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Serological Detection of *Dioscorea alata potyvirus* on White Yams (*Dioscorea rotundata*) in Ghana

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Abstract: A baseline survey was conducted in ten important yam growing districts in Ashanti and Brong Ahafo regions of Ghana during June/July, 2004 to detect the incidence of viral diseases of white yam (*Dioscorea rotundata,* Poir). One hundred and seventy six leaf samples were collected and virus-indexed using the Enzyme Linked Immunosorbent Assay (ELISA) technique. Four yam virus antisera homologous to *Yam mosaic virus* (YMV), genus *Potyvirus*, *Cucumber mosaic virus* (CMV), genus *Cucumovirus*, *Dioscorea alata potyvirus* (DAV), genus *Potyvirus* and *Dioscorea alata badnavirus* genus *Badnavirus* were used. None of the antisera raised against *Dioscorea alata badnavirus* (DaV) and *Cucumber mosaic virus* (CMV) reacted positively. Antiserum raised against YMV reacted positively to antigens in 38% of the samples, while 20.5% reacted positively to DAV antigens. Eleven percent of the total samples collected reacted to both YMV and DAV antisera. Forty one percent of the samples however reacted negatively with all the antisera. The detection of DAV on white yam is the first evidence to be reported in Ghana. This finding provides useful information for the management of viral diseases of yam in Ghana.

Key words: White yam, *Dioscorea rotundata*, *Dioscorea alata potyvirus*, *Yam mosaic potyvirus*

INTRODUCTION

White yam *Dioscorea rotundata* is a popular starchy staple in Ghana. One of the major constraints to yam production in general is the effect of pest and diseases including those caused by viruses (Anonymous, 1992). Viruses adversely affect *Dioscorea* species by reducing vigour and subsequently the yield of the tuber (Amusa *et al*., 2003; Mandal, 1993; Coursey, 1967). An estimated yield reduction of 50% was reported by Amusa *et al*. (2003) as a result of YMV infection on *D. rotundata* in Nigeria.

Viruses that infect yams throughout the world belong to five genera, namely, potyvirus, potexvirus, badnavirus, carlavirus and cucumovirus (Brunt *et al*., 1996). In West Africa, however, viruses from the genera, potyvirus is the most widespread (Thouvenel and Fauquet, 1979). Viruses in the other genera have also been detected in the various locations and countries from different yam species.

Symptoms of infection are several; notable among them being the leaf mosaic. Other symptoms include leaf distortion, leaf malformation, chlorosis and in severe cases, shoe-stringing of the vines (Rossel and Thottappily, 1985). Some infections may also be symptom-less or latent.

In Ghana *Yam mosaic virus* (YMV), a potyvirus is reported to be the most commonly detected virus on *D. rotundata* (Olatunde and Hughes, 1999). Thus, in almost all cases, white yam virus disease

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is attributed to YMV alone. However, in the yam belt of West Africa, outside Ghana, other viruses belonging to the genus badnavirus, cucumovirus and other potyviruses have been isolated (Rechaus and Nienhaus, 1981; Hughes, 1986; Fauquet and Thouvenel, 1987). It is possible that some of these viruses may also be present in Ghana due mainly to the exchange of yam planting materials across the sub-region and vector activity.

A study was thus carried out aimed at detecting the existence of any of the known yam viruses other than YMV in Ghana. The findings of this study would be very useful in the management of viral diseases of yam especially the use of virus-free planting materials which will improve yield thus enhancing livelihoods.

MATERIALS AND METHODS

In a survey, leaves of *D. rotundata* showing virus-like symptoms were collected from ten important yam growing districts in Ashanti and Brong Ahafo regions, situated in the forest and forest-transition zones of Ghana. Sampling was done in farms situated at Fumesua and Tanoso (Kumasi district); Ejura and Sekyeduamasi (Ejura-Sekyedumasi district); Mampong (Mampong district) all in the Ashanti region of Ghana. Duayaw Nkwanta and Susanho (Tano North district), Sunyani, Fiapre, Chira and Heman (Sunyani district); Kintampo, Jema, Barbatorkuma and Beposo (Kintampo district), Techiman, Aworowa, Tanoso and Nipahiamoa (Techiman district); Wenchi, Ayayo, Droboso and Buoko (Wenchi district); Nkoranza, (Nkoranza district) and Atebubu, Prang and Amantin (Atebubu district) all in the Brong Ahafo region. In each town 3-4 farms were visited. Leaf samples were collected in the months of June/July, 2004 when plants were about four months old. Eight to ten plants were sampled from each site visited and leaves showing viral symptoms collected. They were labeled and then kept in an ice chest at a temperature of about 4°C and then sent to the laboratory for sero-diagnosis.

The enzyme linked immunosorbent assay (ELISA) technique was used to virus index the samples. Antibodies homologous to *Yam mosaic virus* (YMV), *Cucumber mosaic virus* (CMV), *Dioscorea alata potyvirus* (DAV), genus *Potyvirus* and *Dioscorea alata badnavirus* (DaV), genus *Badnavirus*, kindly supplied by the virology laboratory of the International Institute of Tropical Agriculture (IITA) were used.

PAS-ELISA Protocol

To detect *Dioscorea alata potyvirus*, genus *Potyvirus*, *Dioscorea alata badnavirus*, genus, *Badnavirus* and *Cucumber mosaic virus*, genus *Cucumivirus*, the protocol followed was by developed by Edwards and Cooper (1985) and modified by Hughes and Thomas (1988). Three replications of each sample were used for the detection.

ELISA microtitre plates (NUNC) were coated with (100 μL) Protein A solution in coating buffer and incubated for 2 h at 37°C after which ground samples (1 g/2 mL of extraction buffer), were added and incubated overnight at 4°C. The detecting antibody was added and incubated for 2 h at 37°C after which Protein-A alkaline phosphatase conjugate diluted in conjugated buffer (1:50,000) was added and incubated for same time period and temperature. Between each step of ELISA procedure (incubation period) the plates were washed thrice at 3 min intervals. 0.01 g/10 mL of p-nitrophenyl phosphate salt in substrate buffer was finally added and incubated overnight at room temperature for one hour.

TAS-ELISA Protocol

To detect YMV in our samples, the triple antibody sandwich ELISA (TAS ELISA) procedure was followed.

This was as described by Adams and Barbara (1982), Barbara and Clark (1982). ELISA plates were coated with 100 µL YMV polyclonal antibody diluted in coating buffer (1:1000) and incubated for 2 h at 37°C after which samples (1 g/2 mL extraction buffer) were added to the wells and incubated overnight at 4°C.

YMV monoclonal antibody (1:1000 in PBS-Tween 20) was then added to wells and incubated for 2 h at 37°C. Goat anti-mouse alkaline phosphatase conjugate diluted in conjugate buffer was added to wells and incubated for 2 h at 37°C. Between each step of ELISA procedure the plates were washed thrice at 3 min intervals. 0.01 g/10 mL of p-nitrophenyl phosphate salt in substrate buffer was finally added and incubated at room temperature and overnight.

For both PAS and TAS ELISA Protocols, Optical Density (OD) values of contents in the wells were determined by using microplate reader at 405 nm wavelength. A sample was considered positive when the OD value was at least twice that of healthy control (Sutula *et al*., 1986).

RESULTS

One hundred and sixty leaf samples were analysed. None of the antisera raised against DaV and CMVreacted positively to antigens in yam samples (Table 1).

However antiserum raised against YMV reacted positively to antigens in 68 (38%) samples, while that raised against DAV reacted positively to 36 (20%) samples. Antiserum raised against YMV and DAV which occurred in a mixture reacted positively to antigens in 20 (11%) samples. In all 72 (41%) samples reacted negatively with antisera raised against YMV, DAV, DaV and CMV even though they showed virus-like symptoms.

The Wenchi district recorded the highest incidence of samples responding to antibody homologous to YMV 75%, followed by the Kumasi district 65%, Kintampo district 65%, Sunyani 40% Ejura/Mampong districts 36.8%, Atebubu/Prang districts 31.5%, Techiman district 20% and Tano North district 10.5% in that order while all the samples collected in the Nkoranza district tested negative

For the *Dioscorea alata potyvirus*, DAV, Techiman district recorded the highest incidence of samples testing positive 80% followed by Wenchi district 60%, Kintampo 20% and Sunyani 20%. Samples from the remaining districts tested negative.

DISCUSSION

The results as shown in Table 1 clearly indicate the widespread nature of YMV in the study areas. This is followed by *Dioscorea alata potyvirus* (DAV), also known as yam virus 1), which is more prevalent around Wenchi, Techiman, Sunyani and Kintampo districts. *Cucumber mosaic virus* and *Dioscorea alata badnavirus*, were not detected in any of the samples tested, possibly indicating their absence in the study areas.

Table 1: Viruses detected with ELISA on *D. rotundata* leaf samples from yam growing districts in Ghana

District	*Yam mosaic virus* (YMV)	*Dioscorea alata potyvirus* (DAV)	*Cucumber mosaic virus* (CMV)	*Dioscorea alata badnavirus* (DaV)	Mixed infection (YMV and DAV)
Kumasi	13/20(65%)	0/20(0%)	0/20(0%)	0/20(0%)	0/20(0%)
Sunyani	8/20(40%)	4/20(20%)	0/20(0%)	0/20(0%)	0/20(0%)
Techiman	4/20(20%)	16/20(80%)	0/20(0%)	0/20(0%)	0/20(0%)
Wenchi	15/20(75%)	12/20(60%)	0/20(0%)	0/20(0%)	0/20(0%)
Kintampo	13/20(65%)	4/20(40%)	0/20(0%)	0/20(0%)	0/20(0%)
Nkoranza	0/19(0%)	0/19(0%)	0/19(0%)	0/19(0%)	0/19(0%)
Atebubu/Prang	6/19(31.5%)	0/19(0%)	0/19(0%)	0/19(0%)	0/19(0%)
Ejura/Mampong	7/19(36.8%)	0/19(0%)	0/19(0%)	0/19(0%)	0/19(0%)
Tano North	2/19(10.5%)	0/19(0%)	0/19(0%)	0/19(0%)	0/19(0%)
Total	68/176(38.6)	36/176(20.4%)	0/176(0%)	0/176(0%)	0/176(0%)

According to Thouvenel and Fauquet (1977), Brunt *et al.* (1996), YMV is the most dominant virus on white yam throughout West Africa. It is not surprising that it was the dominant virus found in most of the samples tested.

Dioscorea alata potyvirus is also common particularly on *Dioscorea alata*, it was first detected in Togo, Rechaus (1986). Hughes (1986) also detected the virus on *D. alata* from the Caribbean and Indonesia. Odu (1997) detected the virus on *D. alata* from Nigeria. Previous findings indicate that DAV has never been isolated or detected on *D. rotundata* in Ghana (Olatunde and Hughes, 1999). The detection of DAV on white yam as has been shown in our results thus contradicts Olatunde and Hughes (1999) who reported that DAV was limited to *D. alata* in Ghana.

In some of the samples YMV and DAV were also found to coexist. This result is again the first report of such infection in Ghana. This finding also contracts Olatunde and Hughes (1999) who reported that YMV infection on white yam never coexisted with DAV. In their report on a comprehensive virus survey carried out in farmers' fields in seven regions of Ghana in 1998 and 1999; two viruses; YMV and DAV were both detected on *Dioscorea* species but not in mixed infections. While YMV was detected on *D. rotundata* and *D. alata* cultivars, DAV was detected only in *D. alata* (Hughes and Olatunde, 1999).

Generally it is not surprising that DAV has transferred from *D. alata* to *D. rotundata*. This is because both species belong to the same genus; *Dioscoreacae*. Again DAV and YMV are different strains of the virus genus belonging to the potyvirus group.

Forty one percent of the samples collected responded negatively to all the antibodies. This could de due to the fact that the virus concentration in them might have been low, probably because of the time of collection. Also, varietal characteristics or strain of virus might have been different, or due to the presence of other viruses, (Van Regenmortel, 1982; Matthews, 1991).

Improvement in the yield of white yams cannot be achieved if viral diseases continue to be prevalent. The most effective way of controlling plant viral diseases is to breed for crop cultivars that are resistant/tolerant to virus infections. The detection of DAV on white yam should signal to yam virus disease researchers and breeders the need to develop comprehensive virus cleaning measures to clean white yam and indeed all yam planting materials from all known yam viruses to realize production of completely virus-free planting materials for better yields to be achieved.

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