Impact of Yam mosaic virus (YMV) on tuber yield of white yam *(Dioscorea rotundata Poir)* in Ghana

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Abstract
The impact of Yam mosaic virus (YMV) on tuber yield of white yam (Dioscorea rotundata Poir) in the forest zone of Ghana was assessed under controlled environment and in the field at Kumasi, Ghana. Two field trials were carried out in 2001 and 2002. The experiment under controlled environment was conducted in 2003. In the controlled environment, white yam developed from tissue culture were mechanically inoculated with sap extracts of YMV infected leaves. The inoculated plants were grown alongside known YMV infected white yams not cleaned with tissue culture as well as non inoculated healthy controls cleaned with tissue culture and virus indexed. In the field trial, data taken were disease severity and yield per plant after harvest. Plants raised from non tissue culture cleaned seed yam showed severe symptoms of YMV with a yield loss of 53% as against 28% for the inoculated plants. In the two field trials in 2001 and 2002, mean yield losses of between 26.0% and 89% were recorded depending on disease severity. This implies YMV on white yam is economically important. This study is the first of such in Ghana. Efforts to control yam mosaic virus disease through the application of biotechnology to produce virus-free planting materials as well as the use of resistant varieties should be intensified to improve yield, thereby increase income of resource-limited yam farmers.

Key words: Dioscorea rotundata, Yam mosaic virus, disease severity, tissue culture, yield

Résumé
L'impact du virus de la mosaïque d'igname sur (VMI) le rendement des tubercules d'igname blanche (Dioscorea rotundata Poir) dans la zone forestière du Ghana a été évalué dans un environnement contrôlé et sur le champ à Kumasi, au Ghana. Deux essais sur le champ ont été réalisés en 2001 et 2002. L'expérience dans un environnement contrôlé a été menée en 2003. Dans l'environnement contrôlé, l'igname blanche développée à partir de la culture in vitro a été mécaniquement inoculé avec des extraits de sève de VMI feuilles infectées. Les plantes inoculées ont été cultivées à côté de VMI ignames blanches infecté non nettoyées avec la culture in vitro ainsi que des témoins sains non inoculés nettoyés avec la culture in vitro et virus indexé. Dans l'essai sur le champ, les données prises étaient infecté gravement de la maladie et le rendement par plante après la récolte. Plantes issues de l'igname sain non nettoyé avec la culture de in vitro ont montré des symptômes sévères de VMI avec une perte de

rendement de 53% contre 28% pour les plantes inoculées. Dans les deux essais sur le champ en 2001 et 2002, la moyenne des pertes de rendement entre 26,0% et 89% ont été enregistrés selon la gravité de la maladie. Cela implique que le VMI sur l'igname blanche est économiquement importante. Cette étude sont les premiers d'une telle au Ghana. Les observations faites dans cette étude sont les premiers d'une telle au Ghana. Les efforts visant à contrôler la maladie du virus de la mosaïque d'igname par l'application de la biotechnologie pour produire du matériel végétal exempt de virus devraient être intensifiés pour améliorer le rendement, ainsi augmenter le revenu des agriculteurs d'igname qui ont des ressources limitées.

Mots clés: Dioscorea rotundata, le Virus de la Mosaïque d'Igname, ELISA, la gravité de la maladie, le rendement, la culture in vitro

Introduction

Food yams are members of the genus *Dioscorea* which contain about 600 species of which only six are important as staples in the tropics (Coursey, 1969; Diehl, 1982). The economically important species grown are *Dioscorea rotundata* (white yam) *D. alata* (yellow yam), *D. bulbifera* (aerial yam) *D. esculenta* (Chinese yam) and *D. dumetorum* (trifoliate yam) (Chinwuba and Odjuvwuederhie, 2006; Konczacki and Konczacki, 2013).

In Ghana however, white yam *(Dioscorea rotundata Poir)* is a very important component of the root and tuber crops which together contribute about 46% of the Agricultural Gross Domestic Product (AGDP) in Ghana (Agriculture in Ghana, 2001). In 2013, Ghana cultivated about 430,000 hectares with a yield per hectare of 15.4 tons (FAOSTAT, 2014). In 2013, Ghana produced over 6.6 million tons compared to Nigeria's 38 million tons and Cote d'Ivoire's 5.8 million tons FAOSTAT (2014). Ghana is the third largest producer of yams in the world after Nigeria and Cote d'Ivoire. In Ghana yams are important staple food crop. It is an important source of carbohydrate for both adults and children. It can be prepared into several local dishes like ampesi, fufu and can be fried with oil and eaten with fish and pepper. One of the major constraints to yam production in West and Central Africa is the adverse effect of pests and diseases with the major one being viruses (Eni *et al.,* 2011; Legg *et al.,* 2007; IITA, 1992). Viruses adversely affect Dioscorea spp. by reducing plant vigor and tuber yields (Eni, 2015; Amusa *et al.,* 2003; Mandal, 1993; Tetteh and Saakwa, 1991).

To date, *Yam mosaic virus* genus *potyvirus,* remains the most important viral disease in several yam producing countries in Africa. The virus was detected in Ghana as early as 1958 when 7% of early planted varieties examined during a survey in Northern Ashanti (forest-savanna transition) were found to be infected (Leather, 1959). Hughes *et al.* (1999) also found YMV to cause very severe infection on yams especially white yam *(D. rotundata)*.Typical symptoms include mosaic patterns, chlorosis and leaf distortions (Eni *et al.,* 2008; IITA, 1993; Theberge, 1985), shoe string, vein clearing, green vein banding and in severe infection the entire plant may appear stunted (Emehute *et al.,* 1998; Rossel & Thottappilly, 1985).

Yield loss due to YMV on white yam has been estimated in Nigeria (Amusa *et. al.,* 2003) who reported over 50% yield loss and elsewhere by Thouvenel and Dumont, (1990) who reported a 25% higher tuber yields using healthy plants of *D. alata* over infected

mosaic plants.

In Ghana, however, information on yield loss induced by YMV on white yam is not available. The objective of this study was therefore to quantify yield loss in white yam caused by YMV.

Materials and methods

Two field experiments in 2001 and 2002 and one experiment in the screen house in 2003 were conducted to determine yield loss caused by YMV.

Experiment 1

Screen house experiment

Tissue culture micro tubers from four *D. rotundata* accessions namely; Pona, TDr 91-93, TDr 179 and TDr 131 were used in the screen house experiment at Crops Research Institute, Kumasi Ghana during the 2003 farming season. Fourteen micro-tubers of Pona, 24 of TDr 91-93, 24 of TDr 179 and 12 of TDr 131 were planted in polyethylene bags measuring 18 cm in diameter x 20 cm in height filled with sterilized topsoil and then kept in the screen house. The design used was completely randomized design. Planting materials for each of the accessions were divided into two equal parts where one-half was inoculated artificially with sap of YMV and the other half left uninoculated to serve as negative control. Infected yam leaves were ground in a 0.05 M phosphate buffer, pH 7.7. The inoculation buffer consisted of 0.01 M sodium phosphate dibasic (Na_2HPO_4), 0.001 ethylenediaminetetracetic acid (EDTA) and 0.0001 M L-cysteine in 600ml of distilled water. The pH was adjusted to 7.7 with 0.01 M potassium phosphate monobasic (KH_2PO_4). Extracts or sap from leaves of a known YMV-infected yam, were rubbed onto 600-mesh carborandum dusted leaves of the healthy plants excluding the controls.

Inoculation was done three weeks after sprouting. Twenty micro tubers (not from tissue culture) of known YMV infected white yams (Pona) accessions were also planted in polybags as indicated above to assess the effects of virus infection on yield. Healthy plants were separated by two metres from diseased plants to avoid cross infections or contamination.

Five grams of NPK 15-15-15 was applied to all plants, including the field infected Pona accessions, four months after planting. The screen house was regularly sprayed (once every month) with *karate* (Lambda cyhalothrin) at 250ml/ha to prevent insect vector transmission of virus to the negative or healthy control plants. Disease assessment was carried out 5 times from one month after inoculation until maturity. Disease assessments were done monthly by visual scoring based on a 1-5 point scale (Kyetere *et al.,* 1999) where 1 represents no infection, 2 mild infection, 3 moderate infection, 4 severe infection and 5 very severe infection or dead. Average mean of disease severity score was calculated by dividing the total severity scores over the number of times disease assessment was carried for each treatment. This mean disease score per plant was correlated with yield to determine disease severity on yield after harvest. Serological diagnosis of plant leaves showing both viral symptoms as well as symptomless leaves was carried out using Protein - A sandwich (PAS) and Triple-antibody sandwich (TAS) enzyme-linked immunosorbent assay (ELISA) as described below.

Tuber yield loss was assessed seven months after planting when all the leaves had senesced and tubers harvested. Each tuber harvested was weighed with a Metler-Toledo™ laboratory weighing balance. The results were analyzed using the MSTAT-C software at 5% significant level to determine the effect on average virus score per plant. Differences were separated using least significance difference (Lsd) at 5% level of

significance. Still photographs were also taken of both control and test treatments.

PAS-ELISA

Protocol used for the Protein A indirect enzyme linked immunosorbent assay technique (PAS ELISA) was described by Edwards and Cooper (1985) and modified by Hughes and Thomas (1988). Antibodies homologous to *Yam virus 1.* (YV1), *Cucumber mosaic virus* (CMV) and *Dioscorea alata badnavirus* (DaV) were used to detect the presence of their corresponding viruses.

ELISA microtitre plates (NUNC™) were coated with 100 µl of Protein A solution in coating buffer, diluted in a ratio of (2 µl/ 5 ml) and then incubated for two hours at 37°C after which wells were thrice washed with phosphate buffered saline-tween (PBS-tween 20) at 3 minutes interval and then tapped dry as done routinely. Each sample was allotted two wells and there were two wells each for both known positive and negative controls. Leaf sap were extracted by grinding leaf samples in extraction buffer in a ratio of 1 g of leaf per 2 mls of extraction buffer and then 100 µl allotted to a well and incubated overnight at 4°C after which wells were washed three times with PBS-tween 20. One hundred micro-litres of the detecting antibodies, diluted in PBS-tween 20 in a ratio indicated by suppliers (IITA) for each antibody were added and then incubated for 2 hours at 37°C. After thrice washing with PBS-tween 20, 100 µl of Protein - An alkaline phosphatase conjugate diluted in conjugate buffer in a ratio of 1:50,000 was added and incubated for another 2 hours at 37oC after which they were again washed with PBS-tween 20. Two hundred micro-litres of p-nitrophenyl phosphate salt diluted in substrate buffer in a ratio of 0.01 g/10 ml was finally added to each well and then left at room temperature for one hour and then overnight at 4°C. At the end of one hour optical density (OD) values of contents in the wells were determined using BIORAD™ microplate reader *model 550* at 405 nm wavelength. A sample was declared positive or infected with the corresponding virus when the mean OD value was at least twice that of the mean of healthy control (Sutula *et.al.,* 1986). Overnight, wells were again read to observe changes if any.

TAS-ELISA

The protocol for the triple antibody sandwich ELISA was as described by Adams and Barbara (1982) and Barbara & Clark (1982). This was used to detect YMV infection in the treatments. The NUNC™ ELISA plates were coated with 100 µl of YMV polyclonal antibody diluted in coating buffer in a ratio of 1:1000 and incubated for 2 hours at 37°C. Wells were thrice washed and then tapped dry after which samples extracted with extraction buffer in a ratio of 1 g per 2 mls of extraction buffer were allotted to the wells. Each sample was allotted two wells and there were two wells each for both known positive and negative controls. Plates were incubated overnight at 4°C after which they were washed with PBS-tween 20 as described above.

One hundred micro-litres of YMV monoclonal antibody diluted in PBS-Tween 20 in a ratio of 1:1000 was then added to wells and incubated for 2 hours at 37°C. Wells were again washed and then tapped dry as described earlier. One hundred micro-litres of goat anti-mouse alkaline phosphatase conjugate diluted in conjugate buffer in a ratio of 1: 40000 was then allotted to each well and again incubated for 2 hours at 37°C after which wells were washed and tapped dry as described earlier. Two hundred micro-litres of p-nitrophenyl phosphate salt diluted in substrate buffer in a ratio of 0.01g salt per 10ml substrate buffer was finally added to each well and then left at room temperature

for one hour and then overnight at 4°C. At end of one hour, optical density (OD) values of contents in the wells were determined using BIORAD™ microplate reader model 550 at 405nm wavelength. A sample was declared positive or infected with YMV when the mean OD value was at least twice that of the mean of the healthy control (Sutula *et.al.* 1986). Overnight, wells were again read to observe changes if any.

Experiment 2:
Assessment of yield loss under field conditions

Yield loss induced in *D. rotundata* (pona accession) by YMV was studied under natural conditions during the main planting seasons in 2001 and 2002 at Kumasi which is located within the forest ecological of Ghana. Seeds of the Pona accession previously screened for YMV infection were used. Pona is a very popular accession of the *D. rotundata* among Ghanaians and its susceptibility to YMV in farmers' field is well known. The area selected for the trial was known to be a hotspot for YMV. The experimental design was RCBD with four replications. Each plot had 15 plants in standard yam mounds. There were two plots in each replication, one for healthy or negative control and the other for infected plants. Data were taken on eight sampled plants in the infected plot. These plants were sampled based on disease symptom expression. Disease severity assessment was scored on a scale of 1-5, (Kyetere *et al.,* 1999) where 1 is no infection, 2 mild infection, 3 moderate infection, 4 severe infection and 5 very severe infection or dead. YMV severity assessment on the treatments was carried out thrice: one month after emergence, 3 months and six months after emergence. Average mean of disease severity score was calculated by dividing the total severity scores over the number of times disease assessment was carried for each treatment. This mean disease score per plant was correlated with yield after harvest to determine the effect of YMV severity on yield per plant. Symptomless leaves as well as those showing viral symptoms were analyzed serologically using PAS and TAS ELISA as described earlier to detect the presence of any of the known common yam virus stated earlier. This was done to verify if other viruses other than YMV infected the treatments. At physiological maturity when plants were harvested, tubers were weighed for each plant and the mean yield for each plant calculated as weight per plant. Data were analyzed using Analysis of Variance (ANOVA) with the MSTAT-C software and LSD calculated at 5% significant level. Yield loss was calculated as a percentage by dividing the mean yield weight per plant of the infected plants for the various severity scores with the mean yield weight per plant of the healthy control. Correlation analyses between mean YMV severity score per plant and mean yield weight per plant was done.

Results
Experiment 1
Screen house experiment
ELISA results

The ELISA results obtained from leaves of micro tubers that were artificially inoculated with YMV detected the presence of yam mosaic virus (YMV). This indicates 100% success of inoculation of the YMV on the test plants. No detection was made on the non inoculated healthy or negative controls that were also symptomless. Antibodies homologous to *Yam virus 1.* (YV1), *Cucumber mosaic virus* (CMV) and *Dioscorea alata badnavirus* (DaV) tested negative and therefore these viruses were not detected in our treatments.

YMV induced loss on yield:

All tubers of accession 131 either failed to sprout or did not grow to maturity after sprouting. Two plants of the artificially inoculated Pona accession and six plants of the field infected Pona micro tubers also did

not grow to maturity so they were dropped from the study. Again for the calculation of yield loss per plant, all accessions were treated as a *D. rotundata*. Thus, no distinction was made between TDr 131, TDr 91-93 and Pona although it was obvious that some accessions performed better but these differences were not significantly different.

The size of yam macro tubers was reduced as a result of YMV infection (plate 1). A more severe tuber yield loss per plant was obtained for the tuber infected planting material compared with the artificially inoculated plants. A 53.4% yield loss per plant was obtained for the tuber/seed infected planting material when compared with the healthy control. However, tuber yield loss of 28.7% was obtained when treatments were artificially inoculated with the YMV.

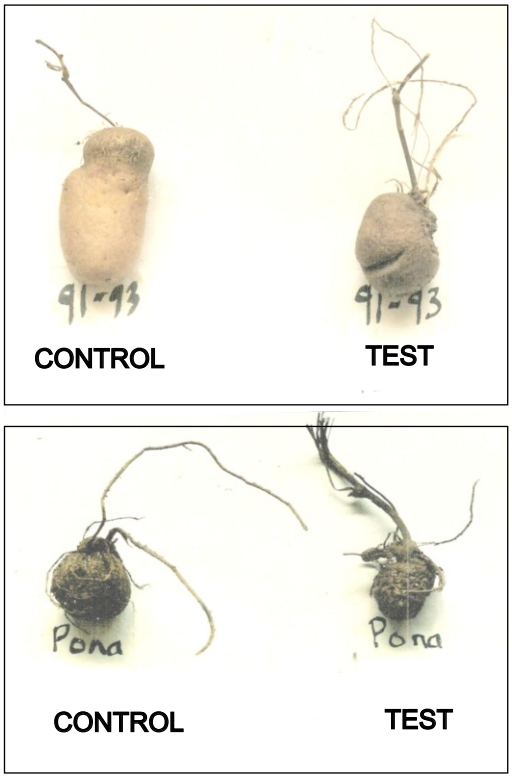

Plate 1: Effect of virus infection on the sizes of yam macro-tuber under screen house conditions: The size of macro-tubers from healthy yam plants accession TDR 91-93 (left) and the size of macro-tuber from virus infected plant (right)

(b).The size of macro-tuber from healthy yam plant, Pona (left) and the size of macro-tuber from virus infected plant, (right)

Severity of disease symptoms showed a direct effect on tuber yield. Overall, YMV disease severity and yield were negatively correlated. Thus as YMV disease severity score increased, yield per plant decreased. Mean macro tuber yield of healthy (control) plants was 419.0 g/plant and a mean disease severity score of 1.0. This was significantly ($p<0.05$) greater than yield of the tuber/seed infected plants which scored a mean disease severity of 3.1 and macro tuber yield weight of 155.5 g/plant. For the artificially inoculated treatments, mean disease severity score of 2.5 was recorded and a macro tuber yield of 319.5 g/plant obtained (figure 1). However,

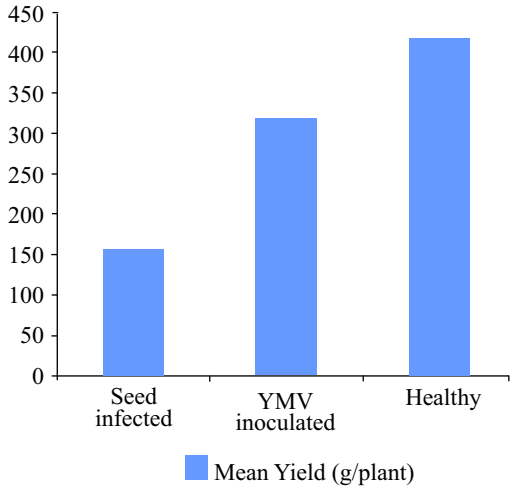

Fig. 1 Effect of YMV on tuber yield of D. rotundata under screen house conditions. Seed infected plants were not cleaned with tissue culture, YMV inoculated seeds were

differences between yield loss of inoculated and the healthy control plants were not significantly different at $p<0.05$.

Experiment 2
Field Trial
ELISA results
ELISA test six months after emergence detected the presence of YMV in infected yam plants used as treatment. None of the other three virus antibodies tested namely, *cucumber mosaic virus* (CMV), yam virus 1 (YVI) and *Dioscorea alata badnavirus* (DaV) reacted positively on any of the treatment samples tested. Healthy and symptomless plants that were used as negative controls tested negative to the ELISA as the OD values of the test samples were less than twice that of the negative control.

YMV induced loss on yield
In both 2001 and 2002 trials, significant yield losses were recorded as a result of YMV infection (Table 1). In 2001 the highest tuber yield obtained was 2.28 kg/plant for healthy controls that were symptomless with a disease score of 1 and also tested negative to YMV with ELISA. Mean tuber yield per plant for various YMV disease severity scores were obtained. For treatments that scored severity of 2 a mean tuber yield of 1.68 kg/plant was obtained. A mean tuber yield of 0.83 kg/plant per YMV severity score of 3 and for those that scored severity of 4 a mean tuber yield of 0.25 kg/plant was obtained respectively. Plants that did not grow to maturity as a result of severe infections were not included because no yield was obtained. Mean tuber yield loss calculated as a percentage of healthy controls showed percentage tuber yield loss of 26%, 64%, and 89% respectively as severity score increased from 2 to 4 (Table 1).

In 2002 the trend was not different. A maximum tuber yield of 2.27 kg/plant was obtained for plants that were symptomless with a severity score of 1 and tested negative to YMV with ELISA. A mean tuber yield of 1.60 kg/plant was obtained for plants that had severity score of 2. Those that had severity score of 3, a mean tuber yield of 0.95 kg/plant was obtained and those that had severity score of 4 a mean tuber yield of 0.30 kg/plant was obtained. Plants that did not grow to maturity as a result of severe infections were not included because no yield was obtained. Mean tuber yield loss calculated as a percentage of healthy controls showed percentage yield loss of 30%, 59%, and 87% respectively as severity score increased from 2 to 4 (Table 1).

Table 1: Disease severity of YMV on the yield of *D. Rotundata,* in 2001/2002 under field conditions.

Disease severity score	*Mean Yield (kg/plant)*	
	2001	*2002*
1	2.28	2.27
2	1.68 (26%)	1.60(30%)
3	0.83(64%)	0.95(59%)
4	0.25(89%)	0.30(87%)
Lsd (5%)	0.62	0.60

Figures in brackets are percentage yield loss per severity score compared with the healthy controls

Discussion
Results from the current study showed the effect of YMV on tuber yield of *D. rotundata* under both controlled and uncontrolled environments. Under both conditions tuber yield loss was significant ($P<0.05$) when compared with the health or negative controls. In the field trial between 26% - 89% tuber yield loss was obtained between the two consecutive years. However, tuber yield loss under screen house conditions was much lower (54% loss) when the planting materials were not cleaned with tissue culture. Artificially YMV infected tissue culture cleaned yam plants did not produce severe disease symptoms and their effect on yield

was lower (28% loss). Eni (2015) indicated that the use of certified virus-free yam planting materials contributes to the management of yam mosaic virus which will consequently impact positively on yields. However, the success of this approach is dependent on the absence of initial sources of yam mosaic virus inoculums and aphid vectors around farm. Agrios (1997) also reported that virus infected crop planting materials when propagated show early viral effects on growth and consequently affects yield negatively. In the screen house, plants were subjected under the same environmental conditions thus any difference in disease severity could be attributed to YMV because disease development started early in the non tissue culture cleaned planting materials thereby impacting heavily on tuber yield compared to the artificially inoculated planting materials obtained from tissue culture which were infected later. Under natural conditions tuber yield losses were much higher than under screen house environment. This could be attributed to adverse effects of the natural environment, resulting from abiotic stresses including climate and soil factors which greatly influenced disease severity.

The use of both PAS and TAS ELISA was very crucial in this study because it accurately diagnosed YMV and complemented the symptom severity scoring. In this study ELISA confirmed that symptom severity on which scoring was made was indeed caused by YMV. Disease symptoms used for scoring included mosaic patterns, chlorosis, leaf distortion, stunting, etc as reported by IITA (1993), Rossel and Thottappilly (1985).

Estimates of yield loss in *D. rotundata* induced by YMV have not been reported in Ghana on which results obtained in this study can be compared. However, Amusa *et. al.* (2003) showed that under severe attack, tuber yield loss of more than 50% could be obtained when *D. rotundata* is infected by YMV in Nigeria. Again, Thouvenal and Dumont (1990) reported a 25% higher tuber yields using healthy plants of *D. alata* over YMV infected plants.

Generally, disease infections in crops are a major worry to both producers and consumers. Farmers lose a chunk of their potential incomes through the impact of diseases. From the results it is important that farmers are provided with improved planting materials tolerant/ resistant to viral diseases and also virus-free planting materials. These can be achieved through the application of biotechnology to improve yield, thereby increase income of resource-limited yam farmers.

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