kwara State was the least (0.0%). A mixed infection of YMV and CMV was the most common mixed infection detected in 2009 and the mixture of YMMV + badnavirus+ YMV was the most common mixed infection in 2010. The report of this study is the first comprehensive result on YMV, CMV, YMMV and badnavirus infecting yam in this zone.

KEYWORDS: Distribution, Incidence, viruses, yam, Nigeria.

INTRODUCTION

Yam (*Dioscorea spp*.) is a major staple food for most people of Nigeria and the country accounts for highest production (65-70%) of the crop worldwide (FAO, 2007). It contributes most of the calorie requirement for each day. The tubers are consumed in several forms: eaten roasted, boiled, fried or as pounded yam and flour. Certain species are used for pharmaceutical compounds. Due to the continued and increasing dependence on yam for food in Nigeria, it is important for food security. Farmers are continually boosting the diversity of their plots by domestication of important high yielding varieties and world species (Dumont and Vernier, 2000). Major yam production is in the forest and the Guinea Savanna zone of Nigeria. *Dioscorea rotundata* (white or guinea yam) is the most important and widely grown yam in the Guinea Savanna with hundreds of hectares of land under yam production each year.

Pests and diseases are major constraints in its production as they have direct negative effects on its yield and quality. The most serious insects on the field and in storage include yam beetles, aphids, scarius flies, weevils and termites (Onwueme, 1978). Diseases caused by viruses, fungi, nematodes and bacteria, either singly or in combination are responsible for yield losses (Onwueme, 1978; Brunt *et al.,* 1990; Hughes *et al.,* 1997; Odu *et al.,* 1999). Viruses are of particular concern because, apart from causing significant reduction in tuber yield and quality, they restrict international exchange of germplasm. Yam viruses have been reported in most of the yam growing areas of West Africa (Thouvenel and Fauguet, 1979; Guoudon *et al.,* 1996; Hughes *et al.,* 1997; Philips *et al.,* 1999; Odu 2002; Eni, 2008).

Viruses infecting yam belong to the Potyvirus, Badnavirus and Cucumovirus genera, while a number of yam viruses remain unclassified. The viruses that have been reported on yam in West Africa are *Yam Mosaic virus* (YMV), Genus Potyvirus, *Yam mild mosaic virus* (YMMV), Genus Potyvirus, *Diosocrea durnetorum virus* (DdV), Genus Potyvirus, *Dioscorea alata bacilliform virus* (DaBV), Genus Badnavirus, *Dioscorea sansibarensis virus* (DSBV), Genus Badnavirus, *Cucumber mosaic virus* (CMV), Genus Cucumovirus and *Dioscorea mottle virus* (DMoV), Genus Cucumovirus. In Nigeria, YMV was the most prevalence in the humid forest, reaching an incidence of 78% (Dongo, 2000; Njukeng *et al.,* 2002 and Atiri *et al.,* 2003). Many of the earlier descriptions of Potyviruses in Nigeria did not provide precise information on the causal viruses and virus identity; and the relationships with other (poty) viruses remain elusive (Hughes *et al.,* 1997; Kondo, 2001; Atiri *et al.,* 2003). Therefore, there is need for the knowledge of distribution and incidence of yam viruses in major yam-producing States of the Guinea Savanna zone of Nigeria.

MATERIALS & METHODS

Survey and Sampling of yam fields in six States of the Guinea Savanna Zone

Survey of farmer's fields was carried out in the months of July and August 2009 and 2010 in six yam-producing areas of the Guinea Savanna zone (Benue, Federal Capital Territory (FCT), Kogi, Kwara, Niger and Nassarawa States) of Nigeria. Three farmers' fields were surveyed in each of three yams producing Local Government Areas in each State using global positioning system (GPS coordinates). Leaf samples were collected from sampled 5 quadrants of 5x5m size, totaling 25 plants per field. Two hundred and twenty-five leaf samples were collected from each State, giving a total of 1,350 leaf samples from 54 farm locations in each of 2009 and 2010 (Figure 1). Leaf samples were collected from symptom-laden (chlorotic spotting, vein-banding and mosaic) and symptomless (latent infection) yam plants. Three leaves were collected from the top, middle and lower portions of each plant. Surface moisture was removed by blotting with absorbent paper as necessary and the leaves slices into pieces were placed on top of cotton wool in a specimen bottle containing Calcium chloride ($CaCl_2$) in the bottom.

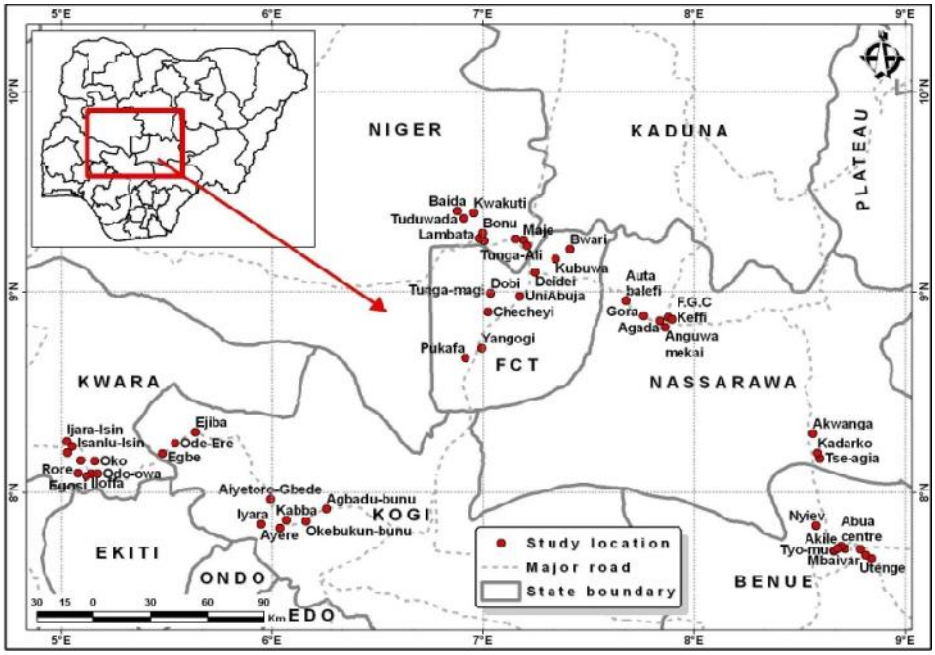

FIGURE 1: Map showing the location of survey areas in five States and FCT

Incidence and Severity of virus diseases

The surveyed fields were assessed and scored for virus incidence and severity of typical yam virus symptoms expressed on the leaves. Three farms were surveyed in each of the three Local Government Areas per state. Twenty-five plants were collected from each farm and the number of plants with symptoms was recorded. The presence of virus was detected using Enzyme linked immunosorbent assay (ELISA) and Polymerase Chain Reaction (PCR) and the incidence (percentage) was calculated from the ELISA and PCR results.

In order to determine the disease severity (DS), twenty-five plants were selected randomly for observation in each farm field. The severity of symptoms was scored on a scale ranging from 1 through 5: 1–No obvious symptoms, 2–Symptoms on 0-24% of leaves, 3–Symptoms on 25%-50% of leaves, 4 – Symptoms on 51%-74% of leaves and 5–Symptoms on 75%-100% of leaves (Eni *et al.,* 2008 modified). The samples collected were tested for yam viruses including Yam mosaic virus (YMV), Cucumber mosaic cucumovirus (CMV), badnavirus and Yam mild mottle virus (YMMV) using ELISA and PCR which were supplied by the Plant Virology Unit of International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Protein A sandwich enzyme linked immunosorbent assay (PAS-ELISA) was used to detect the viruses. The rabbit polyclonal antibodies that were used for ELISA technique were diluted as recommended by the supplier (Sigma) working at 1:2000µl dilution for YMV and 1:3000µl for CMV. IC-PCR was used for the detection of badnavirus and IC-RT-PCR was used for the detection of YMV and YMMV. These were carried out at the Plant Virology Unit of the IITA, Ibadan, Nigeria.

RESULTS

Virus Survey

Seven different symptom phenotypes occurred in different proportions in all the fields surveyed in 2009 and 2010 (Fig. 2). Chlorotic mosaic and mottling were the most common symptoms observed, accounting for 48% of the field symptoms in 2009 and 36% in 2010, while shoe-string, vein-clearing, necrosis, vein banding and stunting accounted for the remaining 52% and 64% of the field symptoms in 2009/2010 (Table 1). Most of these symptoms were associated with YMV, CMV, YMMV and

badnavirus in 2009 and YMV, YMMV and badnavirus in 2010.

a b c d

FIGURE 2: Leaves of Dioscorea species: a – healthy-looking, b – chlorotic mosaic and leaf curling (YMV), c – vein clearing and necrotic mosaic (YMV), d – shoe-stringing and stunting (YMV, Badnavirus).

TABLE 1: Mean number of symptomatic plants, incidence, and severity scores during survey in five States and FCT in Guinea Savanna of Nigeria in 2009 and 2010.

State	2009			2010			Symptoms observed in both years per State
	No. of Sympto-Matic	Mean incidence (%)	Mean severity	No. of Sympto-matic	Mean incidence (%)	Mean Severity	
Benue	19.56a	78.22a	2.59a	3.00a	100a	3.23a	CMO & MOT, SS, GVB, VC & N
Kwara	19.11a	76.44a	2.64a	2.44ab	100a	2.89ab	CMO&MOT, VC&N, N, GVB
Kogi	17.22ab	68.89ab	2.27ab	2.33b	100a	2.73ab	CMO&MOT, VC&N, GVB, N
Niger	16.67ab	66.67ab	2.24ab	2.56ab	100a	2.63b	CMO&MOT, VC&N, N
Nassarawa	15.33b	61.33b	2.18ab	3.00a	100a	2.93ab	CMO&MOT, VC&S, N
FCT-Abuja	12.33c	48.44c	1.57c	1.78c	92b	2.74ab	CMO&MOT, VC, N

Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$, CMO = Chlorotic mosaic, MOT = Mottling, SS = Shoe string, VC = Vein clearing, N = Necrosis, GVB = Green vein clearing and S = stunting.

Leaf samples that showed the expected band sizes of 249 bp, 586bp and 579bp, on gel after PCR, were considered positive for YMMV, YMV and badnavirus respectively (Mumford and Seal 1997; Wylie *et al.,* 1993; Seal and Muller, 2007). Three hundred and twenty five out of 1,350 (24.1%) leaf samples tested in 2009 and seventy three out of 139 (52.5%) tested in 2010, were positive by ELISA and PCR for YMV,CMV,YMMV and badnavirus (Tables 2). YMV was the most prevalent virus in 2009, detected in 217/1350 (16.1%) leaf samples and present in all 54 locations. The occurrence of YMMV was the lowest in the same year being detected in only 4/1350 (0.5%) leaf samples from two locations. In comparison, YMMV was the most prevalent virus infection in 2010, haven been detected in 30/139 (21.6%) leaf samples and in 45 locations while YMV occurred in 20/139 (9.2%) leaf samples. CMV was not detected in any leaf sample or location in 2010 (Table 2).

The incidence of YMV was highest (7.44%) in Kwara State in 2009 (Table 2), followed by Benue State (4.56%), while FCT had the lowest (1.22%). The incidence of CMV was not significantly different at $P \leq 0.05$ among the State in 2009 and did not occur in 2010. YMMV incidence was not significantly different at $P \leq 0.05$ across States. Badnavirus had the highest incidence in Benue (1.33%) but was not significantly among the other State in 2009 (Table 2). In 2010, YMV was not significantly different at $P \leq 0.05$ across States. While YMMV, had highest incidence in Niger (1.00%) and Benue (0.89%) and lowest in Kogi (0.22%) and Kwara (0%), Badnavirus was highest in Niger (1.11%) and lowest in Kwara (0%) (Table 2). Sixty-one out of 1,350 (4.5%) of leaf samples collected had mixed infections of YMV,CMV,YMMV and badnavirus in 2009 and 24 out of 139 (17.3%) in 2010 (Table 3). The percentage of mixed virus infection encountered within various yam species and within the five States and FCT in relation to the number of leaf samples infected ranged from 0-100% for both years. The pattern of distribution of mixed infection across the States was similar for both years. Benue recorded the highest incidence of mixed infection in 2009 (39.6%) and 2010 (45.8%), followed by Kwara (37.8%) in 2009 and Niger (26.7%) in 2010.FCT Abuja had the lowest incidence of

mixed virus infection in 2009 (3.6%) and Kwara (0.09%) in 2010.

TABLE 2: Incidence and distribution of viruses infecting yam (Dioscorea spp.) in five State and FCT of Guinea Savanna of Nigeria in 2009 and 2010

State	2009				2010			
	YMV	CMV	YMMV	BADNAVIRUS	YMV	CMV	YMMV	BADNAVIRUS
Kwara	7.44a	1.56a	0.00a	0.11b	0.00a	0.00a	0.00b	0.00c
Benue	4.56b	2.00a	0.11a	1.33a	0.33a	0.00a	0.89a	0.76ab
Kogi	4.44b	1.78a	0.00a	0.22b	0.11a	0.00a	0.22b	0.11bc
Niger	4.00b	1.67a	0.00a	0.22b	0.33a	0.00a	1.00a	0.67abc
Nassarawa	1.89b	1.56a	0.00a	0.00b	0.44a	0.00a	0.56ab	1.11a
FCT-Abuja	1.22b	0.11b	0.11a	0.22b	0.56a	0.00a	0.56ab	0.56bc

Means followed by the same letter within each column are not significantly different at $p \leq 0.05$

TABLE 3: Multiple infections detected in five States and FCT of the Guinea Savanna zone of Nigeria in 2009 and 2010

State	2009	2010
	Most abundant multiple infections	Most abundant multiple infections
Kwara	YMV + CMV; CMV + BADNAVIRUS +YMV	No mixed infection
Benue	YMV + CMV; CMV + BADNAVIRUS +YMV	YMMV + BADNAVIRUS + YMV
Kogi	YMV + CMV; YMV + CMV + BADNAVIRUS	YMV + BADNAVIRUS
Niger	YMV + CMV	YMMV + YMV; YMMV + BADNAVIRUS
Nassarawa	YMV + CMV	YMMV + YMV; YMMV + BADNAVIRUS
FCT-Abuja	CMV + YMV; BADNAVIRUS + YMV + YMMV	YMV + YMMV

DISCUSSION

This study provides information on the occurrence and distribution of four viruses infecting yam in five major yam producing States and FCT-Abuja of the Guinea Savanna zone of Nigeria. YMV, YMMV and badnavirus were detected in both years in most of the locations in the Guinea savanna, but CMV was not detected in any of the leaf samples from all locations in 2010. These four viruses that were detected in Nigeria have been reported in other countries in the West African yam zone (Thouvenel and Fauquet, 1979; Hughes *et al.,* 1997; Phillips *et al.,* 1999; Odu *et al.,* 1999; Dongo 2000; Eni *et al.,* 2009). The detection of YMV in all the 54 locations in all five yam species used in this study, and its presence in fifteen and five mixed infections with CMV, YMMV and badnavirus in 2009 and 2010 respectively, make this virus very important as it was the widespread yam virus in the Guinea Savanna zone of Nigeria. The observed higher incidence of the YMV in *D. rotundata* compared to other yam species in the Guinea Savanna zone, is similar to the findings of Hughes *et al.* (1997) on the distribution of YMV in Nigeria and in Ghana (Olatunde, 1994). The occurrence of YMV, CMV, YMMV and badnavirus as well as the incidence of the mixed infection observed in most of the five States and FCT surveyed could be attributed to the exchange of infected planting materials between States. This exchange can account for similarities in the incidence and distribution of the viruses in Nigeria (Hughes *et al.,* 1997). The in consisted rainfall may have contributed to in similarities of incidence and severity for the two years since disease is not static and could have also be conducive to disease spread because of the rapid growth of weed hosts of the vectors of disease (Alegbejo, 2001).

Dioscorea alata was the next most heavily infected yam species in this study. Although the production of this variety in the Guinea Savanna zone in under very low acreage, yet the few leaf samples collected had high incidence of the YMV and mixed infections of YMV, CMV, YMMV and badnavirus. Its high susceptibility to diseases is a major limitation to its production (Abang *et al.,* 2003, Rikly *et al.,* 2006). Some leaf samples showing obvious symptoms of virus infection, tested negative to YMV, YMMV, badnavirus and CMV. The symptoms observed on these plants may be caused by other viruses that were not or are yet to be identified. The symptoms may also be due to abiotic agents causing virus-like symptoms. The detection of these four viruses on non-symptomatic leaf samples (latent infection) shows that laboratory diagnosis serves as a more sensitive and conclusive way of affirming the health status of potential breeding or planting materials. Most of the symptoms observed in this study have been reported (Odu *et al.,* 2001; Eni *et al.,* 2009). The severity of the symptoms on green pigmentation of yam leaves observed can lead to severe reduction in the photosynthetic ability of affected plants, which can as well reduce the tuber yield. The extensive spread of yam viruses within the Guinea Savanna zone needs to be addressed further.

CONCLUSION

Obtaining planting materials (seed tubers) from healthy plants for yam cultivation would be a good initial

approach to solving the problem of virus infection of yam in the field. The major challenges faced by farmers and researchers are choosing healthy planting materials and screening of yam genotypes for multiplication. Others include differentiating virus symptoms from those due to nutritional disorder, ability to detect the presence of mixed virus infections and none availability of methods for adequate field diagnosis.

RECOMMENDATION

An adequate rapid field test for the certification of yam planting material will provide a major progress in the management of yam infections. Since these viral diseases are not epidemiologically static, information gathering should be continuous.

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REFERENCES

Abang, M.M., Asiedu, R., Mignouna, H.D., Green, K.R., Wolf, G. A. and Winter, S. (2003) Anthracnose disease of yam in West Africa: Recent advances in research and future perspectives. In: Proceedings of the 8th ISTRC-AB Symp. Ibadan, Nigeria. Pp: 563-571.

Alegbejo, M.D. (2001) Epidemiology of groundnut rosette virus at Samaru, Northern Guinea Savanna of Nigeria. Nigerian Journal of Entomology. 18:95-102

Atiri, G.I., Winter, S., Alabi, O.J. (2003) Yam. In: Virus and virus-like diseases of Major Crops in Developing Countries. By G. Leobenstein and G. Thottappilly eds. Kluwer, Acad Press. Dordrecht, the Netherlands. Pp: 249-267.

Brunt, A.A., Crabtree, K., and Gibbs, A. (1990) Viruses of Tropical Plants Wallingford, CAB International, Oxon. UK. Pp.707.

Dongo, L.N. (2000) Survey of viruses infecting yam and partial characterization of three isomeric virus isolates. Ph.D. Thesis, University of Ibadan. Pp.187.

Dumont, R. and Vernier, P. (2000) Domestication of yam (*D.* cayenensis, *D. rotundata*) within the Bariba ethnic group in Benin. Outlook on Agriculture 29:137-142.

Eni, A.O., Hughes, J.D'A. and Rey, M.E.C. (2009) Survey of the incidence and distribution of five viruses infecting yam in the major yam-producing zones in Benin. Annals of Applied Biology. 153:223-232.

Eni, A.O, Hughes, J.d'A. and Rey, M.E.C. (2008) First report of cucumber mosaic virus in yam (*Dioscorea spp.*) in Ghana, Togo and Republic of Benin in West Africa. Plant Disease 92(5): P. 833.

Food Agricultural Organization (2007) FAO Online publication. FAOSTAT, 2007.

Goudou-Urbino, C., Konate, G., Quiot, J.B. and Dubern, J. (1996) Aetiology and Ecology of a yam mosaic disease in Burkina Faso. Journal of Tropical Science, 36:34-40.

Hughes, J.D.A., Dungo, L. and Atiri, G. I. (1997) Viruses Infecting Cultivated Yam (*Dioscorea alata and D. rotundata)* in Nigeria. Phytopathology 87:545.

Kondo, T. (2001) The 3^1 terminal sequence of Chinese yam necrotic mosaic virus genomic RNA: a close relationship with macluravirus. Archives virology. 146:1527-1535.

Mumford, R.A. and Seal S.E. (1997)I-PCR for the detection of two yam of potyviruses. Virology Methods. 69:73-79..

Njukeng, A.P., Hughes, J.d'A, Atiri, G.L. and Kpo, E.J.A. (2003) Distribution of yam mosaic virus in Nigeria 8th Intern. Plant Virus Epidemiology Symp. Aschersleben Pp. 12-17.

Odu, B.O, Shoyinka, S.A, Hughes J.d'A, Asiedu, R. and Oladiran, A.O. (2001) Yam viruses in Nigeria. Proc. 7th Triennial Symp. Intern.Soc. for Tropical Root Crops 1998, Africa Branch, Cotonou, Benin.

Odu, B.O. (2002) Identification of resistance to yam viruses in Dioscorea species and generic analysis of resistance to yam mosaic virus in *Dioscorea rotundata* Poir. PhD Thesis, University of Ibadan, Nigeria. Pp. 183.

Odu, B.O., Hughes, J. d'A., Shoyinka, S.A. and Dongo. L. N. (1999) Isolation, characterization and identification of a Potyvirus from *Dioscorea alata* L. (water yam) in Nigeria. Annals Applied Biology. 134: 65-71.

Olatunde, O. J. (1994) Viruses of yam in Ghana. M. Sc. Thesis, University of Greenwich, U.K. p75.

Onwueme, I.C. (1978) The Tropical Tuber Crops. John Wiley & Sons, Chichester, UK. 2349pp.

Phillis, S., Briddon, R.W., Brunt, A.A. and Hull, R. (1999) The partial characterization of a badnavirus infecting the greater Asiatic or water yam (*Dioscorea alata*). Journal of Phytopathology 147:265-269.

Riley, C.K., Wheatley, A.O. and Asemola, H.N. (2006) Isolation and Characterization of starches from 8 Dioscorea species *alata* cultivars grown in Jamaica. African Journal of Biotechnology 5(17):1528-1536.

Seal, S, and Muller E. (2007) Molecular analysis of a full-length sequence of a new yam badnavirus from *Dioscorea sansibarensis*. Archives of Virology 152: 819-825.

Thouvenel, J.C. and Fauquet, C. (1979) Yam mosaic, a new Potyvirus infecting *Dioscorea cayenensis* in the Ivory Coast. Annals of Applied Biology. 93:279-283.

Wylie, S, Wilson C. R. C. and Jones, M. G. K. (1993) A polymerase chain reaction assay for cucumber mosaic virus in lupin seeds. Australian Journal of Agricultural research 44:41-51.