VIRUS OCCURRENCE IN YAM (*Dioscorea* spp.) TUBERS AND FIELD LEAF SAMPLES IN A HUMID TRANSITION ZONE OF NIGERIA

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ABSTRACT

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In March 2014 and 2015, 120 tubers of four yam cultivars namely Dioscorea rotundata "Efuru" and "Ikokoro", D. alata "Ewura" and D. cayenensis "Alo" were serologically indexed for viruses using Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) before planting on the field in a Randomized Complete Block Design (RCBD). Leaf samples were collected from the vines four months after planting and indexed for viruses to determine spread of viruses from the tubers and natural field infections. In 2014, in the 60 yam tubers, Cucumber mosaic virus (CMV) was the most prevalent virus occurring in 16 out of 60 (26.7%) then Dioscorea bacilliform virus (DBV) (7/60) 11.6% and Yam mild mosaic virus (YMMV) (3/60) 5.0 %. Yam mosaic virus (YMV) was not detected in the tubers. In 2015, the prevalence of CMV was 30/60 (50.0 %) followed by YMMV 7/60 (11.7 %) and YMV 5/60 (8.3%), and DBV 0.0%. In 2014 leaf samples, YMV, CMV and DBV were the viruses detected in the yam tubers and in the leaves. In 2015, CMV occurred in the tubers and was the most prevalent in leaves occurring in 21 out of 25 (84.0 %) samples, YMV occurred in tubers and in 8 out of 25 leaves (32.0 %). DBV and YMMV which occurred in tubers were not detected in the leaves. YMV was detected in the field leaf samples of tubers not previously infected with YMV. For the effective control of yam viruses resistant yam cultivars should be developed.

INTRODUCTION

Yam, *Dioscorea* spp. is among the major cash and most consumed food crops in West African countries (Babaleye, 2003. Its cultivation is very profitable despite high costs of production and market price fluctuations (Izekor and Olumese, 2010). The production of yam on one hectare of farm field translates to a net profit of ₦450,000.00 equivalent to US $2,000.00 (Asala and Ebukiba, 2016). Yam tubers are consumed in several forms including roasted, boiled, fried, pounded and flour (Amusa *et al*., 2003). Due to the continued and increasing dependence on yam for food in Nigeria, it has been found to be important for food security (Fu *et al*., 2011). They are starchy staple foods, rich in carbohydrates and are also valuable sources of some vitamins, particularly vitamin C, potassium and manganese and low in saturated fat, sodium and cholesterol (Wanasundera and Ravindran, 1994; Walsh, 2003). Yam tubers contain about 13-24.7 mg per 100g ascorbic acid and most of it is retained during cooking (Wanasundera and Ravindran, 1994).

Despite the importance, yam cultivation and storage suffers from many constraints, such as high cost of labour and planting material, difficulty in applying mechanization in planting and harvesting the crop, pests, and diseases (Degras, 1993; Njukeng, 1998; Atiri, *et al*., 2003). Pests and diseases are some of the major constraints to its production as they have direct negative effects on its quality and yield. Over 25% of yield losses are due to diseases and pests (Ezeh, 1998; FAO, 1998). Economically important yam pests include beetles, termites, weevils, scale insects, nematodes, aphids and rodents (Asala *et al*., 2012). Diseases include those caused by nematodes, fungi, bacteria and viruses (Degras, 1993; Hughes *et al*., 1997), singly or in combination, and are responsible for severe yield losses (Hughes *et al*., 1997; Odu *et al*., 1999). These viral symptoms, which mainly affect the foliage, lead to a reduction in the photosynthetic ability of the infected plant with deleterious effects on the tuber yield, quality and, in some instances, death of the plants (Thouvenel and Dumont, 1988; Odu *et al*., 2004).

Viruses infecting yam belong to the *Potyvirus*, *Badnavirus* and *Cucumovirus* genera, while others remain unclassified (Brunt *et al*., 1996; Njukeng *et al*., 2002 and Atiri *et al*., 2003). *Yam mosaic virus* (YMV) is one of the most important viruses infecting yams in sub-Saharan Africa causing very severe losses in yams, for instance yield loss of over 50 % reported was in *D. rotundata* (Amusa *et al*., 2003). Odedara *et al*. 2012 reported several viruses on yam and some occurring in mixed infections in Ogun State Nigeria. Most economic yam species are vegetatively propagated using tubers, tuber pieces, aerial tubers, bulbils and, to a lesser extent, tissue culture (IITA-EIARD, 2013; Aigbewi *et al*., 2014). Where viruses infect the mother plants, the vegetative propagules will be infected. If this goes unchecked, there will be a tendency for buildup of virus diseases (Eni *et al*., 2008). Virus disease build-up can lead to economic losses and shortages in the yam production which can directly or indirect can affect food security. These informed the need to identify viruses in economically important cultivars grown in southwest Nigeria. The objectives of this study were to determine the occurrence of viruses in yam tubers before planting and in the leaves after planting.

MATERIALS AND METHODS

Source of yam tubers and serological indexing of viruses

Four yam cultivars namely *Dioscorea rotundata* "Efuru" (white yam), *Dioscorea alata* "Ewura" (water yam), *Dioscorea rotundata* "Ikokoro" (white yam) were sourced from the Directorate of University Farms Federal

University of Agriculure (FUNAAB) while *Dioscorea cayenensis* "Alo" (yellow yam) was sourced from Ibere kodo local market, Abeokuta in Ogun State of Nigeria. The yam tubers were tested for four viruses before planting. The viruses tested for were *Cucumber mosaic virus* (CMV), *Yam mosaic virus* (YMV), *Dioscorea bacilliform virus* (DBV) and *Yam mild mosaic virus* (YMMV). From each yam tuber a total of 3 samples (about 0.5 cm deep) were collected from the top, middle and bottom of each yam tuber using a sterile lancet. The three samples per yam tuber were bulked together to form a composite sample for virus testing. Specific polyclonal antibodies (IgG) (DSMZ, Germany) to CMV, YMV, YMMV and DBV were diluted in coating buffer according to the manufacturer's specification. CMV, YMV and YMMV tests were carried out using Double antibody sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) (Clark and Adams, 1977) while DBV was done using Tripple antibody sanwich (TAS) ELISA. The tissues were homogenized (1:10 w/v) in extraction buffer (PBS-T pH 7.4 + 2% PVP). With a micropipette 100 µl of extracted saps were added to the wells of the microtitre plates and healthy and diseased controls were added to each plate and incubated overnight at 4^{o}C. The plates were decanted and washed with Phosphate buffered saline with Tween-20 (PBS-T) using a wash bottle. The plates were left flooded with PBS-T for three minutes after each round of washing which was done thrice before blotting dry on tissue paper. The plates were loaded with 100 µl of anti-virus conjugate of the respective viruses and incubated at 37 °C for 2 h. The plates were washed thrice as described earlier, 200 µl aliquots of freshly prepared substrate (*p*-nitro phenol phosphate) was added into each well, and the plates were incubated at room temperature for 1 hour. The ELISA plates were read after 1 hr at room temperature using MINDRAY, MR-96 microplate reader at 405nm. The absorbance reading twice that of the healthy control sample was regarded as positive for the respective virus.

Field experiments and collection of yam leaves for virus indexing

Two field experiments were conducted at the Teaching and Research Farm in Federal University of Agriculture (FUNAAB), Abeokuta, Ogun State in the rainy seasons of March, 2014 and 2015The tubers of the four yam cultivars were planted on the field in a Randomized Complete Block Design (RCBD). Five tubers of each yam cultivar were replicated three times making a total of 15 tubers assessed per cultivar, and 60 for the four yam cultivars on a total land area of 115 m^2 (23 × 5 m^2) in 2014 and 2015 respectively totaling 120 tubers. Heaps (1 *1 m apart) were mulched with dry grass and thereafter bamboo stakes were put in place to support vines at 6 WAP. Four months after planting the yams, when the leaves were fully established, leaf samples of diseased leaf tissue of each cultivar were collected from each plant for virus indexing using ELISA as described previously.

RESULTS

Viruses detected in yam tubers

In 2014, the incidence of CMV was 40.0, 46.7, 13.3, and 6.7% in *D. cayenensis*, *D. rotundata* var Efuru, *D. rotundata* var Ikokoro and *D. alata* yam tubers, respectively. *Yam mild mosaic virus* was also detected in *D. cayenensis* and *D. rotundata* (Efuru) with incidence of 13.3% and 6.7%, respectively. DBV induced 40.0% incidence in *D. alata* and 6.7% in *D. cayenensis* while YMV was not detected in the tubers tested (Table 1). In the 60 yam tubers tested, CMV was the most prevalent virus occurring in 16 out of the 60 (26.7%). This was followed by DBV (7/60) 11.6% while YMMV was (3/60) 5.0%. The mixed infections observed in the tubers were CMV+YMV which occurred in 5/60 (8.3%) and CMV+YMMV 2/60 which was (3.3%). In 2015, CMV was detected in all the yam tubers with the incidence of 66.7, 53.3, 46.7 and 33.3% in *D. rotundata* var Ikokoroand Efuru, *D. alata*, and *D. cayenensis* respectively. Also, YMV also was detected in two cultivars Efuru (6.7%) and *D. cayenensis* (26.7%) (Table 1). Yam tubers of *D. alata* and *D. rotundata* (Efuru) had incidences of 40.0 and 6.7% for YMMV respectively. DBV was not detected in the yam tubers indexed in 2015 while CMV was detected in all (Table 1). The prevalence of CMV was 30/60 (50.0%) followed by YMMV 7/60 (11.7%) and YMV 5/60 (8.3%) respectively.

Viruses detected in yam leaves from the field

In 2014, of the 44 yam tubers that sprouted YMV, CMV and DBV were the viruses detected in the yam tubers and in the leaves but with lower incidences. CMV was detected in *D. cayenensis* and *D. alata* with incidence of 6.7% each (Table 2). However, YMV was not detected in leaves of the four yam cultivars while DBV and YMMV incidences were 6.7% each in *D. cayenensis*. In 2015, 25 yam tubers sprouted, CMV was detected in 13 out of 15 leaves samples with incidence of 86.7% in *D. alata,* (100%), 6 out of 6 (100.0%) in *D. rotundata* (Ikokoro), 1/3 (33.3%) in *D. cayenensis* and 1/1 (100.0%) in *D. rotundata* (Efuru), while DBV and YMMV were not detected in the yam leaves indexed. In the 25 leaves of four yam cultivars collected from the field, CMV was the most prevalent virus occurring in 21 out of 25 (84.0%), YMV was also detected 8 out of 25 (32.0%) leaves.

Spread of viruses from the yam tubers to the leaves

In 2014, the viruses detected in the yam tubers were CMV, DBV, and YMMV. The three viruses occurred in the tubers and leaves of *D. cayenensis* while CMV was the only virus detected in the leaves of *D. alata* although the tubers were also infected with CMV and DBV. Some viruses such as DBV and YMMV which occurred in tubers of *D. alata* and *D. rotundata* (Efuru) tubers were not detected in the leaves collected in the field. The virus CMV occurred in *D. rotundata* (Efuru) tubers but was not detected in the yam leaves (Table 3). In 2015, the viruses detected in the tubers of the four cultivars were CMV, YMMV and YMV. Also, YMMV which occurred in the

tubers of *D. alata* and *D. rotundata* (Efuru) was not detected in field leaf samples. The yam cultivars *D. cayenensis* and *rotundata* var Efuru in which YMV was detected jn their tubers also had the virus detected in their leaves. The virus was also detected in the field leaves samples of *D. alata* and *D. rotundata* (Ikokoro) which was not previously detected in the tubers. The incidences of the viruses in the tubers were higher (between 5 and 38.0%) than in the field leaf samples (between 7 and 20.0%) resulting in lower incidences in the leaf samples (Fig. 1).

Table 1: Incidence of viruses in tubers of four yam (*Dioscorea* spp) cultivars in 2014 and 2015

	2014				2015			
Cultivars	DBV	CMV	YMMV	YMV	DBV	CMV	YMMV	YMV
D. cayenensis	1/15(6.7%)	6/15(40.0%)	2/15(13.3%)	0.0%	0/15	5/15(33.3%)	0/15	4/15 (26.7%)
D. rotundata (Efuru)	0.0%	7/15(46.7%)	1/15(6.7%)	0.0%	0/15	8/15(53.3%)	1/15(6.7%	1/15 (6.7%)
D. alata	6/15(40.0%)	1/15(6.7%)	0.0%	0.0%	0/15	7/15(46.7%)	6/15 (40.0%)	0/15
D. rotundata (Ikokoro)	0.0%	2/15(13.3%)	0.0%	0.0%	0/15	10/15(66.7%)	0/15	0/15

DBV = *Dioscorea bacilliform virus,* CMV = *Cucumber mosaic virus,* YMMV = *Yam mild mosaic virus,* YMV = *Yam mosaic virus*

Table 2: Incidence of viruses in the leaves of four yam (*Dioscorea* spp) cultivars grown in Abeokuta in 2014 and 2015

	2014			2015		
Yam varieties	YMV	CMV	YMMV	DBV	CMV (%)	YMV (%)
Dioscorea cayenensis	0.0	1/15(6.7%)	1/15(6.7%)	1/15(6.7%)	1/3 (33.3%)	2/3 (66.7%)
Dioscorea rotundata (Efuru)	0.0	0.0%	0.0%	0.0%	1/1(100.0%)	1/1 (100.0%)
Dioscorea alata	0.0	1/15(6.7%)	0.0%	0.0%	13/15(86.7%)	4/15(26.7%)
Dioscorea rotundata (Ikokoro)	0.0	0.0%	0.0%	0.0%	6/6(100.0%)	1/6(16.6%)

DBV = *Dioscorea bacilliform virus,* CMV = *Cucumber mosaic virus,* YMMV = *Yam mild mosaic virus,* YMV = *Yam mosaic virus*

Table 3: Carryover viruses from yam tuber to leaves and natural field infections during crop growth in the field in 2014and 2015

	2014		2015	
Yam cultivar	Viruses in Tubers	Viruses in Leaves	Viruses in Tubers	Viruses in Leaves
D. cayenensis	DBV, CMV, YMMV	CMV, YMMV, DBV	CMV, YMV	CMV, YMV
D. rotundata (Efuru)	CMV, YMMV	NIL	CMV, YMV, YMMV	CMV, YMV
D. alata	DBV, CMV	CMV	CMV, YMMV	CMV, YMV
D. rotundata (Ikokoro)	CMV	NIL	CMV	CMV, YMV

DBV = *Dioscorea bacilliform virus*, CMV = *Cucumber mosaic virus*, YMMV = *Yam mild mosaic virus*, YMV = *Yam mosaic virus*

DISCUSSION

Plant viruses are an important constraint to the healthy growth of plants and the production of raw food materials. The production of yam is affected by many factors; among the biological factors are plant virus attacks on the plants which results in severe yield loss. The occurrence of viruses in the yam tubers which are the propagative materials has implications for the epidemiology of the viruses involved. The viruses YMV, YMMV, DBV and CMV were detected in the tubers. Viruses reported to infect yam in West Africa belong to the Potyvirus, Badnavirus and Cucumovirus genera (Seal and Muller, 2007). The effects of virus diseases can be devastating on yams if not prevented (Thottappilly, 1992). YMV infection in *D. rotundata* caused yield loss of 65.4% in TDr 93-31 and 52.6% in TDr 95-127 (Adeniji *et al.,* 2012). The development of effective control measures for the virus diseases should necessarily be based on understanding the mode of spread and survival of the pathogens involved. The effective control of the virus diseases would take into consideration the primary sources of the viruses involved. In other words, the spread or carryover virus from storage (tubers) to field is an important aspect in the development of control strategies against the virus diseases.

Field control of the virus diseases would not be sufficient if due consideration is not given to the control of viruses in tuber which are the propagative materials for the crop. In this study it was observed that the tubers exhibited higher percentage incidences of the viruses than in the field leaf samples. Séka *et al.* (2009) reported that virus accumulation in yam tubers or leaves was dependent on the growth stage of the plant and the virus involved. YMV was higher in tubers than in the leaves with age up to seven months after infection. Also, CMV after three months of infection did not accumulate in the leaves, vines and tubers but that the CMV was highest in the tubers and lowest in the leaves after seven months of CMV infection.

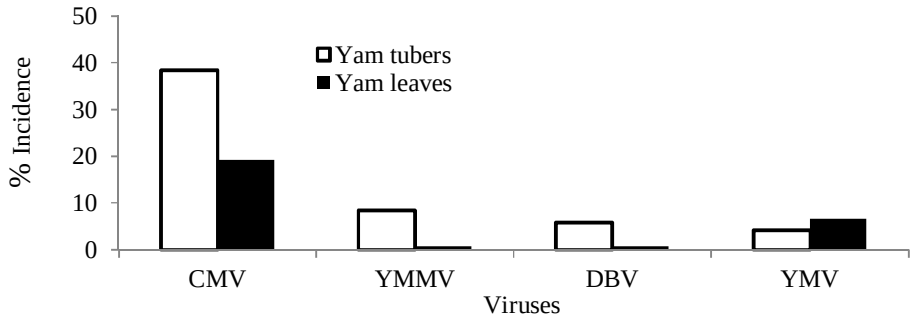

Fig. 1: Percentage occurrence of viruses in the tubers and leaves of four yam cultivars

The viruses which occurred in the tubers but were not detected in the leaves included YMMV, DBV and sometimes CMV. YMV was detected in the tubers and in the leaves. The virus in some cases was detected in the leaf samples and not in the tubers prior to planting, thus implying vector transmission in the field. The virus is the most prevalent virus of yam in the humid forest, reaching an incidence of 78% (Dongo, 2000; Njukeng *et al.*, 2002; Atiri *et al.*, 2003). The occurrence of YMMV and DBV in the yam tubers and not the leaf samples four months after planting might majorly be as a result of vector activities than spread from the tubers. Thus field management is important for the control of such viruses. Higher incidences of CMV were recorded in the tubers than in the leaves, this is in accordance with the report of Séka *et al.* (2009). The virus occurred in both tubers and leaves. CMV is responsible for losses in yam ((Thouvenel and Dumont, 1990; Asiedu *et al*., 1998; Séka *et al.*, 2009). It is aphid transmitted and can also be spread to other crops within and around the yam fields by the aphids. CMV causes diseases of economic importance in vegetables.

Yam cultivars such as *D. cayenensis* and *D. rotundata* (Ikokoro) had higher virus incidences and mixed viral infections. Virus infections are known in all these species but most prevalent in *D. rotundata* and *D. alata*, the two most predominant species covering >70% of the cultivated area in West Africa (Kumar, 2015). Yams are grown annually by farmers through yam tubers, seed yams, tuber etc. which are purchased from local markets. The sources of the yam planting materials to the market place are usually from nearby or distant places. The implications are that yam viruses are moved across different locations, agro-ecologies and indeed the country at large. Farmers also milk their yams to allow for the seed yam production for use as planting materials in the next season. Thus such virus infected materials will be propagated year in and out which may result in build-up and spread of viruses. Viruses infecting yam are systemically distributed in all plant tissues, including tubers. Consequently, tubers, setts, or any plant tissue from infected plants serves as a source for virus spread through vegetative propagation (Kumar, 2015).

The production of yam is limited by virus infections resulting in yield losses (Thouvenel and Dumont, 1990; Asiedu *et al.*, 1998; Séka *et al.*, 2009). The occurrence of viruses in the yam tubers will also be a hindrance to yam as an export commodity due to phytosanitary issues thereby hindering international trade. Solutions to effectively eradicate and or prevent yam tuber infections will be important in controlling yam virus diseases which will enhance yam as a major export commodity for international trade. Virus-free yam planting materials for farmers are not available or limited, similarly to the best of our knowledge virus resistant yam has not been reported. Evaluation of yam germplasm for resistance against the viruses may reveal sources of resistance. The control of virus vectors in the field will help to eliminate or lower virus incidence and further carryover of the viruses to the tuber and vice versa.

REFERENCES

Adeniji, M. O., Shoyinka, S. A., Ikotun, T. Asiedu, R., Hughes, J. d'A. and Odu, B. O. 2012. Yield loss in guinea yam (*Dioscorea rotundata* poir.) due to infection by *Yam mosaic virus* (YMV) genus Potyvirus. *Ife Journal of Science* 14 (2):237- 244.

Aighewi, B. A., Maroya, N. G. and Asiedu. R. 2014. Seed yam production from minisetts: A training manual. IITA, Ibadan, Nigeria. 40 pp.) alata tubers.

Amusa, N. A., Adegbita, A. A., Muhammed, S. and Daiyewu, R. 2003. Yam diseases and its management in Nigeria. *African Journal of Biotechnology* 2: 497-502.

Asala, S.W. and Ebukiba, E. S., 2016. Profitability of yam production in southern guinea savanna zone of Nigeria. *Net Journal of Agricultural Science* 4(1): 9-14.

Asala, S., Alegbejo, M.D., Kashina, B., Banwo, O. O., Asiedu, R. and Lava- kumar, P. 2012 Distribution and incidence of viruses infecting yam (*Dioscorea* spp) in Nigeria. *Global Journal of Bioscience and Biotechnology* 1(2):163-167.

Asiedu, R., Ng, S. Y. C., Bai, K. V., Ekanayake, I. J. and Wanyera , N. M. W. 1998. Genetic Improvement. In: Food yams Advances in Research. Orkwor, C. G., Asiedu, R., Ekanayake, I. J., Wanyera, N. M. W. (Eds.) IITA/NRCRI pp 63-104.

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

Babaleye, T. 2003. West Africa: Improving yam production technology. ANB-BIA, 463: 56–59.

Brunt A A, K Crabtree, M Dallwitz, A J Gibbs, L. Watson. 1996. Viruses of plants. Description and lists from the Vide Database. CABI International. Cambridge University Press. Cambridge. 148 p.

Brunt, A., Crabtree, A., Dallwitz, K. Gibbs, A. J. and Watson L. 1996. Viruses of plants. Description and lists from the Vide Database. CABI International. Cambridge University Press. Cambridge. 148p.

Clark, M. F. and Adams, A. N. 1977. Characteristics of the micro plate method of enzyme–linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* 34: 475 – 483

Degras, L. 1993. The Yam, a tropical root crop. London, UK. 40pp.

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

Eni, A. O. Hughes, J. d'A. Asiedu, R. and Rey, M. E. C. 2013 Incidence and diversity of mixed viruses lower in yam tubers and tuber sprouts compared with field leaf samples: Implications for virus-free planting material control strategy p 3065.

Eni, A.O. 2008. Epidemiology, Diagnostics and Molecular Studies of Yam Viruses in Ghana, Togo and Benin. PhD Thesis. University of Witwatersrand. Johannesburg, South Africa. pp200.

Ezeh N.O. 1998. Economics of production and Post-harvest Technology In: Orkwor G. C., Asiedu, R. and Ekanayake, I. J. (eds) Food yam; Advances in Research IITA and NRCRI, Nigeria, pp. 187-214.

FAO. 1998. Food and Agriculture Organisation of the United Nations. FAO Statistics.

Fu, R. H. Y., Kikuno H and Maruyama M. 2011. Research on yam production, marketing and consumption of Nupe farmers of Niger State, central Nigeria. *African Journal of Agricultural Research* 6(23): 5301-5313

Hughes, J.d'A., Dongo, L.N. and Atiri, G.I. 1997. Viruses infecting cultivated yams (*Dioscorea alata* and *D. rotundata*) in Nigeria. *Phytopathology* 87: S45.

IITA-EIARD. 2013. Healthy yam seed production. Yam for Improved Nutrition and Food Security in West Africa. A Project study produced by WRENmedia, funded by the Swiss Agency for Development and Cooperation (SDC) and implemented by the European Initiative on Agriculture Research for Development (EIARD) and International Institute of Tropical Agriculture (IITA).

Izekor, O. B. and Olumese, M. I., 2010. Determinants of yam production and profitability in Edo State, Nigeria. *African Journal of General Agriculture* 6(4): 205-210.

Kumar, L. 2015. Viral disease threats to yam in West Africa. IITA Research for Development (R4D) Review. Issue 11

Njukeng., A . P. Atiri, G. I. Hughes, J.d'A., Agindotan, B .O. Mignouna, H.D. and Thottappilly, G. 2002. A sensitive TAS-ELISA for the detection of some West African isolates of Yam mosaic virus in *Dioscorea spp. Tropical Science* 42(2): 65-74.

Odedara,O. O., Ayo-John, E. I., Gbuyiro, M. M., Falade, F. O. and Agbebi, S. E. 2012."Serological detection of yam viruses in farmer's field in Ogun State". *Archives of Phytopathology and Plant Protection* 45(7):840-845

Odu, B. O. Hughes, J. d'A. Asiedu, R. Ng, N. Q., Shoyinka, S. A. and Oladiran, O. A. 2004. Responses of white yam (*Dioscorea rotundata*) cultivars to inoculation with three viruses. *Plant Pathology* 53: 141–147.

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

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

Séka, K., Diallo, A., Kouassi, K. and Aké, S. 2009. Screening ten yam (*Dioscorea* spp.) varieties for resistance to *Yam mosaic virus* and *Cucumber mosaic virus* in Côte d'Ivoire. *African Journal of Plant Science and Biotechnology 3: 36-43.*

Thottappilly, G. 1992. Plant virus diseases of importance to African Agriculture. *Phytopathology* 134: 265-288.

Thouvenel, J. C. and Dumont, R. 1990. Pertes du rendement de l'igname infectée par le virus de la mosaïque de l'igname en Côte d'Ivoire. *Agronomie Tropicale* 45: 125-129.

Thouvenel, J. C., and Dumont, R. 1988. An epidemiological Approach to the study of yam mosaic virus in the Ivory Coast. In: Howeler RH, ed. Proceedings of the International Society for Tropical Root Crops, Bangkok, Thailand. 643-648.

Walsh, S. 2003.Plant Based Nutrition and Health. Vegan Society Ltd. 240pp.

Wanasundera, J. P. D. and Ravindran, G. 1994. Nutritional assessment of yam (*Dioscorea alata*) tubers. *Plant Foods for Human Nutrition* 46 (1):33-39.