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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

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Millions of people around the world, particularly in West Africa, depend on yam for food and income, however global yam production has been fluctuating since 2007. Virus infections contribute to yam yield losses and the occurrence of mixed virus infections is potentially catastrophic. Planting of certified virus-free/resistant tubers is advocated therefore knowledge of the role of yam planting material in the virus dynamics in yam fields is crucial for effective yam virus control. In this study, yam tubers bought from markets in six West African countries were planted in an insect proof screen-house. Leaf samples from the tuber sprouts were tested by ELISA and/or IC-PCR/IC-RT-PCR to determine the incidence of Yam mosaic virus, Yam mild mosaic virus, cucumber mosaic virus, and Dioscorea Bacilliform viruses. Yam tubers from Nigeria and Ghana, as well as yam leaves collected from yam fields in Nigeria were also tested. All the viruses assayed for were detected. Most of the virus infections detected in the tuber (83%) and tuber sprouts (95%) were single infections of either *Dioscorea bacilliform* viruses (DBV) or *Yam mosaic* virus (YMV). The incidence of mixed infection in the field samples (49.3%) was about 3 times and 10 times more than those detected in the tubers (17%) and the tuber sprouts (5%). These results suggest that other factors other than the tubers used as planting materials contribute to the vast incidence of mixed virus infections in yam fields. These factors must be properly appraised and be factored into any yam virus control strategy equation in order to achieve a sustainable yam production in West Africa in particular and the world in general.

Key words: Yam mosaic virus, yam mild mosaic virus, cucumber mosaic virus, Dioscorea Bacilliform viruses, mixed virus infection.

INTRODUCTION

The key to sustainable yam virus control and attendant boosting of world yam production maybe intricately knit between vector control, improved cultural practices and the use of virus-free/resistant planting materials. Yams are the starchy underground tubers produced by several species of *Dioscorea* (Coursey, 1967) and millions of

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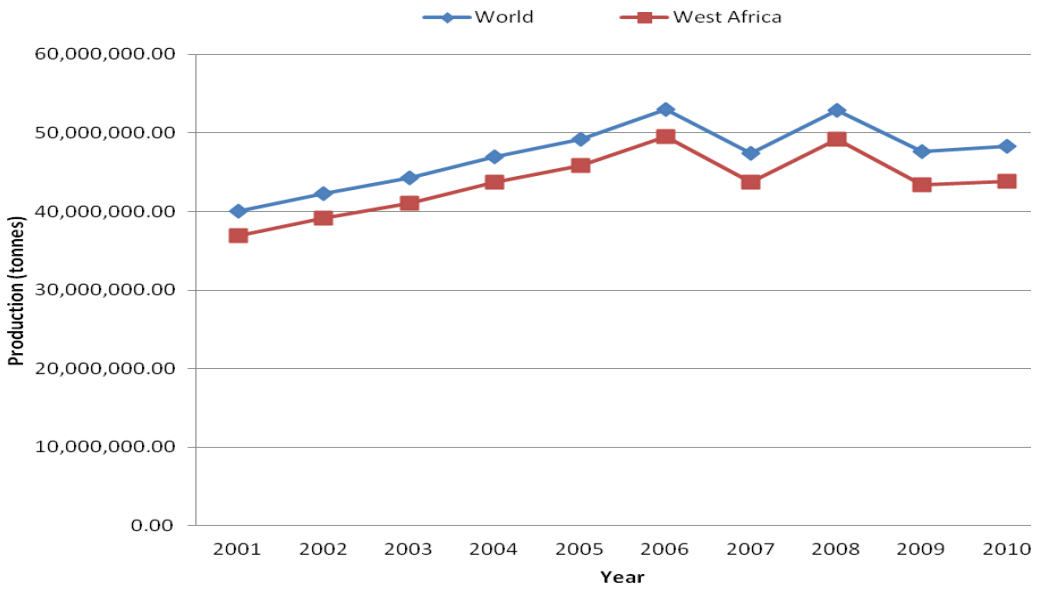

Figure 1. Changes in global yam production between 2001 and 2010 as determined by yam production in West African. Source: FAO (2012).

people in the tropical and sub-tropical regions of the world depend on these tubers for food (FAO, 2012). Yam is also a major source of income for resource poor farmers in West Africa where over 90% of world yam is produced annually (FAO, 2012). The fall and subsequent fluctuations in West African and global yam production since 2007 (Figure 1) (FAO, 2012), resulted in yam tuber demand outpacing supply and thus contributed to the 2008 food price crisis in the West African sub-region. Unfortunately, yam tuber prices in West Africa and around the world have remained high till date. Yam tuber yield depends on the ability of healthy yam leaves to efficiently trap, convert and sink the sun's light energy into chemical energy in tubers during photosynthesis (Nweke et al., 1991). During growth, some farmers guide their yam vines onto stakes or nearby intercropped plants for adequate exposure of leaves to sunlight for enhanced photosynthesis and thus enhanced tuber yield (Nweke et al., 1991; Otoo et al., 2008). Yam virus diseases which results in varying shades of mosaic and chlorotic leaf discoloration and malformation symptoms reduce the photosynthetic efficiency of infected plants and thus reduce tuber yield and quality (Amusa et al., 2003). Furthermore, the presence of yam viruses in tubers hinder the international trading of yam tubers and the international movement of yam germplasm required for research and improvement purposes (Brunt et al., 1989).

Viruses belonging to the *Potyvirus, Badnavirus, Cucumovirus, Comovirus, Potexvirus* and *Macluravirus* genera infect yam worldwide (Kenyon et al., 2001). The more commonly encountered yam viruses in West Africa are Yam mosaic virus (YMV), genus *Potyvirus* (Thouvenel and Fauquet, 1979), Yam mild mosaic virus (YMMV), genus *Potyvirus* (Hughes, 1986) Cucumber mosaic virus (CMV), genus *Cucumovirus* (Eni et al., 2008a) and several species of *Dioscorea bacilliform* viruses (DBV), genus *Badnaviruses* (Briddon et al., 1999; Seal and Muller, 2007; Kenyon et al., 2008; Eni et al., 2008b). These viruses are widespread in yam fields in major yam producing countries in the region both in single and varying combinations of mixed infections, with some plants being infected with all 4 viruses (Odedara et al., 2011; Eni et al., 2010, 2008c; Oppong et al., 2007). In our previous studies, very high incidences of mixed virus infections were observed in yam fields in 2004 and 2005. We found that 42.3, 43.6 and 45.6% of infected yam leaves from yam fields in the Republic of Benin, Ghana and Togo, respectively were infected with a mixture of two or more viruses (Eni et al., 2010, 2008c).

Virus-virus interaction within a host during mixed infection may result in either synergism or antagonism (Carrillo-Tripp et al., 2007; Murphy and Bowen, 2006), however, co-infection of a plant by different species of viruses often results in synergistic interactions that may result in more severe leaf symptoms culminating in greater yield losses (Vance et al., 1995; Anjos et al., 1992). Furthermore, virus-virus interaction of yam viruses in mixed infections enhances the probability of genomic recombination which may result in more virulent strains of existing viruses or entirely new virus species. The devastating cassava mosaic pandemic which ravaged several countries in East Africa in the 1990s resulted from such genomic recombination between geminiviruses in mixed infection (Pita et al., 2001). Synergistic interaction between genomic segments of these geminiviruses was reported to suppress posttranscriptional gene silencing aimed at generating geminivirus resistance in cassava (Vanitharani et al. (2004). Thus synergistic

interaction between yam viruses in mixed infection may frustrate efforts aimed at engineering virus resistance in yam.

Natural transmission of viruses is mainly through infected planting materials, by vectors and through mechanical transmission (Brunt et al., 1989). A clear knowledge of the actual dynamics of the various interplaying factors that ultimately results in the occurrence of mixed virus infections in yam fields is crucial to proffering effective yam virus control strategies. The speculations that the use of certified virus-free planting materials and/or resistant varieties for cultivation would halt the menace of yam viruses may be a myth if the virus infections occurring in yam fields were not originally present in the parent planting material. This study reports the incidence of the common viruses infecting yams in West Africa in yam tubers, tuber sprouts grown in an insect proof screen-house and leaf samples collected from yam fields. The incidence and varieties of multiple infections detected are highlighted.

MATERIALS AND METHODS

Sample collection

Yam tubers bought from yam markets in Mali, Burkina Faso, Cote d'Ivoire, Ghana, Togo and Republic of Benin were planted in sterile soil in an insect proof-screen house at the International Institute of Tropical Agriculture (IITA), Republic of Benin. Leaves were collected from tuber sprouts 8 weeks post planting. The leaf samples were dried over anhydrous calcium chloride and transported to the Virology laboratory of the IITA in Nigeria for virus testing. Yam tubers collected from the IITA yam barn and from yam markets in Ghana as well as yam leaves collected from yam fields in IITA Nigeria were also tested.

Virus testing

All the samples (tubers, leaves from tuber sprouts and field leaf samples) were tested for CMV, DBV, YMMV and YMV, the most commonly occurring yam viruses in West Africa, by Enzyme-linked immunosorbent assay (ELISA) and Immunocapture-polymerase chain reaction (IC-PCR) or immunocapture-reverse transcription-polymerase chain reaction (IC-RT-PCR). All the samples were tested both by serological (ELISA) and molecular (PCR) techniques to ensure accuracy in the data presented. The more sensitive molecular techniques would ensure virus detection in plants with low virus loads which may not be detected by ELISA while on the other hand, the ELISA tests will compliment the molecular techniques because polyphenols and glutinous polysaccharides contained in yam leaves and tubers sometimes interfere with PCR. Immunocapture of whole viruses was favored over nucleic acid extraction for the molecular detection techniques because it reduces the risk of RNA denaturation as 3 (CMV, YMMV and YMV) of the viruses being assayed for in this study are RNA viruses. Although the DBV are DNA viruses, immunocapture was necessary to ensure the amplification and hence the detection of only episomal viruses but not integrated sequences since *Badnaviruses* are reported to be integrated into the genomes of their host (Geering et al., 2005).

For ELISA test, Protein-A sandwich (PAS) ELISA was used for the detection of DBV, YMMV and CMV while Triple antibody-sandwich (TAS) ELISA was used for the detection of YMV. ELISA methodologies and the antibodies used are as previously described (Eni et al., 2008c). Each sample was tested in duplicated and the absorbance (A_{405}) of each well in the microtitre plate was measured in a Dynex MRX microplate reader after 1 h of substrate incubation.

For molecular detection, IC-PCR was used for the detection of the DBV which are DNA viruses while IC-RT-PCR was used for CMV, YMMV and YMV which are RNA viruses. IC-PCR and IC-RT-PCR were carried out as previously described (Eni et al., 2008c) using the following primer pairs; CMV 1 5' GCC GTA AGC TGG ATG GAC AA 3' and CMV 2 5' TAT GAT AAG AAG CTT GTT TCG CG 3' (Wylie et al., 1993), DBV F 5' ATG CCI TTY GGI ITI AAR AAY GCI CC 3' and DBV R 5' CCA YTT RCA IAC ISC ICC CCA ICC 3' (Seal and Muller, 2007), YMMV F 5'-GGC ACA CAT GCA AAT GAA RGC 3' and YMMV R 5' CAC CAG TAG AGT GAA CAT AG 3' (Mumford and Seal, 1997), YMV F 5' ATC CGG GAT GTG GAC AAT GA 3' and YMV R 5' TGG TCC TCC GCC ACA TCA AA 3' (Mumford and Seal, 1997). Amplification products (10 µL) were analyzed in 1.5% agarose gels and the 1 Kb plus DNA ladder (Invitrogen, USA) was ran along with the PCR products for size estimation.

Pretested virus-free yam leaves used as negative controls and the positive controls used for the respective viruses, were from IITA, Nigeria.

RESULTS

A mean absorbance value (A_{405} nm) that was twice or more than that of the healthy control was considered to be virus infected for the ELISA tests (Thottappilly et al., 1998) while IC-PCR/IC-RT-PCR amplicons of the expected band sizes of 500, 249, 586 and 579 bp were considered positive for CMV, YMMV, YMV and DBV respectively (Wylie et al., 1993; Mumford and Seal, 1997; Seal and Muller, 2007).

In all, 187 samples comprising 43 yam tubers, 45 leaves collected from tuber sprouts in an insect proof screen house and 99 leaves collected from yam fields in IITA, were tested. All the viruses assayed for in this study, were detected but the occurrence and incidence of each virus varied in each group of sample. All the 4 viruses, CMV, DBV, YMMV and YMV, were detected in the leaf samples collected from yam fields whereas 3, DBV, YMMV and YMV, were detected in the tubers. Laboratory results further showed that only DBV and YMV were detected in the leaves collected from the tuber sprouts in the screen-house (Figure 2). CMV was not detected in any of the tubers or tuber sprouts tested and YMMV was not detected in any of the tuber sprouts. Overall DBV had the highest incidence and was detected in 49.2% of the 187 samples, followed by YMV (43.3%), YMMV (6.4%), and CMV (2.7%).

A closer look at the various groups of samples revealed that most of the virus infections detected in the tubers (83%) and tuber sprouts (95%) were single infections of either DBV or YMV (Figure 4 and 5). On the contrary, approximately half (49.3%) of the 77 infected yam plants in the fields were simultaneously infected with a mixture of 2 or 3 viruses (Figure 3). The incidence of mixed infection in the field samples was about 3 times and 10 times more than those detected in the tubers (17%) and

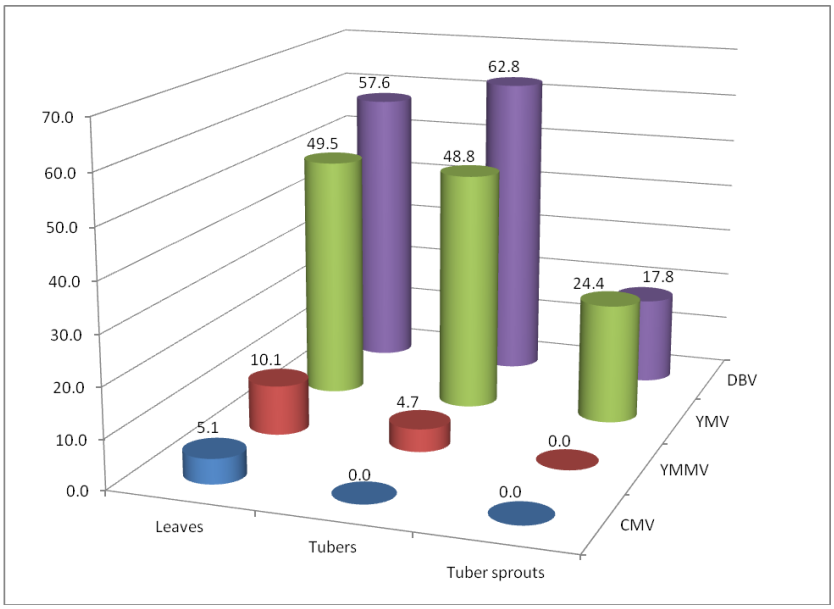

Figure 2. Incidence of Cucumber mosaic virus (CMV), Yam mild mosaic virus (YMMV), Yam mosaic virus (YMV), and *Dioscorea bacilliform* virus (DBV) in yam leaf samples collected from yam fields in IITA, yam tubers collected from the IITA yam barn/yam markets in Ghana and tuber sprouts from yam tuber collected from 7 West African countries and grown in an insect proof screen house in IITA.

tuber sprouts (5%) respectively (Figures 3, 4 and 5). Furthermore, the diversity of mixed infections detected in the field samples were also higher than those detected in the tubers and the tuber sprouts. A total of 5 different types of mixed virus infections were detected in the field samples, comprising three variations of double infections and 2 variations of triple infections (Figure 3), whereas two types of double infections were detected in the tubers (Figure 4). A mixed infection of DBV and YMV was the only mixed infection type detected in the tuber sprouts (Figure 5). Triple virus infections similar to those detected in the field samples were not detected in any of the tubers or tuber sprouts grown in the screen house (Figures 4 and 5).

Of the 4 viruses assayed for, only YMV and DBV were detected in single infections. All the YMMV and CMV detected in this study occurred in mixed infection with other viruses. Notably, the DBV were detected in mixed infection in all the three groups of samples and were present in all the five different mixed infection combinations detected (Figures 3, 4 and 5). The dual infection of DBV and YMV was the most frequently detected mixed infection in all the three groups of samples being present in the field samples, the tubers, and the tuber sprouts in the screen-house.

DISCUSSION

Integrated pest management approaches has often resulted in the most sustainable virus control efforts for several crops however, a proper understanding of the specific contributions of the various interplaying factors in a given disease situations would appropriately guide resource allocation decisions and ultimately result in more effective virus control strategy. Although yam tubers, which serve as planting materials for the next planting, are presumed to accumulate viruses over the years, results from this study suggests that yam tubers may not be solely accountable for the high incidence and diversity of mixed virus infections often detected in yam fields both in this study and in other previous studies across West Africa (Odedara et al., 2011; Eni et al., 2010; Kenyon et al., 2008; Eni et al., 2008c; Oppong et al., 2007). Although all the yam tubers used in this study (either directly tested or sprouted in the screen-house before testing), were originally collected from yam fields in the various countries, the lower incidence of mixed virus infections in these samples compared to field leaf samples suggests that virus accumulation in yam tubers maybe selective such that not all viruses infecting a plant gets transferred to the tuber. The lower incidence and variety of mixed virus infections detected in the yam tubers and tuber sprouts grown in the insect proof screen house also suggests that other biotic and abiotic factors contribute significantly to the high levels of mixed infections present in yam fields. A chief biotic factor worthy of consideration would be vector transmission. All of the 4 viruses indexed for in this study are vector transmitted. YMV, YMMV and CMV are aphid transmitted

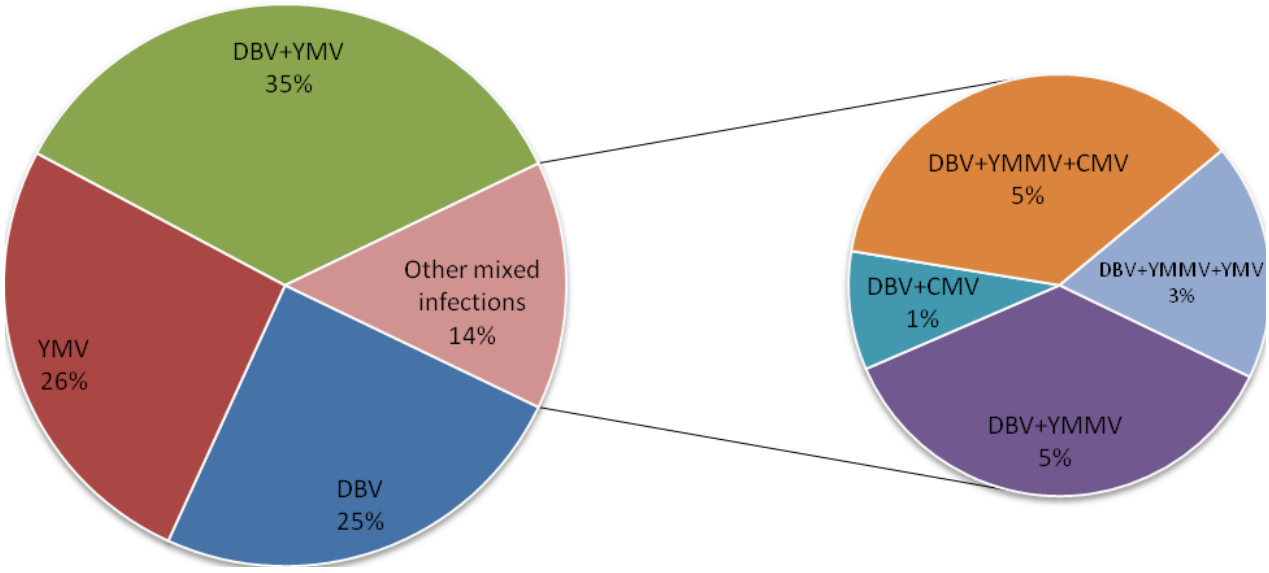

Figure 3. Incidence of single and mixed infections Cucumber mosaic virus (CMV), *Dioscorea bacilliform* virus (DBV), Yam mosaic virus (YMV), and Yam mild mosaic virus (YMMV) in yam leaf samples collected from yam fields in IITA.

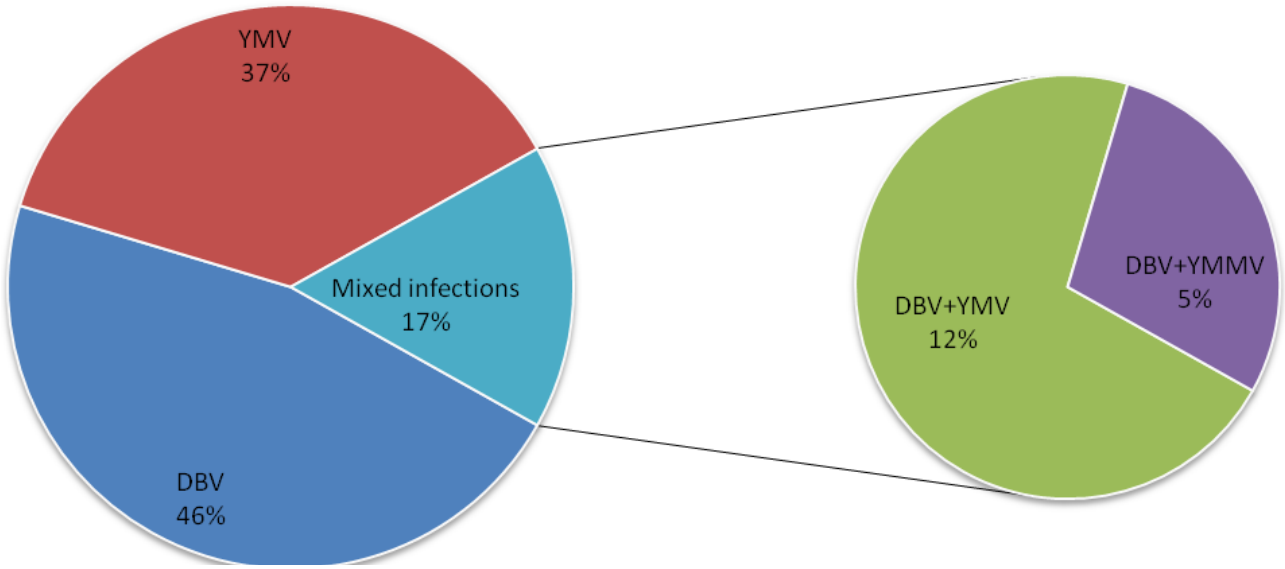

Figure 4. Incidence of single and mixed infections of *Dioscorea bacilliform* virus (DBV), Yam mosaic virus (YMV), and Yam mild mosaic virus (YMMV) in yam tubers collected from the IITA yam barn and yam markets in Ghana.

(Odu et al., 2004; Palukaitis et al., 1992) while the DBV are transmitted by several species of mealybug (Odu et al., 2004). Although plants grown from singly infected tubers may serve as initial source of virus inoculum, the vectors responsible for virus spread in the field must be appropriately targeted to achieve effective yam virus control. Unfortunately, YMV, YMMV and CMV are transmitted in a non-persistent stylet-borne manner by their aphid vectors so the use of insecticides as a means of control may not be efficient as most insecticides are systemic and do not act quickly enough to prevent transmission of stylet-borne viruses which are acquired and transmitted within seconds or minutes. The use of various types of mulches for the effective control of non-persistent stylet-borne plant viruses have been reported (Cradock et al, 2001), such mulches maybe useful for the control of aphid transmitted yam viruses. The exclusion of migrant aphids by the use of non-susceptible barriers plants may also minimize cross transmission. This is particularly important during the early growth stages of the yam plant since delayed infection would reduce the overall effect of the virus infection on tuber yield.

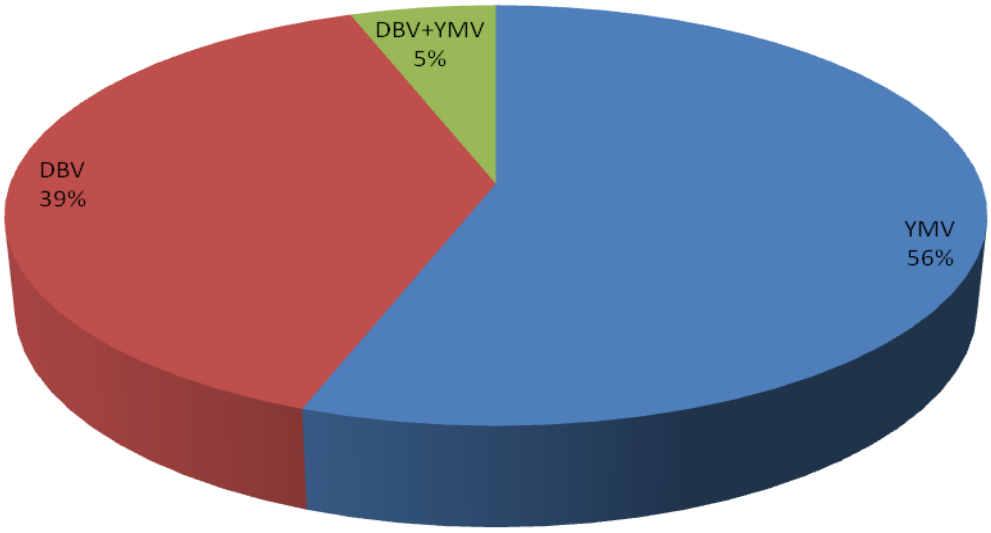

Figure 5. Incidence of single and mixed infections of *Dioscorea bacilliform* virus (DBV) and *Yam mosaic* virus (YMV) in tuber sprouts from yam tuber collected from 7 West African countries and grown in an insect proof screen house in IITA.

Mealybug transmission of the *Badnaviruses* is also very important as mealybugs are reported to spread *Badnaviruses* viruses over short and long distances (Olunloye, 2002). Although adult mealybugs are sedentary and inactive, the movement of young active nymphs between interlocking branches of adjacent cocoa plants facilitated the spread of cocoa swollen shoot *Badnavirus* (CSSV). Similar movement of active virulferous mealybug nymphs will significantly contribute to the spread of the DBV in yam fields particularly in fields where the plants are not staked and the yam vines interlock on the ground. Long distant spread of DBV may also result from "jump spread" by wind borne viruliferous small first instar mealybugs nymphs as observed in the transmission of CSSV (Strickland, 1950; Cornwell, 1956). Therefore, previously described effective chemical and biological mealybug control methods (Teshiba et al., 2012; Aggarwal et al., 2009; Neuenschwander and Herren, 1988) should be explored for the control of the mealybug vectors of DBV in yam fields.

The none detection of CMV in any of the tubers or tuber sprouts tested in this study suggests that the CMV infection detected in the field samples may have been acquired from any of the several vegetables often intercropped with yam in the field. Although the incidences of CMV detected in yam in this study and in other previous studies were low (Odedara et al., 2011; Eni et al., 2008a), CMV has been implicated in several virus epidemics and crop yield losses in several economically important crops worldwide (Palukaitis et al., 1992). Furthermore, CMV is reported to be transmitted by several insect vectors thus a proper assessment and due consideration of all CMV transmitting vectors must be included in future yam virus control plans.

The high incidence of DBV observed in this study and their occurrence in all the mixed infection combinations identified in this study buttresses the importance of these viruses to yam production. Although YMV which was previously considered the most important yam virus, remains of serious concern for yam production, the DBV have recently emerged as the most important viruses infecting yam worldwide with astronomical incidences and widespread distribution in yam fields around the world (Odedara et al., 2011; Kenyon et al., 2008; Eni et al., 2008b). The occurrence of several characterized and uncharacterized species and strains of DBV in yam is also a huge concern since the chance of genomic recombination is higher during co-infection of viruses within the same plant. The massive molecular variability among DBV may well have resulted from such virus-virus genomic interactions. Genomic recombination of viruses bears the risk of generating more virulent and catastrophic new viruses as was observed with the devastating cassava mosaic disease pandemic that swept through at least nine countries in East and Central Africa in the 1990s (Harrison et al., 1997; Pita et al., 2001). The need for an effective control measure for DBV is therefore crucial and urgent.

The most common method of transferring virus from plant to plant is on contaminated hands and tools. Cutting of yam tubers into smaller setts before planting and the periodic weeding of yam fields during cultivation are two other factors that may account for a fraction of the mixed virus infection situation in yam fields. Both processes require the use of mechanical farm implements which creates wounds, thus an infected knife can transfer viruses from an infected to an uninfected plant. Cross

infection during weeding is more likely where yam vines are not staked unfortunately, some farmers view vine staking as an unnecessary additional production cost particularly where available intercrops are not useful for staking.

Despite the possible drawbacks, tuber cutting is necessary to increase the quantity of yam planting materials and weeding remains a crucial farming practice aimed at increasing crop yield by reducing the competition of nutrients and other growth factors. Very simple measures such as hand washing and tool decontamination, especially during multiplication of yam planting materials, would reduce cross contamination and the resultant mixed virus situation prevalent in yam fields.

Researchers at the IITA and other research institutes around the world have invested and are still investing massive resources into breeding and/or engineering yam for virus resistant. In addition, huge efforts towards the development of appropriate and sensitive virus detection/certification methods are also currently ongoing in research institutions around the world. These efforts to control yam viruses through the use of certified virus-free or virus resistant planting materials could become easily frustrated if all these interacting factors responsible for virus transmission and hence the high incidence of mixed virus infections that occur in yam fields are not critically analyzed and considered in the initial planning of these yam virus control efforts.

Conclusion

Virus vector control and the use of clean knives/hoes for tuber cutting and for weeding purposes may be as important as the use of certified virus-free or virus resistant planting materials in the war against yam viruses. Our results suggest that other factors other than tubers used as planting materials contribute to the frequency and variety of mixed infections present in yam fields. However, further work involving the assessment of field and screen house leaf samples generated from pre-tested yam tubers would be done to confirm this. Extension programs aimed at educating yam farmers in West Africa of the existence of the other interplaying factors involved in the yam virus situation, would ultimately improve cultural practices, reduce the incidence of mixed virus infections in particular and ultimately contribute to boosting yam yield.

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