Addis Ababa University

School of Graduate Studies

Institute of Biotechnology

DETECTION AND IDENTIFICATION OF YAM MOSAIC AND YAM MILD MOSAIC POTYVIRUSES IN MAJOR YAM (*Dioscorea* spp.) GROWING AREAS OF SOUTH-WESTERN ETHIOPIA

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This is to certify that the thesis entitled with Detection And Identification of Yam Mosaic and Yam Mild Mosaic Potyviruses in Major Yam (*Dioscorea* Spp.) Growing Areas of South-Western Ethiopia is prepared by Demilew Deres and submitted in partial fulfillment of the requirement for the degree of Master of Science in Biotechnology complies with the regulation of the university and meets the accepted standards with respect to originality and quality.

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LIST OF ABBRIVATIONS

ACP-ELISA	Antigen coated plate ELISA
BARC	Bako Agricultural Research Center
CP	Coat protein
CMV	*Cucumber mosaic virus*
DAS-ELISA	Double antibody sandwich ELISA
DaBV	*Dioscorea alata bacilliform virus*
DMoV	*Dioscorea mottle virus*
DsBV	*Dioscorea sansibarensis bacilliform*
HARC	Hawassa Agricultural Research Center
IgG	Immuno-globulin
IITA	International Institute of Tropical Agriculture
JARC	Jimma Agricultural Research Center
JYMV	*Japanese yam mosaic virus*
PBS	Phosphate buffer saline
PB	Phosphate buffer
PVP	Polyvinyl pyrolidone
p-NPP	p- nitrophenyl phosphate
RT-PCR	Reverse transcription-polymerase chain reaction
YMMV	*Yam mild mosaic virus*
YMV	*Yam mosaic virus*
UTR	Untranslated region
VPg	Viral protein genome-linked

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ABSTRACT

Yam is the common name for some plant species in the genus *Dioscorea (*family *Dioscoreaceae)* that form edible tuber. Yams are herbaceous or dioecious annual or perennial vines cultivated for their edible starchy tuber in Africa, Asia, Latin America and Caribbean. Yam production is negatively affected by pests and diseases in the world. Among these, virus diseases are the most important concerns of yam production as they strictly restrict the exchange of germplasms for various purposes in breeding programs. Among viruses reported to be infectious of yam crop in the world the genus *Potyvirus* is the largest group of viruses in *Potyvirideae* family and is the most widespread pathogen of yam virus diseases in the world. *Yam mosaic virus* (YMV) and *Yam mild mosaic virus* (YMMV) are the two commonly reported *Potyviruses* to be widespread across yam growing areas of tropics and subtropics. This study was therefore designed to detect *Yam mosaic* and *Yam mild mosaic Potyviruses* in major yam growing areas of South-Western part of Ethiopia. ACP-ELISA, DAS-ELISA and RT-PCR were used to test a total of 336 yam leaf samples collected from Jimma Agricultural Research Center, Bako Agricultural Research Center and Hawassa Agricultural Research Center for the two common *Potyvirus*es (YMMV and YMV). Among 336 total samples tested in this study 40 (12%) and 32 (9.5%) of them were found positives for potyvirus in ELISA and RT-PCR tests respectively. YMV was found in 30 (75%) and 38 (95%) of samples confirmed positive for potyvirus via DAS-ELISA and RT-PCR respectively, whereas YMMV is detected in none of the samples. This study therefore provides valuable information about YMV, to be the important *Potyvirus* attacking yams in Ethiopia, and is required to devise effective methods for the control of YMV disease on yams.

Key words: Yam, *Potyvirus*, *Yam Mosaic Virus* (YMV), *Yam Mild Mosaic Virus* (YMMV), Reverse Transcription PCR (RT-PCR) and ELISA

1. INTRODUCTION

Yam is the common name for some plants in the genus *Dioscorea* (family *Dioscoreaceae*) that form edible starchy tuber depending on the type species. The species are herbaceous and dioecious annual and perennial vines cultivated for their edible starch tuber in Africa, Asia, Latin America and Caribbean. They are mono-cots and related to grasses, comprising both diploid and polyploidy species. In West Africa yam is a major source of income, and the festival is held annually to celebrate its harvest. Yams (*Dioscorea* spp.) are tuber bearing plant and constitute over 600 species, out of which six are socially and economically important in terms of food, cash and medicine (IITA, 2009). It is widely propagated and distributed throughout tropics and subtropics of the world. Yam is among the most important root and tuber crops cultivated in West Africa yam belt countries including Nigeria, Togo, Cameron, Benin, Ivory Coast, Ghana with over 93% of the total yam production in the world (FAO, 2013; Eny *et al*., 2010). Over 300 million people in the world survive poverty due to yam being as a source of food and means to generate income (Mignouna *et al.*, 2003). It can be processed and used as food in the forms like pounded, boiled, roasted or fried and can be dried to produce yam flour (Ayendoji *et al*., 2012; Oluwolw *et al*., 2013).

It is the best crop to provide carbohydrates, proteins, minerals, and some medicinal important compounds like dioscorin and diosgenin, and helps people in the tropics and subtropics to meet their need for food and health. It is ranked fourth among the most important tuber crops in the world, next to potato, sweet potato and cassava (Levand and Shriver, 1998, cited in Mignouna and Dansi, 2003). It is one of the most important tuber crops contributing vitamin A, zinc and iron towards the dietary demand of the regions fast growing towns and cities (IITA, 2015). Yam production in general is an integral of food security programs and a means of income generation in Africa (Verter, 2014).

As indicated in several accounts, there are three independent areas of tropics including West Africa, Asia and America where different species of yam in the world are originated (Aleander and Courtsey, 1969; Hahn *et al*.,1987). According to FAO (2005), the annual production of yams in West Africa and Ethiopia is 10,088 and 4,065 kg/ha

respectively. Only 10% and 30% of yam species are cultivated in Africa to serve the purpose for food production and extraction of pharmaceutical compounds (discorin and diosgenin) respectively.

According to Zeven and Wet (1982) Ethiopia is the center of origin for one of *Dioscorea* species (*D. abyssinica Hochst.ex Kunth*). Many different accessions of this species are extensively cultivated by subsistence farmers in the Southern, South-western and Western parts of Ethiopia across a range of agro-ecologies (Miege and Sebsebe, 1997). Most importantly people in these parts of Ethiopia use yams as the most preferred food item for distinguishable guests and during the main traditional celebration of Meskel Holiday (Muluneh *et al*., 2005).

The role and production of yam in West Ethiopia is reported to be better than other crops due to its tolerance to drought stress. As a result, yam plantation is conducted when the rain season comes to end in the beginning of October (Gemeda, 2000). The moisture content of soil is enough to support the growth of yams in this potential growing area of Ethiopia. According to the study by Muluneh *et al* (2005) some species are also found in the wild and often collected for food in various localities.

Despite the tremendous importance of yam production for food security and hunger reducing programs and, pharmaceutical factory particularly in the developing countries the production is not as such of its demand, and it has shown fluctuation since 2007 (FAO, 2012). High cost of labor and planting material, lack of mechanization for planting and harvesting the tuber, pests and diseases are accounted to be the constraints of yam production (Atiri *et al*., 2003). Pests and diseases caused by a single or more combination of bacteria, fungi, nematodes and viruses are the major constraints of yam production in the world (Odu *et al*., 1999).

Although pests and pathogenic diseases caused by fungi, bacteria, viruses, and nematodes are responsible for yam yield loss, viruses are particular concern as they affect international exchange of germplasm and also lead to genetic erosion. As with other root and tuber crops, vegetative propagation techniques particularly those

practiced by farmers in *Dioscorea* species is a major reason for the crop to be vulnerable to virus's diseases.

Therefore, seeds and propagative materials serve the virus as important mode of transmission. Viruses belong to the genus *Potyvirus*, *Badnavirus*, *Cucumovirus, Potexyvirus*, *Carlavirus, Comovirus* and others with no established classification have been reported to cause disease in *Dioscorea* species from mild to high severe ranges. Among these, the genus *Potyvirus* have been reported to be highly severe for yam production throughout yam producing zones of West Africa (Seka *et al*., 2014; Adeniji *et al*., 2012). Virus diseases result in decline in tuber quality and quantity as it causes the reduction in photosynthetic efficiency in plants through leaf deformation and discoloration upon their infection (Seka *et al*., 2014).

Yam mosaic virus (YMV) is the top prioritized economically important virus infecting almost all types of *Dioscorea* species non-selectively and is the highest prevalent *Potyvirus* (Amusa *et al.,* 2003). It is therefore, the first-place question to ensure the health status of yam varieties upon their transfer across countries for a specified purpose in research programs. To be successful in creating and maintaining a system for risk free germplasm transfer, it is essential to index planting materials for previously reported viruses. Although considerable research have been done on *Dioscorea* species cultivated in Ethiopia, none of them are on the pathological aspect of the crop that is equally important to research on the genetics of the crop towards improving its production and productivity.

As a result, in Ethiopia there is a paucity of information available on the primary pathogens or pests causing the poor survival or growth of yams. The detection and then identification of the virus causing the disease is a vital comportment of disease control and management strategy development (Yayeh *et al*., 2014). This is the first paper to report the estimated incidence of the most common *Potyvirus*es (YMV and YMMV) in *Dioscorea* species cultivated in Ethiopia.

2. OBJECTIVES

2.1 General Objective

- To detect and estimate the incidence of potyviruses in major yam growing area of South-Western Ethiopia.

2.2 Specific Objectives

- To detect and estimate the incidence of *Yam Mild Mosaic Virus* (YMMV) and *Yam Mosaic Virus* (YMV) from yam leaf samples.

3. LITERATURE REVIEW

3.1. Yam Taxonomy

Phylogenetic relationships of yams have not been well established because of difficulties in species identification due to a high level of polymorphism in morphological characters. There are some features of yams like the presence of non-emergent cotyledon and reticulate-veining of the leaves which are typical of some dicotyledonous plants, that gravitates to the idea that yams have originated from plant forms that occur before differentiation of mono-cots and dicots even though the family *Dioscoreaceae* is classified under monocotyledon (Degras, 1993).

There have been some complications regarding the classification of food yams despite progresses have been made towards the detail understanding of the origin, phylogeny and distribution of *Dioscorea* species until studies on yam diversity and phylogeny using protein and DNA (RFLP) markers were conducted (Dansi *et al.,* 2000; Mougnouna *et al.,* 2005; Wilkin *et al.*, 2005). Phylogenetic analysis of yams based on sequence data of plastid gene (rbcL, matK) using the main old world and selected new world lineages representing 67 *Dioscorea* species revealed that there are two distinct lineages among the endemic Malagasy taxa (Wilkin *et al.*, 2005).

On the other study the phylogenetic analysis of Oceanian staple yams (species of *Dioscorea* section Enantiophyllum) using plastid trnL-F and rpl32-trnL (UAG) sequences and nine nuclear co-dominant microsatellites revealed the natural interspecific hybridization of the greater yam (*D. alata*) (Chair *et al*., 2016)

3.2. Yam Morphology

Yam tubers are enlarged storage organs containing food reserves in the form of starch, and vary greatly in size ranging from small potato size to over 2.5 m in length, and may weigh up to 7.5 kg (RHS, 1992). They are round, cylindrical, oval or flattened (Toyohara *et al*., 2000) and are usually produced underground.

Depending on the variety, the tuber flesh may be various shades of off-white, yellow, purple, or pink, and the skin can be off-white to dark brown. Some species of yam,

such as *D. bulbifera,* produce aerial tubers. Yam shoots are climbing and vine-like thus usually requiring support from either neighboring plants or stakes on which they twine clockwise or anticlockwise depending on the species (Johnson, 2003).

Shoots can be round, winged or ridged, some bearing spines at the base. Leaves are opposite, heart-shaped, ovate to oblong, with a deeply cordate base. Yam is dioecious, producing small inconspicuous flowers about 2-4 mm in diameter. Flowers are white, cream, greenish or brown depending on the species and they are wind or insect pollinated.

Figure 1: Leaf morphology of yam plant (taken at HARC by Demilew Deres).

3.3. Yam Origin and Distribution

Yam belongs to the largest genus *Dioscorea* in the family of *Dioscoreaceae*. It is assumed that the family is among the earliest angiosperms that thought to be originated from Asia. It is also believed that, yams from Asia, to have been carried across Savannah to West Africa by Malaysians from Madagascar and then reached to Caribbean across Atlantic in the late 1450s (Jonson, 2003).

The introduction of yam species from West Africa to America is believed to take place in the 16th century (Coursey, 1967). There are also evidences for the origin and then distribution of the various yam species to have originated in three areas of tropics and subtropics (West Africa, South America and Asia). The two edible yam species (*D.*

rotundata and *D. caynensis*) are assumed to have originated in West Africa and then distributed to some other part of tropics (Coursey, 1976). *D. rotundata* is known to have wide land coverage, and extensively cultivated in West Africa and India, and to some extent in East Africa. This *Dioscorea* species is the most preferred type in West Africa since it produces tuber with high dry-mater content suitable for pounding. This is a clear indication of *D. rotundata* is the best productive potential species of yam.

The introduction of African yams to America occurred in the 16th century. These African yams have a very limited distribution and as a result they are little or not cultivated in Asia. The description of *D. rotundata* had been obtained in 1883 by Poiret before the belief that it is originated in Africa was established (Haman *et al.*, 2001).

There is a report that led to the belief that the water yam (*D. alata*) is originated in Southeast Asia and distributed to all the tropics and subtropics of America and West Africa. The distribution of this yam species to Africa was taken place 2000 years ago, and the introduction to Africa was achieved 1000 years ago. This yam species has become popular in Saharan Africa as it is easy for propagation. However, it is less acceptable to large number of consumers because its high water content creates difficulties during pounding to prepare *fufa* (types of food prepared from boiled yam tuber) in consistence. There are other species grown for other culinary uses in West Africa including the aerial yam (*D. bulbifera*) and the bitter yam (*D. dumetorum*).

3.4. Composition and Uses of Yams

3.4.1. Composition

Yam tubers are comprised of approximately 75.6-83.3 % carbohydrate, 3-7.4 % protein 0.5 -1.5% fiber, 0.7-2.0% ash, and 0.05-0.02% fat. A large proportion (65- 75%) of the yam tuber is made up of water (Degras, 1993).

They are a starchy staple food, rich in carbohydrates and are also valuable sources of some vitamins, particularly vitamin C. Yam tubers contain about 13-24.7 mg/100g ascorbic acid and most of it is retained during cooking (Coursey, 1969; Wanasundera and Ravindran, 1994). They are also very good source of minerals and are high in

dietary fiber, vitamin B6, potassium and manganese and low in saturated fat, sodium and cholesterol (Omonigho, 1988; Wanasundera and Ravindran, 1994; Walsh, 2003).

The high potassium and low sodium content of yam produces good potassium-sodium balance in the human body and so protect against osteoporosis and heart disease (Walsh, 2003). It is also confirmed that yam constitutes steroidal sapogenin diosgenin which serves as a precursor for the synthesis of steroidal drugs (Vendal *et al*., 2006).

Yam products are also reported to have lower glycemic index than potato products. Phytonutrients, minerals and vitamin content analysis and evaluation of some *Dioscorea* species indicates that yams are rich in bio-active compounds comprising of saponins, alkaloids, flavonoids, tannins and phenols in substantial amount, and ascorbic acids, riboflavin, thiamine, niacin, and appreciable quantities of minerals (Calcium, Magnesium, Phosphors, Potassium, and Sodium) (Okwu and Ndu, 2006).

Most of the wild edible yam species are the main source of protein, starch, crude fiber, lipid, minerals and vitamins, and antinutrational like phenolics, tannins, hydrogen cyanide, oxalate, amylase and trypsin inhibitor activities (Shajeela *et al.*, 2011).

3.4.2. Uses

Dioscorea is staple food crop in tropics and subtropics, particularly in poor countries. Yam is propagated in Africa for food, to generate income, celebrate fertility and marriage ceremonies, and in Ethiopia it is food for distinguished guests and Meskel Holiday ceremonies. The tuber part of the crop is the most expensive in the market among tuber crops, and thus it generates income for low income farmers. In Nigeria and Ghana, the two largest producers of yam, 60% of the production is sold for generating income while 40% is consumed at household level (Mignouna *et al*., 2014).

Despite yam is cultivated for its edible tubers, potential reservoir of protein in some extent and mainly carbohydrates of food security and industrial value, the foliage is nowadays also a renewable source of high value compounds (Price *et al*., 2016). Its medicinal importance is also reflected in several research outputs: it is used for blood pressure regulation to overcome hypertension (Liu *et al*., 2009), reduce the risk of

diabetics and obesity (Apriantia *et a*l., 2009), reducing menstrual pains, and it is a source of antioxidant (Lubag *et al*., 2008). The crop has substantial importance in East Africa, America and Asia for medicine, food, bio ethanol (Arnau *et al*., 2010).

3.5. Yam Cultivation, Propagation and Production

3.5.1. Cultivation

Yam cultivation began 11,000 years ago in Africa. Knowledge of the special cultural practices peculiar to yam is required for its successful cultivation. Edible yams are annual crops with basically two growth cycles. During the tropical wet season, the first stage of vegetative growth of vine extension and flowering takes place. During the dry season, most aerial growth stops and the nutrients are moved to the tuber, which grows before entering into a dormant state (Onwueme and Charles, 1994).

Yam requires a fairly high level of soil fertility and well-drained soil of pH 6-7. A minimum of 1000 mm rainfall or an equivalent amount of irrigation water is required over the 5-6 months during the aerial growth stage (Hahn *et al*., 1987; Osagie, 1992). Yam plantation by local farmers is conducted in Africa in February to April, and harvested 180 to 270 days later.

According to Osagie (1992) Land preparation for yam cultivation in West Africa begins with clearing of the vegetation. Mounds are prepared after clearing from early December to about mid-March. Mounds are 0.6-3 m in length and 0.9-1.3 m apart. These operations are completed before the onset of the major rains. Farmers normally plant whole yam called seed yam or cut pieces of yam, which are referred to as setts. Cut pieces used for planting usually have at least 2-3 ‘eyes’ (buds) to ensure sprouting. Cut surfaces of the pieces (setts) are allowed to dry before planting and are sometimes treated with fungicides.

Once planted, the yam remains dormant for some time or may sprout and send out a bare vine which will develop leaves when the rains come. After sprouting, the vines are sometimes trained onto stakes for adequate exposure of leaves to sunlight.

Figure 2: Yam cultivation at HARC (Photo by Demilew Deres)

3.5.2. Propagation and production

Most of edible yams are infertile, and the seed if they do set is not viable. Yams are mainly propagated and multiplied vegetatively through whole tuber (seed yam) saved from previous season, vine cuttings, and tissue culture. The total land area devoted to yam cultivation is about 5 million ha in the world (IITA, 2010). The world total annual production of yam is 56 million tones, 95% of which comes from five countries (Nigeria, Ghana, Benin, Ivory coast and Togo) in yam belt zones of Africa (Demuyakor *et al*., 2013) with Nigeria alone is 67%. The remaining 5% of the world's annual yam production is in other parts of Africa, the West Indies and parts of Asia, South and Central America, the Pacific and Ethiopia.

The land coverage of yam cultivation has also increased in Ethiopia, and Ethiopia became among top five yam producers next to Nigeria, Ghana, Ivory Coast and Benin (FAO, 2013). Muluneh *et al.* (2011) reported that in Ethiopia more specifically the Sidama, Gedeo, Wolayta and Gamo Goffa zones yam accessions with considerable genetic variability are cultivated and preserved by traditional means on farmers' land. In the other study conducted by Yeshetela and Temesgen (2016) yams are cultivated in South, Southwestern and Western part of Ethiopia. They play a role to ensure food security for farmers by continuously supplying food all year long.

Table 1: Mean annual production of yam from 1990 to 2005 (FAO, 2005).

Country of production	Area harvested ('000' ha)	Yield (Kg/ha)	Total production ('000' MT)
World	3,572	9,694	34,355
Africa	3,418	9,708	32,874
West Africa	3,149	10,088	31,388
Ethiopia[+]	68	4,065	277

Table 2: Ten major yam producing countries in the world by 2006 production data (FAO, 2007)

Country of Production	Quantity(Tons)	Area Harvested (Hectares)
Nigeria	36720000	3035000
Ivory Coast	4800000	540000
Ghana	3600000	300000
Benin	2239757	195747
Togo	621055	60246
Central African Republi	350000	58000
Colombia	332862	28011
Cameroon	300000	40000
Brazil	236325	25736
Chad	230000	24000

3.6. Production Constraints of Yams (*Dioscorea* spp.)

The production potential of yam species cultivated throughout the tropics and subtropics of the world is limited in one or more of the following reasons: lack of access to inputs, high cost of inputs, poor producer prices, lack of capital, incidences of pests and diseases, poor transportation facilities and inadequate extension services (Tetteh & Saakwa, 1991; Degras, 1993; Zaknayiba and Tanko, 2013; Reuben and Barau, 2012).

3.6.1. Insect pests and diseases of yam

Insect pests and diseases, grouped together, were ranked the second most important problem and were considered a major reason for the poor yam yields experienced by the majority of farmers in 1997. A total of 94 broad taxonomic diversity of insect pests from six different orders comprised of *Coloeptera*, *Orthoptera*, *Hymenoptera*, *Heteroptera* and *Hemiptera* have been identified as a potential threat of yam production (Asala *et al*., 2016).

Amsu *et al.* (2003) has reviewed in detail that anthracnose is regarded as the most widely spread of all the field diseases, while yam mosaic virus disease is considered to cause the most severe losses in yams, and dry rot is considered as the most devastating of all the storage diseases of yam. One of the major factors affecting yam production is the susceptibility of many of widely cultivated yam species to anthracnose disease. Anthracnose, caused by the fungus pathogen *Collectotricum gloeosporioides* is common in Mango (Kumari *et al.,* 2016*)* and causes huge production loss (90%) in *Dioscorea caynensis* in Nigeria (Aduramigba *et al*., 2010).

This pathogen is one of the frequently reported plant pathogens among genus *Colletotrichum* to infect a variety of host with characteristic symptoms in the world. However, application of fungicide chemicals could help mitigating the effect of this disease on yam tuber yield. There are also efforts made towards understanding of the biochemistry of host pathogen (*C. gloeosporioides*) interaction, *C. gloeosporioides* systematics, epidemiology and population genetics will lead to a much better understanding of yam anthracnose disease (Abang *et al*., 2003). The contribution of these studies for best management of the disease anthracnose was substantial enough.

Unlike bacteria, fungi and viruses are not effectively managed by chemical applications. Prevention has been used as the best approach to control virus infection. Diseases caused by viruses are a particular concern as they restrict international movement and exchange of germplasm of yams and then lead to genetic erosion.

3.6.2. Viruses infecting yam (*Dioscorea* spp.)

Viral pathogens are amongst the most important factors threatening yam production and productivity, and safe movement of germplasms. Viruses reported to have infected yams fall into six major generas: *Potyvirus*, *Potexyvirus*, *Badnavirus*, *Carlavius*, *Cucumovirus* and *Comovirus*. These viruses significantly vary in their incidence, distribution and severity on yams tuber yield and quality based on the agro-ecologies yams are grown (Yayeh *et al*., 2013). The detection for their occurrence and, the assessment for their variability in different agro-ecologies of tropics and sup-tropics is the major area of studies (Lebas, 2002).

A study by Ayo-John *et al.* (2017) on the occurrence of yam viruses in tuber and leaf samples came to reach at the conclusion that sometimes the variation can occur among viruses (*Yam mosaic virus* (YMV), *Yam mild mosaic virus* (YMMV), *Cucumber mosaic virus* (CMV) and *Dioscorea badna virus* (DBV) in the probability of their detection in leaf or tuber samples as a result of vectors activity on filed grown plants. The responses of portions of a tuber for YMV may even vary as to Aihebhoria *et al.* (2017). The virus concentration in the head of a tuber is higher than the tail as photosynthates flow down to the tuber, they carry along the virus particles (Balgun *et al*., 2017).

3.6.2.1. The genus *Potyvirus*

Potyvirus constitutes an important viruses causing severe economic damage to various crops. They are thought to be responsible for about 40% of all plant disease of virus origin. Potyviru*s* particles are fiexuous rods of 680 to 900 nm in length and contain one positive sense single-stranded genomic RNA molecule of approximately 10kb, which is encapsidated by a single type of coat protein (Hollings & Brunt, 1981; Kwak *et al*., 2015). They are characterized by a 5’ untranslated region (UTR), a large open reading fame (ORF), and a 3’ UTR. They are the largest and economically most important genus of the recognized plant virus groups and families.

The genomic RNA has a protein (VPg) covalently attached to its 5' end (Hari, 1981) and a poly(A) tract at its 3' end (Hari, 1979), and is translated into one single polyprotein, which is proteolytically processed into at least eight different proteins (Dougherty *et al*., 1985; Carrington and Dougherty, 1987; Chang *et al*., 1988; Hellmann *et al*., 1988). Additionally, Chung *et al* (2008) have reported the occurrence of a relatively small second ORF named PIPO (pretty interesting Potyvirus ORF) in frame +2 relative to the polyprotein-encoding ORF and overlapping with the P3 cistron by RNA polymerase slippage.

PIPO was found to be translated in the transframe manner P3N-PIPO, though the mechanism of expression remains unknown. More recently, another short ORF, termed PISPO (pretty interesting sweet potato Potyvirus ORF), also resulting from transcriptional slippage, was found to be embedded in the P1 coding regions of two sweet potato potyviruses (Untiveros *et al*., 2016).

Therefore, the ORF of potyvirus consists of 11 functional proteins P1 (protein 1), HC-Pro (helper component proteinase), P3 (protein 3), 6K1 (6K protein 1), CI (cylindrical inclusion protein), 6K2 (6K protein 2), VPg (viral protein genome-linked), NIa-Pro (nuclear inclusion protein a-proteinase), NIb (nuclear inclusion protein b), and CP (coat protein) (Riechmann *et al*., 1992).

Ravelonandro *et al.* (1988) found that the gene in plum pox potyvirus genome that code for capsid protein greatly differ in size than other characterized potyvirus's capsid proteins. They have also found that the low level of homology between the capsid protein amino acid sequence of plum pox potyvirus and others before characterized. The existence of great variation in the size of capsid protein of potyviruses infecting various plant species implies that the potyvirus is the largest group of viruses negatively impacting crop production in the world.

The virus is observed common in Narcissus worldwide including the Netherlands (Asjes 1996; Langeveld *et al*., 1997), the United Kington (Brunt 1980; Brunt *et al*.,

1982), India (Chandel *et al*., 2010), Australia and China (Wylie *et al.*, 2014) and Poland (Sochacki and Chojnowska, 2016).

Potyviruses infect yam foliage throughout the Caribbean and West Africa, often accounting for 25% yield losses in the region (Mohammed and Mantell, 1976; Thouvenel and Dumont, 1990). Potyvirus is the main cause for the development of several mosaic patterns in these crops (Babu *et al*., 2012).

3.6.2.1.1. Functions of potyvirus genes and infection process

The potyviral genome consists the P1, HC-Pro, P3, 6K1, CI, 6K2, NIa, NIb and CP encoding genes from the amino to the carboxy terminus. The potyvirus proteins function in genome amplification and bind RNA except for P3, 6K1 and 6K2. Proteins encoded by genes at the amino terminal region function in virus movement, whereas those at the carboxy terminal regions are involved in replication (Urcuqui-Inchima *et al*., 2001).

The viral genome linked protein (VPg) of potyviruses play an important role for potyvirus genome replication and in the maturation of potyviral poly-protein (Riechmann *et al*., 1992). The interaction of this viral protein with eukaryotic translation initiation factor (eIF(iso)4E) is suggested to have a significant functional role for potyviruses mRNA translation at the expense of host's mRNA translation (Wittmann *et al*., 1997).

It has also been suggested that the role of the VPg in the initiation of translation could be comparable to that of 5'cap structure, serving as an assembly site for the translation initiation factor complex and subsequent recruitment of 40S ribosomal subunits (Lellis *et al*., 2002). It also mediates the degradation of a gene product (SGS3), a key component of the RNA silencing pathway that functions in double stranded RNA synthesis for virus-derived small interfering RNA (vsiRNA) production with potyvirus via both the 20s ubiquitin-proteasome and autophagy pathways for suppressing RNA silencing activities of the host (Cheng and Wang, 2017). In order for potyvirus to

establish infection it must obstacle the challenge of RNA silencing by the host via this protein (VPg).

Similar to other positive-sense ssRNA viruses, potyviruses replicate through a complementary negative strand RNA molecule generated by the viral-encoded RNA-dependent RNA polymerase (RdRp); the nuclear inclusion protein b (NIb). Generation of the negative strand RNA molecule is mediated by the recognition of the 3'end of the positive strand RNA molecule by the RdRp, which probably requires the presence of specific secondary structures in the 3'NTR (Teycheney *et al.,* 2000). Once the negative strand RNA molecule has been synthesized, it is used as a template for the generation of subsequent positive-stranded RNA molecules.

Viral replication occurs in tight association with cellular membranes (Schaad *et al.,* 1997; Bienz, 1992). Various viral-encoded proteins are involved. The RNA polymerase activity of the NIb (Hong and Hunt, 1996) as well as the helicase activity of the CI (Lain *et al.,* 1990) indicate that these proteins are involved in the replication of *Potyvirus*es and the *cis*-replicative function of the NIa (Murphy *et al.,* 1996). The P1 is the amino terminal gene and encodes a trypsin-like serine proteinase that function in autocleavage from the polyprotein. The P1 is not required for viral infectivity rather it stimulates genome amplification and with HC-Pro acts as a pathogenicity enhancer by suppressing gene silencing (Urcuqui-Inchima *et al*., 2001). The functions of the P3 gene product are not well known, but it is believed to function in viral pathogenicity as shown by mutation studies and is involved in inducing the wilting phenotype in plants (Urcuqui-Inchima *et al.*, 2001).

For potyviruses to infect systemically a given host, the virus must move between adjacent cells through the plasmodesmata (cell-to-cell movement) and in the vascular tissue via the phloem (long-distance movement). Cell-to-cell as well as long-distance movement requires specific interactions between viral movement proteins (MP) and host factors. Intracellular movement of viruses from their site of genome replication into plasmodesmata facilitated by their interactions with cytoskeletal elements such as

microtubules and microfilaments (Langford, 1995; Johnston, 1995) takes place before the onset of virus movement into adjacent cells.

Thus, it is conceivable that viruses exploit the normal intracellular trafficking system of the host. It has been proposed that proteins like CP and CI localized close to the plasmodesmata (Rodriguez-Cerezo *et al.,* 1997) of potyvirus infected plants are involved in viral cell to cell movement.

There are several proteins coded by virus involved in long distance movement of viruses. In this case the virus can move from the initial infected cell to conductive cells to spread throughout the parts of the plant. The central region of the HC-Pro is essential for long distance movement. An experiment confirmed the presence of HC-Pro both in inoculated as well as non-inoculated leaves has been a clear indication of this protein plays a role in long distance movement for the virus to achieve systemic infection (Kasschau *et al.,* 1997). As obviously known to be the mechanism for a virus to use a single protein to execute multiple functions to complete its life cycle, the capsid proteins involved in RNA encapsidation, genome amplification and aphid transmission is also needed for long distance movement (Dolja *et al.,* 1995).

The expression of helper component (HP) protein in *Potyvirus* infected plant is appeared to be actively involved in aphid based transmission of the virus into healthy plant (Burger *et al*., 1989). Therefore, each of proteins encoded by potyviru*s* (mentioned below in the figure) is critical importance of the potyviru*s* genome replication and expression, virus packaging, potyviru*s* particle movement within or across cells and spread in plants.

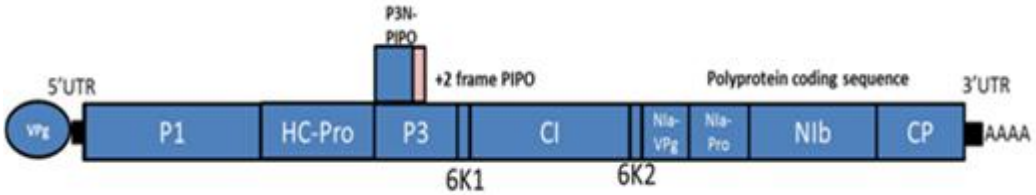

Figure 3: Schematic presentation of the potyvirus genome. A polyprotein is produced and processed into 10 mature proteins by three viral proteinases. A second ORF, *pipo*, overlapping the P3 cistron produces a fusion protein P3N-PIPO. VPg is linked covalently to the 5'-end, while a Poly (A) tail is at the 3'-end of the viral RNA (Lazaro, 2015).

3.6.2.1.2. *Yam mosaic virus* (YMV)

Yam mosaic virus (YMV) is one of the most important viruses infecting yams in sub-Saharan Africa. It was first isolated and characterized in the Ivory Coast by Thouvenel and Fauquet (1979), and has been detected throughout the yam growing regions of Africa (Goudou-Urbino *et al.*, 1996), the Caribbean and the Pacific.

It is a filamentous virus 750 nm in length, is aphid-born in the non-persistent manner and is related to the *Potyvirus* group (Thouvenel and Fouquet, 1979). The virus has been purified and a specific antiserum prepared, as a result it was discovered that the area of distribution of the disease is very large and extends beyond Africa, for the virus have been found in Caribbean and in the Pacific (Thouvenei and Fauquet, 1986).

The detailed analysis of the amino acid sequence alignment and the identity levels observed between the N-terminal part of the coat protein of the six YMV isolates has led to suggestion that they have to be considered as distantly related strains of YMV, but distinct viruses of *Potyvirus* genus (Duterme *et al*., 1996).

This is the most characterized and prevalent *Potyvirus*. The isolation and characterization of YMV isolates from infected yam *Dioscorea cayenensis* in Ivory Coast using serological and molecular techniques revealed that the virus is single strand positive sense RNA genome with high genetic diversity, and infects various species of yam (Bousalem *et al*., 2000).

Yam plants infected with this virus produces environmental dependent symptoms ranges from moderate mosaic to severe shoestring (Fatugoma *et al*., 2014). *Yam mosaic virus* in combination with *Cucumber mosaic viruses* are reported to significantly reduce the yield, quality of tuber, and number of tubers produced per yam plant in Ivory Coast (Seka *et al*., 2014). The quantitative effect of this virus on yield was a bit difficult to determine because of problems associated with the detection of the virus.

According to Thouvenel *et al.* (1990) yam mosaic disease may cause yield losses of 15% in *D. alata* cv. Florido despite the fact that this cultivar is considered to be tolerant to the disease and it was also observed during this study that yam mosaic disease does not affect the whole number of tuber-seed pieces produced by a diseased tuber. This observation has implications for the selection of planting material. Reduced capacity for photosynthesis in YMV infected plants, due to increased diffusive resistance of stomata, as well as reduced leaf area and chlorophyll content, contributed significantly to their reduced tuber yields in Ghana (Adeniji *et al*., 2012).

The economic importance of the virus is thus established as it can result in significant loss to resource-poor farmers. The virus accumulation varies according to the variety, different physiological state and parts of the infected plant (Seka *et al*., 2009). It is transmitted by insect vectors of various aphid species, mechanically and mainly through infected tuber. There are many yam infecting potyvirus isolates related to YMV both in host range and serology. This virus is synonymous with *Dioscorea green banding mosaic virus.*

3.6.2.1.3. *Yam mild mosaic virus* (YMMV)

YMMV is the second most important yam virus next to YMV (Atri *et al*., 2003). Yam mild mosaic virus (YMMV) was originally described as yam virus 1 (YV1) (Hughes, 1986) and subsequently as *Dioscorea alata virus* (DAV) (Odu *et al*., 1999). Mumford and Seal (1997) provided the first molecular evidence for the classification of YMMV as a distinct *Potyvirus*. Analysis of the nucleotide sequence of the coat protein gene of

YMMV revealed that it differs substantially from both YMV and *Japanese yam mosaic virus* (JYMV) (Fuji *et al*., 1999). This virus was detected in *D. alata* from Asia (Huges *et al*., 1986) and Africa (Huges *et al*., 1997).

As to Bousalum *et al* (2003) analyses of the distribution and the prevalence of YMMV in the Caribbean islands of Guadeloupe and Martinique, and in French Guyana revealed that YMMV has a wide reparation and different prevalence on *Dioscorea alata* L. (Asian and Oceanic origin), on *D. cayenensis* Lam. *D. rotundata* Poir. (African origin) and on *D. trifida* L. (Amazon and the Caribbean origin) in this region. The occurrence of this virus mixed together with CMV and JYMV is also confirmed in *Dioscorea* species cultivated in Korea (Lee *et al*., 2017).

Figure 4: Mosaic symptoms of yam plant at JARC by Demilew Deres (**A**), and (**B**) by Adeniji *et al*. (2011)

3.6.2.1.4. *Dioscorea alata virus* (DAV)

Dioscorea alata virus (DAV) probably occurs in all areas where water yam is grown. The virus was previously known to infect only the water yam *(Dioscorea alata*) until it was for the first time detected on *Dioscorea rotundata* in Ghana coexisting with YMV (Oppong *et al*., 2007). Symptoms in water yam range from very mild chlorosis through leaf mosaic to severe stunting though the most common symptom is a mild mosaic. This has led some authors to name the virus *Yam mild mosaic virus* (YMMV).

Sequencing part of the genome of potyviruses from yam suggests that there are two related clades of DAV, one from Africa and the other from Southeast Asia, but isolates

from both clades have been found infecting other *Dioscorea* species like the white yam *D. rotundata* (Oppong *et al*., 2007). It has proved impossible to transmit DAV mechanically by sap inoculation, though several aphid species are efficient vectors (Odu *et al*., 2001).

3.6.2.2. Genus *Badnavirus*

The *Badnavirus*es (Family: *Caulimovirideae*; Genus: *Badnavirus*) are non-enveloped bacilliform DNA viruses with a monopartite genome containing about 7.2 to 9.2 kb of dsDNA with three to seven open reading frames. They are genetically and serologically diverse genera of virus having bacilliform particles of 25–30 × 120 nm. As reviewed by Bahat *et al.* (2016) they are one of the most important plant virus groups and have emerged as serious pathogens affecting the cultivation of several horticultural crops in the tropics, especially banana, black pepper, cocoa, citrus, sugarcane, taro and yam.

Some viruses of genus *Badnavirus* are also known as endogenous viruses integrated into their host genomes and a few such endogenous viruses can be awakened, e.g., through abiotic stress, giving rise to infective episomal forms. In the Caribbean, a bacilliform virus was associated with internal brown spot disease. *Dioscorea alata badnavirus* (DaBV) and the serologically related *Dioscorea bulbifera badnavirus* (DbBV) have been detected in a range of yam species from West Africa using specific antisera.

3.6.2.3. Genus *Cucumovirus*: *Cucumber mosaic virus* (Cmv)

Cucumber mosaic virus (CMV*)*, genus *Cucumovirus*, is a species with several serologically and genetically distinct strains or subspecies. The virus particles are isometric and about 30nm in diameter. The species as a whole has a very wide host range, but there tends to be some specialization within strains or subspecies. CMV infections of yam tend to be sporadic, suggesting they arise from a chance encounter between a viruliferous vector and the yam plant.

However occasionally, CMV incidence in yam can be high locally. Strains of CMV have been reported infecting *D. alata*, *D. trifida*, and *D. rotundata* in West Africa, Caribbean, South America, and the South Pacific (Migliori and Cadilhac, 1976). There were reports that the virus was detected only in three African countries namely Guadeloupe, Ivory Coast and Nigeria (Hughes *et al*., 1997). Surveys in major yam producing agro- ecological zones in Ghana, Togo and Benin revealed, for the first time, the occurrence of Cucumber mosaic virus (CMV) infection in yam (Eny *et al*., 2008).

The detection of this virus in the other three African countries (Ghana, Togo and Benin) brought the need to develop poly clonal antibodies from yam isolates of CMV to ensure the detection of other sero-types of the CMV for which no poly-clonal antibody production was made before. The production of poly-clonal antibody against CMV enhances the monitoring and prevention of the spread of CMV infection in yams across yam growing ago-ecologies (Eni *et al*., 2010).

3.6.2.4. Genus *Potexvirus*: *Dioscorea latent virus* (DLV)

Dioscorea latent virus (DLV) is the only member of the *Potexvirus* genus known to infect yams. It is known to spread in Puerto Rico and causes no symptoms in *D. composita* or *D. floribunda* (Lawson *et al*., 1973). It can be transmitted mechanically to various herbaceous indicator species such as *N. benthamiana* and *N. megalosiphon*, again causing symptomless infection. *Dioscorea latent virus* (DLV) is often found in association with DAV, and detection is usually by ELISA using polyclonal antiserum.

3.6.2.5. Genus *Comovirus*: *Dioscorea mottle virus* (DMoV)

Dioscorea mottle virus (DMoV) belongs to the genus *Comovirus* having particles that are isometric, 20–30 nm in diameter, and a bipartite genome of single stranded RNA. *Dioscorea mottle virus* (DMoV) has been found infecting *D. alata* in Nigeria, and is likely to be distributed across West Africa. Symptoms in *D. alata* include mild chlorosis (mild chlorosis strain), mottling (mottle strain), and necrosis (necrosis strain*)*. *Dioscorea mottle virus* (DMoV) is mechanically transmissible from *D. alata*, and the natural vector is thought to be a beetle. DMoV can be mechanically transmitted to the

indicator plants *Vigna unguiculata*, *Glycine max*, *Chenopodium murale*, *C. amaranticolor*, and *C. quinoa*. Antisera are being developed for use in diagnosis.

3.7. Diagnosis of Yam Virus Disease

Swift and accurate detection of yam viruses is vital for disease management and control, but the diagnosis of yam viruses poses a number of problems. Variability of symptoms caused by changes in environmental factors, differences in the yam cultivars or varieties and/or the strains of the virus(es) make field diagnoses unreliable.

Several methods for the detection and identification of viruses in plant samples, in general, have been developed and used successfully. The choice of which method to use for a specific experiment largely depends on: sensitivity and specificity, the period of time within which the test is completed, availability of facilities and the available information about the pathogen under study. Generally, diagnostic techniques in virus detection fall into two categories due to the intrinsic properties of the virus itself.

Methods are based on the detection of coat proteins and genomic nucleic acids as enzyme-linked immunosorbent assays (ELISA) and immunoblotting (DIBA), whose virus protein limit of detection (LOD) is from 1 to 10 ng/mL of sap, and on the reverse transcription polymerase chain reaction (RT-PCR), the latter being more sensitive than other methods reaching a LOD of 1 fg/mL of virus RNA (Fenby *et al*., 1995).

In general, the different methods for the detection and identification of a pathogen can be grouped into one of the three categories: 1) bio-assay 2) immunoassay 3) nucleic acid based techniques. These methods are reviewed very briefly here, and the detail of each can be found in (Naidu and Huges, 2000; Webster *et al*., 2004; Joung *et al*., 2014 Patel *et al*., 2016; Martenelli *et al*., 2015; Kumlachew Alemu, 2015).

3.7.1. Bio-assay

Until the time when protein and nucleic acid based methods were developed visual observation and use of indicator herbaceous plants had been used for detection and identification of disease causing pathogens (De Leeuw, 1972). Symptomatology and

transmission studies were used as a biological method of yam virus disease diagnosis (Terry, 1976).

Symptoms on plants are commonly used to characterize a disease having viral etiology and for rouging of diseased plants in an attempt to control the disease. Visual inspection is relatively easy when symptoms are clear characteristic of a specific disease. However, many factors such as virus strain, host plant cultivar/variety, time of infection, and the environment can influence the symptoms exhibited (Matthews, 1980).

Besides these, symptoms may be very slight and inconclusive, or infected plants may be symptom-less as a result this method of diagnosis is neither efficient nor consistent enough to be routinely used for virus identification and detection, and are therefore not useful for the certification of yam planting materials.

However, bio-assay through the use of a series of indicator plants remains an indispensable tool for detection and identification of plant viruses and the original symptoms are still of great importance for plant virus denomination (Mulholl, 2009). These methods are still useful in the preliminary stages of research on new viruses.

3.7.2. Immunoassays (Serology)

Serology is used nearly a hundred years ago. Two types of antibodies are used in development of sero-diagnostic tests, polyclonal and monoclonal antibodies, prepared by different protocols. Polyclonal antisera are non-specific and contain antibodies to all the available epitopes on the antigen. This capability makes them useful when the nature of the antigen is unknown and less sensitive to antigen changes and provides more robust signal due to multiple epitopes detection.

Monoclonal antibodies are for serology a much deeper revolution than the polyclonal method. Their principle is: the most often the antigens, even the simplest, such as viruses, have several distinct antigenic sites and stimulate the immune proliferation in several animal clones of antibody-producing lymphocytes B, each corresponding to a particular antigenic pattern.

Immunoassays are a useful tool for detecting and monitoring virus diseases, and particularly those infecting yam, and for routine testing of yam samples for the presence of viruses. It is the best method for detection of only protein infectious agents (prions). Serological detection methods have been become a routine method for detecting plant viruses in the past few decades, and proven to be effective diagnosis methods in field and in virus vectors (Souri *et al*., 2014). It has been demonstrated that dot-ELISA gives the same result as PCR and nucleotide sequencing for *Tomato yellow leaf curling virus (*TYLCV) detection in white-fly vectors (Xie *et al*., 2013).

The most common serological tests for plant viruses include precipitation and agglutination tests, immunosorbent electron microscopy, Enzyme Linked Immunosorbent Assays (ELISA) and dot blot immunoassay. Immunoassays utilize the ability of antibodies raised in animals to bind to the virus of interest. Among the serological tools, ELISA is often the method of choice because of its high sensitivity, simplicity, reproducibility and versatility in screening a large number of samples (Cho, 1990). Also, indirect ELISA based detection of viruses is more sensitive than Dot Immunobinding Assay (DIA) and the sensitivity of DIA is suggested to increase along with increase in sample extract amount (Mervat and Fath-Allah, 2006). The enzyme-linked immunosorbent assay (ELISA) has been very popular for detection of viruses in plant material, insect vectors, seeds, and vegetative propagules since it was introduced to plant virology by Clark and Adams (1977).

Serological tests using ELISA has increased the sensitivity and specificity of detection due to the power of both poly-clonal and monoclonal antibodies produced in animals in response to the pathogens. The use of antiserum produced against the targeted virus simplifies the study to understand the serological relationship of virus isolates (Makkouk and Kumar, 1998).

ELISA works in the principle that antigens (viruses in sample extract) or antibodies are immobilized on the surface solid phase support (nitrocellulose membrane of polystreen

or micro titter plate) and to wash off the unbound antigens or antibodies from the plate or membrane, leaving only the specific substances.

The two forms of ELISA (direct and indirect) vary based on the way an antigen-antibody complex is detected, but the underlying theory and the final results are the same. In a direct ELISA procedure, the antibodies bound to the surface of the microtitre plate wells capture the virus in the test sample and the captured virus is then detected by incubation with an antibody-enzyme conjugate followed by addition of color development reagents. The capturing and detecting antibodies can be the same or from different sources. In the case of indirect ELISA, the antibody in complex with an antigen is detected with enzyme conjugated antibody.

3.7.3. Nucleic acid-based techniques

The most common nucleic acid-based technique is the polymerase chain reaction (PCR). It was first used for the amplification of ß-globulin genomic sequences for diagnosis of sickle cell anemia (Saiki *et al*., 1985), since then, it has been extensively employed for the detection and differentiation of plant pathogens (Lopez *et al*., 2003). It is the consequence of the need for an increased sensitivity and accuracy of detection methods.

Visual assessment of plants for the suspected disease usually gives almost the same result with PCR test if the typical symptom of the disease is observed in the plant. But it is worthy of confirming visual assessment using nucleic acid based tests (PCR) if the disease is not visually detected (Aihebhoria *et al.*, 2017; Njukeng *et al*., 2014).

PCR is a particularly attractive technique for the diagnosis of plant viruses because it is able to amplify the target nucleic acid starting from an extremely low concentration in a complex mixture of heterologous sequences (Henson *et al*., 1993).

The advent of PCR in the diagnosis of plant viruses has greatly improved the sensitivity of virus detection (Choi *et al*., 1999). It is also possible to use the good qualities of ELISA and PCR in-combination to take and make use of the advantages of

the two techniques at a time for detection of pathogens present in the host cell at a very small concentration (Mumford and Seal, 1997).

A single tube multiplex RT-PCR assay has been established for the simultaneous detection of *Yam mosaic virus* (YMV, genus *Potyvirus)*, *Yam mild mosaic virus* (YMMV, genus *Potyvirus)*, *Cucumber mosaic virus* (genus, *Cucumovirus*) and *Dioscorea alata bacilliform virus* (DaBV, genus *Badnavirus*) (Maroya *et al*., 2014).

3.7.4. Electrical impedance method

The development of modern multinational biologically-oriented platforms for a sensitive, reliable, and rapid detection of plant viruses play a critical role in virus disease management. Based upon the polarization properties of viruses recently techniques for the detection differentiation and identification of any viruses using electrical parameters are developed. Ahemad *et al.* (2014) has clearly demonstrated that this methodology provides a better combination of high sensitivity, selectivity, quick response, low cost, high throughput, and ease of use without the need of any bio-marker or labeling techniques.

This label free method of virus detection and identification is nowadays in wide application for the detection of viruses present at extremely low concentration from symptom-less plants and low cost. Textured silicon device consists of a droplet suspension embedding two representative purified plant viruses i.e., Tomato mosaic virus and Turnip yellow mosaic virus, put in contact with a highly hydrophobic plasma textured silicon surface and a high sensitivity of the system towards the virus particles with an interestingly low detection limit, from tens to hundreds of attomolar corresponding to pg/mL of sap, which refers, in the infection time-scale, to a concentration of virus particles in still-symptomless plants is confirmed (Ambrico *et al*., 2016). Electrical impedance was used to distinguish droplets containing DNA, amplified through PCR, from those containing DNA that had not been amplified (Simon *et al*., 2014). This technique provides a label-free scheme for detection of the success of PCR that may be easily integrated into a microfluidic device containing a module for conducting PCR.

4. MATERIALS AND METHODS

4.1. Source of Samples

Leaves from a total of 336 *Dioscorea* individual plants, with 56 from Jimma Agricultural Research Center, 93 from Bako Agricultural Research Center and 187 from Hawassa Agricultural Research Center preserved on station were collected during April to May, 2017. Samples were kept in 50 ml falcon tube containing silica gel and then transported to Holetta National Agricultural Biotechnology Research Center, Plant Molecular Biology Laboratory and subjected to ACP-ELISA test for the targeted potyvirus detection as a preliminary screening for RT-PCR test based analysis of both YMV and YMMV according to the protocol described by Eni *et al.* (2012) and Boeswelm *et al*. (2003) respectively.

4.2. ELISA test

4.2.1. ACP-ELISA

Antigen coated plate ELISA (ACP-ELISA) was carried out to primarily screen samples for potyviru*s* using *Potyvirus* genus specific antibodies (DSMZ, German Resource Center for Biological Material) following the instruction of the manufacturer and the protocol described by Clark and Admas (1997). Microtiter wells were coated with 100 µl aliquots of the test sample extracted 1/20 (w/v) in freshly prepared coating buffer. The plates were left sealed over night at 4°C. The plates were then washed with phosphate buffer saline-tween (PBST) using wash bottle, and soaked for a few minutes.

The plates were then washed three times until any vapor at the surface of the wells removed and blotted by tapping upside down on tissue paper. One hundred micro liter of the *Potyvirus* group specific MAb appropriately diluted in conjugate buffer (1:100) was added to each well of the microtiter plate. The plate was sealed and incubated at 37 °C for 2-4 hours, and washed three times. One hundred micro liter of rabbit anti mouse- alkaline phosphatase conjugate (RAM-AP) diluted 1:1000 in conjugate buffer was added to each well, and the plate was sealed and incubated at 37 °C for 1 hour. It was followed by washing three times. Two hundred micro liter aliquots of freshly prepared substrate (1mg/ ml para-nitrophenyl- phosphate in substrate buffer) was

added to each well and the plate was sealed and incubated at 37°C for 30-60 min. Then the result was assessed both by visual observation and Spectrophotometric measurement of absorbance at 405 nm.

The absorbance of each sample in duplicate was read at 405 nm using ELISA micro-titter plate reader. The average absorbance value of duplicates for a sample was calculated and considered to identify samples as positive or negative to the test virus based on Thottappilly *et al*. (1998).

4.2.2. DAS-ELISA

Samples confirmed to be positive to potyvirus were selected and tested for *Yam Mosaic Virus* (YMV) via Double Antibody Sandwich ELISA (DAS-ELISA) following the instruction of DSMZ, Germany and the protocol by Clark and Admas (1977). The kit bought from DSMZ was provided with yam mosaic virus specific antibodies used for detecting YMV in this study (**App. 2**).

4.3. RNA Extraction and cDNA Synthesis

4.3.1. RNA Extraction

The total RNA extraction was performed from samples of 100 mg -300 mg each in which potyvirus was detected using plant and fungal RNA mini column extraction kit following the instruction and the protocol of the manufacturer and Sambrook *et al*. (1989): One milli liter of RNA-XPress Reagent was added to the already ground sample and mixed thoroughly. The mixture was transferred to a 2.0 ml capped collection tube. The sample was then incubated for 5 minutes at room temperature to permit the complete dissociation of nucleoprotein complexes. Two hundred µl of chloroform per ml of RNA-XPress Reagent was then added, and the sample was covered tightly and shacked vigorously for 15 seconds. The sample was incubated for 5-10 minutes at room temperature then followed by centrifugation of the resulting mixture at 12,000 x g (13,000 rpm) for 15 minutes at 4°C.

The upper phase containing RNA was separately transferred in to 2 ml fresh centrifuge tube, and 1.0 ml of binding buffer provided in the kit was added to facilitate the selective binding of RNA to the silica membrane. The solution mixed thoroughly by

gentle pipette before transfer to 15 ml tube. Half volume (usually 775 µl) of ethanol (96-100%) was then added to this solution, and mixed immediately by gentle pipetting. Seven hundred micro liter of the solution was immediately applied to the HiElute Miniprep Spin Column (Capped) placed in a 2 ml uncapped collection tube.

The tube containing the solution was tightly closed, and centrifuged for 1 minute at 8000 x g (10,000 rpm), and the flow-through was discarded. Seven hundred micro liter of Prewash Solution (RW1) was added into the HiElute Miniprep Spin column, and then centrifuged for 1 minute at 8000 x g (10,000 rpm). Again, the flow through was discarded and 500 µl of diluted Wash Solution (WS) (DS0012) was added to the collection tube and the tube was closed gently before centrifugation for 1 minute at 8000 x g (10,000 rpm) was performed to wash the column.

The flow-through was discarded, and another 500 µl of diluted Wash Solution (WS) (DS0012) was added to the HiElute Miniprep Spin Column. The tube was closed gently and centrifuged for 2 minutes at 8000 x g (10,000 rpm). The column was then transferred to a new uncapped 2 ml collection tube, and 30-50 µl Elution Solution (RNase-Free Water) was added directly onto the spin column. This was followed by centrifugation for 1 minute at 8000 x g (10,000 rpm) to eluate, and this step was repeated until the expected RNA yield is <20 µg. Then, finally the eluate was transferred into 2.0 ml fresh capped collection tube for long term storage.

Both the quality and concentration of RNA was measured using nanodrop spectrophotometer, and RNA with the absorbance ratio of 260 to 280 nm in between 1.8 and 2.2 was selected for normalization of the concentration to 20 ng for cDNA synthesis. The RNA was then incubated at 70 ^{o}C for 5 minutes and cooled on wet ice for 2 minutes and used as a template for cDNA synthesis (Farrell *et al*., 1998).

4.3.2. cDNA Synthesis

cDNA synthesis was carried out using FIREscript RT cDNA synthesis KIT according to the manufacturer's instructions. The reaction mixture for cDNA synthesis was prepared by using 0.5µl dNTP mix (20mM), 1µl oligo (dT) primer (100mM), 1 µl

random primers (100mM), 1µl FIREscript RT enzyme, 2µl of 10x RT Reaction buffer with DTT, 0.5µl RiboGrip RNase inhibitor (40U/µl, 14µl nuclease free water and 20ng of 2.5 µl total RNA extract template of virus in 20µl final volume.

This mixture and nuclease free water were heated at 25 ^{o}C for 8 min to allow the primers anneal to the template RNA. The mixture was then heated at 50^{o}C for 25 min and finally the reaction was stopped by heating at 85^{o}C for 5min. cDNA was then kept at 4 ^{o}C for subsequent use in PCR.

4.4. RT-PCR

The RT-PCR reactions for further analysis of potyvirus detection using NIb2 primers (Table 3) were done using the protocol described by Sokhandan-Bashir *et al.* (2013).

PCR reactions were done in 25 µl containing 10 Pmol of each primer, 1 unit of *Taq* DNA polymerase (Fermentas, Lithuania), 0.2 mM each dNTP, 2 mM $MgCl_2$ and 2 µL of the synthesized cDNA. PCR with NIb2F/NIb2R was therefore performed with 94 ^{o}C for 2 minutes initial denaturation followed by: 35 cycles of 94^{o}C for 45 second, 45 ^{o}C for 45 second, 72 ^{o}C for 45 second and final extension at 72 ^{o}C for 10 minutes.

The procedure described by Eni *et al.* (2012) was followed to perform PCR using YMV primers to identify type of *Potyvirus*: initial denaturation at 94 ^{o}C for 4.5 minute followed by: 35 cycles of 94 ^{o}C for 30 second, 55 ^{o}C for 1 minute, 72^{o}C for 1 minute and then final extension at 72^{o}C for 10 minutes.

For PCR using YMMV primers (Table 3) the amplification scheme that Boeswelm *et al.* (2003) has developed was used as follow: template denaturation at 95 ^{o}C for 4.5 minutes followed by: 38 cycles of 30 s at 95^{o}C, 30 s at 55 ^{o}C, 1 min at 72 ^{o}C followed by a last elongation for 10 min at 72°C.

Finally, 6µl aliquots of the final PCR amplification product were analyzed on 1.5% agarose gel in 1%TAE, stained with gel red and photographed under UV-gel doc system. One Kb ladder (MBI fermants Inc,USA) was used as size standard.

Table 3: Primers used in this study for the detection of YMV and YMMV

Viruses	Designation	Sequences	Expected size	Source
Potyvirus	NIb2F	5‘-GTITGYGTIGAYGAYTTYAAYAA-3‘	350 bp	Sokhandan-Bashir *et al* (2013)
	NIb2R	5‘-TCIACIACIGTIGAIGGYTGNCC-3‘		
YMV	YMVF	5’-ATCCGGGATGTGGACAATGA-3’	586 bp	Mumford&Seal (1997)
	YMVR	5’TGGTCCTCCGCCACATCAAA-3’		
YMMV	YMMVF	5’-GGCACACATGCAAATGAAAGC-3’	276 bp	Mumford & Seal (1997)
	YMMVR	5’-CACCAGTAGAGTGAACATAG-3’		

5. RESULTS

Both ELISA and PCR tests of a total of 336 samples showed that the presence of potyvirus in 40 (12%) of total samples tested via ACP-ELISA. The same tests of potyvirus positive samples for yam mosaic virus indicate the presence of this virus in almost all potyvirus positive samples (30/40). No sample is found positive to *Yam mild mosaic virus* (YMMV*)* in any of the tests. Mixed infection was not found in these tests. A test is considered positive to the virus subjected to ELISA test if the absorbance at 405 nm is twice and greater than the absorbance of negative control.

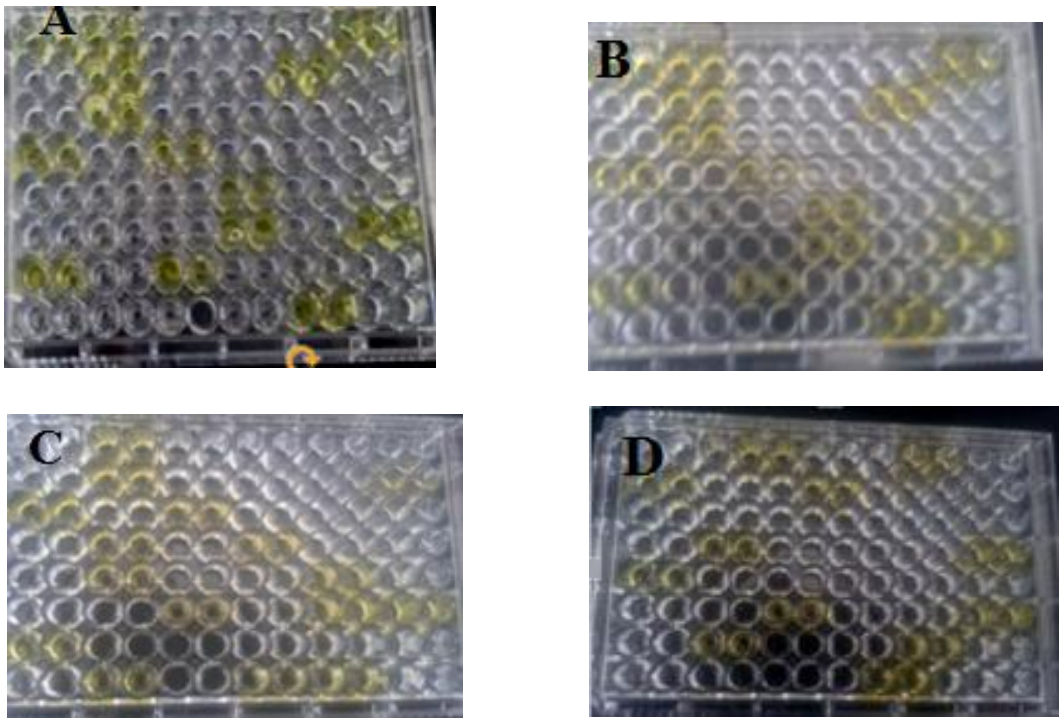

Figure 5: Results of ACP-ELISA tests (A & B) and DAS-ELISA tests (C&D)

Samples positively reacted to antibodies of potyvirus are indicated in the figure (A&B) in yellow color whereas those remained white color signifies the absence of potyvirus in their cell.

The samples positively reacted to potyvirus by ELISA were confirmed by RT-PCR, and a sample was considered positive for potyvirus and *Yam mosaic virus* and *Yam mild mosaic virus* if the specific band with the expected size of 350 bp (Fig. 6, 7) and 586 bp (Fig. 8) and 279 bp (Fig. 9) is produced respectively (Mumford & Seal 1997;

Sokhandan-Bashir *et al.,* 2013). The production of a specific band from test samples in parallel with a band from YMV positive control was also used in this study to judge whether a sample is positive or not for YMV via RT-PCR test.

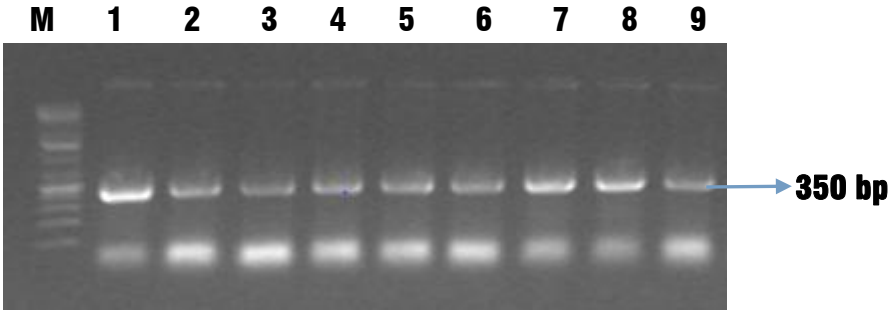

Figure 6: Agarose gel electrophoresis of RT-PCR product of *Potyvirus* infected yam samples using *Potyvirus* specific NIb2 -350 primers
M: 1Kb ladder
1-9: Test samples positive to *Potyvirus*

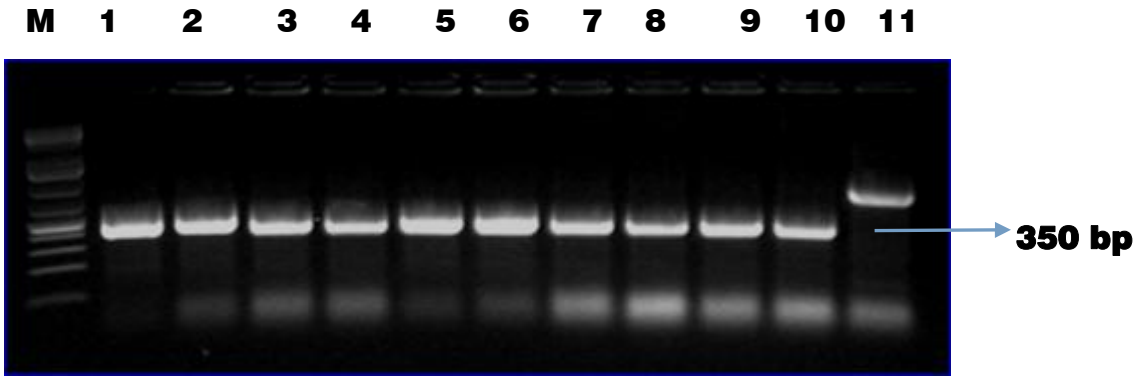

Figure 7: Gel image of RT-PCR products using NIb2-350 *Potyvirus* primers

M: 1Kb ladder

1-10: Test samples positive to *Potyvirus*

11: Test sample negative to *Potyvirus*

Figure 8: Gel electrophoresis image of RT-PCR products using YMV primers

M: 1Kb DNA ladder

1, 5, 8, 11, 10: Test sample negative to YMV

2, 3, 4, 6, 7, 9: Test samples positive to YMV

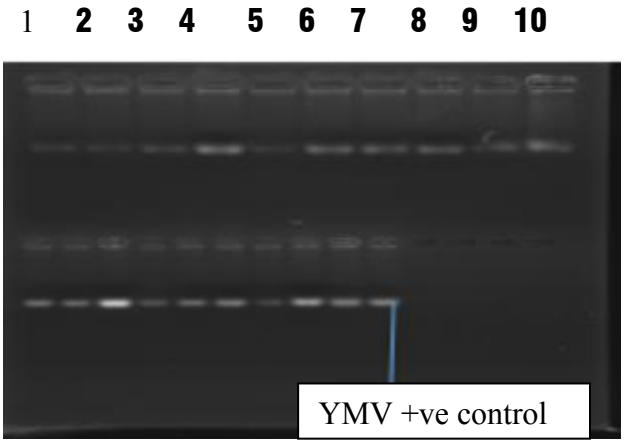

Figure 9: The top 1-10 and the bottom 1-6 are YMV +ve samples

The sample indicated by the arrow is YMV +ve control

Figure 9 is the indication of 19 samples produced a band with a size (586 bp) expected to be generated if and only if a sample is positive for yam mosaic virus. A band indicated by the arrow was used as positive control, and the samples were found positive to yam mosaic virus because they have produced a band with the same size produced from the positive control.

Among a total of 56 samples collected from Jimma Agricultural Research Center 25 of them were found potyvirus positive in ACP-ELISA test whereas only 20 of them were further analyzed and confirmed potyvirus positive in RT-PCR test. Fifteen samples of total 93 samples collected from Bako Agricultural Research Center were found

infected with potyvirus in ACP-ELISA whereas only 12 were confirmed positive to potyvirus infection in RT-PCR test. None of samples collected (187 total) from Hawassa Agricultural Research Center were found infected with potyvirus in any of the two tests. Among a total of 336 leaf samples collected from three locations (Table 4) potyvirus was found in 40 samples in ACP-ELISA test. The further analysis of those 40 samples in RT-PCR test confirmed that only 32 were infected with potyvirus. *Yam mosaic virus* was found in 30 and 38 number of samples among potyvirus was detected in, in DAS-ELISA and RT-PCR tests respectively. However, yam mild mosaic virus was found in none of samples infected with potyvirus.

Table 4: **Summarized result of both ELISA and PCR tests from three potential yam growing areas in Ethiopia.**

Locations	**Potyvirus**		**Yam mosaic virus (YMV)**		**Yam mild mosaic virus(YMMV)**	**Mixed (YMV& YMMV)**
	ELISA	PCR	ELISA	PCR	PCR	No
JARC	25/56	20/25	20/25	23/25	None	
BARC	15/93	12/15	10/15	15/15	None	
HARC	0/187	0	0	0	None	
Total	**40/336**	32/40	30	38	None	

BARC: Bako Agricultural Research Center

HARC: Hawassa Agricultural Research Center

JARC: Jimma Agricultural Research Center

6. DISCUSSION

6.1. Potyvirus Detection

In this study randomly collected yam leaf samples were tested in two subsequent steps for potyvirus detection and then the commonly reported mosaic causing potyviruse*s* in yams. The present study provides a little piece of information on the occurrence of potyviruses, particularly YMV and YMMV in major yam growing areas of South-Western Ethiopia.

Both ACP-ELISA and RT-PCR tests were used and found efficient for the detection of potyvirus as a preliminary screening of samples for YMV and YMMV detection in the present study. Potyviru*s* was detected in 40 of a total of 336 tested samples via ACP-ELISA (Table 4). But the further analysis of 40 samples reacted positively to potyvirus group specific antibodies by RT-PCR has confirmed the presence of potyvirus only in 32 samples in this study. Konate *et al*. (2016) found a result that demonstrates RT-PCR is more sensitive than ELISA. PCR and RT-PCR based tests were used to generate more reliable data about the presence of potyvirus in yam leaf samples (Sipahioglu, 2005; Liebenberg *et al*., 2009).

Potyvirus was detected in West Africa yam belt countries where the largest production of yam in the world is reported (Eny, 2008; Odedara *et al*., 2011; Oppong *et al*., 2007) and in Iran (Sokhandan-Bashir *et al*., 2013). The present study reported that potyvirus has also been found in South-Western Ethiopia. ACP-ELISA and RT-PCR tests based indexing of *Dioscorea* leaf samples using potyvirus group specific antibodies and degenerate primers discovered this virus previously in yam producing areas (Wulandari & Ermayanti, 2011).

In this study the detection of potyvirus in 40/336 samples in ACP-ELISA and 32/40 (Table 4) in RT-PCR signifies the greater sensitivity of ACP-ELISA than RT-PCR for the detection of potyvirus. But it is demonstrated that RT-PCR is more sensitive and used to produce reliable data than ELISA (Sipahioglu, 2005; Liebenberg *et al*., 2009: Konate *et al*., 2016). The possible reasons for the result sometimes to appear this way is pointed out here: (1) this may probably be due to either plant pigments have

contributed towards false positive result development in ACP-ELISA test. (2) PCR was inhibited by the metabolites of yam leaf. Lebas *et al.* (1999) obtained a result that shows the greater sensitivity of ELISA while working with yam from the South Pacific islands. The last reason may be the variability in the virus genomes (Lebas *et al*., 1999; Wilson, 1997).

In this study potyvirus is detected in samples expressed mosaic symptoms in South-Western part of Ethiopia. In Brazil the virus is known to cause mosaic and flower variegation in *Catharanthus roseus* (Macie *et al*., 2011). The virus is often associated with diseased plants showed typical symptoms of yellow mosaic without malformation of the leaves, pale green mosaic with malformation of the leaves, severe mosaic, thickened leaves or stunted and retardation of the plant growth in patchouli (*Pogostemon cablin* (Blanco) Benth.) according to Noveriza *et al.* (2012).

Thirty-two of yam samples confirmed potyviru*s* infected in this study (Table 4) are among those collected from Jimma Agricultural Research Center and Bako Agricultural Research Center. The virus has been reported in different species of crops, particularly those of asexually propagated ones using *Potyvirus* genus specific primers (Langeveld *et al*., 1991; Lunello *et al.*, 2005; Kwak *et al*., 2015; Ghasemi, 2011). This virus had also been detected in Benin, Guinea, Nigeria, Togo (Phillips *et al*., 1999; Odu *et al*., 1999; Eny, 2008).

The detection of potyvirus in samples collected from a specific area of farming or research center may be due to the use of infected planting materials to serve the production or research purpose for long period of time. The accumulation of the virus in the infected plant cells is a result of the repeated use of infected planting materials as seed. Seka *et al.* (2014) reported that virus accumulation in cells of infected yam plant results in reduction in quality and quantity by reducing the photosynthesis efficiency via leaf deformation and malformation.

Serological or PCR based diagnosis of varieties before they are delivered to farmers and also the checkup of randomly collected samples from areas of production where

released varieties are to be delivered at a certain interval may be the best way of identifying the viruses' origin and distribution area. The appearance of a band with a size out of the expected for potyvirus using potyvirus specific degenerate primers (NIb-350) but negative to YMV using YMV specific primers and YMMV is confirmed in the present study for the band to be non potyviru*s* origin. Potyvirus is detected in none symptomatic samples subjected to both ELISA and PCR test in this study.

Thus, this study provides the evidence for the essence of applying ELISA and PCR tests for samples of healthy looking samples. Similarly, Filloux and Girard (2006) have indicated that indexing and elimination of viruses infecting yams (*Dioscorea* spp.) is essential for the safe movement of germplasms**.** It is essential to test yam planting material ready either for transfer across country or plantation. (Aihebhoria *et al.*, 2017; Njukeng *et al*., 2014) have obtained the idea that it is worthy of confirming visual assessment using nucleic acid based tests (PCR) if the disease is not visually detected

6.2. YMV and YMMV Detection

The result of DAS-ELISA and RT-PCR test of samples for yam mosaic virus shows that the virus is present in 38 samples among 40 samples in which *Potyvirus* is detected. The number of samples positive to YMV test shifts from 30 by DAS-ELISA to 38 by RT-PCR. This happens when the virus (YMV) concentration in these samples may probably be too low to be detected by the maximum sensitivity level of DAS-ELISA, but the virus presents in the concentration that can be detected by RT-PCR (Mumford and Seal, 1997; Njukeng *et al*., 2005; Brunt *et al*., 1990).

Furthermore, the greater sensitivity of RT-PCR than DAS-ELISA is showed by Bernik *et al.* (2009). In the laboratories where molecular facilities to run PCR is not available, ELISA techniques like IC-PCR is recommended for a greater sensitivity and accuracy in the detection of YMV (Eny *et al*., 2012). The detection of YMV in 38 of 40 *Potyvirus* positive samples indicates that the YMV is the most likely of *Potyvirus* infecting yams in Ethiopia. The result of the present study concurs with previously reported research outputs that the YMV is the most common potyvirus infecting *Dioscorea* species across yam growing areas of tropics and subtropics, particularly

West Africa (Thouvenel and Fauquet 1979; Eny, 2009; Hughes *et al.*, 1997; Olatunde, 1999; Wulandari and Ermayanti, 2011).

Furthermore, similar results of the present findings were reported in West Africa particularly in Ghana that YMV was detected in 38% of 176 total tested yam leaf samples (Oppong *et al*., 2007), eight years after this virus had been reported to be the most frequent *Potyvirus* infecting *Dioscorea rotundata* species of yam in Ghana (Olantunde and Hughes, 1999). *Yam mild mosaic virus* (YMV) is detected in 38/40 (95%) of samples confirmed potyviru*s* positive in this study (Table 4). Despite the fact that YMV causes several symptoms including mottling, leaf and vein chlorosis, leaf mosaic, leaf distortion and malformation, shoe stringing of leaf as well as plant stunting (Odu *et al*., 2011) the detection of YMV on non-symptomatic leaf samples, shows that absence of visual symptoms on yam leaves may not be indicative of absence of virus infection, but laboratory diagnosis serves as a more sensitive and conclusive way of affirming the health status of potential breeding or planting materials (Eny, 2008).

The present study supports the suggestion made by Eny (2008), and has demonstrated the need to assess yam planting materials either ready to come to Ethiopia for various specific purposes for mainly YMV of *Potyvirus*. But YMV free certified yam planting materials cannot be guaranteed in this study to be free of other potyviruses as the detection of potyvirus in two samples finally tested negative for YMV and YMMV suggests the possible presence of potyviruses other than the two *Potyviru*ses (YMV and YMMV) for which the test is not done in this study. YMMV is detected in none of samples potyvirus is detected in. Almost all of samples found infected with YMV were not symptomatic.This may be due to the virus present in the plants at low concentration to induce the symptom but at the level of detection by RT-PCR (Njukeng *et al*., 2014).

It is also in agreement with the findings of Eny (2008) that among the asymptomatic leaf samples, 95/169 (56.2%) from Ghana and 33/57 (57.8%) from Togo, tested positive to at least one virus subjected to the test (YMV, YMMV, CMV). The result of this study supports the suggestion of Odu *et al.* (2004) that rapid, reliable and robust

screening methods for indexing yam plants are needed for the regulation of international exchange and production of virus-free germplasm. Infected but symptomless genotypes that pass through without detection may become sources of inoculum to susceptible genotypes in another locality.

7. CONCLUSIONS AND RECOMMENDATIONS

Based on the results obtained from this study the following conclusions are drawn, and recommendations are indicated.

7.1. Conclusions

In this study the occurrence of yam mosaic virus and yam mild mosaic virus in the potential yam growing area of South-western Ethiopia was investigated. These viruses reported to be common in yam growing area of tropics and subtropics were assessed for their occurrence in Ethiopia, and the high level of YMV occurrence is recorded. *Yam mild mosaic virus* (YMMV) is detected in none of samples assessed in this study, and the virus is less important as a problem than YMV for yam production in Ethiopia. The detection of YMV in samples of not symptomatic in this study is an indicative of plants of healthy looking are not necessarily free of YMV infection. It is essential to apply both ELISA and PCR based diagnostic techniques to ensure that yam plants are free of YMV infection.

The occurrence of YMV in almost potyvirus infected plants led to draw a conclusion that YMV is the most common potyvirus infecting yam plants in Ethiopia. The detection of YMV on yams cultivated in Ethiopia is a signal for yam virus disease researchers and yam breeders the need to comprehensive virus cleaning measures to clean yam planting materials from YMV. Therefore, the need to perform diagnostic tests for samples from asymptomatic yam plants for potyviruses mainly YMV at the early stage of infection is vital for the management of the disease.

7.2. Recommendations

Based upon the result of the present study the following recommendations are made.

- YMV isolates from samples tested positive for this specific virus in Ethiopia need to be characterized.
- There is a need to investigate the prevalence of YMV in major potential yam growing area of Ethiopia.
- Assessment of the disease incidence and severity of potyviruses other than YMV and YMMV and other viruses reported to be infectious of the yam plant in the world need to be done.
- Determining the quantitative effect of YMV on *Dioscorea* species in Ethiopia is important.
- There is a need to analyze resistance of *Dioscorea* species cultivated in Ethiopia to mosaic virus.
- It is needed to consider phytosanitary issues upon germplasms transfer across countries.

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9. APPENDICES

9. 1 Appendix 1: Antigen Coated Plate Enzyme Linked Immuno Sorbent Assay (ACP-ELISA) buffers and their preparation.

Buffers used

1. **Coating buffer (pH 9.6)**

   1.59 g Sodium Carbonate (Na_2CO_3)

   2.93 g Sodium Bicarbonate ($NaHCO_3$)

   0.20 g Sodium Azide (NaN_3)

- Dissolve in 900 ml H2O, adjust pH to 9.6 with HCl and make up to 1 L.

2. **PBS (pH 7.4) phosphate buffered saline**
   8.0 g Sodium Chloride (NaCl)

   0.2 g Monobasic Potassium Phosphate (KH_2PO_4)

   1.15 g Dibasic Sodium Phosphate (Na_2HPO_4)

   0.2 g Potassium Chloride (KCl)

   0.2 g Sodium Azide (NaN_3)

   - Dissolve in 900 ml H_2O, adjust pH to 7.4 with NaOH or HCl and make up to 1 L.

3. **PBS-Tween (PBST)**

   PBS + 0.5 ml Tween 20 per liter

4. **Conjugate buffer**

   PBST + 2% PVP + 0.2% egg albumin

5. **Substrate buffer**

   97 ml Diethanolamine

   600 ml H_2O

   0.2 g Sodium Azide (NaN_3)

- Adjust to pH 9.8 with HCl and make up to 1 liter with H_2O

9.2. Appendix 2: Double Antibody Sandwich Enzyme Linked Immuno Sorbent Assay (DAS-ELISA) buffers and their preparation.

Buffers used

1. Coating buffer (pH 9.6)

1.59 g Sodium Carbonate (Na_2CO_3)

2.93 g Sodium Bicarbonate ($NaHCO_3$)

0.20 g Sodium Azide (NaN_3)

- Dissolve in 900 ml H_2O, adjust pH to 9.6 with HCl and make up to 1 L.

2. PBS (pH 7.4) phosphate buffered saline

8.0 g Sodium Chloride (NaCl)

0.2 g Monobasic Potassium Phosphate (KH_2PO_4)

1.15 g Dibasic Sodium Phosphate (Na_2HPO_4)

0.2 g Potassium Chloride (KCl)

0.2 g Sodium Azide (NaN_3)

- Dissolve in 900 ml H_2O, adjust pH to 7.4 with NaOH or HCl and make up to 1 L.

3. PBS-Tween (PBST)

PBS + 0.5 ml Tween 20 per liter

4. Sample extraction buffer (pH 7.4)

PBST + 2% PVP (e.g. Serva PVP-15 Polyvinyl Pyrrolidone)

5. Conjugate buffer

PBST + 2% PVP + 0.2% egg albumin

7. Substrate buffer

97 ml Diethanolamine

600 ml H_2O

0.2 g Sodium Azide (NaN_3)

- Adjust to pH 9.8 with HCl and make up to 1 liter with H_2O

9.3 Appendix 3: ELISA and PCR pictures of samples tested for potyvirus and YMV

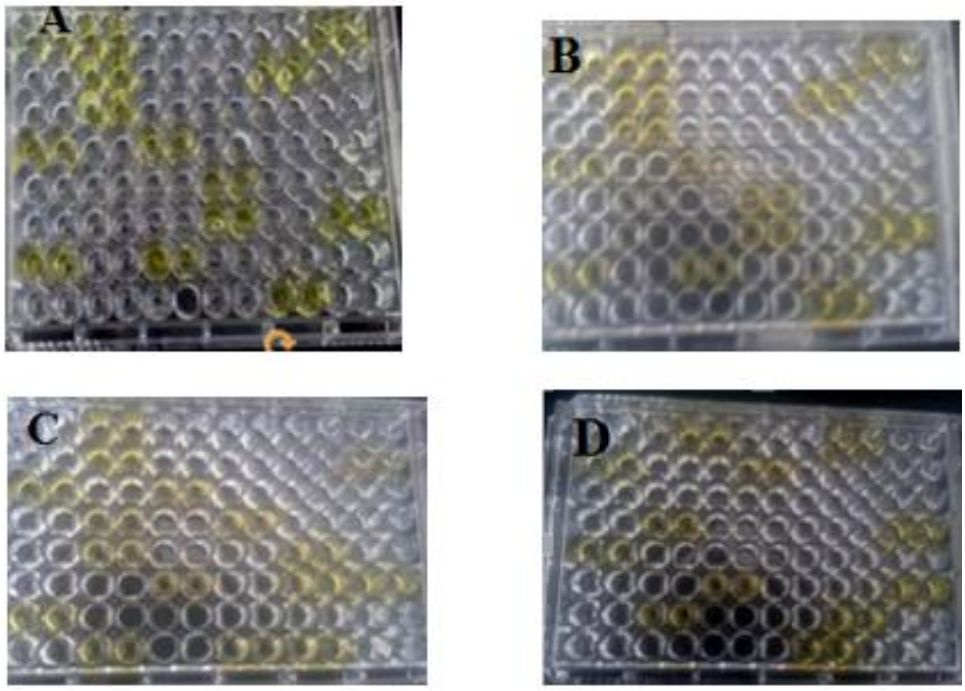

Figure 5. ELISA test results of samples for potyvirus (A and B) and YMV (C and D)

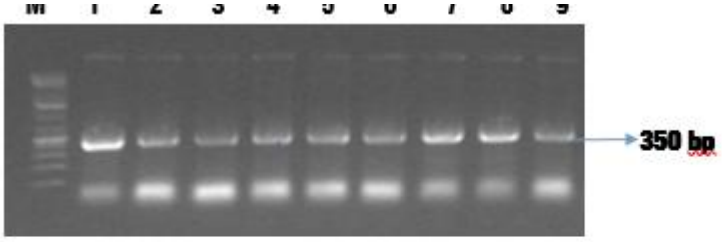

Figure 6: Agarose gel electrophoresis of RT-PCR product of *Potyvirus* infected yam samples using *Potyvirus* specific NIb2 -350 primers

M: 1Kb ladder

1-9: Test samples positive to *Potyvirus*

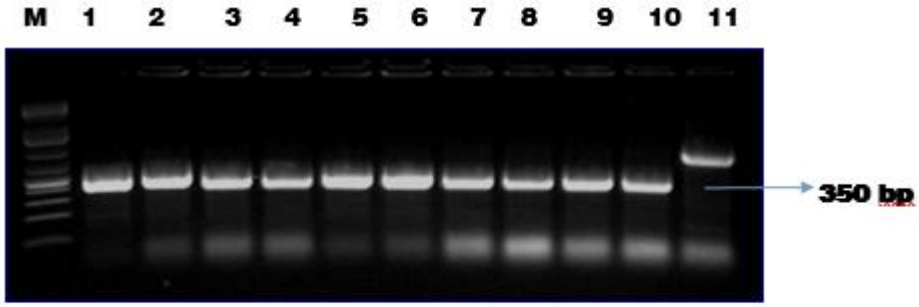

Figure 7: Gel image of RT-PCR products using NIb2-350 *Potyvirus* primers

M: 1Kb ladder

1-10: Test samples positive to *Potyvirus*

11: Test sample negative to *Potyvirus*

Figure 8: Gel electrophoresis image of RT-PCR products using YMV primers

M: 1Kb DNA ladder

1, 5, 8, 11, 10: Test sample negative to YMV

2, 3, 4, 6, 7, 9: Test samples positive to YMV

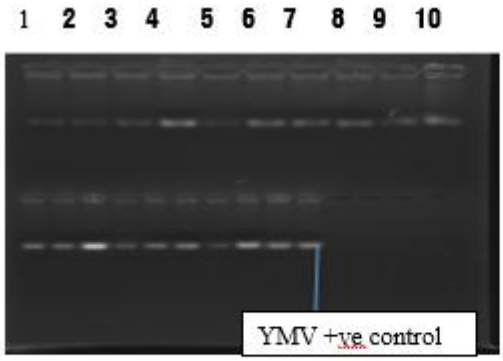

Figure 9: The top 1-10 and the bottom 1-6 are YMV +ve samples

The sample indicated by the arrow is YMV +ve control