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VIRUSES IN WEEDS IN *Dioscorea* YAM FIELDS IN NIGERIA

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ABSTRACT

The presence of viruses in yam leaves and the presence of weeds in close proximity to yam fields have been shown to be associated with diminished tuber yield. But the precise role of weeds as alternative hosts of viruses infecting yam has not been systematically studied. Therefore, leaf samples of weeds were collected from *Dioscorea* yam fields in FCT-Abuja, and five States of the Guinea Savanna agro-ecological zone of Nigeria in 2009 and 2010, and analysed by Enzyme-Linked Immuno-absorbance Assay (ELISA) and Polymerase Chain Reaction for viruses. Fifty-four and 70% of samples collected during the two years, respectively, were infected with *Yam mosaic virus*, *Cucumber mosaic virus*, *Cowpea mild mottle virus*, *Pepper venial mosaic virus*, *Telfeiria mosaic virus*, *Cowpea yellow mosaic virus* and *Badnavirus* (DaBV). The weeds and viruses were *Hibiscus esculentus* Moench (YMV, CMV and CPMMV), *Amaranthus spinosus* Linn (CMV, YMV), *Physalis angulata* L (YMV, CMV), *Procumbane* Linn (CMV), *Phyllanthus amarus* Shum (YMV, CMV, CPMMV), *Ludwigia abyssinica* A. Rich (YMV), *Galinsoga culiata* L. (YMV), *Eclipta prostrate* Linn (YMV), *Justicia flara* Vahl (YMV, CMV), *Euphorbia heterophylla* Linn (YMV, CMV), *Melanpodium divaricatum* L. (YMV, CMV) and *Saccivlepsis Africana* Hubb (YMV), *Melanpodium divaricatum* L. (CPMMV), *Crotalaria rutusa* L. (YMV, CMV), *Aspelia bussei* O. Hoffin *(CPMMV), Aneilema acquinotide* P. Beauv (CPMMV), *Pueraria phaseloides* Linn (YMV), *Platostoma Africana* P. (YMV), *Conyza summtrensis* Retz (YMV, BCMV, PVMV, TeMV), *Chroniolea oduratiu* L. R (YMV, CYMV), *Mitracarpus villosus* D.C(CMV) and *Sclerocarpus africanus* Jacq (BCMV, PVMV, TeMV, Badnavirus). Weeds surrounding yam plants may serve as alternative hosts of viruses.

Key Words: *Cucumber mosaic virus, Dioscorea* yam, *Hibiscus esculentus*

RÉSUMÉ

La présence de virus dans yam laisse et la présence de mauvaises herbes dans une proximité immédiate avec l'igname champs auraient dû être divulgués de s'associer avec un rendement de tubercules diminuée. Mais le rôle précis des mauvaises herbes comme hôte alternatif de virus infectant yam n'a pas été systématiquement étudié. Par conséquent, les échantillons de feuilles des mauvaises herbes ont été recueillies lors des relevés des champs igname *Dioscorea* en FCT-Abuja et cinq États de la zone agro-écologique savane de Guinée du Nigéria en 2009 et 2010 et analysés par Enzyme-Linked Immuno-absorption Assay (ELISA) et réaction en chaîne par polymérase pour virus. Cinquante-quatre et 70 % des échantillons prélevés au cours des deux années ont été infectés par le virus de la mosaïque Yam, Cucumber mosaic virus, virus doux de marbrure du niébé, virus de mosaïque véniel poivre, Telferia virus de la mosaïque, virus de mosaïque jaune de niébé et mosaïque (DaBV). Les herbes et les virus ont été *Hibiscus esculentus* Moench (Vitrothèque, CMV et CPMMV), *Amaranthus spinosus* Linn (CMV, Vitrothèque), *Physalis angulata* L. (Vitrothèque, CMV), Procumbane Linn (CMV), *Phyllanthus amarus* Shum (Vitrothèque, CMV, CPMMV), *Ludwigia abyssinica* A. Rich (Vitrothèque), culiata L. *Galinsoga*. (Vitrothèque), *Eclipta prosterner* Linn (Vitrothèque), Justicia flara Vahl (Vitrothèque, CMV), *Euphorbia heterophylla* Linn (Vitrothèque, CMV), divaricatum Melanpodium L. (Vitrothèque, CMV) et *Saccivlepsis Africana* Hubb

(Vitrothèque), *Melanpodium divaricatum* L. (CPMMV), *Crotalaria rutusa* L. (Vitrothèque, CMV), *Aspelia bussei* O. Hoffin (CPMMV), *Aneilema acquinotide* P. Beauv (CPMMV), *Pueraria phaseloides* Linn (Vitrothèque), *Platostoma Africana* P. (Vitrothèque), summtrensis de Conyza Retz (VMC, Vitrothèque, PVMV, TeMV), *Chroniolea oduratiu* L. R (VitrothèqueCYMV), *Mitracarpus villosus* D.C(CMV) et *Sclerocarpus africanus* Jacq (VMC, PVMV, TeMV, mosaïque). Les mauvaises herbes autour d'igname peuvent servir des hôtes alternes de virus.

Mots Clés: *Cucumber mosaic virus,* igname *Dioscorea*, *Hibiscus esculentus*

INTRODUCTION

Yam (*Dioscorea* spp.) is the fourth most important root crop with a worldwide production estimate of about 3600 million tonnes in 1999 (FAO, 2002). It is cultivated mainly in West Africa and parts of East, Central and Southern Africa (FAOSTAT, 2009), which contribute about 95% of the world yam production; followed by Southern Asia including China, Japan and Oceania (FAOSTAT, 2009). It is extensively used for consumption and animal feeds. Certain cultivars are used in the production of contraceptives (Komesaroff *et al.*, 2001). The toxic properties of some yams are valuable in tanning leathers (*Dioscorea tannis*), insecticides, and fishing (*Dioscorea saponins*) and animal baiting (*Dioscorea alkaloids*) (Coursey, 1967; Degras, 1993).

Yam is one of the principal root crops of the Nigeria economy in terms of land under cultivation, volume and value of production (Bamire and Amujoyegbe, 2005). In many yam-producing areas of Nigeria, it is said that 'yam is food and food is yam'. According to FAOSTAT (2011), Nigeria produces 37,889,500 tonnes of yam per year on 2, 889,050 hectare of land.

Yield losses due to pests/diseases and weed interference (Akinlosotu, 1985; Unamma and Akobundu, 2006; Okoroafor, 2009) constitute a problem during yam cultivation. Known viruses infecting yam are Potyvirus, Macluravirus, Badnavirus, Cucumovirus and Potyvirus. The major modes of transmission and survival are through seeds, propagative materials and weeds. A virus may survive within a dormant seed, propagative material or weed, infecting the seedlings that develop after germination during the next growing season (Alegbejo and Kashina, 2000; Odedara *et al.*, 2008).

Studies by Gumedzeo (2002), Moran *et al.*, (2002) and Amusa *et al.* (2005) identified viruses in weeds that were not associated with yam fields. However, Mukhtar *et al*. (2011) identified Yam mosaic virus in 16 weed samples in and around yam fields. Most other studies on weeds were in relation to growth performance and yield (Akobundu, 1981; Orkwor *et al.*, 1994; Unamma and Akobundu, 2006). Therefore, the objective of this study was to determine the alternative weed hosts of yam viruses in and around yam fields.

MATERIALS AND METHODS

This study was conducted in the Guinea Savanna zone, which is the major yam producing area of Nigeria. This zone lies within latitudes 8° 4'N, and 11° 3'N, and longitudes 2° 41'E and 13° 33'E; with bimodal rainfall ranging from 1000 to 1300 mm per *annum*.

Weed species with or without virus-like symptoms were sampled at random from heaps at the outer portion and centre of the farm and also from neighbouring yam fields during the rainy and dry seasons. Whereas wet season yam cultivation existed in all six locations in the study area, dry season (Fadama) farming was available only in Kogi and Kwara out of a total of 5 States. Specimens of the weed species were identified in the Agronomy Unit of the Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria in Nigeria.

Leaf samples of weed plants were taken from the top, middle and lower parts of the plants and preserved in specimen bottles which contained calcium chloride. The calcium chloride removed moisture from the leaves without affecting the viruses in the leaves. The samples were serologically indexed for viruses using the Polymerase Chain Reaction (PCR) technique at the Virology Laboratory of International Institute of Tropical Agriculture (IITA), Ibadan. Twenty-

one viruses were tested for, out of which seven were detected in the weed samples. Immunocapture PCR (IC-PCR) was used to detect Badnavirus and Immunocapture reverse transcript PCR (IC-RT-PCR) for YMV and YMMV. Protein A sandwich ELISA (PAS), as described by Edwards and Copper (1985), was used to detect Yam mosaic virus (YMV) and Cucumber mosaic virus (CMV). Antigen-coated plate enzyme-linked immunosorbent assay (ACP-ELISA) was used for the detection of Cowpea mild mottle virus (CPMMV), Bean common mosaic virus (BCMV), Pepper veinal mottle virus (PVMV), Telfairia mosaic virus (TeMV) and Cowpea yellow mosaic virus (CYMV). Mean absorbance values (A405) greater by two times that of healthy control sample were considered positive.

RESULTS

About 54.12% of weed samples collected during the wet season were positive for one or more viruses; while 70% in the dry season were positive for one or more viruses. Tables 1 and 2 show the lists of weeds that were found in and around yam field locations. YMV, CMV and CPMMV were the three viruses detected in the weeds during the wet season. YMV, CMV, CPMMV, PVMV, BCMV, TeMV and CYMV were present in the weeds during the dry season.

Generally, YMV showed the widest distribution among the weeds. CMV showed a high presence in weeds in Kwara and Niger States during the wet season (Tables 1 and 2). The YMV had the highest incidence of infection in the weeds in Kwara and Kogi States during the two

TABLE 1. Weed hosts of yam viruses in the wet season of 2009 in northern Nigeria

State in northern Nigeria	Weed	Virus detected by ELISA		
		YMV	CMV	CPMMV
Benue (Makurdi)	*Phyllanthus amarus* Shum.T.	+	–	+
FCT Abuja (Kwali)	*Phyllanthus amarus* Shum.T.	–	+	–
FCT (Kubuwa)	*Saccivelepsis Africana* Hubb	+	–	–
Kogi (Yagba West)	*Ludwigia abyssinica* A.Rich	+++	–	–
Kogi (Kabba-Bunu)	*Amaranthus spp* Linn	+++	–	–
Kogi (Kabba-Bunu)	*Amaranthus spp* Linn	++	–	–
Kogi (Ijumu)	*Galinsoga Culiata* L	+	–	–
Kogi (Ijumu)	*Galinsoga culiata* L	+	–	–
Kogi (Yagba West)	*Ludwigia abyssinica* A.Rich	+++	–	–
Kwara (Isin)	*Eclipta Prostrata Linn*	+++	–	–
Kwara (Irepodun)	*Euphorbia heterophyilla Linn*	+	+	–
Kwara (Isin)	*Justicia Flara* Vahl	+	+++	–
Kwara (Oke-Ero)	*Euphorbia heterophylla Linn*	+++	+++	–
Nassarawa (Kaura)	*Melanpodium divaricatum* Rich	+	+	–
Nassarawa (Keana)	*Tridax procumbens* Linn	–	+	–
Niger (Gurara)	*Hibiscus esculentus* Meonch	+++	+	+
Niger (Suleja)	*Amaranthus spinosus* Linn	–	++	–
Niger (Suleja)	*Physalis angulata* L.	+	+	–

+ = ELISA mean absorbance value two times greater than that of healthy sample
++ = ELISA mean absorbance value three times greater than that of healthy sample
+++ = ELISA mean absorbance value four times greater than that of healthy sample
Names in parenthesis are local administrative areas (Local Government Areas)
YMV = Yam Mosaic Virus, CMV = Cucumber Mosaic Virus, CPMMV = Cowpea Mild Mottle Virus, FCT = Federal Capital Territory

TABLE 2. Weed hosts of yam viruses in the dry season (Fadama) of 2010 in northern Nigeria

State in northern Nigeria	Weed	Viruses detected by ELISA						
		YMV	CMV	CPMMV	PVMV	BCMV	TeMV	CYMV
Kogi	*Heterotis rotundifolia* Jacf	-	-	-	-	-	-	-
Kogi	*Aspilia bussei* O. Hoffin.M	-	-	+	-	-	-	-
Kogi	*Crotalaria retusa* Linn	++	+	-	-	-	-	-
Kogi	*Melampodium divaricatum* Rich	-	-	++	-	-	-	-
Kogi	*Anaillema acquinoctide* Beauv	-	-	+	-	-	-	-
Kogi	*Cenchus biflorus* Roxb	+	-	-	-	-	-	-
Kogi	*Pueraria phaseloides* Linn	+	-	-	-	-	-	-
Kogi	*Eupharbia hurta* Linn	-	-	-	-	-	-	-
Kogi	*Chromolaena odorata* L.	+	-	-	-	-	-	-
Kogi	*Chamaerista mimosoides* L	-	-	-	-	-	-	-
Kwara	*Phyllanthus amarus* Shum	+	-	-	-	-	-	-
Kwara	*Croton hirtus* L	+	-	-	-	-	-	-
Kwara	*Hyptis lanceolata* Poir	++	-	-	-	-	-	-
Kwara	*Platossma africanna* P	+++	-	-	-	-	-	-
Kwara	*Conyza summrensis* (Retz)	+	-	-	+++	+	+	-
Kwara	*Chromolaena odorata* L.	+	-	-	-	-	-	+
Kwara	*Pteridium aquilinum* Kuhn	-	-	-	-	-	-	-
Kwara	*Eclipta alba* (L.)	+	-	-	-	-	-	-
Kwara	*Mitracarpus villosus* SW	-	+	-	-	-	-	-
Kwara	*Sclerocarpus africanus* Jac	-	-	-	+	+	+	-

+ = ELISA mean absorbance value two times greater than that of healthy sample
++ = ELISA mean absorbance value three times greater than that of healthy sample
+++ = ELISA mean absorbance value four times greater than that of healthy sample
YMV = Yam mosaic virus; CMV = Cucumber mosaic virus; CPMMV = Cowpea mild mottle virus

TABLE 3. Viruses detected in leaf samples of weeds collected during wet season of 2009 in northern Nigeria

States in northern Nigeria	Weed types	+ve samples	Infection (%)	YMV	CMV	CPMMV
Benue	8	2	25.0	12.5(1/8)	0.0	12.5(1/8)
FCT Abuja	5	2	40.0	20.0(1/5)	20.0(1/5)	0.0
Kogi	18	7	38.9	38.9(7/18)	0.0	0.0
Kwara	7	7	100.0	57.2(4/7)	42.8(3/7)	0.0
Nassarawa	4	2	50.0	25.0(1/4)	25.0(1/4)	0.0
Niger	6	6	100.0	33.3(2/6)	50.0(3/6)	16.7(1/6)

YMV = Yam mosaic virus; CMV = Cucumber mosaic virus; CPMMV = Cowpea mild mottle virus

TABLE 4. Viruses detected in leaf samples of weeds collected during the dry season from yam fields in northern Nigeria

State	Weed type	Positive samples	% infection	YMV	CMV	CPMMV	PeVMV	BCMV	TeMV	CYMV
Kogi	10	8	80.0	40.0(4/10)	10.0(1/10)	30.0(3/10)	0.0	0.0	0.0	0.0
Kwara	10	15	100.0	70.0(7/10)	10.0(1/10)	0.0	20.0(2/10)	20.0(2/10)	20.0(2/10)	10.0(1/10)

YMV = Yam mosaic virus; CMV = Cucumber mosaic virus; CPMMV = Cowpea mild mottle virus, BCMV = Bean common mosaic virus, PVMV = Pepper veinal mottle virus, TeMV = Telfaria mosaic virus and CYMV = Cowpea yellow mosaic virus

years of investigation (Tables 3 and 4). The incidence of CMV in the weeds was highest in Kwara State, followed by Kogi State during the wet season.

DISCUSSION

The observed high occurrence of YMV and CMV on weeds (Tables 1 and 2) makes them (viruses) very important as they are widely spread in most crop-growing areas and continents. These viruses may be ubiquitous in nature, occurring in almost every yam field, in a wide range of weeds present either in yam farms or their neighbouring fields. The presence of virus on weeds may also account for the high incidence of diseases during growing seasons; which is in agreement with Gumedzeo (2002) and Moran *et al*. (2002). Their adverse effects on epidemiology of viral diseases on tuberous crops cannot be underestimated. This result has proved a possibility that weed species growing among yam can be alternative hosts of virus, by harbouring insect vectors. Infection of weeds in yam fields by virus can be a serious threat to crops due to the close proximity of weeds to yam. Weeds could serve as initial and primary sources of inocula to the healthy yams, or as alternative hosts of virus with insect vectors within the yam field and neighbouring crops. Some of these weeds had several viruses in mixed infections similar to the case reported by Mukhtar *et al*. (2012). Yam is a significant and outstanding crop for agriculture in the tropics and subtropical Africa. Therefore, the presence of viruses in yam leaves and weeds, have the potential to interfere with maximal production of the crop in the field (Unamma and Akobundu, 2006; Moran *et al*., 2002).

The diversity in the incidence of these viruses in the middle and southern Guinea Savanna might be due to differences in the surrounding vegetation and climate in the two Zones (Tables 3 and 4). While the middle Savanna is characterised by monomodal rainfall and grass vegetation, the southern Savanna has bimodal rainfall and thick tree forest, which can support the activities of most insect vectors (Odedara *et al.*, 2008). The consequence of having virus infection in weed species that inhabit yam or

surrounding fields is that such weeds constitute sources of infection of the yam leaves and crop. Furthermore, such weeds in Fadama environment survive the dry season and transfer their viruses to germinating yam crops and weeds during the following farming season. In this way, there is a persistent reservoir of viruses in the vicinity of growing yam and these are transmitted from weed to yam, and from weed to weed (Alegbejo and Kashina, 2000; Odedara *et al.*, 2008). Kwara and Kogi States where the incidence of viruses in weeds was highest are not major yam producers compared with other Guinea Savanna States such as Benue, Nassarawa and Niger, where virus incidence in weeds is lower. Therefore, the etiology and epidemiology of weeds as hosts of viral diseases of yam, and especially how this interaction may impact on the productivity and yield of yam should be given more attention in Nigeria.

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