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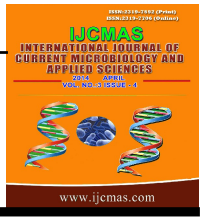

Original Research Article

Survey of the incidence and distribution of two viruses infecting yam (*Dioscorea* spp) in two agro-ecological zones of Cameroon

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A B S T R A C T

Yam is an important income generating food crop to millions of people around the world, particularly Sub-Saharan Africa. Virus infections are important constraint to its production. A survey was carried out in May and June 2009 to improve on yam production and safe germplasm movement, by determining the incidence and distribution of two viruses infecting yam in two major yam-producing agro-ecological zones of Cameroon. Six hundred yam leaves sampled from 10 farmers' fields (60 from each field), on *D. rotundata and D. cayenensis* in the Northwest and Southwest Regions were tested for *Yam mosaic* virus (YMV), Yam badnavirus (YBV) and co-infection of both viruses, using TAS-ELISA. Thirty of the samples were asymptomatic, 30 symptomatic and 540 collected randomly. 490 out of 600 (81.7 %) leaf samples tested positive for YBV, YMV and co-infection of both viruses. Out of 600 leaf samples tested, 312(52 %), 397(66.17 %) and 217(36.17 %) were positive for YMV, YBV and co-infection of both viruses respectively. The incidence of YMV was significantly higher in the Southwest Region (humid forest agro-ecological zone) than in the Northwest Region (Western highlands savannah agro-ecological zone). Out of the 8 types of symptoms observed on leaves, mosaic was the most common. Both symptomatic and asymptomatic leaf samples tested positive for YMV, YBV and co-infection. Use of symptoms is not a reliable tool for diagnosing yam virus infections, but can only accompany more sensitive and reliable virus indexing techniques.

Introduction

Yam (*Dioscorea* spp) is an economically important staple food crop of tropical and sub-tropical areas of the world. It is one of the most important food crops cultivated in the West African yam belt, comprising of Cameroon, Nigeria, Ghana, Benin, Togo and Côte d'Ivoire with over 93 % of total yam production (FAO, 2013; Eni *et al.*, 2010). Of the 56.6 million tonnes of total yam production worldwide, West Africa accounts for 52.5 million tonnes with Nigeria producing 37 million tonnes while Cameroon produced only 510000 tonnes and is ranked 6th in the world (FAO, 2013).

In Cameroon, yam is ranked third after cassava and cocoyam, among root and tuber crops and, it is cultivated in all agro-ecological zones of the country (Ngue *et al.,* 2007)). The most important species cultivated and consumed in the West African yam belt are *D. rotundata, D. cayenensis* and *D. alata,* and in Cameroon, the main cultivated species are *D. rotundata, D. alata, D. cayenensis* and *D. dumetorum.* The crop is particularly important because of its underground tubers and aerial tubers (called bulbils) which are an important source of carbohydrates, proteins, minerals, vitamins and have a low glycemic index which gives better protection against obesity and diabetes (Bell, 1983; Holford, 2008; Eni, 2008). In addition, crop also has socio-cultural importance and pharmaceutical properties, and its cultivation and sale serves as a major income generating activity for the people in yam-growing areas. Yam cultivation therefore provides multiple opportunities for poverty alleviation and nourishment.

Despite its 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 disease are some of the major constraints to its production as they have direct negative effects on its quality and yield. Some of the most serious pests include insects such as beetles, aphids, scale insects, weevils and termites (Asala *et al.,* 2012). Singly or in combination, diseases caused by Nematodes, Bacteria, Fungi and viruses are responsible for very serious yield losses (Hughes *et al.,* 1997; Odu *et al.,* 1999). Viruses are of particular importance because; in addition they also restrict international exchange of yam germplasm (Brunt *et al.,* 1989). Many surveys have reported the presence of several viruses and serious virus diseases on yams in the yam belt of West Africa, and other yam-growing areas of the world (Thouvenel and Fauquet, 1979; Eni *et al.,* 2010). Viruses infecting yam belong to the Potyvirus, Badnavirus and Cucumovirus genera, while others remain unclassified. Viruses that have been reported on yam in the African yam belt include *Yam mild mosaic virus* (YMMV), Genus Potyvirus, *Yam mosaic virus* (YMV), Genus Potyvirus, *Dioscorea dumetorum virus* (DdV), Genus Potyvirus, *Cucumber mosaic virus* (CMV), Genus Cucumovirus, *Dioscorea mottle virus* (DMoV), Genus Cucumovirus, *Dioscorea alata bacilliform virus* (DaBV), Genus Badnavirus, and *Dioscorea sansibarensis bacilliform virus* (DsBV), Genus Badnavirus. Amongst these, *Yam mosaic virus* (YMV), causes very severe losses in yams, with yield loss of over 50 % reported in *D. rotundata* (Amusa *et al.,* 2003). Yam badnavirus (YBV) has also recently been reported as being the most prevalent in Benin and other countries of West Africa (Eni, 2008*).* These viruses spread through seasons and fields, mostly through infected planting material, and their accumulation paralyses yam germplasm movement worldwide, by hindering national and international exchange of selected yam varieties, and also reduces yam production and productivity (Brunt *et al.,* 1989; Eni *et al.,* 2008). Yam badnavirus and *Yam Mosaic virus* are also spread in a semi-persistent/persistent and non-persistent manner, by mealy bugs and aphids respectively (Brunt *et al.,* 1996). Unfortunately, virus diseases cannot be controlled by chemical treatment unlike those caused by fungi and bacteria, but through the principle of exclusion (Walkey, 1991). They may infect plants singly or mixed, producing varying symptoms which make identification of specific causal

viruses, by use of symptoms difficult and unreliable (Offei, 2003; Njukeng *et al.*, 2005; Eni, 2008). This lends credence to the necessity of applying more sensitive immunological and molecular indexing techniques, such as TAS-ELISA and IC-RT-PCR in diagnosing yams for virus infections. Research on yam virus diseases in Cameroon is very scanty and previous work has concentrated on assembling yam germplasm, increasing vegetative planting material and studying morphological diversity amongst species (Sato, 2001; Ngeve and Nolte, 2001; PNDRT, 2007). This might have accounted for the relatively low level of yam production in the country, compared with other nations of the yam belt of West Africa. The aim of this work is to contribute in the improvement of yam production through enhancement of safe germplasm movement and virus disease control efforts in Cameroon, by establishing a baseline data for *Yam mosaic virus* and Yam badnavirus in the country. Therefore, there is need for the knowledge of a survey of the incidence and distribution of yam viruses in two major yam-producing agro-ecological zones of Cameroon.

Materials and Methods

Location of the study site

The survey was carried out in two main yam-growing areas of the Northwest and Southwest regions of Cameroon, in May and June 2009. Cameroon is situated between latitudes $1^{o}22'$ and $13^{o}17'$ N and longitudes $8^{o}13'$ and $16^{o}14'$ E, and has five main agro-ecological zones. The two regions share a common border and are situated between longitudes $8^{o}13'$ and $11^{o}14'$ E, and latitudes 3^{o} 48' and $6^{o}11'$ N, but fall under two different agro-ecological zones: Western highland (NW and W Regions) with an average annual rainfall of 2000 mm and humid forest agro-ecological zone (SW and Littoral Regions) with monomodal rainfall and an average annual rainfall of 3000 mm (IRAD, 2005). Five farmers' fields were surveyed in the Southwest (Idenau, Bokuva and Ekona, Banga-Bakundu and Mbonge-road) and five in the Northwest (Batibo, Mankon, Bambui, Ndu and Misaje). The location of the study area and the altitude of each field were obtained and recorded using the global information system (GIS) (Figure 2).

Collection and preservation of leaf samples

During the survey, a yam leaf sample each was collected from 60 separate plants (*Dioscorea rotundata* and *Dioscorea cayenens*) in each of the 10 farms visited, by putting a polyethylene bag over the leaf and detaching it at the petiole, without direct contact between the hand and the plant. In each field, prominent symptoms of YMV and YBV infections on these plants were recorded and photographs taken using a photographic camera. Three symptomatic and three asymptomatic plants were also selected in each field and a leaf sample harvested from each and labelled, to serve as positive and negative controls, respectively. To collect the remaining 54 leaf samples, large farms were divided into groups of 162, and balloting was done to select one group. In farms that did not have more than one group of 162 yam plants, samples were collected from the first 162 plants. Once a group was selected, the plants were numbered from 1 to 162 and a raffle draw was done, to choose the first plant from which the first leaf sample was collected. From this plant, leaf samples were randomly collected from the field, following "sampling with a skip two" systematic random sampling method, as described earlier. The same procedure was repeated in each farm visited and a total of 600 leaf samples were collected throughout

the survey. Therefore, out of this total number of leaf samples, 30 were symptomatic, 30 asymptomatic and 540 randomly harvested. When harvested each sample was accurately labelled, and immediately placed in a cool box (maintained cold by means of ice blocks) in which they were transported to the Laboratory of Virology in the University of Dschang and stored at 0 ^{o} C in a freezer. The samples were immediately tested for YMV and YBV, using the triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA).

Virus testing: Enzyme linked immunosorbent assay (ELISA)

The TAS-ELISA procedure followed was the one developed from the TAS-ELISA procedure for detection of peanut clumps virus in Clark & Adams (1977) and Njukeng (1998). Two micro-titre plates were appropriately labelled (one for YBV and another for YMV), for testing the 60 samples from each field for the presence of YBV and YMV, respectively. Yam badnavirus and YMV rabbit polyclonal antisera (IgG) were separately diluted in ELISA coating buffer at a ratio of 1/1000 and 1/2000, respectively. 100 µl of the diluted antibodies were separately dispensed into each of the 60 internal wells of their respective micro-titre plates. The plates were incubated in the fridge at 4 ^{o}C overnight. They were then emptied and the wells washed three times, by flooding them with washing buffer (PBS-T), and allowing for three minutes before emptying them quickly. Two grams of each yam leaf sample was separately ground in a polyethylene bag which was sealed at one end and accurately, using a roller so as to extract sap. 1000 µl of grinding buffer at 1/500 w/v dilution were added into each polyethylene bag and the solution homogenised thoroughly. 100 µl of the sap so extracted from each sample were dispensed into corresponding internal wells of each micro-titre plate. The 60 internal wells of each plate therefore received 100 µl of sap from the 60 samples collected from each farm in an orderly, and a corresponding manner. The plates were then incubated at 4 ^{o}C in a fridge overnight, after which they were emptied and washed with PBS-T, as described earlier. The 60 internal wells of each micro-titre plate were each, further filled with 200 µl of blocking solution (PBS-T, containing 5 % w/v of non-fat skimmed milk), and incubated at 37 ^{o}C for 45 - 60 minutes. The plates were then emptied and 100 µl of the second antibody, YMV monoclonal antibody (Mab) at 1/500 dilution and YBV monoclonal antibody (Mab) at 1/100 dilution in PBS-T, were dispensed into each of the internal wells of their respective plates. The plates were then incubated in a fridge at 4 ^{o}C overnight, after which they were washed as described earlier. 100 µl of antibody conjugate (goat mouse immunoglobulin conjugated to alkaline phosphate GAM (Ram-Ap) for YMV and YBV at a 1/1000 dilution each in conjugate buffer (with 2 g of skimmed milk added per litre of buffer), were dispensed into each of the internal wells of their respective micro-titre plates. These micro-titre plates were further incubated in a fridge at 4 ^{o}C overnight after which they were washed as described earlier. 100 µl of freshly prepared substrate (10 mg of paranitrophenyl phosphate tablet dissolved in 10 ml of substrate buffer) were added to each internal well of the plates. The plates were then incubated at room temperature for about 30 - 60 minutes to obtain a clear reaction. Through visual observation, the results were recorded with a yellow/orange colour change indicating a positive reaction, marked (+) and no colour change indicating no reaction, marked (-) (Figure 1).

Mixed infection and incidence of virus diseases

The surveyed fields were assessed and scored for incidence of *Yam mosaic virus*, Yam badnavirus and mixed infection of both viruses. For samples from each field corresponding wells of the two plates which reacted positive for the two viruses were considered as mixed infection by the two viruses. These triple antibody sandwich (TAS)-ELISA results were used to calculate the incidence of virus diseases, as a percentage of virus-positive counts per field. Average incidence (%) was calculated from the separate incidence obtained in each of the 10 fields.

Results and Discussion

Virus survey

A total of 600 yam leaf samples were collected from two yam species (*D. rotundata and D. cayenensis*) in 10 fields visited in the two ago-ecological zones of Cameroon. Eight different kinds of symptoms were recorded in different proportions in all the fields surveyed. Mosaic was the most common symptom observed in all fields while Puckering was the least common symptom observed only in Bokuva. Other symptoms observed include chlorosis, shoe-stringing, vein-banding, stunting, chlorotic spotting, and leaf crinkling and distortion (Table 1; Fig 3).

The incidence of YMV ranged from 13.3 to 73.3 %, at Ndu and Ekona while that of YBV ranged from 25 to 93.3 % at Batibo and Mankon respectively. Both YMV and YBV were very prevalent, detected by TAS-ELISA in single or co-infections in yam (*D. rotundata and D. cayenensis*) leaf samples from all the yam fields visited. Four hundred and nine[B]out of 600 (81.7 %) leaf samples tested were positive by TAS-ELISA, for YBV, YMV and co-infection of both viruses. Three hundred and twelve out of 600 (52 %), 397/600 (66.17 %) and 217/600 (36.17 %) of leaf samples tested positive for YMV, YBV and co-infection of both viruses respectively. The incidence of YMV and YBV infections were not significantly different but the incidence of the two viruses were significantly higher (P = 0.05) than that of mixed infection (Table 1).

Distribution of the virus infections within and across agro-ecological zones

In the two agro-ecological zones, 189(63 %), 179 (59.7 %) and 120 (40 %) out of 300 leaf samples harvested in the Southwest Region, as well as 123 (41 %), 218 (72.7 %) and 97 (32.3 %) out of the 300 leaf samples harvested in the Northwest tested positive for YMV, YBV and co-infection of both viruses respectively (Table 2). There was no significant difference between the incidence of YBV and co-infection (YMV +YBV) in the Southwest, compared with the Northwest Region. However, the incidence of YMV infection was significantly higher (P = 0.05) in the Southwest than Northwest regions (Table 2). Within the Southwest Region, the incidence of YMV and YBV were significantly higher (P = 0.05) than that of co-infection of both viruses. This was also true of YBV in the Northwest Region where its incidence was significantly higher than that of YMV and Co-infection of both viruses.

Distribution of virus infections within and across yam species

Virus infection in the two yam species showed that out of 300 leaf samples collected, 184 (61.3 %), 178 (59.3 %) and 120 (40.0 %) (*D. rotundata*) and 128 (42.6

%), 213 (71 %) and 95 (32.3 %) (*D. Cayenensis*) tested positive for YMV, YBV and co-infection of both viruses, respectively (Table 2). There was no significant difference between the incidence of YBV and co-infections on *D. rotundata*, compared with *D. Cayenensis* but, the incidence of YMV infection was significantly higher (P = 0.05) for *D. rotundata* than *D. cayenensis.* However, incidence of YMV and YBV infections on *D. rotundata* was also significantly higher (P = 0.05) than that of co-infection of the two viruses. This was true for YBV on *D. cayenensis* whose incidence was also significantly higher (P = 0.05) than that of YMV and co-infection for both viruses (Table 2).

Distribution of virus infection across symptomatic and asymptomatic yam leaf samples

Both symptomatic and asymptomatic leaf samples tested positive for YMV, YBV and co-infection of both viruses. Out of the 30 leaf samples tested, virus infection showed that 7 (23.3 %), 20 (66.7 %) for asymptomatic and 5 (16.7 %) and 26 (86.7 %), 22 (73.3 %) and 19 (63.3 %) for symptomatic, all tested positive for YMV, YBV and co-infection respectively. The incidence of YMV and mixed infection (YMV and YBV) were significantly higher (P = 0.05) in symptomatic leaf samples than asymptomatic. Although no significant differences were observed in the incidence of YBV infection for both asymptomatic and symptomatic samples, its infection on both symptomatic (77.33 ±8.30%) and asymptomatic (66.67 ± 12.17 %) was very high (Table 2).

This study provides information on the incidence and distribution of two viruses infecting yam in two major yam-growing agro-ecological zones of Cameroon. Yam mosaic virus, YBV and co-infection by both viruses were detected in the two agro-ecological zones with very high incidence. These viruses amongst others have been detected on yam in other countries of the West African yam belt and else where (Phillips *at al.,* 1999; Eni, 2008; Asala *et al.,* 2012). This finding buttresses previous report of YMV and YBV being the most widespread and important yam viruses, with YBV emerging recently as the most important viral threat to yam systems, occurring in very high incidence and widespread distribution in yam fields throughout the world (Njukeng *at al.,* 2.002; Eni, 2008; Kenyon *et al.,* 2008; Odedara *at al.,* 2011).

The high incidence and widespread distribution of these two viruses in the study can be attributed to unchecked local and international exchange of yam germplasm through permeable land borders. This exchange also accounts for the similarly high incidence of these viruses in all countries of the West African yam belt (Hughes *et al.,* 1997; Eni, 2008).

These infected planting materials have continued to be distributed because of the long absence of sensitive and reliable field diagnostic tools for yam viruses (Njukeng *et al.,* 2005). It may also be attributed to the fact that the two yam species (*D. cayenensis and D. rotundata*) from which leaf samples were collected have their probable centre of origin in the West African yam belt and are susceptible hosts of the two viruses (Coursey, 1967; Brunt *et al.,* 1996). In addition, there are reports that YMV originated from West Africa on these yam species followed by independent transfer to *D. trifida* and *D. alata* during virus evolution (Bousalem *et al.,* 2000; 2009).

Figure.1 Micro-titre plates (A) virgin (B) showing positive reactions at the end of TAS-ELISA procedure

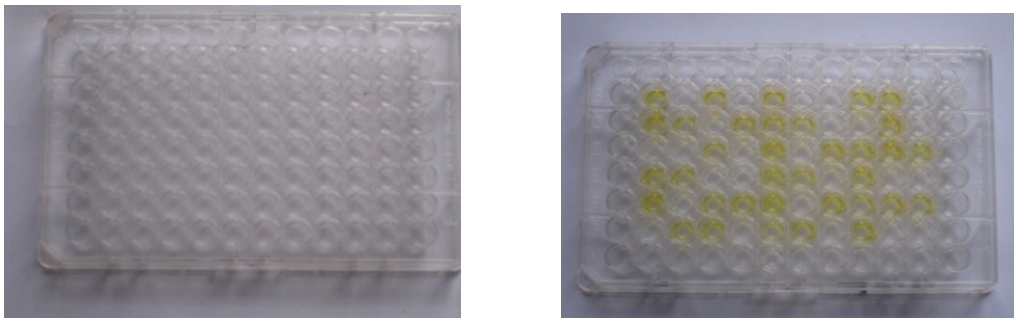
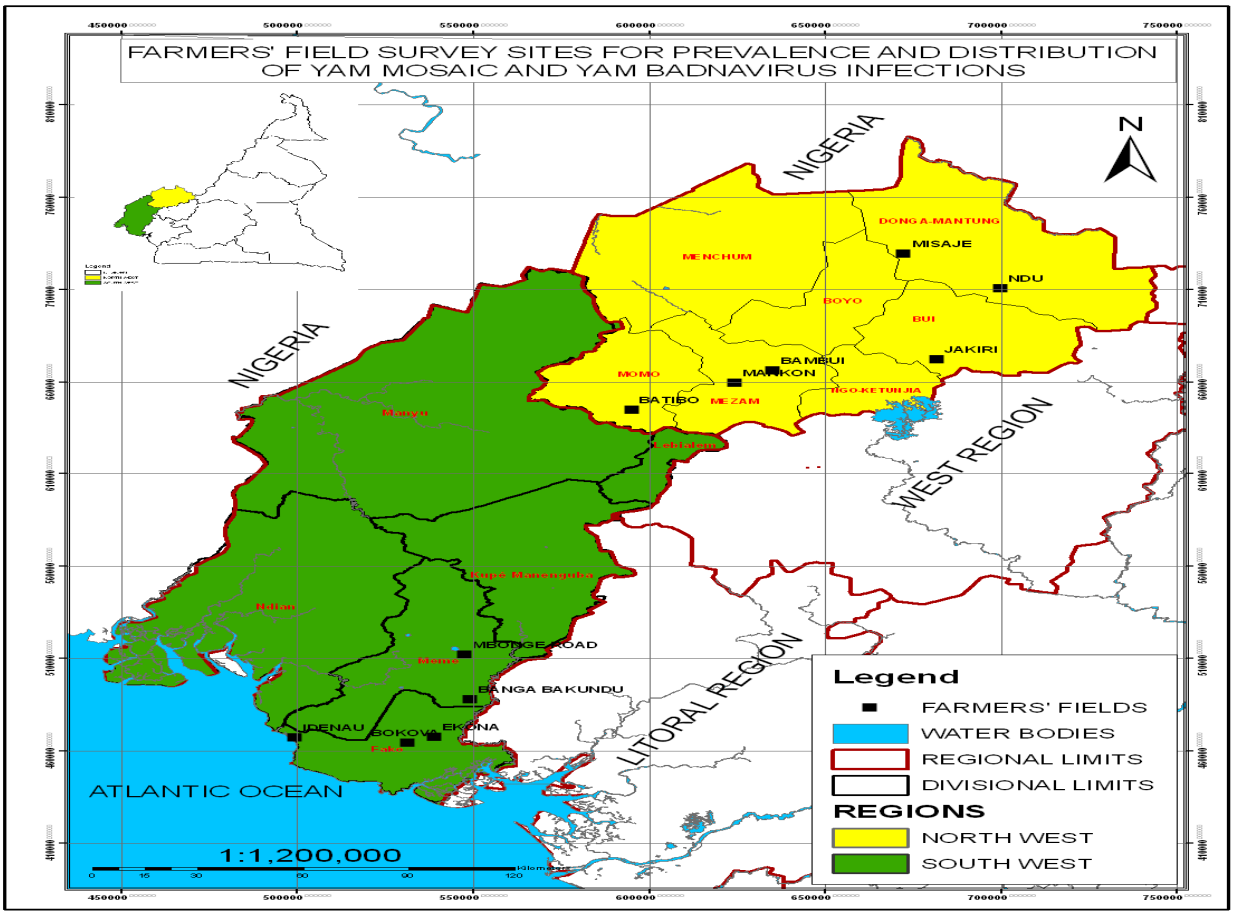


Figure.2 Map of Cameroon showing the study area and location of virus survey sites in the two regions (agro-cological zones)



(Source: GIS unit, MINFOF-Buea, Cameroon. May 2010)

Table.1 Incidence and symptoms of YMV and YBV observed on a total of 60 plants/field in 2009 on two yam species during a survey in two agro-ecological zones of Cameroon

Agro-ecological zone	Site/ Field	Incidence			Symptom types observed	Yam species
		YMV	YBV	Co-infection (YMV+YBV)		
SW Region	Idenau	40 (66.6)	26 (43.3)	20 (33.3)	Ss,Ms.Cl	*D. rotundata*
	Bokuva	32 (53.3)	44 (73.3)	24 (40.0)	Cls,Ms,Pc	*D. Cayenensis*
	Ekona	44 (73.3)	33 (55.0)	24 (40.0)	Ms,St,Cl	*D. rotundata*
	Banga-Bakundu	36 (60.0)	35 (58.0)	24 (40.0)	Ms,Ss,Lc/d	*D. rotundata*
	Mbonge Road	37 (61.6)	41 (68.3)	28 (46.6)	Ms,Ms,Gvb	*D. rotundata*
Total	**05**	**189/300 (63)**	**179/300 (59.7)**	**120/300 (40.0)**	**-**	-
					-	-
NW Region	Misaje	27 (45.0)	43 (71.6)	24 (40.0)	Ms,Ms,Lc/d	*D. rotundata*
	Batibo	24 (40.0)	15 (25.0)	10 (16.6)	Cl,Ms,Ms	*D. Cayenensis*
	Mankon	35 (58.0)	56 (93.3)	33 (55.0)	Gvb,Ms,Lc/d	*D. Cayenensis*
	Ndu	08 (13.3)	54 (90.0)	08 (13.3)	Ms.Cls,Ms	*D. Cayenensis*
	Bambui	29 (48.3)	50 (83.3)	22 (36.6)	St,Ms,Cls	*D. Cayenensis*
Total	**05**	**123/300 (41.0)**	**218/300 (72.7)**	**97/300**	**-**	-
	-			**(32.3)**	**-**	-
General total	**10**	**312 (52.0)**	**397 (66.2)**	**217 (36.2)**	**08**	**02**
Mean Incidence (Mean ± SE):		**52.00 ± 5.35 [b]**	**66.17 ± 6.73 [b]**	**36.17 ± 3.98 [a]**		**-**
-						

***** Means (on the last row) followed by different letters are significantly different at P=0.05 (DMRT); SE = Standard error. Incidence values out of brackets are positive counts, and those in brackets are in percentage; **Symptoms:** Ss = Shoe-stringing, Ms = Mosaic, Cl = Chlorosis, Pc = puckering, St = Stunting, Lc/d = leaf, Gvb = Green vein-banding, Cls = Chlorotic spotting

Figure.3 Some symptoms of YMV and YBV infections on yam leaves

A*^

B*

C*^

D*

E*

H*^

F*^

I*^

(***** = Symptom common in *Yam mosaic virus*; ^ = Symptom common in Yam badnavirus; A = Puckering; B = Shoe-stringing; C = Stunting; D = Mosaic; E = Mild mosaic; F = Severe Chlorosis; H = Chlorotic spotting**;** I = Leaf distortion / crinkling)

Table.2 Relative incidence and distribution of YMV and YBV across two yam species, symptomatic and asymptomatic leaf samples, and two agro-ecological zones of Cameroon

Distribution of viruses within and across yam species				
Virus infection	N° and % of positive counts		Incidence (Mean ± SE)	
	D. rotundata	*D. cayenensis*	*D. rotundata* (%)	*D. cayanensis* (%)
YMV	(184/300) 61.33	(128/300) 42.67	61.33 ± 4.70[b]	42.67 ± 7.93[a]
YBV	(178/300) 59.33	(213/300) 71.0	59.33 ± 5.05[b]	72.67±12.45[b]
Co-infection	(120/300) 40.00	(97/300) 32.33	40.67 ± 2.11[a]	32.33 ± 7.75[a]

Distribution of the virus infections within and across agro-ecological zones				
Virus infection	N° and % of positive counts		Incidence (Mean ± SE)	
	Southwest	Northwest	Southwest (%)	Northwest (%)
YMV	(189/300) 63.00	(123/300) 41.00	63.00 ± 3.34[b]	41.00 ± 7.54[a]
YBV	(179/300) 59.67	(218/300) 72.67	59.67 ± 5.26[b]	72.67 ± 12.48[b]
Co-infection	(120/300) 40.00	(97/300) 32.33	40.00 ± 2.12[a]	32.33 ± 7.74[a]

Distribution of virus infection across symptomatic and asymptomatic yam leaf samples				
Virus infection	N° and % of positive counts		Incidence (Mean ± SE)	
	Asymptomatic	Symptomatic	Asymptomatic	Symptomatic
YMV	(7/30) 23.33	(26/30) 86.67	23.33 ± 5.11 [a]	86.67 ± 5.43 [b]
YBV	(18/30) 60.00	(22/30) 73.33	66.67 ± 12.17 [a]	73.33 ± 8.30 [a]
Co-infection	(5/30) 16.67	(19/30) 63.33	16.67 ± 5.57 [a]	63.33 ± 7.77 [b]

***** For each row, means followed by different letters are significantly different at P = 0.05 (T-test); and, for each column (under distribution across agro-ecological zone and yam species), means followed by the different letters are significantly different at P=0.05 (DMRT); Values in brackets are positive counts and those out of brackets are incidence values; SE= Standard error

The incidence of YMV was significantly higher in the southwest than northwest region. This may be due to the fact that *D. rotundata* (from which 240/300 leaf samples were collected in the Southwest) is more susceptible to YMV than *D. cayenensis* (from which 240/300 leaf samples were collected in the Northwest) (Hughes *et al.,* 1997; Njukeng, 1998; Opong *et al.,* 2007).

Despite the fact that these viruses are mostly transmitted through infected planting material, insect vectors which spread them in the field also need to be targeted so as to permit effective virus control. YMV is transmitted by aphids in a non-persistent stylet-borne manner. This makes the use of insecticides inefficient in controlling the spread of the virus since most insecticides will not act fast enough to prevent yam virus transmission by this means. The use of non-susceptible barrier plants and mulches (Walkey, 1991; Cradock *et al.,* 2001) may help to exclude aphids and minimise spread of yam viruses.

Yam badnaviruses are transmitted by mealy bugs in a persistent and semi-persistent manner (Brunt *et al* 1996) over short and long distances (Eni *et al.,* 2013). Active movement of young viruliferous nymphs also greatly contribute in the transmission of YBV between interlocked yam branches particularly in unstaked yam fields (Eni *et al.,* 2013). The use chemical and biological control methods may effectively limit transmission of YBV in yam fields.

Symptoms observed in the study have previously been reported by other researchers (Offei, 2003; Eni, 2008). Yam badnavirus, YMV and co-infection of both viruses were detected on leaf samples showing obvious symptoms as well as asymptomatic ones. Some of the leaf samples showing obvious symptoms did not test positive for these viruses. The symptoms might have been caused by nutritional disorders/deficiencies, other viruses for which test was not done or which are yet to be identified or infestation by some other pests and pathogens.

Further, the detection of these viruses on asymptomatic leaf samples may be due to latent infection or tolerance by some plants (Goudou-Urbino, 1995; Odu *et al.,* 2004) and shows that use of symptoms is a very unreliable tool for indexing yam for viruses. This is even further compounded by the fact that two or more viruses can produce the same symptom and the same virus can produce varying symptoms on yam leaves. Use of symptoms can only aid laboratory diagnosis which serves as a more sensitive, reliable and conclusive way of affirming the health status of potential planting material. The high incidence of YBV compared with YMV on asymptomatic leaf samples may be due to the fact that symptoms caused by YBV on yam leaves maybe persistent, very seasonal or disappear soon after infection (Brunt *et al.,* 1996; Odu et al., 2004). These symptoms and their effect on the green pigmentation of plant's leaves can lead to a severe reduction in the photosynthetic ability of the infected plants, with a consequent reduction in the tuber yield, quality and in some instances death of the plant (Thouvenel & Dumont, 1998; Eni, 2008).

Viruses are ubiquitous on yam in the two agro-ecological zones, as in other countries of the West African yam belt. Use of virus-free planting material and virus-resistant varieties, non-susceptible plant barriers and mulches, as well as use of insecticides may greatly reduce virus infection on yam in the field. Unfortunately selection of healthy planting material is a major challenge to farmers and researchers because it requires rapid sensitive and reliable field diagnostic tools which are absent. Difficulties involved in differentiating symptoms of yam virus infection from those caused by nutritional disorders, some pests and pathogens is also a major challenge to researchers.

Recommendations

Achievement of real progress in the management of yam virus research requires a holistic approach involving continuous gathering of information, development of adequate field diagnostic tools, extension of research to all areas of yam cultivation in the world, selection of virus-resistant yam varieties, use of virus-free certified planting material and intensive education of the farmers on the economic importance of yam viruses and the interplaying factors involve in its management.

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