PREVALENCE AND IDENTIFICATION OF YAM VIRUSES RESPONSIBLE FOR SEED YAM DEGENERATION IN THE EJURA-SEKYEDUMASE AND ATEBUBU-AMANTIN DISTRICTS OF GHANA

BY

ESTHER AGYEMANG MARFO

BSc (Hons) Agriculture

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A THESIS SUBMITTED TO THE DEPARTMENT OF CROP AND SOIL SCIENCES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF PHILOSOPHY IN CROP PROTECTION (PLANT VIROLOGY)

November, 2018

DECLARATION

I wish to declare to the best of my knowledge that the research presented in this thesis is original and conducted by myself under supervision and has not been presented for a degree award or any other institution of higher learning before except where due acknowledgement has been made in the text.

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ABSTRACT

Yam viruses are reported to be widespread in all areas where yam is grown. In Ghana, viral diseases are known to cause about 50% of yield reduction on yam. It was for this reason that this research was carried out to manage yam viruses in Ghana. Surveys were conducted in the 2015 and 2016 cropping seasons in two major growing districts, Ejura-Sekyedumase and AtebubuAmantin of Ghana to determine the prevalence of yam virus. There was the need to identify the specific viruses producing the symptoms that were observed during the survey, as such symptomatic leaf samples were taken for analysis at the laboratory using RT-PCR. Out of the 40 leaf samples collected from each district, Ejura-Sekyedumase District had six mixed infections for both Yam mosaic virus (YMV) and Yam mild mosaic virus (YMMV) while eight single infections were recorded for YMMV. Atebubu-Amantin District had 10 mixed infections for both viruses and eight single infections for YMV. Molecular-based diagnostics techniques were also employed to monitor the health status of seven plants (*Diosecorea rotundata* Poir) established from positive selection, of which five of them did not amplify for any of the two viruses tested while two amplified for both viruses that were tested. Seed yams ('Dente', 'Pona' and 'Laribako') selected in 2015 from symptomless or mildly infected plants (positive selection), as well as those purchased from the Ejura market (farmer practice) and those selected from field diseased plants were established in field experiments in 2016 and 2017 cropping seasons at Ejura and Fumesua using a 3 x 3 factorial in randomized complete block design. The performances of the three seed yam sources were compared for their reaction to yam mosaic virus infection and tuber yield. The three white yam (*D. rotundata*) varieties used were; 'Dente', 'Pona' and 'Laribako'. Plants raised from positive selection performed significantly ($P<0.05$) better with least virus percentage infection and disease severity scores irrespective of the variety. Positive selection Dente out-yielded farmer practice

Dente and diseased Dente by 35 and 66.7% respectively in the 2016 cropping season at Ejura. Similar result was obtained at Fumesua with positive selection Dente out-yielding farmer practice and diseased seed yams by 32.6 and 60.7% respectively. In the 2017 cropping season, even though there was general yield reduction indicating loss of seed yam quality with time, similar trend occurred with positive selection plants performing significantly ($P<0.05$) better with least virus incidence and severity scores at both locations. Positive selection Laribako produced the highest yield at both locations. With farmers' current practice of recycling seed yams from one season to another, this study showed that positive selection was a good approach to reducing virus load in farmers' farms as well as reducing seed yam degeneration while maintaining fairly good yields.

DEDICATION

I dedicate this thesis to my parents, Mr. and Mrs. Marfo and also to lovely husband, Mr. Isaac Oheneba Ntim and my children, Samuel Oheneba Ntim and Ivan Oheneba Ntim for their love, support and encouragement throughout my study.

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CHAPTER ONE

1.0 INTRODUCTION

Yam, *Dioscorea* species in the family *Dioscoreaceae* is an important food crop which is second after cassava among the root and tuber crops. There are about 600 species within the family but those which are of economic importance include *Dioscorea rotundata* Poir*, D. alata* L*, D. cayenensis* Lam*, D. dumetorum* (Kunth) Pax*, D. bulbifera* L., *D. trifida* L. *and D. esculenta* (Lour) Burkill (Lebot, 2009). *Dioscorea rotundata* also referred to as white yam, is most widely cultivated and preferred by consumers and for the export market as well in West Africa (Aighewi *et al.*, 2014). The bulk of the world's production of yam is in West Africa with about 93% of the world's total yam production by five countries namely, Nigeria, Ghana, Côte d'Ivoire, Benin and Togo (Asiedu and Sartie, 2010). Out of this, Nigeria produces about 68% making it the world's leading producer (Sanginga and Mbabu, 2015; FAO, 2013). Ghana became the second leading producer of yam after Nigeria with a total production of 4,044,025.62 tonnes in 2015 (FAO, 2015). Ghana is the leading exporter of yam with 4% global market share in the West Africa's "Yam Belt" and Africa as a whole (Asante *et al.,* 2014).

Yam serves as source of food for about 150 million people in West Africa (FAOSTAT, 2015; www.itegratedbreeding.net). It provides income to both resource-poor farmers and women who are into marketing of yam and yam products (Sanginga and Mbabu, 2015). With a longer shelf life (3-4 months) than other root and tuber crops, yams contribute to food supply during periods when other foods are scarce and thus, it is referred to as a food security crop (IITA, 2013).

In terms of nutrition, yam is highly rich in carbohydrate and dietary fibre. The complex carbohydrate it provides helps to regulate steady rise in blood sugar levels (Iwu *et al.,* 1990).

Yam is also an excellent source of protein and vitamins. The vitamins function as a mediator of some metabolic functions in the body, anti-aging, immune function booster, wound healing and for bone growth in human's development (http://www.stylecraze.com). Some yam species also produce a chemical known as dioscin, the active ingredient of birth control pill. Several drugs are produced from yam-based ingredients for both allopathic and homeopathic medicine and also as nutraceutic products (Chandrasekara and Kumar, 2016).

Yam production is faced with many constraints, the key among them are: scarcity and high cost of quality seed yam of both local popular and improved varieties (this accounts for 63% of total variable cost of production), high levels of post-harvest losses (almost 40%), high production costs (high cost of seed yam, labour for land clearing and harvest and staking, all contributing almost 70% of the total production costs), low and declining soil fertility, moisture stress as well as pests and diseases, mainly viruses, fungi and nematodes (Sanginga and Mbabu, 2015). All these factors go a long way to adversely affect yam production. Also these obstacles undermine yam production and farmers' ability to generate sustainable incomes.

Diseases and pests on yam over the years have proven to be far more difficult to address wherever the crop is produced. This has been attributed partly to the wide range of organisms involved (including viruses, fungi, bacteria, nematodes and insects) and also to their persistence through the cultivation, storage and marketing periods (Coyne *et al.,* 2006; Aboagye-Nuamah *et al.,* 2005). Most especially, for virus infection of yam, once the infection occurs, they are automatically transmitted into the planting material (Ng, 1992). There is therefore the need for farmers to use clean seed yams for planting to avoid this constraint but the availability of clean or good quality

seed yam has always been a major constraint to the production of yam. As a result, farmers still use disease and pest infested seed yam to plant each planting season.

In Ghana, there are very few farmers who are into seed yam production and as a result, farmers do 'milking' and leave yam plant to produce seed yams that are used for planting in subsequent season. This makes quality seed yam scarce and where they are available the cost is very high in Ghana. Orkwor *et al.* (1998) reported that farmers put aside as much as 30% of their harvest as seed yams for the next cropping season because of the scarcity and high cost of seed yam.

Most of the edible yam species are relatively infertile and if or when they do set true seed, most seed is not viable. Thus, most propagation and multiplication of yam is by vegetative means through the planting of small tubers (seed yams) or pieces of tuber (setts). This vegetative propagation allows the perpetuation and accumulation of some diseases, including those caused by viruses. The planting of smaller tubers saved from the previous harvest may in effect be selection of the most infected lines (Kenyon *et al.,* 2001). Since there is no selection of diseasefree plants or seed yams to be used for planting in the following season and the same diseaseinfected seed yams are replanted year after year, there is high incidence of yam diseases in areas where the crop is grown in Ghana.

In Ghana, knowledge exists about the presence of viruses in yam growing areas and also the effect of viral diseases on the yields of yam. Despite this knowledge, there is inadequate recent empirical data on the prevalence of viral diseases in important yam growing areas such as the Atebubu-Amantin and Ejura-Sekyedumase Districts located in the Forest-Transition zone of Ghana. There was therefore the need to conduct disease diagnostic survey in this zone. Baseline

information on the incidence and severity of viral diseases of yam in these locations was needed to support the development of any future disease management strategies for such communities.

It is also important to know how long it will take farmer-selected seed yams (traditional method such as milking) or seed yams from other sources such as apparently healthy-tagged plants (positive selection) to lose their quality as a result of recycling of seed yams.

The main objective of this study was to identify seed sources for healthy *D. rotundata* seed yam production for improve yield to enhance farmers' livelihood.

The specific objectives were to:

i. determine the incidence and severity of viruses infecting yam in selected yam growing areas,

ii. assess seed yam quality loss among the different seed yam sources, and iii. identify the yam viruses causing quality loss of seed yam in selected yam growing areas using reverse transcription polymerase chain reaction (RT-PCR).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and distribution of yam

The genetic information of yam suggests that there may be different places of origin depending on the species. It can therefore be said that yam originated from Asia, West Africa and tropical America (IITA, 1995). *D. rotundata* (white yam), *D. cayenensis* (yellow yam) and *D. dumetorum* (trifoliate yam) are believed to have originated from Africa whiles *D. alata* (water yam) and *D. esculenta* (Chinese yam) also originated from Southeast Asia and were introduced to West Africa. *D. alata* is the most widely distributed in the world. *D. trifida* is also known to have originated from tropical America (Coursey, 1975). In Africa, the crop is produced in areas within 15℃ of the equator. Thus, the crop is cultivated in fairly high rainfall areas with distinct dry and wet seasons in West Africa.This area extends from the Savanna to the Guinea Savanna zones (Nweke, 1981).

2.2 Production of yam in Ghana

Yam serves as an important staple food in the tropics especially in West Africa for millions of people. Although Ghana is the second largest world producer of yam, it is the leading exporter of yam in Africa and among the five largest exporters globally (Asante *et al.,* 2014)

In Ghana, the crop is cultivated in all the ten regions except Upper east and Greater Accra but the bulk of the production comes from Brong Ahafo and Northern Regions with 2,171,341 MT and 2,005,607 MT respectively in 2011 (MOFA/SRID, 2012). Brong Ahafo and Northern regions produce about 71% of the total yam produced in Ghana.

Table 2.1 Top five yam producing countries in the world

Rank	Country	Production (MT)	% of world total
1	Nigeria	40,500,000	64.2
2	Ghana	7,074,574	11.2
3	Cote d'Ivoire	5,731,719	9.09
4	Benin	3,177,265	5.03
5	Ethiopia	1,191,89	1.89

Source: FAOSTAT (2015)

2.3 Diseases and pests of yam

Yam is associated with several diseases by viruses from the field to the post-harvest period. The pests and diseases have direct and indirect negative impact on the quality and yield of the crop, thereby reducing farmers' expected income.

2.3.1 Pests of yam

Among the major production constraints of yam in Ghana are insects, nematodes and diseases which attack yam both on the field and in storage (Asante *et al.:* 2007). Insects that infest and cause serious economic damage include mealy bugs (*Rastrococus* sp.), scale insects (*Aspidiella hartii*), Greater yam beetle or yam tuber beetle (*Heteroligus meles*), yam moth (Pyralid moth worms), termites and defoliating caterpillars. Mealy bugs form white powder on the yam tuber surface and they cause complete necrosis on sprouts thereby preventing the use of such tubers as seed yams. The greater yam beetle is known to cause serious havoc to yams particularly, in West Africa. They create big holes in tubers from germination to the time of harvesting (Onwueme and

Charles, 1994). The adult yam tuber beetles cause field infestation during their feeding migration from swampy areas. Their infestation and damage are caused by boring holes and tunnels into the tubers (Obeng-Ofori, 1998). Not only do the holes and tunnels affect the quality, they also affect the market value of yam tubers. Scale insects of yam mainly attack tubers in storage by sucking the sap thereby causing shriveling. This promotes attack by fungal rot and can inhibit subsequent sprouting of tubers (Obeng-Ofori, 1998; Mishra *et al.*, 1989; Ikotun, 1983). According to Asante *et al.* (2007), scale insects, termites, yam tuber beetles, mealy bugs, leaf beetles and millepedes are field and storage pests that cause serious damage to yam. Damage of yam tubers caused by nematodes infestation is generally noticed at harvest. They cause yam dry rot with characteristic cracking of the tuber periderm (Obeng-Ofori, 1998; Mishra *et all.*, 1989; Ikotun, 1983). *Scutellonema bradys* (yam nematode), *Meloidogyne incognita* (root knot nematode) and *Pratylenchus coffeae* (lesion nematode) have been identified as the three most damaging nematodes of yam (Bridge *et al.,* 2005). They cause direct damage both in the field and in storage. Their damaging activities favour the development of secondary fungal and/or bacterial rots on tubers (Castagnone-Sereno, 2006). These lead to firstly, yield and quality reduction of yam resulting in low market value of tubers and secondly, transmission of inoculums to the soil when infested planting materials are used (Kusi *et al.,* 2013). The yam nematode has also been reported to cause dry rot disease of yam in storage. This type of rot occurs only in the outer 1 to 2 cm of tubers. Symptoms of dry rot of yam include necrotic lesions beneath the skin, followed by yellow lesions below the outer skin of the tuber. External cracks appear in the skin of the tuber. The infestations created by the nematode can serve as external opening facilitating fungi and bacteria colonization, causing wet rot (Bridge *et al.,* 2005).

2.3.2 Fungal and bacterial diseases of yam

The main fungal diseases associated with yam are anthracnose, tuber rot and leaf spots caused by various types of fungi. The causative fungus of anthracnose is *Collectotrichum gloesporioides* Penz. The symptoms appear on the leaves, stem and petioles of infected cultivars as small dark brown or black lesion. The lesion is often surrounded by an enlarged chlorotic halo leading to extensive necrosis of the leaves and die-back of the stem (Amusa, 1997, 1991). This makes the leaves and stems to appear as withered and burnt and thus the name 'scorch disease' (IITA, 1993).

There are three main types of tuber rots in yam and these are mostly caused during storage. They are dry, soft and wet rots. Dry rot is reported to be caused by some species of *Penicillium, Aspergillus, Lasiodiplodia* and *Fusarium* (Morse *et al.,* 2000; IITA, 1993). Depending on the type of pathogen causing the rot, there are various degrees of symptoms. Fungal pathogens associated with soft rot disease of yam are *Rhizopus* spp.*, Mucor circinelloides* Tiegh.*, Sclerotium rolfsii* Sacc. and *Rhzoctonia solani* Kuhn (Amusa and Baiyewu, 1999; Green *et al.,* 1995). Wet rot is the type of rot in yam that is characterized by the oozing of whitish fluid out of the tuber when pressed. This symptom is associated with a bacterium called *Erwinia carotovora* pv. *caratovora* Jones (IITA, 1993; Amusa and Baiyewu, 1999).

2.3.3 Viral diseases of yam

Cultivation of yam is being threatened by diseases caused by viruses in all areas where the crop is grown (Asiedu *et al.,* 1998). Various viruses belonging to different types of virus genera have been identified to cause viral diseases in cultivated and wild yams especially *D. rotundata*, *D. cayenensis* and *D. praehensilis* Benth. The different virus genera include *Potyvirus, Badnavirus, Cucumovirus, Potexvirus, Comovirus* and *Carlavirus* (Wang *et al.,* 2015; Filloux and Gerard, 2006: Kenyon *et al*., 2001). They produce symptoms such as mosaic, vein clearing, vein banding,

chlorosis, mottle, stunting and leaf distortions in their host plants (Séka *et al.,* 2009; Kenyon *et al.,* 2001).

In Africa, there are six main yam viruses known to cause serious economic damage to yam, namely, Yam mosaic virus (YMV), Yam mild mosaic virus (YMMV), Cucumber mosaic virus (CMV), Dioscorea mottle virus (DMV), Dioscorea alata Badnavirus (DaBV) and Dioscorea alata virus (DAV) also known as Yam virus 1(YV1) (Kenyon *et al.,* 2001). Although they are different viruses, the symptoms produced are similar and as such it is difficult to distinguish among them. The expression of symptoms may differ based on the genotype, time of infection, environmental conditions and cultivar (Kumar, 2015).

2.3.3.1 Potyviruses

This genus belongs to family Potyviridae and it is a member of the group of viruses that have not been assigned to an order (ICTV, 2017; Porth *et al.,* 1987; Thouvenel and Fauquet, 1979). Potyvirus is named after Potato virus Y and it is the largest group and economically most important of plant viruses (Shukla *et al.,* 1994). The most economically important viral disease by far characterized are caused by members of this group (Silva *et al*., 2015). They have flexuous filamentous particles ranging from 720-900 nm in length and 11 nm in diameter depending on the sub-group it belongs. They sediment at 150S and have a buoyant density in caesium chloride (CsCl) of 1.31 g/cm^3. The particles of potyviruses consist of about 2000 subunits of a single protein species (Molecular weight of 32 to 34×10^3) arranged as a helix enclosing the genome. The genome is a single molecule of single standed RNA with a molecular weight of 3.0 to 3.5×10^6 and constitute about 5% of the particle weight (Huttinga and Mosch,

1974; Damirdagh and Shepherd, 1970). Potyviruses usually have thermal inactivation point (TIP) of 55 to 60℃ (for 10 min) but it can range from 50 to 75℃. Their longevity *in vitro* (LIV) is 1 to 50 days but usually two to four days and have dilution end point (DEP) of 10^{-1} to 10^{-6} but mostly 10^{-3} to 10^{-4}.

They induce the formation of characteristic conical/cylindrical cytoplasmic inclusion (CCI) bodies in their hosts. They scatter randomly throughout the cytoplasm and sometimes within the plasmodesmata of infected cells. The CCI is believed to be concerned with the intercellular transport of virus and or their nucleic acid and protein components (Edwardson, 1966; Rubio Huertos and Lopez-Abella, 1966).

They are transmitted mainly by aphids (*A. gossypii* Glover*, A. craccivora* Koch*, Toxoptera citricida* Kirkaldy and *Rhopalosiphum maidis* Fitch) in a non-persistent manner but there are mites and whitefly transmitted groups. Potyviruses are also transmitted by inoculation of sap. The viruses survive in perennial or vegetative propagated crops for which yam is one. The most dangerous virus sources are infected planting material or infected volunteer plants from previous crops (https//www.dpvweb.net; Thouvenel and Fauquet, 1979)

Yam mosaic virus (YMV) and Yam mild mosaic virus (YMMV) or Dioscorea alata virus (DAV) belong to this group. YMV has linear monopartite single stranded positive sense RNA genome (Alemaner-Verdaguer *et al.*, 1997). Its virus particle is encapsidated by approximately 2000 copies of a 34 kDa coat protein and measures about 785 nm in length. Thouvenel and Fauquet (1979) reported that YVM identified in Cote d'Ivoire had a dilution end point of 10^{-2} beyond which point the virus is systemically non-infective. The thermal inactivation point of 55℃ for 10 min and the virus when stored at 25℃ remained infective for 12 h. The ultraviolet absorption spectrum of

purified virus showed a characteristic of that of a nucleoprotein which showed a maximum at 262 and minimum at 247 nm. The absorbance ratio $A_{262/247}$ was 1.13 ± 0.01 and the $A_{260/280}$ was 1.20 ± 0.01 and indicates a nucleic acid content of 6 and 94% of protein content (Brunt *et al.,* 1996; Thouvenel and Fauquet, 1979). YMV is reported to be the most important virus on yam and has been reported in all the yam growing areas in West Africa including

Ghana, Nigeria, Benin, Burkina Faso, Cote d'Ivoire and Togo (Brunt *et al.,* 1996; Thottappilly, 1992; Porth *et al.,* 1987; Thouvenel and Fauquet, 1979;). It was first reported on *D. cayenensis* by Thouvenel and Fauquet (1979) in Cote d'Iviore. It has also been reported on *D. rotundata*, *D. alata* and *D. trifida*. Generally, symptoms observed among infected plants are vein banding, leaf curl, mottling, green spoting, flecking, blistering, vein yellowing and shoe-string under severe conditions (Kenyon *et al.,* 2001; IITA, 1993; Mantell, 1980). These symptoms usually lead to reduced plant growth (stunting) and vigour, thereby resulting in poor yield. IITA (1981) reported that YMV causes about 40% yield reduction in *D. rotundata*. Oppong *et al. (*2007) reported that 38% of the samples collected reacted positive to YMV antigens in Ghana. YMV is reported to be the most commonly detected virus on *D. rotundata* in Ghana (Olatunde, 1999)

YMMV or DAV has been recognized as distinct potyvirus infecting *D. alata* in South Pacific (Fuji *et al.,* 1999; Mumford and Seal, 1997). It is widespread in areas where *D. alata* is grown in Africa (Odu *et al.,* 1999.) The first molecular characterization of the virus showed a significant divergence between isolates from Colombia, Martinique and Papua New Guinea (Bousalem *et al.,* 2003; Dallot *et al.,* 2001; Bousalem and Dallot, 2000). YMMV is reported to infect *D. alata* and *D. cayenensis* (Bousalem and Dallot, 2000; Odu *et al.,* 1999). Atiri *et al.* (2003) described

YMMV as the most prevalent virus on yam after YMV. Odu *et al.* (1999) reported that YMMV has 32100 daltons as molecular weight of the coat protein and it is transmitted by *A. craccivora.*

Oppong *et al.* (1997) reported the detection of DAV on *D. rotundata* in Ghana and found that 20.5% of the samples tested reacted positively to DAV antigens.

2.3.3.2 Badnaviruses

The genus Badnavirus belongs to the family Caulimoviridae which is also referred to as pararetroviruses. They are non-enveloped bacilliform dsDNA viruses with a monopartite genome that contains about 7.2 to 9.2kbp of dsDNA. The virions badnaviruses are about 30 nm in diameter and vary in length between 120 and 150 nm depending on the species. The complete genome is made up of 7200 to 9200bp. They are transmitted by mealybugs *(Planococcus citri)* and a few species are transmitted by aphids in a semi-persistent manner. Badnaviruses are known to be present as integrated sequences in the genome of some host plants and as such are referred to as endogenous badnaviruses. Symptoms caused by badnaviruses include chlorotic mottle or necrotic streaks, leaf deformation and reduced internode length leading to stunting of plants. Symptoms re-emergence and severity increases when plants are subjected to abiotic stress such as lack of nutrients and unfavourable weather conditions. Majority of badnaviruses infect perennial host that are propagated vegetatively (https://www.ncbi.nlm). Dioscorea bacilliform virus is the viral disease on yam caused by badnavirus. The disease produces symptoms such as chloritic mosaic on leaves that leads to reduced sugar formation and minimal starch storage. This leads to reduction in tuber quality and crop yield (Phillips *et al.,* 1999). It was first reported in the Caribbean, a bacilliform virus that was associated with internal brown spot disease in *D. alata* and *D. rotundata* (Mantell and Haque, 1979; Harrison and Roberts, 1973). The isolates from Nigeria on *D. alata* are referred to as *Dioscorea alata* bacilliform virus (DaBV) (Kenyon, 2001; https://www.ncbi.nlm). Based on the current taxonomic criteria, only two are recognized species of badnavirus for which

complete genome sequence data exist, namely, Dioscorea bacilliform alata virus (DBALV) isolated from Nigeria (Briddon *et al.,* 1999) and Dioscorea bacilliform sansibarensis virus (DBSNV) in wild *D. sansibarensis* Pax from Benin (Seal and Muller, 2007). Badnavirus sequences have also been found to be integrated in the yam genome hence the name endogenous pararetrovirus sequences (EPRVs) or endogenous yam badnaviruses (eYBVs) and they have been detected in almost all species grown in West Africa, the Carribbean and South-Pacific region (Seal and Muller, 2014).

2.3.3.3 Potexvirus

Generally, potexviruses are non-enveloped and have flexious filamentous particles that are 470580 nm in length and 12-13 nm in diameter and sedimenting at 114-130 S. Each particle of potexvirus consist of 1000 to 1500 protein subunits of a single protein species with a molecular weight of 1.8-2.7×10^4 , arranged as a helix enclosing the genome that happens to be a positive sense single-stranded RNA (ss-RNA+) (Adams *et al.,* 2004). The ss-RNA constitutes about 57% of the particle weight of the virus. Most potexviruses have proteins that degrade partially *insitu* during purification leading to multiple proteins that can be resolved by denaturing gel electrophoresis. The thermal inactivation point (TIP) ranges from 60-80℃ whiles their longetivity *in vitro* (LIV) is usually several weeks to months. Normally their dilution end-point ranges from 10^5 to 10^6. The main symptoms produced by potexviruses on their limited host ranges are mosaic or ringspot in wide range of monocotyledonous plants. Virus particles, frequently in large aggregates, occur in the cytoplasm and occasionally are also found in the nuclei of their host plants (https://www.dpvweb.net; https://en.wikipedia.org/wiki/Potexvirus).

Dioscorea latent virus (DLV) was detected in naturally infected *D. composita* Hemsl. and *D. floribunda* M. Martens and Galeotti (medicinal yams); was shown to occur in high concerntration in those infected plants but not on any of the cultivated cultivars such as *D. rotundata, D. alata* and *D. esculenta.* DLV is reported to produce symptomless infection in its natural hosts (Phillips *et al.,* 1986; Waterworth *et al.,* 1974). Philips *et al.* (1986) reported that DLV was neither transmitted in a persistent manner nor in a non-persistent manner from infected to healthy *Nicotiana benthamiana* and *N. megalosiphon* seedlings by either *A. gossypii* or *Myzus persicae.* No transmission of DLV occurred from infected to healthy *Nicitiana megalosiphon.*

2.3.3.4 Carlavirus

The Carlavirus group is slightly flexuous filaments that are normally 610-700 nm long and 12 – 15 nm in diameter. They usually seem to have curved to side and sedimenting at 147-176 S. The particles are made of about 1600-2000 subunits of a single protein species with a molecular weight of $3.1\text{-}3.4\times10^4$ and arranged as a helix with a pitch of 3.3-3.45 nm enclosing the genome which is a single-stranded RNA that has molecular weight of $2.3\text{-}3.45\times10^6$ and constitutes normally 5-7% of the particle weight (Adams *et al.,* 2004). The proteins of some carlaviruses can become partially degraded in the assembled particles. They have TIP of 55-70°C and longevity in sap is only a few days. The dilution end point of carlaviruses usually ranges from $10^3\text{-}10^4$ but occasionally it can go up to 10^6. The ultra violet absorbance spectra of carlaviruses are typical of nucleoproteins, having a maximum of 258 to 260 nm and a minimum of 243 to 248 nm (Adams *et al.,* 2004) and A_{max}/A_{min} ratios of 1.1 to 1.2. The A_{260}/A_{280} ratios of 1.1 to 1.3 indicate a nucleic acid content of 5-7%. The absorption coefficient A_{260}(0.1%; 1cm) which has been calculated for only a few carlaviruses, ranges from 2.1 to 2.8. The particles of carlaviruses have buoyant density in caesium chloride

(CsCl) of 1.31 to 1.33g/cm³. Most of them have restricted host range but different viruses occur in a wide range of monocotyledonous and dicotyledonous host. Usually, they produce latent infections in their natural hosts but sometimes mosaic symptoms are produced. The virus is transmitted mechanically and often in a non-persistent manner by aphids. There are some that are seed transmitted. The virus particles occur in the cytoplasm of their hosts as single or aggregates which are sometimes banded (https://www.dpvweb.net). Carlaviruses are noted to induce little or no symptoms in infected plants. For those that cause mild symptoms, they usually occur in the early stages of infection in certain plants. This has led to the common name of carlaviruses as latent as in Carnation latent virus (CLV) (Foster, 1992). Under natural conditions, carlaviruses often occur jointly with potyviruses, probably because they have the same mode of transmission (https://www.dpvweb.net/dpv/showdpv).

Chinese yam necrotic mosaic virus (ChYNMV) is the carlavirus that infect Chinese yam (*D. opposita* Thunb) in Asia. It was first reported by Fukumoto and Tochihara (1978) in Japan. The vectors, *M. persicae* and *A. gossypii* are responsible for their transmission in a non-persistant manner. The virus is transmitted by mechanical inoculation but not transmitted by contact between plants. Infected yam plants show chlorotic and necrotic spot or netting (Brunt *et al.,* 1996). ChYNMV is reported to cause to yield loss of as much as 30-45% if the seed tubers have been infected (Tochihara, 1993).

2.3.3.5 Cucumoviruses

Cucumovirus is a virus genus that belongs to the family Bromoviridae. The virus particles are isometric (Kenyon *et al*., 2001). The virus has three functional pieces of single –stranded RNA

(ss-RNA) and package in three classes of icosahedral particle of about 28 nm in diameter. The ss-RNA takes about 18% of the particle weight. The buoyant density of formaldehyde-fixed virions ranges from 1.35 to 1.37 gcm^{-3} in caesium chloride (CsCl) and they sediment at 63 to 99S. The virus is transmitted in a non-persistent manner by more than 60 species of aphids. Transmission efficiency varies with the aphid species and the host plant but the *A. gossipii* and *M. persicae* are known to be efficient transmitters. Virus can be acquired by all instars within 5 to 10 seconds but their ability to transmit the virus to a healthy plant declines after about 2 min and is usually lost within 2 h. Transmissions through seed occur to varying degrees in 19 species. The particles precipitate on exposure to physiological salt solutions and mild heating and therefore serological test are usually done by agarose double-diffusion tests in buffers of low molarity or in water. In terms of stability, it is relatively unstable in plant extracts, being unable to withstand high temperatures in excess of 70℃ for 10 min. Infectivity is lost within a few days and in some instances hours at room temperature (https://talk.ictvonline.org; http://www.dpvweb.net). The species as a whole has a very wide host range but there tends to be some specialization within strains or subspecies. CMV infections on yam are sporadic, thus, they result from a chance encounter between a viruliferous vector and the yam plant, but occasionally, it can be high locally. Usually, CMV infection of yam causes severe leaf chlorosis and mosaic symptoms; it may also cause leaf distortions and stunting (Kenyon *et al.,* 2001).

Cucumber mosaic virus is the type within the genus cucumovirus that causes infection to yam. Eni (2008) reported the first occurrence of CMV in Ghana, Togo and Benin but before then, it was restricted to three countries, namely Guadeloupe (Migliori and Cadilhac, 1976), Côte d'Ivoire (Fauquet and Thouvenel, 1987), and Nigeria (Hughes *et al.,* 1997). This indicates a 50% increase

in the number of countries worldwide where CMV infection in yam have been reported (Eni *et al.,* 2013)

2.3.3.6 Comoviruses

The genus *Comovirus* is classified into the family *Secoviridae* and subfamily Comovirinae under the order *Picornavirales*. They belong to the positive sense single stranded RNA (ssRNA+). Members belonging to this genus have segmented and bipartite linear genome composed of RNA-1 with 6 to 8kb and RNA-2 having 4 to7kb. They are non-enveloped with about 28 to 30 nm in diameter with an icosahedral capsid. The virus is transmitted mechanically by beetles form one plant to the other and replication occurs in the cytoplasm of their hosts (ViralZone; Comovirinae)

The species within genus Comovirus that causes infection is known as *Dioscorea* mottle virus (DMoV). DMoV has isometric particles with 20-30 nm in diameter and a bipartite genome of single stranded RNA (https://talk.ictvonline.org/ictv-reports/ictv_online). DMoV was found on *D. alata* in Nigeria and it is likely to be distributed across West Africa. Symptoms in *D. alata* include mild chlorosis caused by the mild chlorosis strain, mottling by the mottle strain and necrosis caused by the necrosis strain. The natural vector of DMoV is thought to be a beetle which mechanically transmits the virus (ICTV Taxonomy history for *Comovirinae*). The virus can also be mechanically transmitted to the indicator plants such *as Vigna unguiculata* L. Walp*., Glycine max* L. Merr*., Chenopodium murale* L*., C. amaranticolor* Coste and Reyn and *C. quinoa* Willd (Kenyon *et al.,* 2001).

2.4 Management of yam viruses

Over the years efforts have been made to control or manage the high viral disease incidence and severity at various locations where the crop is grown. The management of viral diseases on yam

that is also propagated clonally and transmitted by insect vectors requires a multiple approach including: field phytosanitation to reduce virus inoculum and replacement of infected seeds with virus-free planting materials, the use of resistant cultivars, and control insect pest to prevent further spread of the disease (Kumar, 2015). The above -mentioned strategies have not being successful due to unavailability of virus-free planting materials from tissue culture and/or aeroponics techniques and in the case where they are available, adoption and affordability become a problem on the part of yam farmers (personal observation). It is as a result of this that the CAY-Seed project funded by Bill and Melinda Gates foundation seeks to manage the situation by the introduction of positive selection to prevent recycling of severely infected seed yams. This will help to eliminate seed yams with high virus concentration as well as those infected with multiple viruses.

2.5 Seed yam degeneration and positive selection

Gildemacher *et al.* (2007) defines seed degeneration as the build-up of diseases over seasons as a result of replanting tubers that are infected with viruses, fungi, bacteria and other seed-borne diseases. In West Africa, particularly Ghana, farmers use seed yams obtained from their own previous harvest, purchase from the market or collect from their neighbours. This practice contributes to the accumulation and perpetuation of tuber-borne diseases especially viruses (Kumar, 2015). This means the continual use of infected-seed yams contribute to high virus incidence in Ghana and West Africa.

Positive selection also referred to as Select the best means selecting the best looking plants as source of seed for the next planting season (Gildemacher, 2007). Positive selection involves identification, tagging and monitoring healthy-looking plants during growth until they are harvested and tubers kept and used as seed for the next season (Kakuhenzire *et al.,* 2013; Schulte-

Geldermann *et al.,* 2012). Positive selection has been proven to be a promising complementary practice for smallholder farmers in Kenya, in addition to seed production and marketing by specialized seed growers (Gildemacher *et al.,* 2011). Positive selection has an advantage of increasing yield of smallholder potato farms without monetary investment and the important mechanism behind the effect of positive selection is the reduction of virus infection in plant population (Schulte-Geldermann *et al.,* 2012). Kakuhenzire *et al.* (2013) reported that positive selected seed showed 12.6% latent Bacterial Wilt infection compared to 44.7% from farmer selected seed on potato. All samples from positive selected seeds were free from Potato leaf-roll virus (PLRV) and Potato Virus Y (PVY), and had lower infection incidence with Potato Virus S and Potato Virus X than farmer selected seeds. In Uganda, the incidence of Bacterial wilt symptomatic potato plants in progeny crops did not exceed 3% in positive selected seeds compared with 6.7% in farmer selected seeds and incidence did not significantly differ from Basic Seed. Bacterial wilt incidence in positive selected seeds and farmer selected seeds in Kenya was 12.6 and 40.8%, respectively. Positive selected seed had significantly higher yield than farmer selected seeds and did not significantly differ in yield from Basic Seed in Uganda. Positive selection in both Uganda and Kenya increased yield of potato between 19 and 52% over farmer selected seeds. Overall, positive selected seeds were superior to farmer selected seeds and were comparable in terms of quality and performance to basic seeds or certified seeds which are produced under highly controlled conditions. Schulte-Geldermann *et al.* (2012) observed 30% average yield increase of positive selected potato seeds and also 28.1% virus incidence reduction of three potato viruses (PLRV, PVX, PVY). The use of positive selection would increase the yield of potato resulting in 29% maximum increase in gross revenue of farmers when they adopt the technique (Gunadi *et al.,* 2017).

2.6 Reverse transcription polymerase chain reaction (RT-PCR)

RT-PCR is an excellent method for analysis of RNA transcripts, especially for measuring lowabundance species or working with limited amounts of starting material. RT-PCR couples the tremendous DNA amplification powers of the PCR with the ability of reverse transcriptase (RT) to reverse transcribe small quantities of total RNA (Coleman and Tsongalis, 2016).

The first step is to convert isolated RNA to a complementary DNA (cDNA) molecule using an enzyme known as reverse transcriptase during a process called reverse transcription (RT). The complementary DNA can then be used as any other DNA molecule for PCR amplification as the second step. The RT reaction consists of (1) cDNA synthesis primer, (2) an appropriate RT reaction buffer, (3) dNTPs, (4) RNA template (total RNA or mRNA), and (5) RT enzyme (Coleman and Tsongalis, 2016).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Survey to determine incidence and severity of yam viruses in selected communities within Ejura-Sekyedumase and Atebubu-Amantin Districts in 2015 and 2016 cropping seasons

A baseline disease diagnostic survey was conducted in Ejura-Sekyedumase (7^0 23' N, latitude and 1^0 21' W longitude) and Atebubu-Amantin (7° 45' N, latitude and 0° 59' W longitude) Districts in 2015. This was necessary to determine the prevalence of yam viruses in the yam growing areas and also provide justification for the field trial under natural conditions. Four yam growing communities were visited, Bisiw and Nyinasae in Ejura-Sekyedumase, Mem and Abour in Atebubu-Amantin. In each community, ten farms were visited.

A follow-up survey was conducted in 2016 in other farming communities in the Ejura-Sekyedumase and Atebubu-Amantin Districts in the Forest-Transition agro-ecological zone of Ghana. In each district, four yam growing communities were selected; Asanteboa, Watro, Ahotor and Densi in Atebubu-Amantin and Kramokrom, Mesuo, Nokwareasa and Kasei in EjuraSekyedumase. Ten farms from each community were assessed. On each farm, 30 white yam plants were sampled and assessed for disease incidence and severity by walking across the diagonals of the farm. Each of the 30 white yam plants was assessed for the presence of yam virus symptoms and their severity scored on a modified five-point scale of 1-5 (Where, a score of 1 represents no obvious symptoms, 2 represents symptoms on 1-24% of leaves, 3 represents symptoms on 25-50% of leaves, 4 represents symptoms on 51-74% of leaves and 5 represents symptoms on 75-100% of leaves (Eni *et al*., 2008). Ten virus symptomatic leaf samples were collected from each community using a pair of forceps. Each sample was put in a 2-ml labelled

eppendorf tube and frozen immediately in liquid nitrogen for molecular laboratory analysis. In all, 80 symptomatic leaf samples were collected from the survey for laboratory analysis.

3.2. Total nucleic acid (DNA and RNA) extraction of white yam leaf samples collected from the survey and experimental fields

Total nucleic acid was extracted using modified CTAB (Cetyl trimethylammonium bromide) and Musa protocol (Doyle, 1990) at the Biotechnology laboratory, CSIR-Crop Research Institute (CSIR-CRI) Fumesua, Kumasi.

Each leaf of 0.2 g sample collected from the survey was weighed into 2 ml eppendorf tube and placed in liquid nitrogen. The leaf of each sample was ground in mortar using pestle. Freshly prepared 1 ml CTAB extraction buffer was added onto the ground leaf sample in eppendorf under a fume hood. The contents were then vortexed using a vortex mixer for 1 min and incubated in a water bath at 65℃ after 25 min with 5 min interval gently mixing the samples in the tubes. The eppendorf tubes were removed from the water bath after 25 min and allowed to cool for 3 min. They were then centrifuged at 13000 rpm for 10 min. The aqueous phase (600 µl) was then transferred into a new 2 ml eppendorf tube. Chloroform isoamyl (24:1) (600 µl) was added under a fume hood and mixed gently by inverting the tubes until mixture turned milky and were centrifuged at 13000 rpm for 10 min. Upper layer (550 µl) was picked using a pipette without disturbing the middle layer and were put into a new labelled 2 ml eppendorf tube. About 825 µl (1.5 times) of ice cooled absolute ethanol was added plus 82.5 µl of 3M sodium acetate to each sample in the 2 ml eppendorf tube. The resultant solution of each sample was mixed 10 times by inverting and then centrifuged at 13000 rpm for 10 min. The DNA pellet of each sample in an

eppendorf tube was washed using 1 ml 70% ethanol and centrifuged at 13000 rpm for 10 min. The ethanol was discarded and pellet dried for 30 min. After drying, the pellet was dissolved in 500 µl of low salt TE buffer and 250 µl of 7.5M ammonium acetate was added to the total nucleic acid (DNA and RNA) and mixed. The resultant mixtures were then incubated on ice for 5 min and then centrifuged at 13000 rpm for 10 min. The supernatant was transferred into a new labeled 1.5 ml eppendorf tube. Isopropanol (700 µl) was added and mixed by inversion and incubated at -20℃ for 1 h and then centrifuged at 13000 rpm for 5 min. The supernatants were discarded and the nucleic acid pellets were washed with 80 % ethanol by centrifuging at 13000 rpm for 5 min. The alcohol was discarded and the pellets were dried at room temperature. Dried nucleic acid pellets were then dissolved in 100 µl low-salt TE buffer. The total nucleic acid quality was checked on 0.8% agarose gel electrophoresis. The nucleic acid of each sample was quantified using Nanodrop 2000c spectrophotometer (Thermo Scientific, USA). In all, 127 samples had their nucleic acids extracted; 80 from the 2016 survey, 40 symptomatic (showing viral symptoms) and 7 asymptomatic (positive selection) leaves from the experimental fields at Fumesua and Ejura.

3.3 Multiplex reverse transcription polymerase chain reaction (RT-PCR) of leaf samples from survey and experimental fields

Reverse transcription polymerase chain reaction in a multiplex was done using RT-PCR kit (Protoscript® II RT-PCR kit, New England Biolabs Inc.). RT-PCR amplifications were set up in 12.5 ul reactions containing 6.25 ul of one taq one step reaction mix (2x), 0.5 ul one tag one step enzyme mix (25x), 1.0 ul each of primer mix YMV (F+R) and YMMV (F+R) (Table 3.1), 1.75 ul PCR water and 2 ul of nucleic acid template (Appendix 1). The cycler used, AB Applied Biosystem

PCR thermal cycler, had the following conditions: 42℃ for 30 min for reverse transcription, 92℃ for 5 mins followed by 35 cycles at 94℃ for 40 s, 55℃ for 40 s, 72℃ for 5 min and final extention at 72℃ for 5 min. Amplification products were analysed by agarose gel electrophoresis in 1x Tris-Boric acid-EDTA (TBE) buffer to observe the expected amplifications at the exppected band sizes; 586bp for YMV and 249bp for YMMV.

Table 3.1: Primers and their sequences used in this study

Primer name	**Sequence**
YMV F	ATCCGGGATGTGGCAATGA
YMV R	TGGTCCTCCGCCACATCAAA
YMMV F	GGCACACATGCAAATGAARGC
YMMV R	CACCAGTAGAGTGAACATAG

(Inqaba Biotechnical Industries (Pty) Ltd, Hatfield 0028, South Africa)

3.4 Generation of seed yams from positive selection and field infected plants

As a prelude to the seed yam degeneration experiments, there was the need to generate seeds from positively selected plants (tagged healthy plants) and also field infected plants in the 2015 cropping season. These were used as sources of seed (treatments) for the degeneration trials which commenced in 2016 cropping season.

3.4.1 Land preparation and planting for positive selection and field infected plants

Multiplication fields were established at the two locations, Fumesua and Ejura during the major season of 2015. A field size of 100 m×50 m (5000 m^2) was ploughed, harrowed and ridged using a tractor at both sites. Seed yams of three varieties of *D. rotundata,* Pona, Dente and Laribako were purchased from Ejura yam market. The authenticity of the yam varieties were confirmed by the CSIR-CRI Yam Breeding Team. They were cut into 70 g minisett sizes and treated with a cocktail of 70 g mancozeb and 50 ml of Karate (lambda-cyhalothrin) in 10 l of water. The treated minisetts were dried under shade for 24 h prior to planting. Planting was done at Fumesua on 23rd April, 2015 and that of Ejura was done on 1st May, 2015. The experimental fields were established at a spacing of 1.0 m × 0.5 m. Weeding was done as and when it was necessary using hoes.

Yam vines were directed unto about 2 m bamboo sticks two weeks after sprouting for effective interception of sunlight for photosynthesis, thus to obtain maximum yield as well as reduce the spread of soil-borne diseases from attacking the growing yam vines.

3.4.2 Selection/tagging of symptomless and diseased yam plants

The sprouted plants were tagged using blue and red ribbons. This was done by using a disease severity score on a scale of 1-5 (Eni *et al*., 2008). Yam plants with disease severity scores of 1 and 2 (symptomless and mildly infected) were tagged with blue ribbons and those that scored 4 and 5 (severely infected) were also tagged with red ribbons.

The blue tagged plants were inspected every two weeks in order to ensure that they were not severely disease-infected with passage of time. Any blue tagged plant that scored above 2.0 in the course of the season was rejected and the tag removed accordingly. The symptomless and mildly

infected (blue-tagged plants) of the three varieties represented the positive selection treatment (Plate 3.1) whilst the severely infected (red-tagged plants) plants represented the diseased treatment (Plate 3.2).

Plate 3.1. Tagged healthy yam plant

Plate 3.2. Tagged mosaic-infected yam plant

3.4.3 Harvesting of positive selection and field- infected plants

Harvesting was done on 5th January, 2016 and 7th January, 2016 at Fumesua and Ejura respectively. During harvesting, all the positive selection plants (blue tagged plants) from the three varieties, Pona, Laribako and Dente were harvested first and grouped accordingly with a label followed by the severely infected plant. This gave six out of nine treatments leaving three treatments (the three yam varieties that were purchased from the market) for the field experiment in the 2016 cropping season.

Harvested seed yams were treated with 79 g of mancozeb super (mancozeb and methaxyl) and 50 ml of Karate (lambda-cyhalothrin) mixed in 15 l of water against fungal and insects damage during storage. They were then air dried for 4 h after which they were stored in a bamboo yam barn under ambient conditions at CSIR-CRI, Fumesua.

3.5 Seed yam degeneration studies in 2016 cropping season

The field experiments were carried out in Ejura and Fumesua in the Forest-Transition and the Forest agro-ecological zones of Ghana respectively. Ejura and Fumesua have a slope of 2 to 6%.

Ejura is located on 7^0 23' N latitude and 1^0 21'W longitude whiles Fumesua is on latitude 6^0 41' N and 1^0 28' W longitude. Both locations are characterized by bimodal rainfall pattern with the major season starting from March to mid-August and the minor season from September to November, peaking in October. Fumesua has a higher annual rainfall ranging from 1190 to 1650 mm with an average rainfall of 1345 mm as compared Ejura with an annual rainfall ranging from 1000 to 1200 mm with an average rainfall of 1108 mm (Adu and Asiama, 1992).

3.5.1 Field Preparation and Planting for seed yam degeneration studies

Fields at Fumesua and Ejura were ploughed and poultry manure (at a rate of 6 tons/ha) was spread eveningly on the soil before harrowing. Ridges were made with a distance of 1 m apart. All these preparations were done using a tractor. In all, there were nine treatments made up of three varieties of white yam (Pona, Dente and Laribako) and three different seed yam sources (positive selection, farmer-saved seed yams /seed yams purchased from the market and diseased seed yams). The experimental design was a 3×3 factorial in Randomized Complete Block design (RCBD) with three replications. There were six rows per plot or treatment for all the nine treatments at both

locations. Each row measured 6 m and the three replications were 2 m apart. The seed yams for the various treatments were cut into 70 g minisetts and treated with a cocktail of 70 g mancozeb super and 50 ml of karate in 10 l of water. The treated minisetts were dried under shade for 24 h prior to planting. Planting was done at Fumesua and Ejura in May. The fields were established at a spacing of 1.0 m × 0.5 m with 10 plants per row.

The yam plants were staked using trellis where vines were trailed unto threads supported by bamboo. This was done at both locations, at two weeks after sprouting to give room for effective interception of sunlight for maximum photosynthesis. Manual weeding was done four times from the period after planting to harvesting. However, chemical weed control (herbicide) was employed immediately after planting with Roundup (Glyphosate) in order to prevent damage to newly sprouts or young plants before staking. Ridges were re-shaped as and when they were washed off by rain. Re-shaping of ridges was important because it prevents roots and developing tubers from being exposed.

In December 2016, yams were harvested using pick axe and mattock and they were grouped according to the nine treatments and treated against storage diseases and pests using karate and mancozeb. The seed yams were dried under shade before storage in a yam barn at CSIR-CRI. The same seed yams were used to establish the experiment in 2017 cropping season at Fumesua and Ejura. The methodology used for the 2016 field experiment was applied to the 2017 field experiment that was used to follow the rate of seed yam degeneration among the treatments.

3.6 Screen House Experiment for degeneration studies

In 2016, a seed degeneration experiment was conducted in an insect-free environment (screen house) where the vector, aphid, was eliminated. This was necessary in order to compare the

treatments from the field to that of the screen house if there was any difference in terms of performance amongst them. Using a 3×3 factorial in complete randomized design (CRD) as the experimental design, and replicated three times with three plants per treatment. The yam setts were also treated against fungi and insects attack just as it was done for the field experiment before planting in a small size plastic bucket filled with sterilized top soil. Watering was done immediately after planting and periodically to ensure sprouting. They were staked as and when they were due for staking. This experiment was repeated in 2017.

3.6 Data collected for seed yam degeneration studies in 2016 and 2017 cropping seasons i. Sprouting

The number of seed yam sprouts on the field after three weeks of planting was counted for each of the nine treatments. The total number of seed yam sprouts was collected at the end of the tenth week after which no new sprouts were observed.

ii. Disease assessment

Viral symptoms such as mosaic, necrosis, stunting, leaf distortion, mottling and shoe string incidence and severity were taken on monthly basis. For each treatment, the incidence was obtained by counting the number of plants infected and divided by the total number of plants and multiplied by 100 whilst the severity was obtained using the modified scale of 1-5 by Eni *et al*. (2008). The disease assessment commenced from the third month after planting when the plants were well established following sprouting. This was done three times on the plants in the two middle rows of each treatment before harvesting. The range (lowest and highest disease incidence) within which the viral disease incidence fell

was recorded for the individual communities and their standard deviations were also determined based on the figures obtained.

iii. Yield data

The two middle rows for each treatment were harvested and the tubers weighed using a weighing scale (Salter scale, England) to determine the fresh weight of yam tubers from each of the nine treatments. The values obtained were extrapolated to Megagram/ha to represent the yield. Data collected were analysed using Genstats statistical software, version 12 and least significant difference (LSD) at 5% was used to separate the means. Yield data obtained were used to calculate the percentage increase or gain in tuber yield by using the formular by Kakuhenzire *et al.* (2013) with use of positive selection instead of farmer practice and/or diseased seed yams yield as reference using the formula (Kakuhenzire *et al.,* 2013):

$$\%\ yield\ increase = \frac{mean\ yield\ positive\ selection - mean\ yield\ farmer\ practice}{positive\ selection} x100$$

Yields obtained from interactions between seed sources and varieties were used to calculate the yield increase caused by using positive selection instead of farmer practice seed yams.

CHAPTER FOUR

4.0 RESULTS

4.1 Survey to determine incidence and severity of yam viruses in selected communities within Ejura-Sekyedumase and Atebubu-Amantin Districts in 2015 and 2016 cropping seasons

The main viral symptoms observed among white yam plants in all the farmers' farms that were visited both in 2015 and 2016 cropping seasons included shoe string (Plate 4.1), mottle (Plate 4.2), leaf distortion (Plate 4.2), mosaic (4.3), severe leaf reduction and puckering (Plate 4.4), leaves showing retarded growth (Plate 4.5) and chlorosis (Plate 4.6). The least viral incidence of 10% was recorded at Nyinasae in Ejura-Sekyedumase District during the 2015 field survey. The high viral disease symptoms incidence observed at Ejura-Sekyedumase explains the establishment of the degeneration studies at Ejura.

Plate 4.1 Shoe String

Plate 4.2 Mottle and Leaf distortion

Plate 4.3 Mosaic

Plate 4.4 Severe leaf reduction and puckering

Plate 4.5 Stunted growth and chlorosis

Plate 4.6 Severe chlorosis and mosaic

The mean incidence and severity of viral symptoms on white yam observed at Ejura were 72.8% and 2 respectively and that in Atebubu were 78.2% and 3 respectively during the 2015 survey. The

disease incidence and severity of the 10 farms assessed in each of the four communities are presented in Figure 4.1 to Figure 4.4

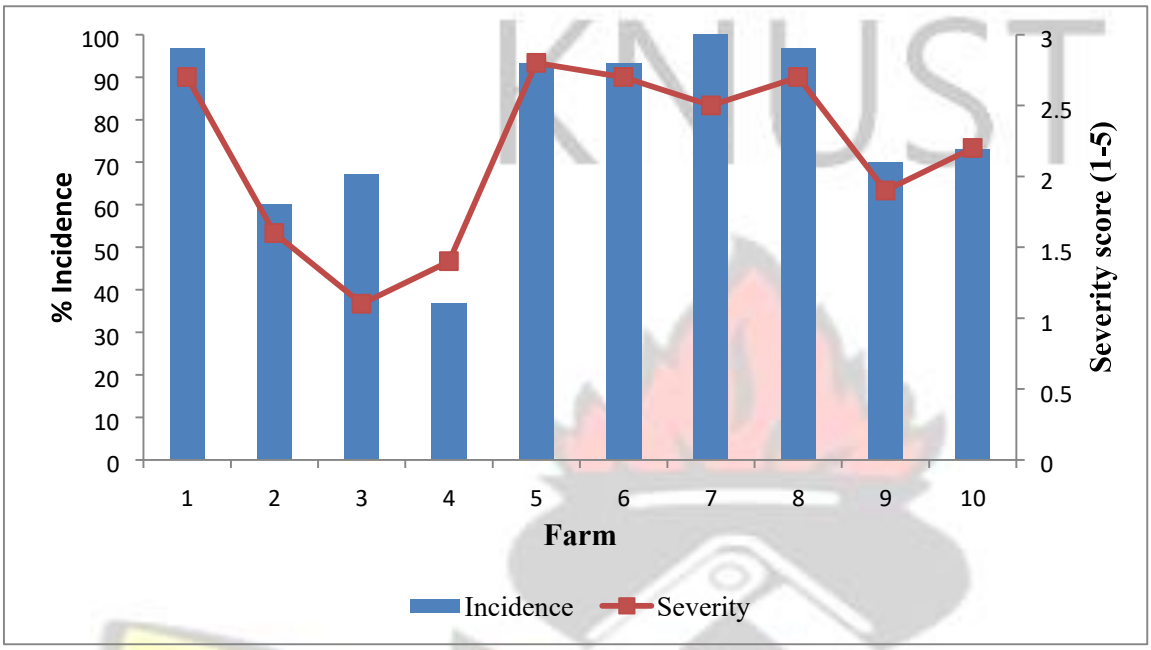

Figure 4.1. Mean incidence and severity of virus disease symptoms on white yam plants in 10 farms at Bisiw in Ejura-Sekyedumase District in 2015 cropping season

The mean virus incidence recorded at Bisiw range from 36.7 to 100.0% whilst that of the severity score were from 1.1 to 3 indicating higher virus percentage incidence with relatively lower disease severity (Fig. 4.1). At Bisiw, only one farm had less than 40% viral disease incidence with all the other farms ranging between 55 to 100%. The community therefore has a high viral disease symptoms incidence since three farms had more than 90% and two having 100%.

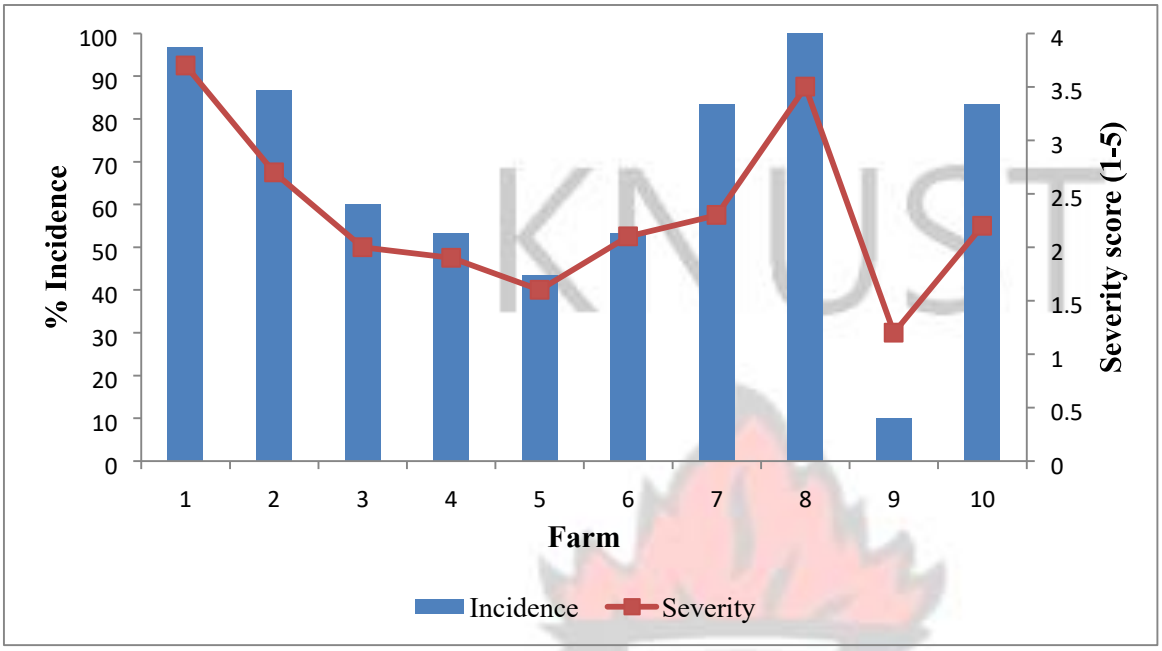

Figure 4.2 Mean incidence and severity of virus disease symptoms on white yam plants in 10 farms at Nyinasae in Ejura- Sekyedumase District in 2015 cropping

Among the four communities visited in 2015, the least percentage viral disease incidence and severity of 10% and 1.2 was recorded in a farm at Nyinasae (Fig. 4.2). This makes this particular farm better in terms lower viral disease incidence and severity.

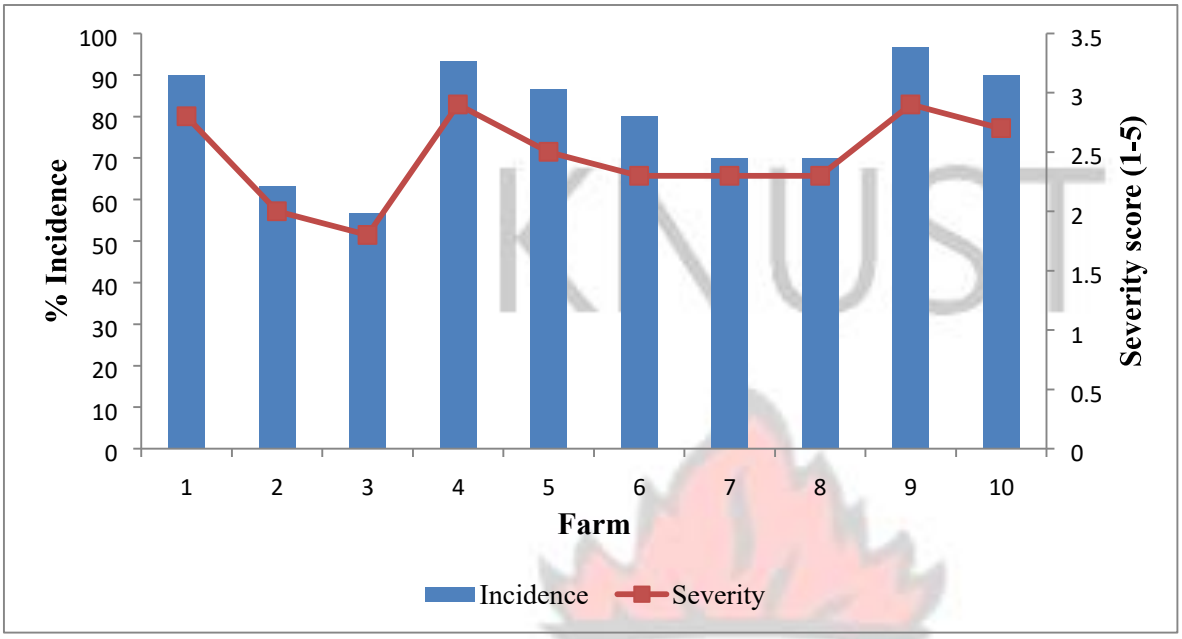

Figure 4.3 Mean incidence and severity of virus disease symptoms on white yam plant in 10 farms at Mem in Atebubub-Amantin District in 2015 cropping season

Viral disease incidence observed at Mem ranged from 56.7 to 96.7% and the severity score from 2 to 3 (Fig. 4.3). This shows that more than 50.0% of yam plants found at Mem were virus infected. Mem had the highest viral disease symptom incidence as compared to Bisiw, Nyinasae and Abour.

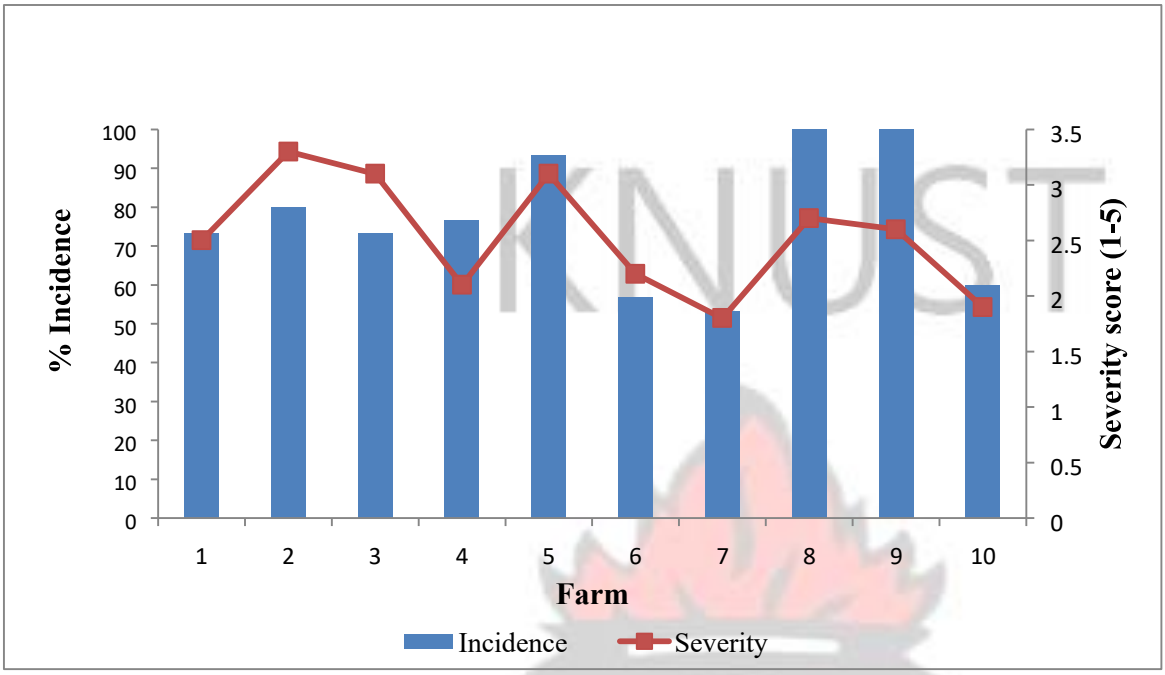

Figure 4.4 Mean incidence and severity of virus disease symptoms on white yam plants in 10 farms at Abour in Atebubu-Amantin District

The viral disease incidence and severity ranged from 53.3 to 100% and 1.8 to 3.3 respectively (Fig. 4.4). All the farms assessed at Abour had more than 50% viral symptom incidence with the entire yam plants in two farms showing viral symptoms.

In 2016, Ejura had a mean virus incidence and severity of 78.67% and 2.25 respectively while Atebubu had mean virus incidence and severity of 78.2% and 2.52. The percentage virus incidence and severity in each farm and location have been presented in graphs (Appendix 2).

At Atebubu, the percentage incidence and severity scores of yam viruses among the communities were determined for all the 40 farms visited. Asanteboa had the lowest mean percentage disease incidence

and mean severity score of 59.3 and 2.3 respectively while Ahotor has the highest with 89.3% and 2.5 respectively. The results are represented in the Table 4.1.

Table 4.1: Percentage white yam viral disease symptom incidence and severity scores at Atebubu-Amantin District in 2016 cropping season

Community	% Mean yam virus incidence (range ± SD)	M virus disease score (1-5) severity
Asanteboa	59.3 (13.3 – 93.3 ± 26.9)	2.3
Watro	82.0 (56.7 – 100 ±15.5)	2.7
Ahotor	89.3 (66.7 – 93.3 ± 8.7)	2.5
Densi	79.7 (83.3 – 100 ± 8.7)	2.5
Mean	77.6 (59.3 – 89.3 ± 12.9)	2.5

The mean yam viral disease symptom incidence and severity of the four communities at Ejura is represented in Table 4.2 with Mmesuo having the highest and Kramokrom having the lowest percentage incidence and severity scores.

Table 4.2: Percentage white yam viral disease symptom incidence and severity scores at Ejura-Sekyedumase District in 2016 cropping season

Community	% Mean yam virus incidence (range ± SD)	Mean yam virus severity score (1-5)
Kramokrom	76.0 (63.0 – 80 ± 6.8)	2.3
Mmesuo	99.7 (96.7 – 100 ± 1.1)	3.0
Nokwareasa	87.4 (76.9 – 100 ± 8.9)	2.9
Kasei	92.0 (76.7 – 100 ± 9.2)	2.7
Mean	88.8 (76.0 – 99.7 ± 9.9)	2.7

The highest mean viral disease incidence was recorded at Mmesuo whilst the lowest was from Kramokrom (Table 4.2)

Comparing the mean viral disease incidence for Atebubu-Amantin and Ejura-Sekyedumase Districts in the 2016 cropping year, Atebubu-Amantin District had a wider viral disease incidence range of 59.3 to 89.3% than Ejura-Sekyedumase District with a range of 76.0 to 99.7% but numericaly,s there was higher mean viral disease incidence at Ejura-Sekyedumase District than Atebubu-Amantin District (Table 4.1 and 4.2).

4.2 Multiplex reverse transcription Polymerase Chain Reaction (RT-PCR) of leaf samples from survey and experimental fields

Among all the 127 white yam leaf samples collected during the survey and the experimental fields that were analysed at the laboratory, 43 amplified for the two primers, YMMV and YMV.

There were 27 amplifications of co-infections, eight leaf samples each for YMMV and YMV single infections. Out of the 40 leaf samples collected from Ejura-Sekyedumase District, there were six mixed infections for both YMV and YMMV (Plates 4.9 and 4.10) and eight single

infections for YMMV (Plate 4.7). Leaf samples from Atebubu-Amantin District showed 10 mixed infections of YMV and YMMV (Plates 4.10 and 4.11) and eight single infections were shown for only YMV (Plate 4.11). From the experimental fields, 11 leaf samples amplified for both viruses but there were no single infections from either of the viruses that were tested.

Out of the seven samples collected from plants that were labeled on the experimental field at Fumesua as positive selection, two of the samples were positive for both YMV and YMMV whiles five did not show any amplification for the two primers (Plates 4.8 and 4.9). In all, 27 (62.7%) out of 43 white yam leaf samples that amplified showed mixed infections for the two viruses. Tables presenting the scores for the gel images showing the presence or absence of YMV and YMMV for all the samples that were tested have been presented at appendix 3.

Plate 4.7 Multiplex RT-PCR products on agarose gel for samples 1 to 26 sampled from Ejura-Sekyedumase District

Note: well 1: ladder, well 2: space, well 3: YMV positive sample and well 4: YMMV positive sample. Well 5 to 30 are leaf samples tested that were for presence or absence of YMV and YMMV and well 12, 15, 19, 20, 22, 23, 26, and 28 showed positive for YMMV only (Appendix 3.1).

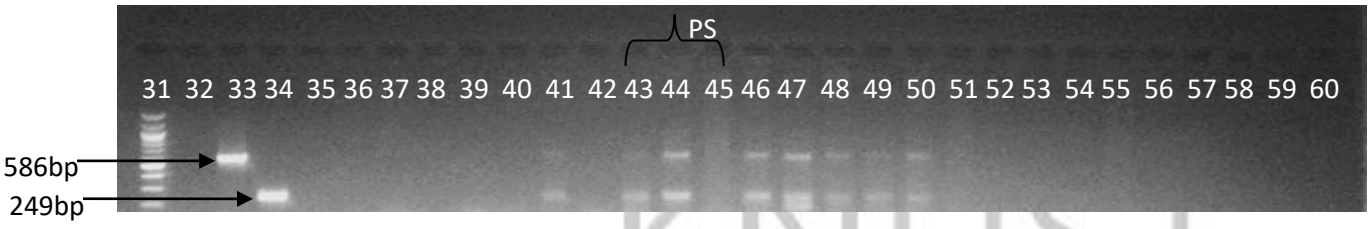

Plate 4.8 Multiplex RT-PCR products on agarose gel for samples 27 to 52 sampled from white yam experimental fields at Ejura and Fumesua

Note: well 31: ladder, well 32: space, well 33: YMV positive sample and well 34: YMMV positive sample. positive selection = positive selected samples for which 2 showed amplification for both YMV and YMMV and 1 did not amplify for any of the two viruses (well 43, 44 and 45).

Well 41, 46, 47, 48, 49 and 50 showed positive for both YMV and YMMV (Appendix 3.2).

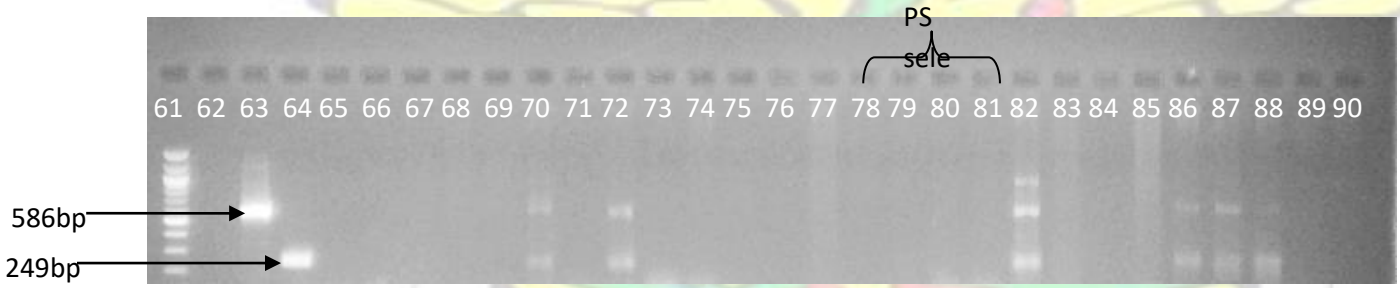

Plate 4.9 Multiplex RT-PCR products on agarose gel for leaf samples 53 to 78. Sample number 53 to 73 are from Ejura and Fumesua white yam experimental fields and sample 74 to 78 are from Ejura –Sekyedumase District

Note: well 61: ladder, well 62: Space, well 63: YMV positive sample and well 64: YMMV positive sample. positive selection = positive selected samples showing no amplification for both YMV and YMMV (well

78, 79, 80 and 81). Well 70, 72, 82, 86, 87 and 88 showed positive for both YMV and YMMV (Appendix 3.3).

Plate 4.10 Multiplex RT-PCR products on agarose gel for white yam leaf samples 79 to 104. Sample 79 to 87 are from Ejura-Sekyedumase District and sample 88 to 103 are from Atebubu Amantin District.

Note: well 91: ladder, well 92: space, well 93: YMV positive sample and well 94: YMMV positive sample. Well 95, 96, 99, 106, 107, 110 and 112 showed positive for YMV and YMMV (Appendix 3.4).

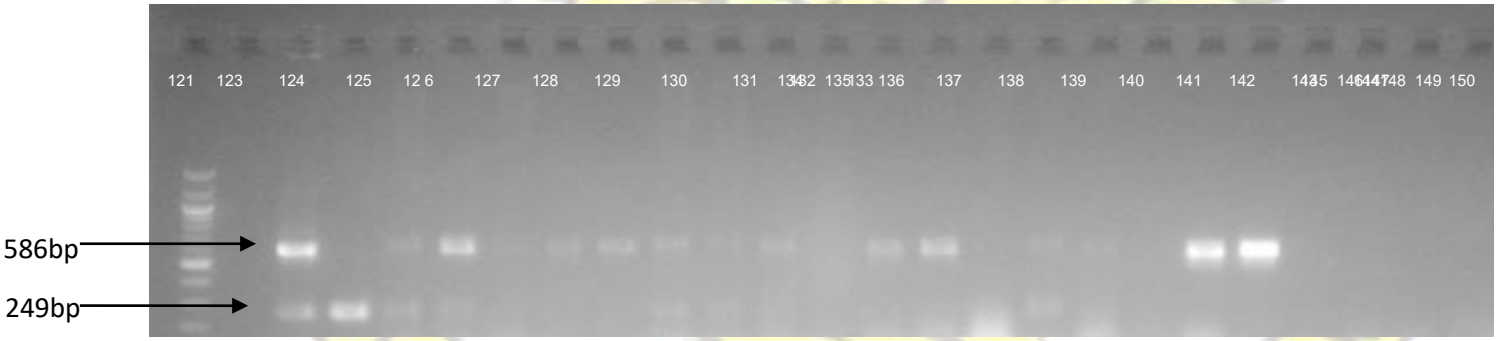

Plate 4.11 Multiplex RT-PCR products on agarose gel for leaf samples 105 to 127 are from Atebubu-Amantin District.

Note: well 121: ladder, well 122: space, well 123: YMV positive and YMMV positive sample and well 124: YMMV positive sample. Well 125, 126, 130, 131, 132 and 137 showed positive for YMV and

YMMV and well 127, 128, 129, 134, 135, 138, 140 and 141 showed positive for YMV only (Appendix 3.5).

4.3 Generation of seed yams from positive selection and field infected yam plants

In all, 45 plants from Laribako, 32 from Dente and 40 from Pona all of which were positively selected were harvested. Also 60 field infected plants were each harvested for the three varieties from both Ejura and Fumesua in the 2015 cropping season. These seed yams served as seed stock for the 2016 field and screen house experiments.

4.4 Seed yam degeneration studies in 2016 cropping season at Ejura and Fumesua

At Ejura, with regard to sprouting, virus disease symptoms incidence, virus disease symptom severity and tuber yield, there were no significant differences ($P>0.05$) among the yam varieties for sprouting and yield but there were significant differences ($P<0.05$) in both the virus disease symptoms incidence and disease severity among the varieties (Table 4.3). Although Dente had the least percentage viral disease incidence, it recorded the highest viral disease severity and the opposite was observed in Laribako (Table 4.3).

On the different seed sources, positive selection materials irrespective of the variety, performed significantly better ($P< 0.05$) than farmer practice seed yams and also diseased seed yams under all the four parameters (number of sprouts, viral disease symptoms incidence, viral disease symptom severity and tuber yield). Positive selection materials had the highest number of sprouts, least viral symptoms incidence and disease severity score and highest in terms of yield (Table 4.3).

For the interaction between variety and seed sources, there were significant differences among the treatments. In terms of sprouting, Dente positive selection had the highest number of sprouts, least percentage viral disease incidence and disease severity resulting in highest yield among the treatments followed by Pona positive selection and Laribako positive selection (Table 4.3).

Table 4.3: Performance of yam variety, seed source and interaction of variety and seed sources at Ejura for the 2016 cropping season.

Treatment	Mean no. of sprouts	Mean viral disease incidence (%)	Mean viral disease severity (scale:1-5)	Mean tuber yield (Mg/ha)
Variety Dente				
	10.0	84.3	3.4	7.0
Laribako	9.30	89.5	2.8	8.0
Pona	8.90	86.0	3.0	7.5
LSD(5%)	NS	NS	0.2	NS
CV(%)	8.50	2.20	2.8	6.1
Source				
Positive selection	11.6	62.30	2.2	10.1
Farmer Practice	8.40	97.50	3.0	7.90
Diseased	8.20	100.0	4.0	4.40
LSD (5%)	0.90	4.60	0.3	0.50
CV(%)	8.50	2.20	2.8	6.10
Variety*Source				
Dente positive selection	12.3	56.70	2.3	10.5
Dente farmer practice	8.30	96.30	3.6	6.80
Dente diseased	9.30	100.0	4.2	3.50
Laribako positive selection	11.3	72.20	2.0	9.70
Laribako farmer practice	8.30	96.30	2.7	8.10
Laribako diseased	8.70	100.0	3.8	6.10
Pona positive selection	11.0	58.00	2.2	10.0
Pona farmer practice	8.70	100.0	2.7	8.80
Pona diseased	7.00	100.0	4.0	3.60
LSD (5%)	NS	7.10	0.50	1.1
CV (%)	9.6	5.10	10.2	7.1

At Fumesua, there were significant differences ($P<0.05$) among the yam varieties under all the four parameters measured. Dente had the highest number of sprouts followed by Pona and Laribako. Numerically,s the highest viral disease incidence and disease severity were recorded on

Laribako resulting in the least yield produced by Laribako. Highest yield was obtained in Pona followed by Dente (Table 4.4).

There were significant differences ($P<0.05$) among the different seed yam sources. Positive selection, among the different seed sources, performed significantly ($P<0.05$) better than farmer practice seed yams and diseased plants in terms of sprouting, lower percentage virus incidence and severity as well as tuber yield (Table 4.4).

For the interactions between variety and source, there were significant differences ($P<0.05$) between the treatments. Varieties from positive selection performed significantly ($P<0.05$) best among the treatments. Positive selected Dente gave the best performance in terms of all the four parameters assessed. This was followed by Pona positive selection and positive selection Laribako (Table 4.4).

Table 4.4: Performance of variety, seed source and interaction of variety and seed source at Fumesua for the 2016 cropping season

Treatment	Mean no. of sprouts	Mean viral disease incidence (%)	Mean viral disease severity (scale: 1-5)	Mean tuber yield (Mg/ha)

Variety Dente				
	12.0	86.3	3.1	9.3
Laribako	8.30	94.3	3.2	8.5
Pona	10.4	84.8	2.8	9.6
LSD (5%)	1.60	6.90	0.4	0.3
CV (%)	6.80	3.40	6.6	1.6
Source				
Positive selection	12.1	74.9	2.4	12.5
Farmer practice	10.4	91.1	2.6	9.40
Diseased	8.20	100.0	4.1	5.50
LSD (5%)	0.90	7.20	0.3	0.60
CV (%)	6.80	3.40	6.6	1.60
Variety*Source				
Dente positive selection	14.0	68.30	2.3	13.5
Dente farmer practice	11.7	96.30	3.6	9.10
Dente diseased	10.3	100.0	4.2	5.30
Laribako positive selection	11.3	91.30	2.0	11.0
Laribako farmer practice	8.00	96.30	2.7	9.60
Laribako diseased	5.70	100.0	3.8	4.90
Pona positive selection	11.0	65.00	2.2	13.0
Pona farmer practice	11.7	100.0	2.7	9.60
Pona diseased	8.70	100.0	4.0	6.30
LSD (5%)	1.80	11.20	0.5	0.90
CV (%)	8.80	7.90	10.9	6.40

At Fumesua in the 2016 planting season, Pona had the highest yield (9.6 Mg/ha) followed by Dente (9.3 Mg/ha) and then Laribako (8.5 Mg/ha) (Table 4.4). Laribako had the highest viral disease incidence and severity at Fumesua and this explains the least yield obtained (Table 4.4). the of this occurred at Ejura, Laribako had the least incidence and severity score (Table 4.3) giving the highest yield among the varieties. Among the interactions, at Fumesua in the 2016 cropping season,

positively selected plants had the highest yield but positively selected Dente had the highest yield followed by Pona and then Laribako (Table 4.4) and similar observation was also made Ejura. Using Shculte-Geldermann et al. (2012) formula for calculating the yield difference, Positive selection Dente out-yielded farmer practice Dente and diseased Dente by 35 and 66.7% respectively in the 2016 cropping season at Ejura. Similar result was obtained at Fumesua with positive selection Dente out-yielding farmer practice and diseased seeds by 32.6 and 60.7% respectively.

4.5 Seed yam degeneration studies in 2017 cropping season at Ejura and Fumesua

Generally, for all the seed sources, there was reduction in the number of sprouts and increase in the viral disease incidence and disease severity score; consequently there was total yield reduction (Table 4.5).

At Ejura, among the varieties, there were significant differences ($P<0.05$) with Laribako performing significantly best ($P<0.05$) with respect to sprouting, virus incidence, virus severity and total yield production (Table 4.5).

Among the seed yam sources, there were significant differences ($P<0.05$) with positive selection performing best as compared to farmer practice and diseased seed sources considering with regard to the four parameters assessed. There were significant differences ($P<0.05$) among the treatments with respect to viral disease incidence and tuber yield but there were no significant differences among the treatments considering sprouting and virus severity score. Positivelyselected plants gave the best yield and least percentage viral disease symptoms incidence and disease severity. Laribako produced from positive selection gave the highest yield followed by positive selected Pona and then positive selected Dente (Table 4.5).

Table

4.5: Performance of yam variety, seed source and interaction of variety and seed yam source at Ejura for the 2017 cropping season

Treatment	Mean no. of Sprout	Mean viral disease Incidence (%)	Mean viral disease Severity(scale: 1-5)	Mean tuber yield (Mg/ha)
Variety				
Dente	3.22	90.67	4.27	1.76
Laribako	4.78	92.22	3.27	4.62
Pona	3.78	92.78	3.50	4.02
LSD (5%)	1.00	1.79	0.59	0.52
CV(%)	11.3	0.9	7.1	6.6
Source				
Positive selection	5.11	75.67	3.22	5.17
Farmer practice	4.00	100	3.72	3.55
Diseased	2.67	100	4.11	1.67
LSD (5%)	0.83	7.16	0.25	0.53
CV(%)	11.3	3.4	7.1	6.6
Variety*Source				
Dente positive selection	4.33	72.00	4.00	3.96
Dente farmer practice	3.33	96.29	4.33	0.93
Dente diseased	2.00	100	4.50	0.40
Laribako positive selection	6.00	76.67	2.66	6.16
Laribako farmer practice	5.00	96.29	3.33	5.13
Laribako diseased	3.33	100	3.83	2.56
Pona positive selection	5.00	78.33	3.00	5.4
Pona farmer practice	3.67	100	3.50	4.60
Pona diseased	2.67	100	4.00	2.06
LSD (5%)	NS	3.41	NS	0.83
CV(%)	20.80	2.40	6.7	15.0

At Fumesua, there were significant differences ($P<0.05$) among the varieties regarding sprouting and yield but there were no significant differences ($P>0.05$) among them with respect to virus incidence and severity. The highest yield was obtained in Laribako, even though it had the least number of sprouts (Table 4.6).

There were significant differences ($P<0.05$) among the three seed sources with respect to sprouting, virus severity and yield but there were no significant differences ($P>0.05$) among them with respect to virus incidence (Table 4.6).

For interaction between variety and seed sources, there were significant differences between the treatments with regards to viral disease severity and yield but there were no significant differences ($P>0.05$) among the different treatments with respect to sprouting and virus incidence. Positive selection materials performed significantly ($P<0.05$) better than the other treatments but positive selected Laribako gave the highest yield among the treatments followed by positive selection Dente and positive selection Pona (Table 4.6).

Table

4.6: Performance of variety, seed source and interaction of variety and seed yam sources at Fumesua for the 2017 cropping season

Treatment	Mean no. of Sprout	Mean viral disease incidence (%)	Mean viral disease severity(scale: 1-5)	Mean tuber yield (Mg/ha)
Variety				
Dente	2.90	97.20	3.7	3.1
Laribako	1.80	97.80	3.7	3.9
Pona	2.30	100.0	2.8	3.2
LSD (5%)	0.60	NS	NS	0.5
CV (%)	11.7	2.6	11.9	6.2
Source				
Positive selection	3.00	95.0	2.40	5.6
Farmer practice	2.10	100.0	3.90	2.4
Diseased	1.90	100.0	4.10	2.1
LSD (5%)	1.00	NS	0.40	0.4
CV (%)	11.7	2.6	11.9	6.2
Variety*Source				
Dente positive selection	3.0	91.7	2.2	5.4
Dente farmer practice	3.0	100.0	4.7	1.8
Dente diseased	2.7	100.0	4.5	2.0
Laribako positive selection	3.3	93.3	2.7	6.2
Laribako farmer practice	1.0	100.0	4.3	2.7
Laribako diseased	1.0	100.0	4.3	2.7
Pona positive selection	2.7	100.0	3.0	5.1
Pona farmer practice	2.33	100.0	2.8	2.7
Pona diseased	2.0	100.0	3.3	1.7
LSD (5%)	NS	NS	1.0	0.6

CV (%)	14	6.3	12.0	15.0

In 2016, at Ejura, the percentage tuber yield gained by using positive selection instead of farmer practice seed yams was 22% and in 2017, 31% of yield was gained by using positive selection (Table 4.7).

Table 4.7: Effect of using positive selection, farmer practice and diseased seed yam sources on the yield of yam at Ejura for 2016 and 2017 cropping seasons

Source of seed yam	2016 cropping season		2017 cropping season	
	Mean yield (Mg/ha)	Yield loss (%)	Mean yield (Mg/ha)	Yield loss (%)
Positive selection	10.1	-	5.2	-
Farmer Practice	7.9	22.0	3.6	31.0
Diseased	4.4	56.0	1.7	67.0

Similar observation was also made from Fumesua where the percentage yield gained was even higher as compared to that of Ejura. Positive selection saved 25 and 57% of yield that would have been lost as a result of using farmer practice in 2016 and 2017 respectively (Table 4.8).

4.8: Effect of using positive selection, farmer practice and diseased seed yam sources on the yield of seed yam at Fumesua for 2016 and 2017 cropping season

Table

Source of seed yam	2016 cropping season		2017 cropping season	
	Mean yield	Yield loss (%)	Mean yield	Yield loss (%)
Positive selection	12.5	-	5.6	-
Farmer Practice	9.40	25.0	2.4	57.0
Diseased	5.50	56.0	2.1	63.0

From the interaction Tables (Tables 4.3, 4.4, 4.5 and 4.6), yields obtained from positively selected Pona, Dente and Laribako and farmer practice Pona, Dente and Laribako were used to calculate the yield increase caused by using positive selection instead of farmer practice seed yams. The results are represented in Table 4.9.

Table 4.9: Yield from positive selection compared with farmer practice for the three local varieties from Ejura for the 2016 and 2017 cropping seasons

2016 Cropping Season					2017 Cropping season Yam Yield		Yield increase	Yield
Yield Yield increase								
variety	positive selection (Mg/ha)	farmer practice (Mg/ha)	Mg/ha	%	positive selection (Mg/ha)	farmer practice (Mg/ha)	Mg/ha	%
Dente	10.5	6.8	3.7	53.0	4.0	0.9	3.0	325.0
Laribako	9.70	8.1	1.6	19.0	6.2	5.1	1.0	20.0
Pona	10.0	8.8	1.2	13.0	5.4	4.6	0.8	17.0

In 2016, in terms of yield performance of the interaction between variety and seed yam sources at Ejura, positive selected Dente recorded the highest yield of 10.5 Mg/ha, followed by Pona with

10.0 Mg/ha and then Laribako with 9.7 Mg/ha whereas in 2017, positive selected Laribako gave the highest yield of 6.2 Mg/ha followed by Pona positive selection (5.4 Mg/ha) and Dente positive selection (4.0 Mg/ha) (Table 4.9). For the interaction between the yam varieties and two seed yam sources, positive selection and farmer practice, (Table 4.9), the percentage tuber yield increase was 53, 19 and 13% for Dente, Laribako and Pona respectively in 2016 cropping season while in 2017 cropping season, the percentage tuber yield increase was 325, 20 and 17% for Dente, Laribako and Pona respectively. The highest yield increase for using positive selection was observed on positive selection Dente. Similar observation was also made at Fumesua (Table 4.10).

Table 4.10: Yield from Positive selection compared with Farmer practice for three local varieties from Fumesua for the 2016 and 2017 cropping seasons

2016 Cropping season					2017 Cropping season			
Yam variety	Yield positive selection (Mg/ha)	Yield farmer practice (Mg/ha)	Yield increase		Yield positive selection (Mg/ha)	Yield farmer practice (Mg/ha)	Yield increase	
			Mg/ha	%			Mg/ha	%
Dente	13.5	9.1	4.4	48.4	5.4	1.8	3.6	200.0
Laribako	11.0	9.6	1.4	14.0	6.2	2.7	3.5	130.0
Pona	13.0	9.6	3.3	35.0	5.1	2.7	2.4	90.0

For Fumesua, in 2016 cropping season, Dente gave the highest yield increase of 46% followed by Pona with 35% yield increase and Laribako gave the least percentage yield increase of 14%

while in the 2017 cropping season, the percentage yield increase was 193, 130 and 90% for Dente, Laribako and Pona respectively. The results for the 2017 cropping season followed the trend of that of Ejura (Table 4.9).

4.4 Screen house experiment for degeneration studies

Among the nine treatments, positively selected ones had the least viral disease symptom incidence and disease symptom severity in 2016 cropping season. In the 2017 cropping season, all treatments became infected but farmer practice and diseased materials had higher virus severity score as compared to plants raised from positive selection (Table 4.11).

Mean viral disease incidence for positive selection was lower in the screen house as compared to that of the field experiments at Ejura and Fumesua. There were however no differences in the disease severity between those in the field and those in the screen house (Tables 4.3, 4.4, 4.5, 4.6 and 4.11).

Table 4.11: Percentage mean yam virus incidence and severity score for 2016 and 2017 cropping season in the screen house experiments

	2016 cropping Season	2017 cropping season

Treatment	% Mean virus incidence	Mean virus severity (1-5)	% Mean virus incidence	Mean virus severity (1-5)
Dente positive selection	50.8	2.7	100.0	3.0
Dente farmer practice	75.0	2.6	100.0	3.5
Dente diseased	100.0	3.7	100.0	4.3
Laribako positive selection	58.3	2.3	100.0	2.5
Laribako farmer practice	83.3	2.1	100.0	2.8
Laribako diseased	100.0	4.3	100.0	4.8
Pona positive selection	65.8	2.5	100.0	2.2
Pona farmer practice	100.0	3.3	100.0	3.8
Pona diseased	100.0	4.3	100.0	4.8

CHAPTER FIVE

5.0 DISCUSSION

5.1 Survey to determine yam viral disease symptoms incidence and severity in selected communities in Ejura-Sekyedumase and Atebubu-Amantin Districts in the 2015 and 2016 cropping seasons

The main yam virus symptoms observed during the survey were mosaic, chlorosis, shoe string, mottle and reduced leaf sizes. Similar observations were made by Eni *et al.* (2012) and Eni (2009) in disease diagnostic survey conducted in the Guinea-Savanna and the Forest-Savanna agro ecological zones of Ghana. The overall percentage mean viral disease symptoms incidence and disease severity score obtained for both Ejura-Sekyedumase and Atebubu-Amantin districts, showed that there was high incidence of yam viral disease symptoms in these yam growing areas. These two districts are among the known yam growing areas in Ghana and this gives an indication of the need to manage yam viruses to improve yam production. The results obtained for the survey at Ejura, also provided a strong justification for conducting the yam degeneration studies under natural conditions at Ejura and Fumesua since they serve as hotspot for yam viruses due to continuous planting of the crop at both locations. There was higher viral disease incidence and severity at Abour as compared to that of Mem (both in the Atebunu-Amantin District) because there was 100% viral disease incidence and a severity score of 3.3. Comparing the viral disease symptoms incidence and disease severity at both Ejura- Sekyedumase and Atebubu-Amantin Districts during the 2015 survey, Atebubu-Amantin had a higher viral disease incidence and severity than Ejura-Sekyedumase. Lower viral symptom incidence was observed at Asanteboa in the Atebubu-Amantin district with three of the farms having less than 30% viral symptom incidence and a mean severity score of 2. This means that the yam plants in this community are mildly infected with viruses and as such it would be a good starting point for positive selection to

be practiced by farmers and the seed yam be used for the subsequent cropping season in order to reduce the virus load associated with the crop.

5.2 Laboratory Analysis to identify the yam viruses causing quality loss of seed yam using reverse transcription polymerase chain reaction (RT-PCR).

Out of the 127 leaf samples tested, less than 35% of the samples tested positive for the two primers, YMV and YMMV. The higher number of mixed infections raises much concern to yam production in those yam growing areas in Ghana. Mixed infections often produce synergistic effects of viruses resulting in higher virus accumulation and movement with a significant yield reduction (Eni *et al.,* 2013; Gutierrez *et al*., 2003; Pio-Ribeiro *et al.,* 1978). YMV and YMMV single infections recorded confirmed observation made by Eni *et al*. (2010) that there was high incidence of YMMV and YMV in the Forest-Transition zone of Ghana. This result gives an indication that YMV and YMMV are still prevalent in this area. YMV is considered to be the most important yam virus in Ghana and in the West Africa sub-region as a whole (Legg *et al.,* 2007; Oppong *et al*., 2007; Olatunde, 1999). This calls for pragmatic measures to manage this virus in yam growing areas and positive selection can be considered as one of the viable options. The use of virus-free yam seeds could help eradicate yam mosaic disease (Thouvenel *et al.,* 1989).

5.3 Seed yam degeneration studies in 2016 cropping season

The viral disease symptoms incidence was higher at Ejura compared to Fumesua and as such, the total tuber yield obtained from Fumesua was higher than that of Ejura. This may imply that, the higher the viral disease incidence on yam plants, the lower the total tuber yield. This confirms that

yam viruses are able to cause significant reduction in the tuber yield to as high as 50% as reported by Amusa *et al.* (2003).

Among the varieties that were planted, the performance of Dente was outstanding in terms of tuber yield in the 2016 cropping season at both locations but tuber yield at Fumesua was better than Ejura and likewise the yam virus disease incidence and severity. Perhaps Dente produce higher yield than other white yams considering the assertion by Ennin *et al.* (2009) that Dente out-performed Pona in their report. This means that with a reduced virus incidence and severity, Dente as a variety, did better in terms of tuber yield when compared with Pona or Laribako. However, in 2017 cropping season, when yam virus incidence and severity increased as a result of accumulation of virus over time, Laribako gave the best tuber yield followed by Pona, then Dente in both Ejura and Fumesua. Probably, Laribako and Pona showed higher level of tolerance in the presence of high viral infection as compared to Dente that could not produce appreciable yields.

The increase in viral incidence in the 2017 cropping season, especially with the positive selection plants probably indicated higher re-infection through aphid vectors that transmit the mosaic virus. The general increase in viral disease symptoms severity from 2016 to 2017 probably indicates that there was increase in the concentration levels of viruses with time in the seed yams, thus continuous use of the same seed yam, season after season causes loss of quality of seed yam as a result of the yam seed becoming infected (Kenyon *et al.,* 2001). This shows degeneration of the seed within a space of one year with more than half of the yield realized in 2016 cropping season lost. It is therefore recommended that positive selection be practiced every season to avoid recycling of infected seed yams in subsequent seasons.

The tuber yield at Fumesua being better than that of Ejura could be as a result of inadequate rainfall at Ejura as compared to the annual rainfall at Fumesua (Adu and Asiama, 1992). This affected the total number of sprouts of all the treatments at Ejura and thus a reduction in yield since yield is a function of plant population (Akbar *et al.,* 2010). Also, the higher prevalence of virus incidence and severity recorded at Ejura also had direct negative effect on the tuber yield there.

The outstanding tuber yield out-put and lower viral disease incidence and viral disease severity recorded by positive selected plants irrespective of the variety compared to farmer practice was in line with the observations made by Gunadi *et al.* (2017), Kakuhenzire *et al.* (2013), Gildemacher *et al.* (2012) and Schulte-Geldermann *et al.* (2012) that positive selection has the potential of reducing disease incidence thereby increasing yield.

The importance of positive selection cannot be over emphasized, in that, the difference or percentage tuber yield loss as a result of using farmer practice seed yams as opposed to positive selection was higher for 2016 and 2017 cropping seasons at Fumesua compared to Ejura. The practice of positive selection reduced virus infection thereby reducing tuber yield loss that would have been caused by those yam viruses. It therefore implies that farmers can maintain appreciable tuber yields to sustain their livelihood if positive selection is practiced.

Although there was a sharp decrease in tuber yield from 2016 to 2017 cropping seasons, that of Dente was exceptional for both positive selection and farmer practice seed sources. Similar observation was made at Fumesua. This clearly showed the effect of recycling of seed yam. Increase in viral disease symptoms incidence causes adverse effect on tuber yield of Dente as compared to Laribako and Pona. Recycling of seed yam causes the quality of the seed to degenerate as result of tuber-borne diseases mainly viruses accumulate and cause the yield potential of seed

yam to diminish (Struik and Wiersema, 1999; Salazar, 1996). Tuber yield loss can be avoided by regularly replenishing seed yam stocks by high-quality seed yam with little virus infection, a situation that positive selection sought to address.

The seven white yam positive selected leaf samples out of which five did not test positive for any of the primers means that the probability of selecting a healthy or virus-free plant is very high. As such the continued use of positive selection with time would help to bring the yam virus disease load to the barest minimum. The two samples that tested positive for both viruses could be as a result of latent infection where an infected plant may not show any visible symptoms (Eni *et al.*, 2010). It can therefore be said that those plants carry some level of tolerance that is why they were not showing the symptoms or the virus concentration is low and as such it is unable to produce symptoms. Currently, there are no resistant varieties of yam to these yam viruses so the practice of positive selection could make available seed yams that are tolerant to yam viruses and at the same time, the virus load at the yam growing areas could also be reduced in the long run. This will eventually increase the income of the resource-poor farmers for a better livelihood.

The high number of symptomatic leaf samples that did not show any amplification for the two primers tested also suggests that there could be other yam viruses such as CMV and Badnaviruses (Yeyeh *et al*., 2014) that were not tested for in this work but their incidence were reported in Ghana by Eni *et al.* (2010) and Olatunde (1999). The symptoms could also be due to some abiotic agents. It also implies that laboratory diagnostics is the best means by which the health status of a plant can be declared (Yeyeh *et al*., 2014).

5.4 Screen house experiment for degeneration studies

The results from the screen house study suggest that yam viruses (YMV and YMMV) are seedborne viruses that was why the plants in the screen house still showed symptoms even in the absence of the aphid vector. In 2016 cropping season, positive selected plants had lower percentage virus incidence and severity score as compared to farmer practice seed yams. This indicated the reduction of viral disease incidence and severity among plants that were obtained from positive selection. However, in 2017 cropping season, all the plants from all the nine treatments became infected but the differences were in their severity scores with positive selected plants having the least severity scores. This also explains that the severity of infected seed yams increased as the seed yams were recycled season after season thereby increasing the virus load. That is why the positive selected plants (mildly infected) with time had increased virus severity (Gunadi *et al.,* 2017).

Yam virus incidence for the screen house experiment compared to the field study was lower especially for the positive selection plants. This is possibly due to higher re-infection by aphid vector under field conditions.

5.5 CONCLUSION AND RECOMMENDATIONS

The study has shown that there was high prevalence of mosaic viruses on yam in the EjuraSekeyedumase and Atebubu-Amantin which are important yam growing areas in Ghana. It therefore raises concern for the situation to be salvaged.

This high virus load in areas where the crop is grown could be reduced if farmers are trained to adopt the use of positive selection as it was found from this study that positive selection to reduced viral disease incidence and severity and thereby increased tuber yield. Efforts had been made to manage the yam virus disease by developing disease resistant varieties and also produce planting

materials from tissue culture, but currently, there are no available resistant yam varieties and adoption and promotion of tissue culture planting materials have been low due to their unavailability and high cost. The availability of tolerant seed yam using positive selection technique looks the most viable alternative to reduce the prevalence of yam viruses in yam growing areas within the country.

The positive selection technique is therefore highly recommended to farmers because it is simple, cost effective, reduces virus disease incidence thereby increasing yield to ensure food security and also improve farmers' income for a better life. Positive selection can also reduce the rate of seed degeneration if it is practiced season after season.

The degeneration studies showed that recycling of seed yam from one cropping season to another without selecting healthy looking or mildly viral infected plants for planting the subsequent cropping season, produced high viral infection, reduced number of sprouts and reduced tuber yield even among positive selection.

The detection of YMV and YMMV in single and mixed infections in white yam leaf samples using RT-PCR suggests the prevalence of yam viral disease in the major yam growing areas in Ghana remain same, perhaps even more severe because of the presence of mixed infections.

Another survey should be conducted in all yam growing areas across the country to assess the current viral diseases situation. During the survey, symptomatic and asymptomatic leaf samples should be taken for laboratory diagnostics using primers such as YMMV, YMV, Badnaviruses and Cucumber mosaic virus (CMV) that have been reported within the 'Yam Belt' of West Africa. This will help to know the current state of yam virus diseases prevalence in Ghana.

Seed yams raised from virus indexed tissue culture or aeroponics materials should be included as seed source for future studies. This will provide a holistic picture for seed quality degeneration with time using different seed sources.

Positive selection technique should be practiced by farmers every season in order to ensure that seed yam for subsequent planting season is healthy or mildly infected in order to improve the tuber yield for both local and export markets.

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APPENDICES

Appendix 1

PCR Components

	x1/µl PCR
water	1.75
One Taq One step reaction mix(2x)	6.25
One TaqOne Step enzyme mix(25x)	0.5

Primer mix	YMV(F+R)	1
	YMMV(F+R)	1
RNA Template		2
		12.5µl

Thermal Cycle Conditions

42˚C ---- 30mins

92˚C ----- 5mins

94˚C ----- 40sec }

55˚C ----- 40sec } 35cycles

72˚C ----- 40sec

72˚C ----- 5mins

4˚C -------α

Appendix 2

The virus incidence and severity in all the 40 farms visited in the Atebubu-Amantin District are presented in the graphs below:

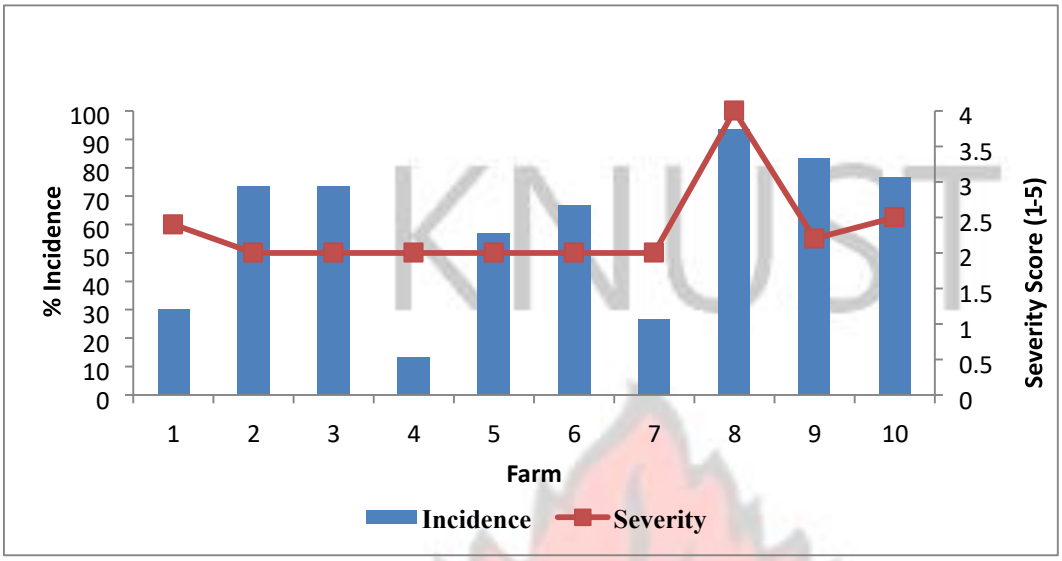

Figure 4.5 Mean incidence and severity of virus disease symptoms in 10 farms at Asanteboa in Atebubu-Amantin District

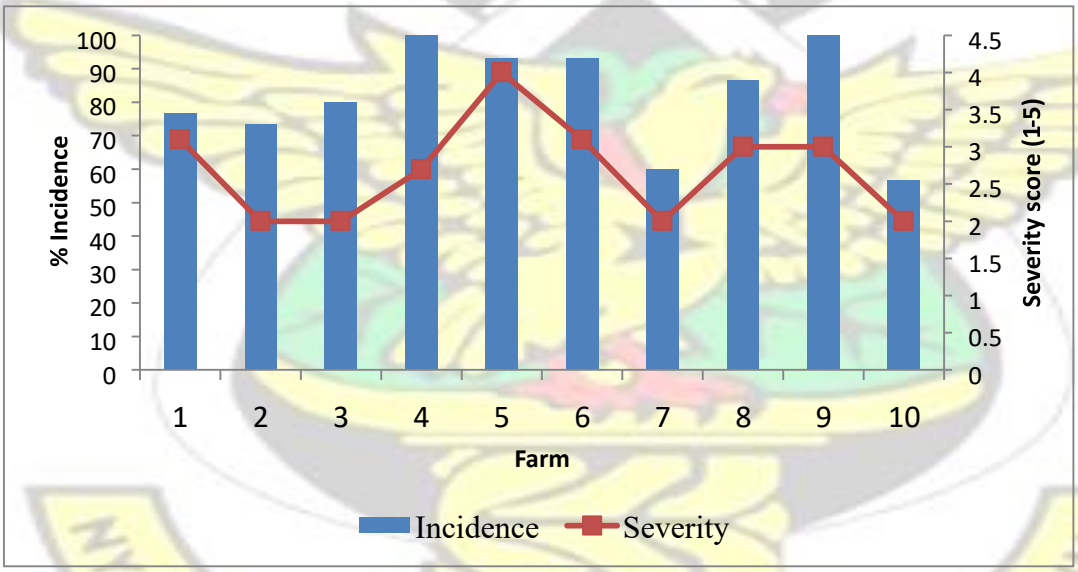

Figure 4.6 Mean incidence and severity of virus disease symptoms in 10 farms at Watro in Atebubu-Amantin District

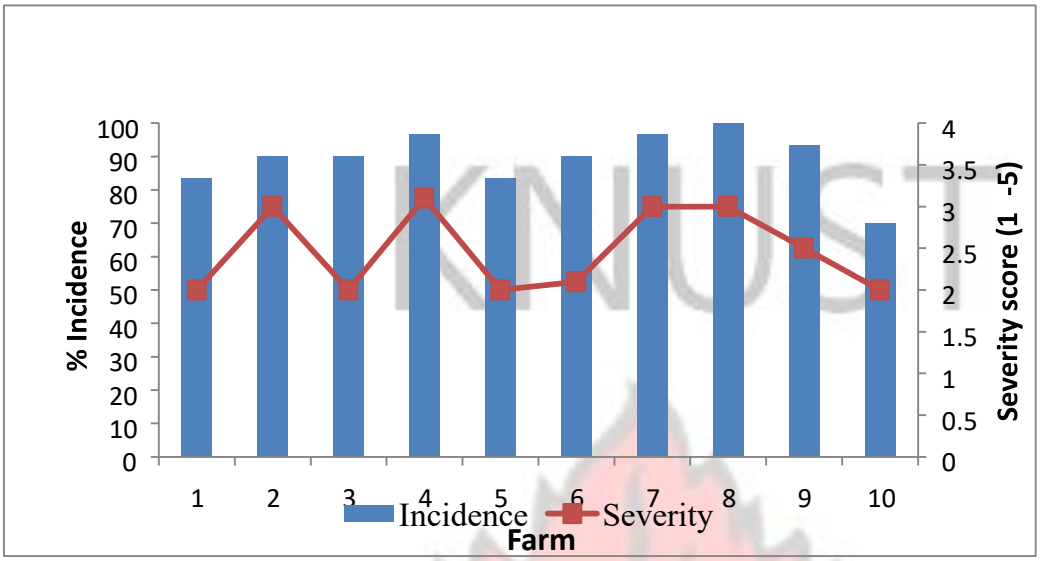

Figure 4.7 Mean incidence and severity of virus disease in 10 farms at Ahotor in Atebubu-Amantin District

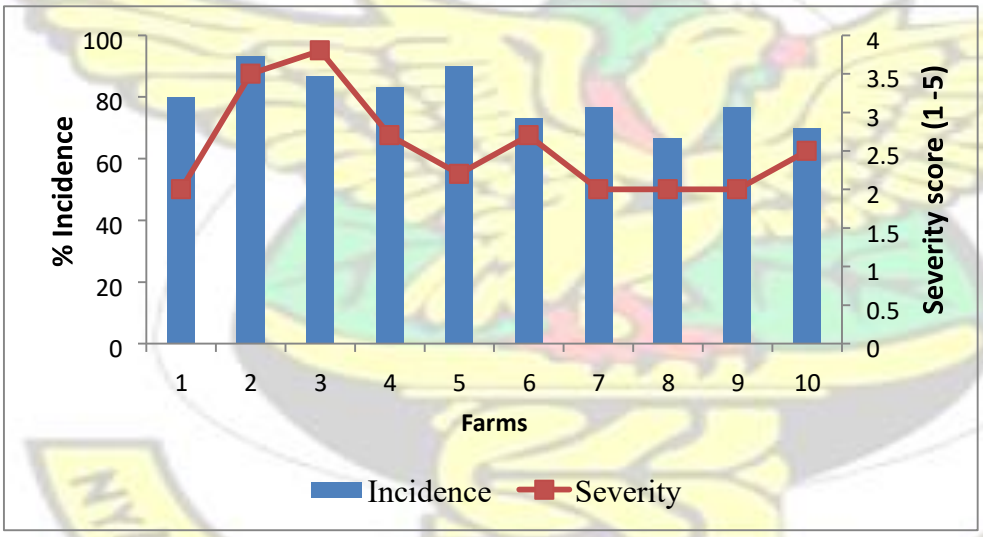

Figure 4.8 Mean incidence and severity of virus disease in 10 farms at Densi in Atebubu-Amantin District

The virus disease incidences and severity scores for the 40 farms visited in the EjuraSekyedumase District have been represented in the graphs below:

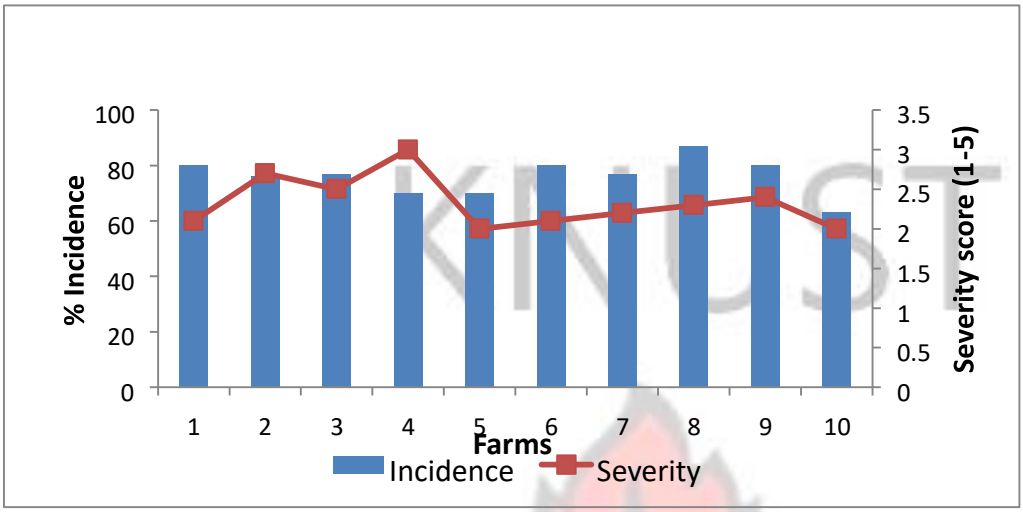

Figure 4.9 Mean incidence and severity of virus disease in 10 farms at Kramokrom in EjuraSekyedumase District

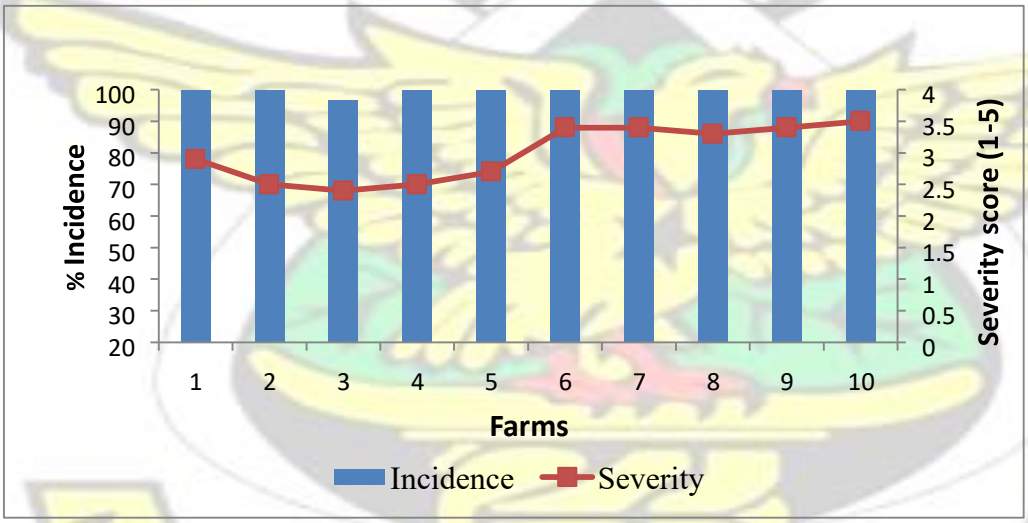

Figure 4.10 Mean incidence and severity of virus disease in 10 farms at Mmesuo in Ejura-Sekyedumase District

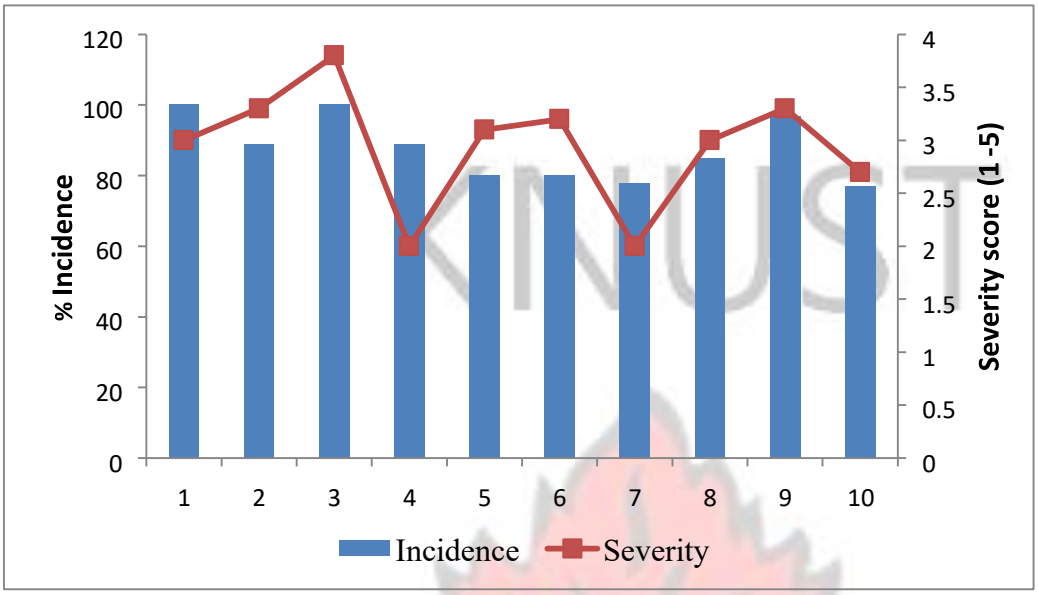

Figure 4.11 Mean incidence and severity of virus disease in 10 farms at Nokwareasa in Ejura Sekyedumase District

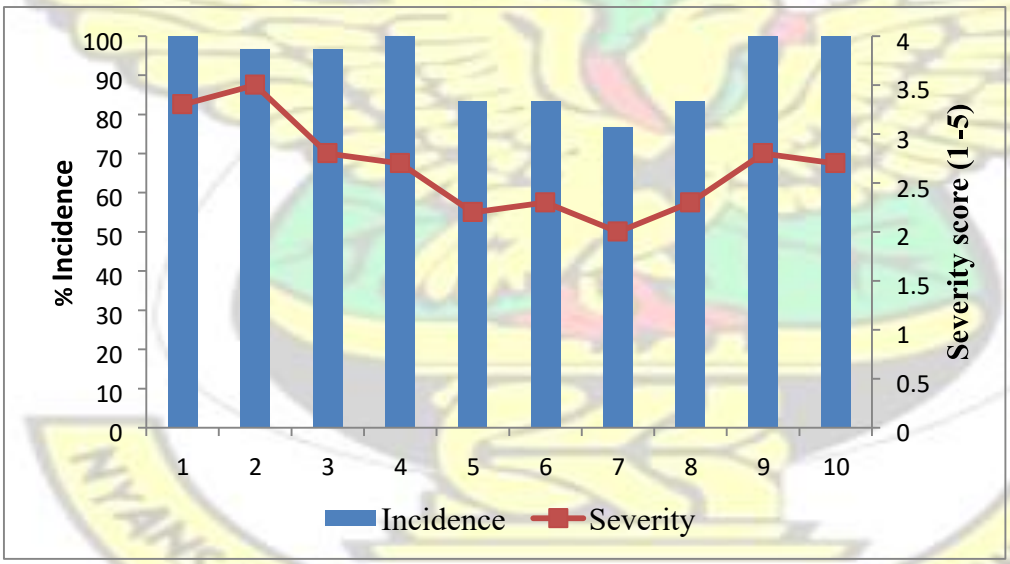

Figure 4.12 Mean incidence and severity of virus disease in 10 farms at Kasei in Ejura-Sekyedumase

Appendix 3

3.1. Table representing scores from gel image for Plate 4.7

Sample no.	well number	YMV score	YMMV score
100bp	1		-
SPACE	2		-
YMV	3	+	-
YMMV	4	-	+
1	5	-	-
2	6	-	-
3	7	-	-
4	8	-	-
5	9	-	-
6	10	-	-
7	11	-	-
8	12	-	+
9	13	-	-
10	14	-	-
11	15	-	+
12	16	-	-
13	17	-	-
14	18	-	-
15	19	-	+
16	20	-	+
17	21	-	-
18	22	-	+
19	23	-	+
20	24	-	-
21	25	-	-
22	26	-	+
23	27	-	-
24	28	-	+
25	29	-	-
26	30	-	-

Note: "+" means presence of virus, while "–" means absence of virus

Sample number 1 to 26 are from Ejura-Sekyedumase District

3.2. Table representing scores from gel image for Plate 4.8

Sample no.	well number	YMV score	YMMV score
100bp	31		
Space	32		
YMV	33	+	-
YMMV	44	-	+
27	35	-	-
28	36	-	-
29	37	-	-
30	38	-	-
31	39	-	-
32	40	-	-
33	41	+	+
34	42	-	-
35	43	+	+
36	44	+	+
37	45	-	-
38	46	+	+
39	47	+	+
40	48	+	+
41	49	+	+
42	50	+	+
43	51	-	-
44	52	-	-
45	53	-	-
46	54	-	-
47	55	-	-
48	56	-	-
49	57	-	-
50	58	-	-
51	59	-	-
52	60	-	-

Note: "+" means presence of virus, while "–" means absence of virus

Sample number 27 to 52 are from the experimental fields; Ejura and Fumesua

3.3. Table representing scores from gel image for Plate 4.9

sample no.	well number	YMV score	YMMV score
100bp	61		
Space	62		
YMV	63	+	–
YMMV	64	-	+
53	65	-	-
54	66	-	-
55	67	-	-
56	68	-	-
57	69	-	-
58	70	+	+
59	71	+	+
60	72	-	-
61	73	-	-
62	74	-	-
63	55	-	-
64	76	-	-
65	77	-	-
66	78	-	-
67	79	-	-
68	80	-	-
69	81	-	-
70	82	+	+
71	83	-	-
72	84	-	-
73	85	-	-
74	86	+	+
75	87	+	+
76	88	+	+
77	89	-	-
78	90	-	-

Note: "+" means presence of virus, while "–" means absence of virus

Sample number 53 to 73 are from the experimental fields; Ejura and Fumesua

Sample number 74 to 78 are from Ejura-Sekyedumase District

3.4. Table representing scores from gel image for Plate 4.10

Sample no.	well number	YMV score	YMMV score

100bp	91		
Space	92		
YMV	93	+	-
YMMV	94	-	+
79	95	+	+
80	96	+	+
81	97	-	-
82	98	-	-
83	99	+	+
84	100	-	-
85	101	-	-
86	102	-	-
87	103	-	-
88	104	-	-
89	105	-	-
90	106	+	+
91	107	+	+
92	108	-	-
93	109	-	-
94	110	+	+
95	111	-	-
96	112	+	+
97	113	-	-
98	114	-	-
99	115	-	-
100	116	-	-
101	117	-	-
102	118	-	-
103	119	-	-
104 (Negative Control)	120	-	-

Note: "+" means presence of virus, while "–" means absence of virus.

Sample number 79 to 87 are from Ejura-Sekyedumase District

Sample number 88 to 103 are from Atebubu-Amantin District

3.5. Table representing scores from gel image for Plate 4.11

Sample no.	well number	YMV score	YMMV score
100bp	121		
Space	122		
YMV and YMMV	123	+	+
YMMV	124	-	+
105	125	+	+
106	126	+	+
107	127	+	-
108	128	+	-
109	129	+	-
110	130	+	+
111	131	+	+
112	132	+	+
113	133	-	-
114	134	+	-
115	135	+	-
116	136	-	-
117	137	+	+
118	138	+	-
119	139	-	-
120	140	+	-
121	141	+	-
122	142	-	-
123	143	-	-
124	144	-	-
125	145	-	-
126	146	-	-
127	147	-	-
128	148	-	-
129 (Negative Control)	149	-	-

Note: "+" means presence of virus, while "–" means absence of virus.

Sample number 105 to 128 are from Atebubu-Amantin